Targeted Application of Dietary Fibers for Selective Modulation of the Gut Microbiota and Improved Human Health

By

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ABSTRACT

Obesity and associated comorbidities have reached epidemic proportions worldwide. Observational studies provide consistent evidence that plant-based diets rich in dietary fibers (DFs) reduce chronic disease risk. Mechanistic studies have established processes by which DFs improve health, with one emerging mechanism being the modulation of gut microbiota composition and its functions. However, results from human interventions with purified DFs remain inconsistent with extensive between-study heterogeneity. Therefore, questions remain as to whether DFs can exert reliable health effects when a reductionist approach is used with purified DFs, what the efficacious doses are, and if such doses are tolerable. The overall goal of this dissertation was to explore the potential of purified DFs to improve human health through more targeted approaches. This was achieved by the following objectives.

The first objective of this dissertation was to summarize the effects of purified DFs on immunometabolic disease markers in humans and consider the role of DF dose, physicochemical properties, intervention duration, and the placebo. Systematic review of 77 publications revealed that purified DFs reduced markers of insulin resistance and cholesterol in 36-49% of interventions, while <20% of interventions reduced dysglycemia and inflammation measures. A higher proportion of interventions showed an effect if they used higher doses for C-reactive protein (CRP) and total cholesterol (40-63%), viscous and mixed plant cell wall DFs for total cholesterol (>50%), and longer durations for CRP and glucose (50%). Although additional research is needed, a more targeted application of purified DF with specific physicochemical properties at higher doses and for longer durations shows promise for improved clinical efficacy.

As both the dose and physicochemical properties of DF were relevant to health, the second objective was to characterize the effects of high doses of soluble, fermentable

arabinoxylan (AX) on fecal microbiota composition and short-chain fatty acids (SCFA), as compared to insoluble, non-fermentable microcrystalline cellulose (MCC), and integrate findings using an ecological framework. Using a randomized controlled design, we showed that AX exerted global shifts to bacterial community composition (AX-vs-MCC: baseline p=0.17, week 6 p=0.019, PERMANOVA), promoted *Bifidobacterium longum* and *Prevotella copri* (q<0.15, Wilcoxon test), and increased propionate (p=0.012, Friedman's test); bacterial taxa and a SCFA previously linked to immunometabolic benefits. SCFA responses to AX were individualized and linked to compositional shifts and its baseline composition (q<0.05, MLR models), providing evidence that such responses might be predictable.

The third objective of this dissertation was to evaluate the gastrointestinal tolerance of fermentable AX at efficacious amounts relative to non-fermentable MCC and systematically investigate links to fecal microbiota and diet. This study showed that AX increased symptoms during the first three weeks of supplementation relative to MCC (p<0.05, Mann-Whitney tests), but subjects 'adapted' with symptoms reverting to baseline levels towards the end of treatment. Adaption responses were individualized and correlated with the relative abundance of *B. longum* at baseline (r_s=0.74, p=0.002), within the bacterial community that actively utilized AX *ex vivo* (r_s=0.69, p=0.006), AX-induced shifts in acetate (r_s=0.54, p=0.039), and greater habitual consumption of meat/meat alternative relative to whole grains (r_s=-0.54; p=0.042) and cholesterol (r_s=-0.58; p=0.027). These findings provide a basis for the development of strategies for improved tolerance of efficacious DF doses.

The final objective was to determine if specific doses of discrete DF structure could be used to direct changes in fecal microbiota composition and its output of beneficial SCFAs. Using a dose-response trial with three type-IV resistant starches we found that crystalline and

phosphate-cross-linked starches induce divergent effects on the gut microbiota, promoting either *Eubacterium rectale* (q=0.007, two-way repeated measures ANOVA) and butyrate (p=0.05) or *Parabacteroides distasonis* (q=0.005) and propionate (p=0.04), respectively. These effects were dose-dependent plateauing at 35 g/day and remarkably consistent with respective *E. rectale* and *P. distasonis* enrichments detected in all subjects. Overall, these findings support the potential of using discrete DF structures to achieve targeted manipulations of the gut microbiota and its functions relevant to health.

Together findings in this dissertation provide evidence that purified DFs could exert more reliable effects in humans; however, targeted approaches are needed that apply higher doses of specific DF structures and consider individualized responses. Findings also provide a basis for the development of more precise nutritional strategies based on purified DFs that selectively modulate the gut microbiota and improve immunometabolic outcomes.

PREFACE

This doctoral dissertation is an original work by Edward C. Deehan. The research projects, of which this thesis is a part, were conducted at the University of Alberta and received research ethics approval from the University of Alberta Health Research Ethics Board **(Chapters 4 and 5**: Project Name 'The Alberta FYBER (Feed Your gut Bacteria morE fibeR) Study', Pro00050274, 10/31/2016; **Chapter 6**: Project Name 'Gastrointestinal Assessment of Three Novel RS4', Pro00069884, 08/18/2017). These projects were supported by funding from Ingredion Incorporated, a joint European Research Area and Joint Programming Initiative-A Healthy Diet for a Healthy Life (ERA-HDHL) grant (Biomarkers for Nutrition and Health; The FiberTAG Project), the Canadian Institutes of Health Research, and the Campus Alberta Innovation Program. The doctoral program was also supported by the Queen Elizabeth II Graduate Scholarship, the Frederick Banting and Charles Best Canada Graduate Scholarship, the Walter H. Johns Graduate Fellowship, the Dr. Michael E. Stiles Graduate Scholarship in Applied Microbiology, the Fisher Scientific Graduate Scholarship, the Elizabeth Russell MacEachran Scholarship, and the Anthony Fellowship in Human Nutrition.

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A.M.A. and E.C.D. are co-first authors thus contributed equally to this work, while E.C.D. and J.W. are co-corresponding authors. A.M.A. developed the systematic review protocol, performed eligibility screening and data extraction, performed stratification analyses, wrote the initial manuscript, and had primary responsibility for the final manuscript. E.C.D. designed the research, developed the systematic review protocol, performed eligibility screening and data extraction, performed stratification analyses, supervised the project, and wrote the initial manuscript. J.V.T. and S.J.H. developed the systematic review protocol. J.W. designed the research, developed the systematic review protocol, supervised the project, wrote the initial manuscript, and had primary responsibility for the final manuscript.

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analysis. C.M.P. developed methodology, obtained grant funding, and supervised the human trial. J.W. conceptualized the idea, developed methodology, obtained grant funding, supported the fecal microbiota and statistical analyses, supervised the project, and wrote the manuscript. All authors edited the final manuscript.

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methodology, obtained grant funding, supported statistical analyses, supervised the project, and wrote the manuscript.

Dedication

To my wife Ana Rose and my children Edmond and Rosalie,

I am sincerely grateful for your constant support throughout this journey.

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LIST OF ABBREVIATIONS

AICc	Akaike information criterion
ANOVA	Analysis of variance
AUC	Area under the curve
AX	Arabinoxylan
AXOS	Arabinoxylan oligosaccharide
BMI	Body mass index
BCFA	Branched short-chain fatty acid
C-DHQ II	Canadian diet history questionnaire II
CLR	Centered log-ratio
CNCD	Chronic noncommunicable disease
CARG	Co-abundance response group
CRP	C-reactive protein
DF	Dietary fiber
FDR	Benjamini-Hochberg's false discovery rate
GEE	Generalized estimated equation
GOS	Galactooligosaccharides
GI	Gastrointestinal
GLP-1	Glucagon-like peptide-1
GNG	Gluconeogenesis
HOMA-IR	Homeostatic model assessment of insulin resistance
HDL-C	High-density lipoprotein cholesterol
IL-6	Interleukin 6
ISAPP	International Scientific Association for Probiotics and Prebiotics
IGN	Intestinal gluconeogenesis
LPS	Lipopolysaccharide
LDL-C	Low-density lipoprotein cholesterol
NDC	Non-digestible carbohydrate
NDFC	Non-digestible fermentable carbohydrate
MAC	Microbiota-accessible carbohydrate

MCC	Microcrystalline cellulose
MLR	Multiple linear regression
OTU	Operational taxonomic unit
PYY	Peptide tyrosine tyrosine
PC	Principal component
PCA	Principal component analysis
PERMANOVA	Permutational multivariate analysis of variance
rANOVA	Repeated-measures analysis of variance
RCT	Randomized controlled trial
RS	Resistant starch
RS2	Resistant starch type-II
RS3	Resistant starch type-III
RS4	Resistant starch type-IV
SCFA	Short-chain fatty acid
TC	Total cholesterol
TG	Triglycerides

CHAPTER 1: Literature Review

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Subchapter 1.1: The Fiber Gap and the Disappearing Gut Microbiome: Implications for Human Nutrition

1.1.1 Lifestyle-Induced Microbiome Depletion and its Implications for Health

Humans have evolved with dense microbial populations that colonize their gastrointestinal tract and are integral to our biology, for example, through the provision of signals that aid the development of the immune system. There is convincing evidence from research in animal models that a disruption of this host-microbiome symbiosis leads to an increase in immune-mediated pathologies related to chronic non-communicable diseases (CNCDs), such as obesity, cardiovascular disease, colon cancer, allergies, other atopic diseases (including asthma), autism, and autoimmune diseases[1]. The role of the gut microbiome in CNCDs is difficult to test in humans, but disease risk is epidemiologically linked to practices that disrupt the establishment of the gut microbiota early in life (such as caesarean sections, antibiotics, formula feeding), and pathologies are often associated with an aberrant microbiome. Importantly, most CNCDs have increased substantially within the past decades, suggesting that modern lifestyle might have led to a loss of bacterial symbionts that are protective[2]. In fact, comparisons of the gut microbiota in unindustrialized rural human communities from South America, Africa, and Papua New Guinea (which generally have a low prevalence of CNCDs)

with that of communities in the USA and Europe provide compelling evidence for a substantial decline of gut microbiome diversity through industrialization[3].

1.1.2 A Low-Fiber Diet is a Key Driver of Microbiome Depletion

It is likely that a combination of factors (antibiotics, modern clinical practices, sanitation, dietary habits) have caused the decline in gut microbiome diversity. However, the only factor that has been empirically shown to be important is a diet low in microbiota-accessible carbohydrates (MACs), which are indigestible dietary carbohydrates that become available to the microbes that colonize the intestine. Research in mice showed that feeding a diet low in MACs substantially depleted gut microbiota diversity over the duration of only a few generations[4]. Intake of dietary fiber (DF), which is the main source of MACs in the diet of adult humans, is negligibly low in the Western world when compared with both the diet consumed in non-industrialized societies and that of our ancestors[5]. Such a low-fiber diet provides insufficient nutrients for the gut microbes, leading not only to the loss of species reliant on these substrates but also to a reduction in the production of fermentation end products with important physiological and immunological functions[6]. In other words, by shifting to a diet that is fundamentally different to the diet under which the human-microbiome interrelationship evolved, we might have disrupted this symbiosis, reducing or removing the evolutionary routed benefits provided by the microbes. The notion that this process might have contributed to the rise of CNCDs and a substantial degree of morbidity and mortality provides a strong incentive to consider attempts to conserve and potentially restore the gut microbiome.

1.1.3 DF Can Increase Microbiome Diversity and Prevent CNCDs, but Consumption Is Not Sufficient

DF and whole grains have been shown to increase diversity of the human fecal microbiota[7,8]. Epidemiological studies further consistently show significant inverse associations between DF intake and microbiome-associated CNCDs, and all-cause mortality, and research in animal disease models supports a beneficial role. Human intervention studies are often inconclusive, but inconsistencies may stem from a variety of reasons that have not yet been sufficiently considered. First, inter-individual differences in composition of the gut microbiome, which is especially pronounced in industrialized societies[9], results in an individualized response

to DF that may increase variation in the findings from human trials[10]. Second, most human intervention studies are performed with daily amounts of DF that are much lower than those consumed by our ancestors, and therefore might not lead to detectable physiological changes. Accordingly, moving African Americans to a traditional South African diet with a daily dose of 55 g of DF was efficient to improve markers of colon cancer within two weeks[11].

The available data indicate a considerable potential for DF to elevate microbiome diversity and prevent CNCDs, but consumption is on average only half of what is recommended in dietary guidelines, which is referred to as the 'fiber gap'[12]. Given that host-microbiome symbiosis evolved with a diet that contained substantially more DF than what is currently recommended, the real 'fiber gap' for optimal health and conservation of microbial diversity might be even larger than currently appreciated[5]. These evolutionary considerations, the appreciation of the gut microbiome's role in human health, and especially the recent findings on the 'disappearing microbiome', now provide a clear incentive for society-wide efforts to fundamentally change the Western diet and significantly increase the consumption of DF.

1.1.4 What Can Be Done to Conserve and Restore the Human Microbiome?

Virtually all nutritional organizations encourage consumption of DF. Despite these efforts and a general understanding of the benefits, average DF intake remains low – thus changes in dietary recommendations alone are unlikely to result in significant changes in consumer behavior. What is necessary is an integrated effort that involves academics, the food industry, economics, nutritional policy makers, and regulatory organizations with the goal to systematically enhance the DF content of the food supply. In the USA, white flour provides the largest portion of DF, even though it is a vastly suboptimal source, with around 80% being removed during processing[13]. Fiber supplementation of white flour and white flour-rich food products, which are 'overused' and the dominant component of the Western diet, therefore constitute an untapped opportunity to considerably increase DF consumption. A variety of purified DFs and fiber-rich raw materials, as well as prebiotics with established MACs, are already commercially available to produce DF-enriched food products (**Table 1.1**). Several of these DFs (such as acacia gum, polydextrose, resistant starches, and soluble corn fiber) have been shown in human trials to be well tolerated at daily doses of at least 50 grams and could therefore be used to substantially enhance DF consumption without adverse effects.

What is needed are clinical evaluations of fiber types, mixtures thereof, and acceptable and palatable fiber-enriched food products and diets through well-designed and rigorous randomly controlled trials with clear health- and microbiome-related outcomes. Unfortunately, regulatory policies, especially in the USA and Canada, make it extremely difficult to obtain disease-oriented health claims for food ingredients or products and to communicate those to the consumer, discouraging research and product development in this area. The preservation of the microbiome will require regulatory policies specifically for foods and independent to those for drugs that, while aspiring for stringent scientific standards, permit innovative research and an effective communication of validated health benefits to society. However, even if such attempts were successful, the additional costs associated with high-DF diets would still likely prevent them from being broadly embraced, as indicated by the inverse relationship between socioeconomic status and DF intake[14]. One avenue could be to subsidize food products with established health benefits. If done right, the costs associated with promoting a healthy diet are likely low compared with the healthcare expenditures that could be saved[15].

Although a strategy to boost the consumption of MACs alone would likely be beneficial and could be immediately implemented, this might not restore microbiome diversity completely without parallel efforts to reintroduce microbes that were lost through industrialization[4]. The latter could be achieved through probiotics (food) or live biotherapeutics (drugs). The development of such strategies will be challenging from a safety perspective, and it will likely take decades (and adjustments in the regulatory policies) for the first products to be implemented. Even if such products would become available, microbes can only be 'reintroduced' if humans consume a diet that supports their growth, providing another rational to envisage a fundamental change to human diet as described above.

1.1.5 Closing Thoughts

The depletion of the gut microbiome might well be one of the 21st century challenges to modern society as it is likely to contribute to growing disease pandemics, with clear implications for public health[1], clinical practices[2], and human nutrition[6]. There are already avenues available to enrich the food supply with DF (**Table 1.1**) in an attempt to restore composition and function of the gut microbiome. However, their successful implementation will require a society-wide effort and essentially a transformation of human nutrition away from a discipline that focuses

merely on meeting the nutritional needs of the human host to one that is concerned with also nourishing the symbiotic microbial communities that are so essential in health.

Table 1.1. A non-exhaustive list of dietary fibers available on the market, product names, and food products in which these fibers could be used

Dietary Fibers	Fiber Products	Potential Food Products
Resistant starch	ActiStar® RM Fibersym® RW [a] Hi-MAIZE® 260 [a] PENFIBE® RS4	Flour-based foods, breads, pastries, pasta, snacks
Arabinoxylan	Biofiber Gum NAXUS®	Flour-based foods, beverages, soups/sauces
β-Glucan	B-CANTM PromOat® [a] Wellmune® Yestimun®	Flour-based foods, beverages, soups/sauces
Cellulose	GRINDSTED® MCC MICROCEL Solka-Floc® Vitacel®	Flour-based foods, dairy products
Inulin/oligofructose	Actilight® [a] Frutalose® L90 [a] NUTRAFLORA® [a] Oliggo-Fiber® DS2 [a] Orafti® Synergy1 [a]	Beverages, confectionery, preserves, dairy products, flour-based foods, soups/sauces
Galactooligosaccharide, xylooligosaccharide	Bimuno® [a] BIOLIGO® GL Vivinal® GOS [a] Longlive XOS [a] NovaGreen XOS	Beverages, confectionery, preserves, dairy products, flour-based foods, soups/sauces
Human milk oligosaccharides	Mum's Sweet Secret Glycom	Infant formula
Polydextrose	STA-LITE® [a] Litesse® II [a] NUTRIOSE® FB [a]	Beverages, confectionery, preserves, dairy products, flour-based foods, soups/sauces
Soluble corn fiber	PROMITOR® [a]	Beverages, confectionery, preserves, dairy products, flour-based foods, soups/sauces
Alginate	AlgogelTM [a] KIMICA ALGIN Manugel DMB [a]	Beverages, confectionery, preserves, dairy products
Pectin	Citrus Pectin USP GENU® Pectin C74 [a] Unipectine®	Confectionery, preserves, dairy products

Gum arabic/acacia gum	Agri-Spray Acacia® EmulGold® [a] FibregumTM [a] Gum Arabic SD	Beverages, confectionery, preserves, dairy products, soups/sauces
Guar gum	GuarNT® Ricol Rg-250 ViscogumTM Guar Gum [a]	Confectionery, preserves, dairy products, soups/sauces
Fiber-rich raw materials	'Best' Pea Fiber [a] Corn Z-Trim® Cranberry Fiber Fibrex® Sugar Beet [a] FIBRIM® Soy Unicell® WF	Beverages, confectionery, preserves, dairy products, flour-based foods, soups/sauces

[a] Dietary fiber with established microbiota-accessible carbohydrates (MACs).

Subchapter 1.2: Modulation of the Gastrointestinal Microbiome with Non-digestible Fermentable Carbohydrates to Improve Human Health

1.2.1 The Gastrointestinal Microbiome and Chronic Non-Communicable Diseases

Vertebrates have evolved with dense microbial populations in their gastrointestinal (GI) tract (referred to as the GI microbiome) that contribute to the performance and health of the host[16]. Although symbiotic in nature, animal experiments have established that the GI microbiota plays a causative role in the development of chronic non-communicable diseases (CNCDs), such as obesity, diabetes, cardiovascular disease, colon cancer, autism, autoimmune diseases, allergies, and other atopic diseases including asthma (**Figure 1.1**)[17]. CNCDs are often associated with microbial dysbiosis, which is typically characterized by a reduced diversity, a bloom of facultative taxa (such as enterobacteria), and a lower output of beneficial metabolites[18]. These associations provide a clear rationale for the development of strategies that modulate GI microbiome structure and function for the prevention of CNCDs[19].

Various strategies have been developed for the introduction of live microbes to modulate the GI ecosystem, through either probiotics, live biotherapeutics, or fecal transplants. These approaches have generated tremendous interest[20]. However, diet has also been shown to readily alter GI microbiome structure and function[21-23], making it a particularly promising modifiable lifestyle factor of interest for the treatment of CNCDs. In addition, dietary supplements that employ non-digestible carbohydrates (NDCs) have been developed for several decades as prebiotics, targeted to support the growth of beneficial GI microbiota in an attempt to improve health[24].

Interestingly, the incidence of most microbiome-associated CNCDs has substantially increased in recent decades in industrialized countries[25,26], suggesting that practices associated with industrialized lifestyles predispose to disease. Although the exact factors that drive disease development are unknown and likely complex, the lack of NDCs is one contributing factor[27,28]. Humans likely evolved consuming more than 100 grams of NDCs daily, and nonindustrialized communities still exist today that have intakes that parallel those of our ancestors[29]. In the 1970s, Denis Burkitt and colleagues compared industrialized (USA and UK) and nonindustrialized (rural Africa) populations, and found strong epidemiological links between urbanization and a reduction of dietary NDC intake[28,30]. These lifestyle shifts were associated with an increased

prevalence of CNCDs[28,30]. Recent research demonstrated that a diet low in NDCs that are accessible to the GI microbiota (*i.e.* non-digestible fermentable carbohydrates [NDFC]) resulted in reduced production of fermentation end products that provide important physiological and immunological functions to the host[6,31]. Moreover, a diet low in NDFCs has been implicated in the depletion of microbiome diversity observed in industrialized societies[9,32-35] and the loss of bacterial species that rely on them for growth[4]. Therefore, a possible explanation for the rise in CNCDs is that by shifting away from a diet in which our human-microbiome interrelationship evolved, we have essentially disrupted this symbiosis, ultimately reducing or removing the evolutionary routed benefits provided by the microbes[27].

The connections described above provide a rationale for the application of NDFCs to modulate the composition and/or function of the GI microbiome to benefit host health. In **Chapter 1.2**, we discussed i) the concepts by which the GI microbiome can be modulated through the intake of NDCs that are fermentable (or accessible to the microbes), ii) the effects of these strategies on the GI microbiota, iii) the mechanisms by which these strategies promote health, and iv) the future research needed to optimize these strategies for their use in the field of human nutrition. Specific attention is given to strategies that would allow for the systematic increase of NDFCs in the human adult diet by means of supplements such as prebiotics, fermentable dietary fiber (DF), and microbiota-accessible carbohydrates (MACs), and not through whole food sources such as fruits and vegetables. Supplemental NDFCs could provide a promising avenue for targeted modulation of the GI microbiota once they are understood at a mechanistic level[36]. They could also provide an applicable strategy for increasing NDFC consumption within the context of an industrialized lifestyle, since current whole-food-focused strategies encouraged by nutritional organizations have shown little success in increasing NDFC intake[12,37]. Furthermore, even though NDFCs such as human milk oligosaccharides are important for infant growth and development and have tremendous potential to be included in infant formula[38], **Chapter 1.2** will focus on the application of NDFCs in weaned children and adults.

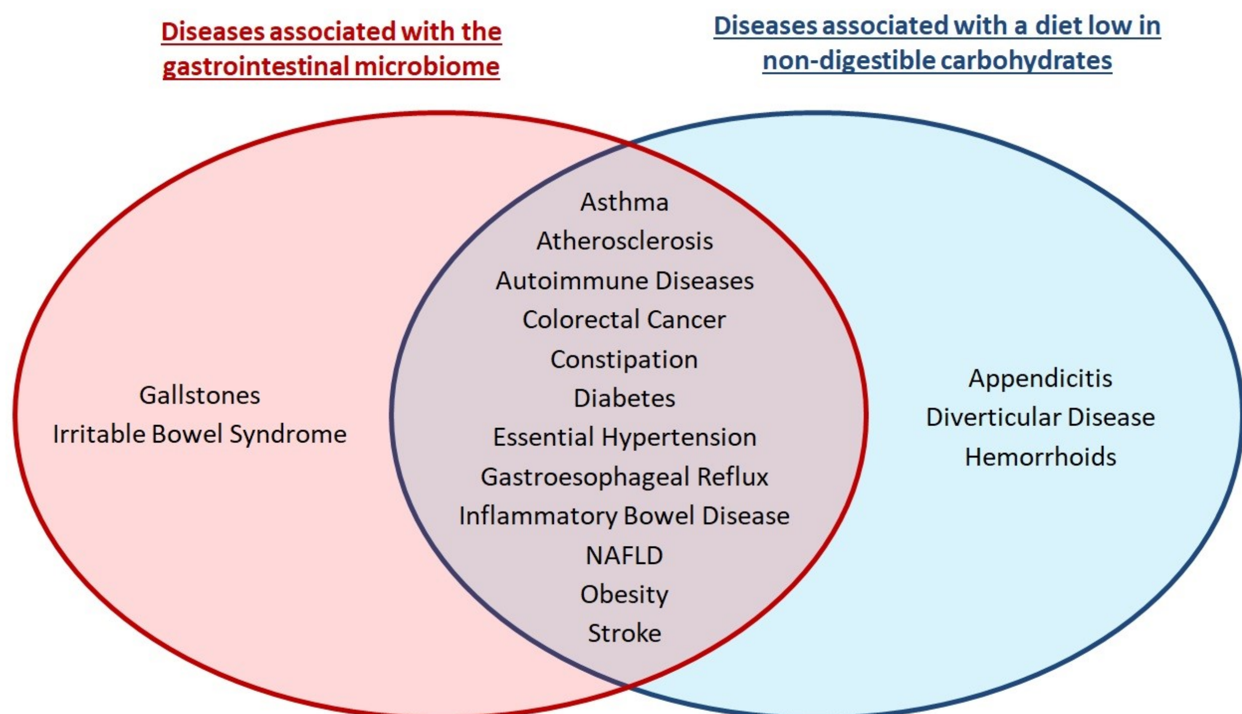

Figure 1.1. Chronic non-communicable diseases (CNCDs) that are associated with the gastrointestinal (GI) microbiome and diets low in non-digestible carbohydrates (NDCs). An industrialized lifestyle is associated with an increased prevalence of multiple CNCDs[30]. Most of these diseases have now clearly been associated with the GI microbiome (pathology in animal models is dramatically different under germ-free conditions, and the GI microbiome displays a dysbiosis in humans suffering from the disease). The Venn diagram designates CNCDs that are associated with the GI microbiome[17,39-41] and a diet low in NDCs[28,42,43]. NAFLD, nonalcoholic fatty liver disease.

1.2.2 Modulation of the GI Microbiome through NDFCs: Three Concepts

1.2.2.1 Prebiotics

Although the potential to modulate the human GI microbiome through NDFCs had been recognized decades earlier (especially in Japan), the term 'prebiotics' was first coined by Gibson and Roberfroid in 1995. A prebiotic was initially defined as a 'nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria already resident in the colon, and thus attempt to improve host health'[24]. The definition has been adjusted various times[44], with the International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus panel proposing the most recent definition: 'a substrate that is selectively utilized by host microorganisms conferring a health benefit'[45]. Currently, the most commonly used definition is 'a selectively fermented ingredient

that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health'[46]. Although, most definitions do not restrict prebiotics to carbohydrates, currently only lactulose, inulin-type fructans, and trans-galactooligosaccharides are considered prebiotics[46]. Polydextrose, glucooligosaccharides, lactosucrose, soybean oligosaccharides, and xylooligosaccharides have been further proposed as 'candidate prebiotics'[46]. However, the criteria that these carbohydrates would have to fulfill to move beyond the status of mere candidates have not been clearly established[44].

To date, most definitions, including the recent definition brought forward by the ISAPP consensus panel[45], require that prebiotics have to be 'specific' for or 'selective' towards health-promoting taxonomic groups. Conceptually, the idea is to shift the GI microbial community towards a more 'healthy' state. This concept is derived from early findings from culture-based and later probe- and primer-based studies that found bifidobacteria and lactobacilli (putatively health-promoting organisms) to be selectively stimulated through prebiotics such as inulin and galactooligosaccharides[47]. However, even though there is a strong rationale to specifically target the beneficial components and functional attributes of the GI microbiome, the prebiotic concept as it currently stands has been repeatedly criticized for being poorly defined[44,48,49] and/or scientifically outdated[44,50,51]. Some scientists have suggested revisions to the concept[51], while others consider it completely obsolete[20,50]. Criticism is primarily focused on the concept of 'selectivity' and the question of how to identify beneficial microbiota that should be targeted[44,51].

First, based on the most current scientific understanding, it is too simplistic to categorize GI microbes as either 'good' or 'bad'. Microbes can possess both beneficial and detrimental traits, strains of one species can differ widely in their attributes, and their role is highly context-dependent (*e.g.* host genetic predisposition, host physiology, GI microbial ecology, diet, etc.). Second, it has been argued that the specific targets should go beyond that of *Bifidobacterium* and *Lactobacillus* species, because many bacterial genera (*Akkermansia, Eubacterium,* and *Faecalibacterium)* have been linked to health benefits[8,52-54], including taxa previously considered to be detrimental, such as *Clostridia* and *Bacteroides*[55,56]. Third, given that the GI microbiota functions as a complex community, it may be imperative to support community characteristics, such as diversity, stability, and ecosystem functionality (*e.g.* short-chain fatty acids [SCFAs] output), which have all been positively correlated with health[57-59]. To this end, the formation of SCFAs does not rely on selective fermentation. Moreover, although some strains of

bifidobacteria and lactobacilli have been reported to produce butyrate and propionate[60], which are the SCFAs with the most evidence for health effects[31], from the metabolism of amino acids, the amounts produced (<150 μM) are less than 1% of what bacteria produce from the fermentation of carbohydrates[60,61]. Bifidobacteria and lactobacilli lack the biosynthetic pathways to produce butyrate and propionate from the fermentation of carbohydrates[62-65] and therefore cannot be the target of prebiotics that aim at boosting these SCFAs. Fourth, human studies using next-generation sequencing have shown that the response of the GI microbiome to NDFCs that are currently regarded as prebiotics, such as inulin, is not as selective as previously believed[66], while NDFCs that were considered to be broadly fermented result in restricted shifts of the GI microbiome[67-69]. In this respect, it is important to consider that no carbohydrate is fermented solely by one or two species (partially because bacterial traits are shared between bacteria through horizontal gene transfer[70]), and no carbohydrate is broadly fermented, especially not under the competitive conditions within the GI tract[71]. So the recent proposition by the ISAPP consensus panel that selectivity 'could extend to several microbial groups, just not all'[45], would essentially mean that any carbohydrate would qualify as a prebiotic.

The notion that carbohydrates currently accepted as prebiotics are not utilized differently by the GI microbiota when compared to 'regular' DFs has recently been demonstrated using *in vitro* fecal fermentations of inulin (a well-described prebiotic) and pectin (not considered a prebiotic)[72]. Both carbohydrates induced multiple substrate-specific compositional shifts. The fermentation of inulin resulted in the increased abundance of five taxa, while the fermentation of pectin resulted in the enrichment of seven different taxa. Nevertheless, both carbohydrates resulted in comparable amounts of SCFAs[72]. This illustrates that both inulin and pectin can lead to a specific enrichment of different bacterial species among the GI microbiome, which would allow targeted modulation of GI microbiota. In spite of this, the rationale for why only one of them is considered a prebiotic is not obvious from these findings, especially considering that selectivity, according to the ISAPP consensus panel, 'could extend to several microbial groups'[45]. What constitutes a specific fermentation, therefore, remains ill-defined.

In this respect, it is important to point out that the specificity of a prebiotic has, to date, been exclusively established only by the determination of compositional shifts. However, species that utilize a prebiotic might produce metabolites without becoming enriched. For example, *Bacteroides* numbers often decrease after the administration of prebiotics even if they are able to

utilize them[73,74], likely since their growth is negatively affected through a reduction in pH that results from the production of SCFAs[72,75,76]. To truly establish whether a prebiotic is selectively fermented would require the use of techniques such as stable isotope probing[77] that allow for the identification of all microbes that are able to utilize the substrate within a complex microbial community, including those that do not become enriched. Finally, there are often substantially different inter-individual responses within the GI microbiome toward a prebiotic, yet this variation has not been considered in the concept at all[51].

Overall, in agreement with recently published statements[20,44,48-50], the prebiotic concept remains ill-defined and based on outdated scientific views. To address these inconsistencies, Bindels and colleagues proposed updating the definition of a prebiotic to 'a non-digestible compound that, through its metabolization by microorganisms in the GI tract, modulates composition and/or activity of the GI microbiota, thus conferring a beneficial physiological effect on the host'[44]. By removing the requirement of specificity, this definition embraces the complexity of host-microbe metabolic interactions and focuses on ecological and functional features of the microbiota that are more likely to be relevant for host physiology, such as the production of SCFAs. Still, consensus on the definition of a prebiotic has not been reached[48], and some scientists prefer to abandon the term altogether[50]. However, the term prebiotic does remain helpful, as it has become well known in the scientific community and is recognized by regulators, industry, consumers, and health care professionals. Therefore, the prebiotic concept could remain valuable after the inconsistencies in the definition have been resolved.

1.2.2.2 Fermentable DF

Based on current definitions, most prebiotic carbohydrates are DFs, but not all DFs are considered to be prebiotics[78,79]. Nonetheless, a clear-cut distinction between prebiotics and nonprebiotic DF is not possible[49], and it is increasingly recognized that the fermentation of DF by the colonic microbiota contributes to human health.

The term 'dietary fiber' was initially coined in 1953 by Eben Hipsley to describe the non-digestible components of the plant cell wall[80]. Later in the mid-1970s, Trowell and colleagues refined the definition to 'remnants of plant cells resistant to hydrolysis by the alimentary enzymes of man, the group of substances that remain in the ileum but are partially hydrolyzed by bacteria in the colon'[81]. Since then, the definition has undergone several revisions. The most widely used definition was put forth in 2009 by the Codex Alimentarius Commission, a joint

principal branch of the Food and Agriculture Organization and the World Health Organization, which defines DF as 'carbohydrate polymers with ten or more monomeric units, which are not hydrolyzed by the endogenous enzymes in the human small intestine and belong to the following categories:

1) edible carbohydrate polymers naturally occurring in the food as consumed,
2) carbohydrate polymers which have been obtained from raw materials by physical, enzymatic, or chemical means and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities, and
3) synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities'[82].

The decision to consider carbohydrates with three to nine monomeric units (oligosaccharides) as DF was left to individual country authorities[12]. Recently, the Food and Drug Administration in the United States updated its food labeling regulations to model the Codex definition by requiring that 'isolated' and 'synthetic' NDCs added to foods must first demonstrate a beneficial physiological effect in humans prior to being permitted as a DF on a food label[83].

DFs can be found in plants, bacteria, and fungi and can be chemically synthesized (**Figure 1.2**)[84,85]. Plant-derived non-starch polysaccharides display a substantial variety of chemical structures due to the diverse functional roles that they play in plants[85]. DFs also include starches that are resistant to human digestion (resistant starches [RSs]), which are divided into five subtypes based on the mechanism responsible for their inaccessibility to host digestion[86]. Given the heterogenic chemical structures found in DFs, their utilization within the GI tract often requires a diverse array of enzymes distributed amongst various microbial members. The extent by which different DF types are utilized or fermented by the GI microbiota is therefore structure-dependent and relies on the metabolic capabilities of an individual's microbiome, which ultimately determines the bacterial metabolites produced from the fermentation[36].

The chemical structure of a DF also determines other important physicochemical properties such as solubility and viscosity[87], which influence its accessibility to microbes. Most linear DFs form crystalline structures (like cellulose), which significantly reduces their solubility

in water. Meanwhile, DFs with charged moieties or structural irregularities in the sugar backbone and side chains (like β-glucan) tend to increase in solubility, which is often correlated with fermentability[88]. However, one common exception would be RSs, which are typically insoluble in water yet highly fermentable[49]. Furthermore, most soluble DFs tend to also be viscous in water[49], which relates to their ability to render a solution gelatinous via the absorption of water[88]. Solubility and viscosity are important characteristics that influence the functionality of a DF, but for the purpose of modulating the GI microbiota, fermentability is of particular relevance.

'Dietary fiber' is a widely accepted and useful term to describe NDCs that influence health benefits. Most consumers recognize the term, because it is a required part of the nutrition facts label of processed food. However, to describe NDFCs that are intended to modulate the GI microbiome, the term has obvious limitations. Various mechanisms (*e.g.* reduced absorption through viscosity, bile acid binding, stool bulking, etc.) have been identified by which DF can benefit human health completely independently of their effects on the GI microbiome[87]. In addition, as described above, not all DFs are fermented by the microbiota. The definition of a prebiotic proposed by Bindels and colleagues would encompass DFs for which there is evidence that they improve health via the GI microbiota[44], but as described above, there is no agreement on the definition of a prebiotic[48]. What is clear is that the portion of DF that is fermentable or 'accessible' by an individual's microbiota is what determines its ability to modulate the GI microbiome. This notion is central to the concept of microbiota-accessible carbohydrates (MACs).

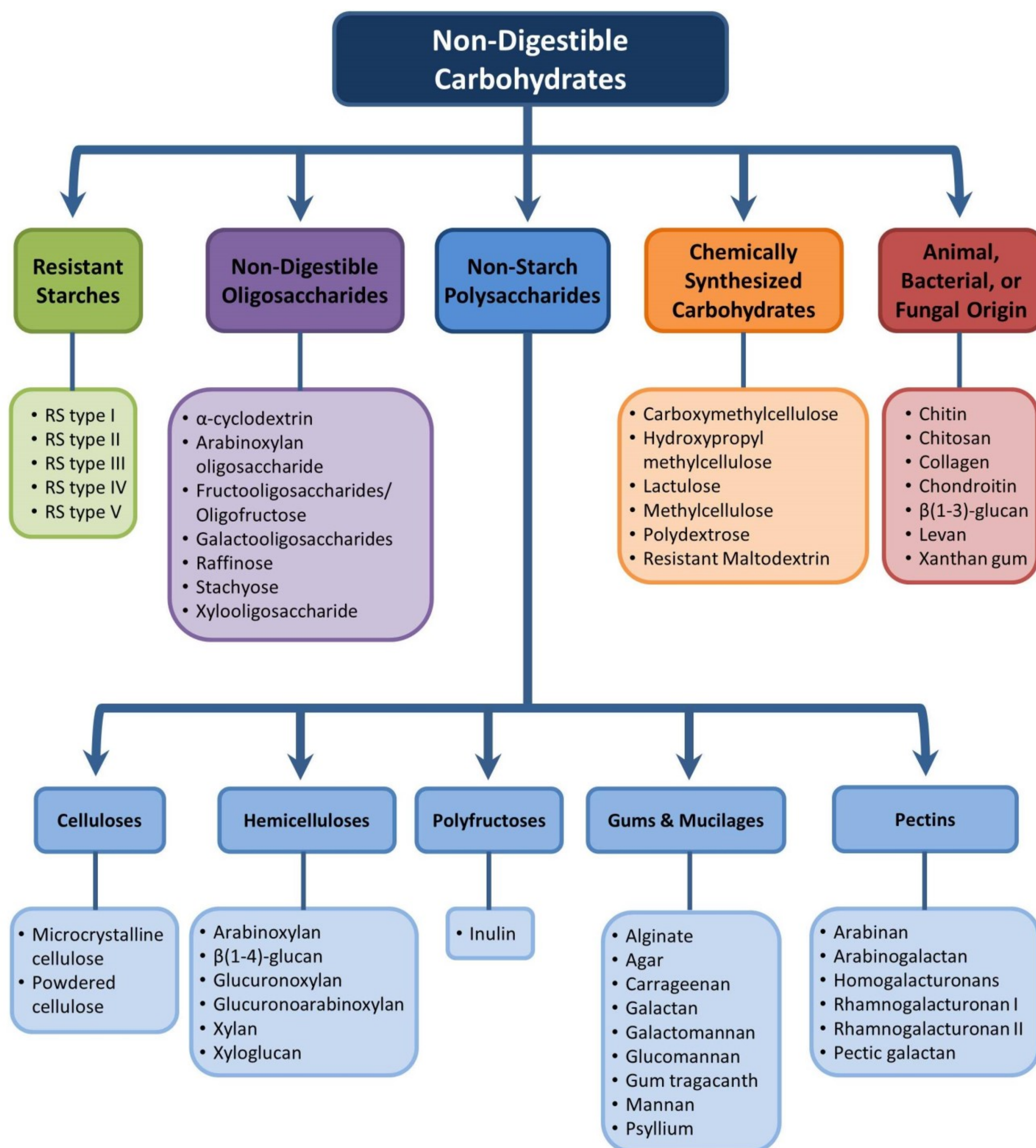

Figure 1.2. Categories of non-digestible carbohydrates (NDCs). NDCs are a heterogeneous group of compounds that display diverse chemical structures, which is the basis for their categorization alongside their origin[36,84,85]. Non-digestible oligosaccharides are NDCs comprised of three to nine monosaccharides and are either from plant or animal origin, as well as chemically synthesized.

1.2.2.3 MACs

To address the limitations of the concepts discussed above, especially as they relate to the inter-individual differences of the human GI microbiome, Sonnenburg and Sonnenburg introduced the concept of MACs[6]. MACs can be divided into dietary MACs (NDFCs such as prebiotics and DF) and host-derived MACs (such as mucosal glycans)[6]. Determining whether a NDFC is considered a MAC is not entirely dependent on the NDFC's physicochemical characteristics, because an individual's GI microbiota must also have the enzymatic capacity to metabolize it[89,90]. For example, cellulose does not qualify as a MAC for humans, because the capacity of the human GI microbiota to ferment cellulose is extremely low[91], while it would qualify as a MAC for other host species (*e.g.* hindgut and foregut fermenters). On the other hand, RS type-III would be considered a MAC for most individuals, as it is readily metabolized by their GI microbiota. However, individuals that lack the keystone species *Ruminococcus bromii* do not have the enzymatic capacity to metabolize RS type-III, and thus for these individuals, it would not be considered a MAC[89]. The concept of MACs is therefore particularly applicable to efforts aimed at personalizing human nutrition, which could customize dietary recommendations toward the goal of incorporating specific NDFCs that are known to be accessible to an individual's GI microbiota.

The concept of dietary MACs is essentially equivalent to that of fermentable DF, with the additional criterion regarding the individuality of the GI microbiota in their capacity to utilize certain NDFCs. Both concepts would include the same types of NDFCs[6], and according to Bindels and colleagues, these carbohydrates could be considered prebiotic if they exert a health benefit via the GI microbiota[44]. Overall, there is currently no unifying concept that describes NDFCs that target the GI microbiota for health purposes.

1.2.3 How do NDFCs Modulate GI Microbiota Composition and Function?

1.2.3.1 Modulation of GI Microbiota Composition and Diversity

Consumption of NDFCs has the potential to improve human health by changing both the composition (structure and diversity) and the function (metabolism) of the microbial communities that reside in the GI tract. Dietary administration of NDFCs alters the nutritional niches in the GI tract by providing substrates for microbial growth. Thus, in general, species that are able to utilize these substrates can expand their populations[92]. For example, administration of

RS has been shown to enrich specific bacterial groups (*Bifidobacterium adolescentis*, *R. bromii*, and *Eubacterium rectale*) in a subset of individuals[67-69]. The taxa shown to be enriched differ between RS type-II (which appears to be similar to RS type-III[89]) and RS type-IV[69], indicating that shifts are dependent on the carbohydrate's chemical structure. Accordingly, the consumption of galactooligosaccharides mainly induces *Bifidobacterium* species that possess the enzymatic machinery to efficiently utilize this substrate[73]. In addition to its enzymatic capacity, the ability of a microbe to 'adhere' to a substrate and to tolerate the environmental conditions generated from fermentation (*e.g.* low pH) determines whether a microbe can become enriched. Although RS is utilized by many members of the human GI microbiota[71,93,94], the species that become enriched under competition (*B. adolescentis*, *R. bromii*, and *E. rectale*) have been shown to directly adhere to this substrate in the human GI tract[94].

Furthermore, the degradation of many complex NDFCs does require a different species with complementary enzymatic repertoires for their degradation, which establishes syntrophic interrelationships within the GI microbiota[95-97]. Primary fermenters directly degrade NDFCs, leading to the release of partial breakdown products and the production of metabolites that can benefit themselves, as well as be beneficial or inhibitory (*e.g.* through acidity) to other taxa[98]. Secondary fermenters are able to benefit through cross-feeding on these partial carbohydrate breakdown products and the metabolic end-products released by primary fermenters[96,99]. Through coculture experiments, *R. bromii* has been identified as a primary fermenter of RS types II and III, whereupon RS degradation-reducing sugars are released that support the growth of secondary fermenters that are not able to degrade RS directly, including *B. adolescentis*, *E. rectale*, and *Bacteroides thetaiotaomicron*[89]. Another cross-feeding relationship exists for hydrogen-consuming species, such as *Blautia hydrogenotrophica*, which flourishes in the mouse GI tract only if in bi-association with *B. thetaiotaomicron* by utilizing the hydrogen generated as an end product of fermentation by the latter[100]. Removal of hydrogen maintains the redox balance (NAD^+/NADH ratio) in the GI tract, providing conditions favorable for fermentation, and can result in the formation of methane by methanogens (such as *Methanobrevibacter smithii*) and acetate by acetogens (such as *B. hydrogenotrophica*), which is further converted to butyrate by other taxa, including *Roseburia intestinalis*[101-103]. Lactate is another metabolic end product of NDFC fermentation that can also be converted to butyrate through cross-feeding between lactate-producing species and lactate-utilizing butyrogenic species[97,104].

While the processes described above lead to the enrichment of GI microbes through the fermentation of NDFCs, with different species being either directly or indirectly stimulated, there are taxa that simultaneously become inhibited through the metabolites produced. For instance, *Bacteroides* species often decrease in number after the administration of NDFCs[8,73,74], even though this group of bacteria is well equipped to utilize these substrates. These inhibitory effects are due to *Bacteroides* having a low tolerance towards acidic conditions generated from the SCFAs produced during the fermentation of NDFCs[72,76].

The impact of NDFCs on the GI microbiome composition displays several consistent characteristics that are important for therapeutic applications. First, the magnitude of the induced changes can be substantial, with specific species becoming enriched to constitute more than 30% of the fecal microbiota[68,69,73], thus providing a potential strategy for the enrichment of minority members of the GI microbiome to become dominant members. However, these changes are only maintained as long as the substrate is consumed. Once the substrate is no longer available, resilience of the microbiome results in a return to the original state. Second, the microbial response to NDFCs is highly individualized, with some individuals showing substrate-specific shifts, while others do not respond at all[69,73]. The reason for this individuality is not yet understood. Individuals might lack keystone species[89] or contain strains with varying enzymatic capacity towards the substrate[105]. Third, although individualized, compositional changes observed after administration of NDFCs remain restricted to certain groups of microbes. This is true for classic prebiotics like inulin and galactooligosaccharides that are supposed to be selectively fermented[73,106], but also applies to substrates that were assumed to be broadly utilized, such as RSs and pectin[68,69,72]. The reason for these observations stems from the highly competitive conditions within the human GI tract, which allow for only certain microbes to benefit directly from the NDFCs[101]. Although central to the original prebiotic concept[46], whether such specific shifts are related to health outcomes still remains to be established.

Most CNCDs are associated with a dysbiosis that displays decreases in bacterial diversity and/or genetic richness of the GI microbiome[18,57,58]. Although it is difficult to prove whether these patterns are the cause or an effect of disease, community ecology theory postulates high diversity as a beneficial trait that is attributed to the stability and functionality of the ecosystem[7,59]. Several independent research groups have consistently shown that individuals from nonindustrialized populations in various parts of the world have greater GI microbiome

diversity when compared to individuals from industrialized regions[3]. This increased diversity is reflected not only in the number of bacterial species (or operational taxonomic units [OTUs])[9,32-35], but also the abundance of genetic functions encoded in the GI microbiome[33]. While there are many possible factors that could cause reduced GI microbial diversity through industrialization (*e.g.* sanitization and antibiotic use), dietary diversity is considered a key mediator[6,107]. Research in humanized mice (mice that have been colonized with a human GI microbiota) demonstrated that a maternal diet low in MACs induced significant depletions in the GI microbiota diversity of the offspring within only a few generations and that this depletion was irreversible even after the re-introduction of MACs[4]. Enrichment of the maternal diet with MACs maintained the GI microbiota diversity in the offspring over multiple generations[4]. Moreover, the transition of non-human primates from wild to semicaptive to captive conditions led to a reduction in bacterial OTU richness. An in-depth analysis of the chloroplast sequences found within the 16S sequences obtained from the fecal samples suggested that this depletion was in part driven by a reduction in the diversity of dietary plant content, and particularly a reduction in the consumption of NDFCs[108].

Overall, there is convincing evidence that a depletion of NDFCs in the diet results in a reduction of microbial diversity within the human GI microbiome, thus providing a rationale for targeting microbial diversity through dietary modulations. An enrichment of both the amount and structural diversity of NDFCs could in theory enhance microbial diversity and gene richness by generating niche opportunities[36]. Cross-sectional assessments of long-term dietary intake in overweight humans have shown that long-term consumption of fruit and vegetables, and therefore NDFCs, is associated with higher GI microbial gene richness and diversity[58,109]. Conversely, short-term dietary intervention studies have produced conflicting results[8,110,111]. Studies supplementing DF-rich whole foods have been shown to increase GI microbial diversity[7,8], while most short-term feeding trials with purified NDFCs[69,73,110,112] or even whole plant-based diets[21] had no effect.

In summary, although clear associations exist between the consumption of NDFCs, GI microbial diversity, and improved metabolic and inflammatory markers of CNCDs, rigorously controlled human intervention studies with NDFCs that assess well-defined clinical and microbial endpoints are needed in order to determine (i) if diversity can be enhanced by NDFCs

and (ii) to what extent this constitutes a microbial-dependent mechanism by which NDFCs can improve human health.

1.2.3.2 Impact of NDFCs on GI Microbiome Function

The provision of NDFCs does not only impact microbiota composition as described above, but also changes the profile of microbial-derived metabolites within the GI tract[113]. The fermentation of NDFCs results in the production of beneficial metabolites (*e.g.* SCFAs) and microbial gases (H_2, CO_2, CH_4), with SCFAs being the main focus of recent research[31,113]. Acetate, propionate, and butyrate are the dominant SCFAs (at >95%)[114]. A significant portion of acetate (around 24%) can support the production of butyrate through microbial cross-feeding[115]. The total amount and proportion of individual SCFAs produced is dependent on the type of NDFC[116], as well as the individual microbiota[117], further reinforcing that the response of the human GI microbiota to NDFCs is individualized.

Fermentation of NDFCs within the GI tract leads to various systemic effects on the host, including an influence on energy homeostasis and metabolism[31]. A majority of the SCFAs produced are rapidly absorbed in the GI tract, with only around 5 to 10% being excreted in the feces[113]. Upon absorption, the majority of butyrate is metabolized by the colonocytes and serves as their major form of energy[118]. Propionate reaches the liver via portal circulation, where it is primarily utilized for hepatic gluconeogenesis (GNG). Acetate, on the other hand, reaches peripheral circulation at extensively higher concentrations than the other SCFAs, where it is metabolized by peripheral tissues for energy, in addition to being utilized by the liver for lipogenesis[113-115]. NDCs provide the host with 0 to 2.5 kcal/g (with digestible carbohydrates providing 4 kcal/g), dependent on their level of fermentability by the GI microbiota[42].

A low intake of NDFCs leads not only to a reduction in SCFAs, but also shifts in GI microbiota metabolism toward the utilization of less favorable nutrients, particularly dietary and endogenously supplied proteins[119]. For instance, moving the diet of humans volunteers from a weight-maintenance diet to a high-protein, low-carbohydrate diet not only significantly reduced the production of total SCFAs and butyrate[120], but also led to an increase in potentially detrimental metabolites derived from the fermentation of amino acids, including branched-chain fatty acids, ammonia, amines, *N*-nitroso compounds, phenolic compounds including *p*-Cresol, and sulfides (**Figure 1.3**)[121,122]. These metabolites are thought to directly contribute to the development of CNCDs, particularly colon cancer[122]. In addition, depletion on NDFCs within

the diet subsequently causes the GI microbiota to shift their glycan-foraging behavior towards utilizing host-derived substrates such as mucins by upregulating the expression of bacterial genes necessary for the metabolization of mucosal glycoproteins[123]. This shift towards the fermentation of mucosal glycans leads to a significant depletion of the epithelial mucus layer[124], which can cause GI inflammation and increases the host's susceptibility to pathogen invasion[125].

In summary, the metabolic effect of NDFCs is central to their importance in human nutrition and their effects when used as supplements[126] in the form of prebiotics or fermentable DF[79]. Although the fermentation of NDFCs is considered beneficial, this subject is not without its controversies. Individuals with obesity tend to have increased fecal SCFAs when compared to their lean counterparts[127], and SCFAs might contribute to weight gain by providing energy. In addition, butyrate has a controversial role in the induction of colon cancer, because it has been shown to fuel the hyperproliferation of colon epithelial cells in a mouse model of the disease[128,129]. However, epidemiological studies consistently report a negative association between DF consumption, which would increase SCFAs, and both obesity[130] and colon cancer[131,132]. Furthermore, as discussed below, the majority of the physiological effects associated with SCFAs are considered beneficial.

1.2.4 Physiological Effects of NDFCs on the Host

Two primary signaling mechanisms have been described by which SCFAs are able to influence the biological responses of the host. First, SCFAs can impose epigenetic regulation through direct inhibition of histone deacetylases activity and expression[133]. Histone deacetylases inhibition has been indicated as a central mechanism by which SCFAs modulate the immune system and inhibit the development of colon cancer[133]. Second, SCFAs can bind to G-protein-coupled receptors (GPRs), with the primary receptors activated by SCFAs being GPR41, GPR43, and GPR109A[133]. GPR41 and GPR43 are coexpressed locally on colonic enteroendocrine L-cells[31,134,135], as well as systemically expressed in white adipose tissue, skeletal muscle, and the liver[31,136,137]. GPR109A (which is also commonly referred to as Niacin Receptor 1 since niacin is its primary ligand[138]) has been shown to be expressed on ileal and colonic enterocytes, adipocytes, and immune cells[138-140]. Agonists for GPR41 can be ranked in the following order based on potency: propionate greater than or equal to butyrate, butyrate greater than acetate; however, these SCFAs exhibit similar potencies for GPR43[136,137]. Butyrate,

on the other hand, is the only SCFA known to bind to GPR109A. Overall, SCFAs are a primary microbiome-dependent mechanism by which NDFCs modulate host heath[31] through the regulation of satiety, glucose and lipid metabolism, as well as systemic inflammation (**Figure 1.3**).

1.2.4.1 Regulation of Satiety

The regulation of the balance between hunger and satiety, which ultimately impacts energy intake, is highly complex and influenced by multiple physiological, psychological, and environmental factors[141]. SCFAs are able to act as physiological regulators of satiety by primarily functioning as signaling molecules through the enhanced production of key anorectic hormones such as peptide tyrosine tyrosine (PYY) and glucagon-like peptide-1 (GLP-1) (**Figure 1.3**)[61]. By means of activating GPR41 and GPR43, SCFAs induce the release of both PYY and GLP-1 from colonic enteroendocrine L-cells into systemic circulation[142,143]. PYY has been shown to promote satiety by acting on the arcuate nucleus within the hypothalamus to suppress neuropeptide Y neurons and activate pro-opiomelanocortin neurons while also delaying gastric emptying[144,145]. GLP-1 similarly influences the hypothalamus by binding to the GLP-1 receptor[146,147] while also inhibiting gastric emptying and the secretion of gastric acid[148,149]. Furthermore, the SCFAs acetate and propionate have been shown to act on white adipose tissue to stimulate the production of leptin, another anorectic hormone involved in the regulation of satiety (**Figure 1.3**)[150,151].

SCFAs can also regulate the interplay between hunger and satiety independent of anorectic hormones. Acetate has been shown to induce satiety through directly eliciting hypothalamic appetite suppression[152]. Furthermore, propionate and butyrate promote an up-regulation of intestinal GNG gene-expression, which positively influences energy homeostasis and promotes satiety by portal vein glucose sensors[153,154]. Besides influencing energy intake, SCFAs may also affect energy expenditure. In rodents, SCFA supplementation promoted an increased rate of oxygen consumption while also enhancing mitochondrial function, adaptive thermogenesis, and fat oxidation[155,156]. However, the extent of this influence and its relevance to humans is not currently known.

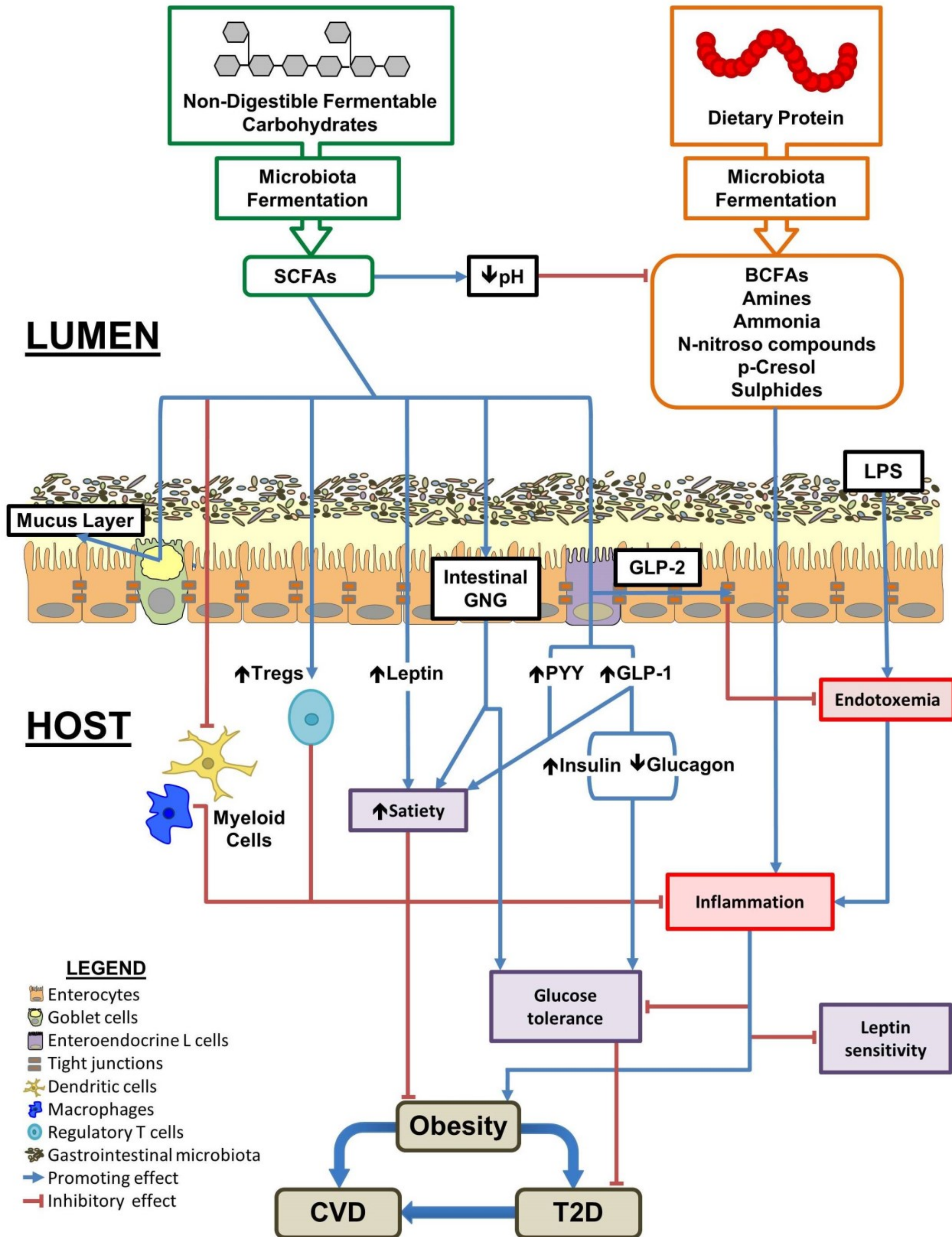

Figure 1.3. Mechanisms by which the metabolism of non-digestible fermentable carbohydrates (NDFCs) by the gastrointestinal (GI) microbiota modulates host health. *Legend continued on the next page.*

NDFCs are fermented by the GI microbiota to SCFAs, which upon absorption into enterocytes can activate intestinal GNG, leading to improved satiety and glucose homeostasis. SCFAs can further stimulate enteroendocrine L-cells to secrete PYY, GLP-1, and GLP-2. Both PYY and GLP-1 act as satiety hormones, while GLP-1 also promotes glucose tolerance. Meanwhile, the secretion of GLP-2 enhances intestinal barrier function by upregulating the expression of tight junction proteins. SCFAs further enhance the intestinal barrier by stimulating mucin secretion from goblet cells, which aids in reducing the translocation of LPS through the intestinal epithelium, consequently reducing inflammation. Additionally, SCFAs exert immunomodulatory effects by regulating both the expansion of regulatory T-cells and myeloid cell function to inhibit inflammation. Moreover, SCFAs signal to organs distant to the GI tract, such as white adipose tissue, where they may act on adipocytes promoting the secretion of leptin, another anorectic hormone. Furthermore, the presence of NDFC inhibits the production of potentially detrimental metabolites from the fermentation of dietary proteins through lowering intestinal pH. BCFAs, branched-chain fatty acids; CVD, cardiovascular disease; GLP-1, glucagon-like peptide-1; GLP-2 glucagon-like peptide-2; GNG, gluconeogenesis; LPS, lipopolysaccharides; PYY, peptide YY; SCFAs, short-chain fatty acids; T2D, type II diabetes; Tregs, regulatory T-cells.

1.2.4.2 Glucose and Lipid Metabolism

Obesity, cardiovascular disease, type II diabetes, and other CNCDs are associated with an altered glucose and lipid metabolism[157,158]. Human intervention studies have shown that increased consumption of NDFCs can improve glucose and lipid metabolism[159-161], thereby providing a mechanism by which they reduce the risk of developing cardiovascular disease, type II diabetes, and other CNCDs[162,163]. As discussed above, activation of enteroendocrine L-cells by SCFAs stimulates the release of GLP-1, which directly acts on pancreatic β-cells to promote insulin and inhibit glucagon secretion[146,164,165]. GLP-1 is also known to improve β-cell responsiveness to glucose, even in glucose-resistant β-cells[166,167]. Additionally, SCFAs themselves, specifically propionate, have recently been shown to act directly on β-cells to stimulate insulin secretion, independent of GLP-1[168]. This would subsequently increase the uptake of glucose by skeletal muscle and adipose tissues while also decreasing hepatic-associated GNG[169]. Further, SCFAs have been shown to inhibit hepatic-associated GNG via the up-regulation of intestinal GNG[153]. This reduction in hepatic GNG is critical because enhanced hepatic production of glucose is linked to insulin resistance and the development of some CNCDs[170,171].

SCFAs may also directly act on the liver to further influence hepatic glucose and lipid metabolism, which may in part be mediated by GPR41 and GPR43 signaling[136]. Dietary administration of SCFAs decreased lipid accumulation within the liver in mice by means of

increased lipid utilization[172]. This occurred primarily by a downregulation of peroxisome-proliferator-activated receptor-γ expression and activity, which stimulated AMP-activated protein kinase-associated fatty acid oxidation within the liver. This SCFA-induced increase in fat oxidation was also observed in adipose tissue[172]. SCFAs may play a further role in lipid metabolism by acting on adipose tissue through both intracellular and extracellular mechanisms. The acute administration of acetate and propionate in humans led to a significant reduction in serum free fatty acid levels[173]. This may be in part due to a GPR43-dependent decrease in the intracellular lipolytic activity of adipocytes[174]. In addition to this, propionate may also have extracellular lipolytic properties by enhancing the activity of adipose tissue lipoprotein lipase[175]. Collectively, the mechanistic involvement of SCFAs in modulating glucose and lipid metabolism has been well established in animal and *in vitro* models, although further research is needed to clarify if these findings translate to humans.

1.2.4.3 Systemic Inflammation

Most CNCDs are characterized by a state of systemic, low-grade inflammation, which contributes to disease progression[157,158,176]. Although the findings are to some degree variable, recent research indicates that NDFCs have anti-inflammatory effects[177,178], primarily through SCFA-dependent mechanisms involving intestinal barrier function and regulation of immune cell responses[31].

1.2.4.3.1 Barrier Function and Endotoxemia

Diminished GI barrier function enhances the translocation of lipopolysaccharide (LPS) and other microbial-derived proinflammatory molecules across the GI epithelial layer. LPS interacts with LPS-binding protein and CD14 to stimulate Toll-like receptor-4 and subsequently promotes a pro-inflammatory response, including a systemic increase in acute-phase proteins and proinflammatory cytokines[179]. Elevated plasma LPS in humans (endotoxemia) is positively correlated with percent body fat, excessive energy intake, metabolic inflammation, insulin resistance, and dyslipidemia[8,180-182]. In mice, endotoxemia has been demonstrated to cause obesity and insulin resistance without influencing food intake[183]. Considering this, promoting GI mucosal barrier function could constitute a strategy to address CNCD-associated metabolic abnormalities.

SCFAs have been shown to enhance GI barrier function through upregulating the expression of tight junction proteins, which occurs through at least two different mechanisms.

First, NDFC-induced SCFA production increases glucagon-like peptide-2 secretion by enteroendocrine L-cells[184,185], which leads to an upregulation in the expression of tight junction proteins (zonula occludens-1 and occludin) and, concomitantly, a reduction in LPS and systemic inflammation[186] (**Figure 1.3**). Recently, Kelly and colleagues illustrated another mechanism by which SCFA metabolism (mainly butyrate) within intestinal epithelial cells creates a state of hypoxia, which enhances intestinal barrier integrity[187], likely through enhanced tight junction protein expression[188].

Other mechanisms by which SCFAs have been shown to improve GI barrier function include butyrate-driven upregulation of epithelial cell proliferation and differentiation[189], enhanced production of antimicrobial peptides including secretory immunoglobulin A (IgA) and intestinal alkaline phosphatase[190], stimulation of goblet cells to secrete mucus that fortifies the GI mucus layer[191,192], and GPR109A-dependent regulation of enterocyte derived interleukin-18, which is essential to maintain mucosal homeostasis and ultimately GI barrier function[193,194].

1.2.4.3.2 Immunoregulation

Subpopulations of immunosuppressive regulatory T-cells (Tregs) play an essential role in both the maintenance of immunotolerance and, ultimately, the prevention of many CNCDs[195,196]. Microbiota-derived SCFAs, particularly butyrate and propionate, have been demonstrated to encourage immunotolerance through an expansion and differentiation of Tregs[196]. This process has been shown to occur through GPR43-dependent signaling[197], as well as through the epigenetic regulation of the Foxp3 promoter through an inhibition of histone deacetylase[198,199]. This systemic immunoregulatory effect is thought to be determined largely by the immunologic context. For example, in a state of infection, SCFAs enhance the differentiation of proinflammatory T-helper cell subsets (*i.e.* Th1 and Th17 cells) instead of Tregs[200]. This suggests that SCFA production from the fermentation of NDFCs may play an intricate role in host immune responses to infection beyond lowering intestinal pH, which inhibits colonization by pathogens[24].

The immunoregulatory effect of SCFAs is, however, not solely reliant upon an expansion of Tregs and can occur in organ systems throughout the body (*e.g.* lungs and skin)[201,202]. SCFAs have also been shown to regulate immune cells of the myeloid lineage (**Figure 1.3**) through facilitating the polarization of macrophages towards an M2 phenotype involved in downregulating an inflammatory response[23], specifically that of GI macrophages to LPS[203].

Moreover, SCFAs modulate neutrophil activity through suppressing their migratory behavior[204] while shifting their microbial killing phenotype towards an increased phagocytic activity with diminished pro-inflammatory cytokine production[205,206]. The activity of dendritic cells has similarly been shown to be influenced by SCFAs, primarily through enhancing their ability to induce the differentiation of Tregs[193] and to promote the production of IgA by plasma cells[207]. Furthermore, SCFAs are able to act directly on B-cells to enhance the production of IgA, both by acting as an energy source and by upregulating the expression of genes necessary for plasma cell differentiation[208]. SCFAs can also act on nonimmune cells to stimulate the release of antimicrobial peptides. Within the pancreas, SCFAs act on pancreatic β-cells in a GPR-dependent manner to enhance the secretion of cathelicidin-related antimicrobial peptide, which ultimately induces Treg cell expansion and protects against the development of autoimmune diabetes[209]. Although additional research is needed, these animal and *in vitro* models elegantly illustrate the central mechanisms by which microbiota-derived SCFAs reduce inflammation.

1.2.5 Evidence for Microbiota-Mediated Health Effects on NDFCs

There is substantial evidence from well-designed animal studies that NDFCs, or the metabolic products that result from their fermentation (*e.g.* SCFAs), have beneficial effects and that these effects are in part due to the GI microbiota. Work originating from Patrice Cani and Nathalie Delzenne's research groups has repeatedly demonstrated, although primarily through associations and not causal assessments, that the GI microbiota is implicated in the ability of NDFCs to improve obesity-associated low-grade inflammation and insulin resistance[54,184-186,210]. Research employing fecal microbiota transplantation methodology supports these findings and further suggests a causative link, in that upon transferring the GI microbiota from mice fed an NDFC (resistant maltodextrin) to antibiotic-treated *db/db* mice (a model of type II diabetes), the improved glucose homeostatic phenotype was likewise transferred[211].

Work with GPR41 and GPR43 knockout (KO) mice, or their specific receptor antagonists, provides additional evidence that the NDFC-associated metabolic health effects described above are mediated by the GI microbiota and specifically through the production of SCFAs. For instance, the ability of SCFAs to stimulate insulin secretion through increase GLP-1 release[212,213], promote satiety through enhanced PYY secretion[214], reduce systemic free fatty acid levels[174], or regulate blood pressure[215] is lost in GPR41 and/or GPR43 KO mice. Moreover, the

combination of GPR41 KO mice with a model of allergic asthma showed that the production of SCFAs was essential for NDFCs to protect against allergic airway inflammation, which occurred by impairing the ability of lung dendritic cells to promote allergen-reactive Th2 responses[202]. A similarly designed study compared the effect of guar gum, a viscous NDFC, in wild-type, GPR43 KO, and GPR109A KO mice treated with dextran sulfate sodium, which induces colitis. The study showed that signaling through GPR43 and GPR109A was essential for the NDFC to protect against the development of colitis[216].

Although these animal studies do illustrate a clear causative link between NDFCs, host health, and GI microbiota composition and function (*e.g.* SCFAs), knowledge on the exact role of the GI microbiota in the health effects of NDFCs remains incomplete. One experimental approach by which this could be resolved is through the comparison of the physiological effects of NDFCs in conventionalized and germfree animals[44]. However, germ-free animals are not without limitations since there are important physiological features that differ from their conventional counterparts, including an immune system that is not fully developed[217,218]. Furthermore, the pathological progression of CNCD-like symptoms in several animal models is often vastly different under germ-free conditions, preventing direct comparison between germ-free and conventional animals[219,220]. To address these limitations, one could alternatively test if the phenotypic effects of NDFCs are able to be transferred through cohousing or fecal microbial transfers. This is based on the premise that if the beneficial effect of the NDFC is caused by a shift in the microbiome, then the same beneficial effect should be seen in the recipient animal[44].

In humans, most microbiome-associated CNCDs are also linked to a low intake of dietary NDFCs, with a substantial degree of overlap between the two (**Figure 1.1**). This suggests that NDFCs may prevent CNCDs through a modulation of the GI microbiome[221]. Epidemiological studies have established convincing associations between DF intake and health[162,163,222], and although findings from human intervention studies are less consistent[222], health claims have been approved for DFs (cancer and cardiovascular disease)[223] and prebiotics (constipation)[224]. Moreover, multiple human intervention studies have been conducted that assess the effect of NDFCs on well-defined clinical outcomes while also characterizing the microbiome for compositional and functional signatures that correlate with these health outcomes[8,66,110,225-227]. For instance, treatment with the prebiotic inulin led to an increase in both *Bifidobacterium* and *Faecalibacterium prausnitzii*, which was inversely correlated with serum LPS levels, indicating

the prebiotic enhanced GI barrier function through modulating the GI microbiota[66]. Furthermore, systematic meta-analyses have shown that prebiotic supplementation is capable of restoring bowel function[228], while also reversing multiple metabolic abnormalities associated with CNCDs, including reducing fasting insulin, triglycerides, and low-density lipoprotein cholesterol levels[159,161,229,230]. Supplementation with lupin kernel fiber, a viscous NDFC, also led to a significant reduction in low-density lipoprotein cholesterol levels, and this response was inversely correlated with the fecal excretion of SCFAs[231]. Although these studies do detect microbial signatures that closely correlate with clinical outcomes of NDFC supplementation, they do not provide direct evidence for a causative role of the GI microbiota[39,44].

Still, some studies have been conducted that do indicate a causative link between the GI microbiota and NDFCs. Work from Fredrik Bäckhed's group paired a human study with a humanized germ-free mouse model to demonstrate that improved glucose metabolism due to whole-grain barley intake was dependent on the presence of *Prevotella copri* within the participant's GI tract[10]. Furthermore, by providing SCFAs directly through colonic infusions, studies have demonstrated that SCFAs do promote a systematic increase in PYY and GLP-1, while also benefiting markers of inflammation[232,233].

Clinical research that establishes clear connections between the health benefits of NDFCs and the GI microbiota, as well as clear evidence for the role of the GI microbiome in the health effects of NDFCs, including the underlying mechanisms involved, is altogether limited. As described above, NDFCs can exert health benefits through microbiome-independent mechanisms such as a reduction in nutrient absorption through viscosity or binding of bile acids and cholesterol[87]. This clearly illustrates a need for future studies that pair rigorously designed human studies with animal models, because this would constitute a unique tool to assess causation in humans[10,221].

1.2.6 Future Directions

1.2.6.1 Assessing the Clinical Efficacy of NDFCs and the Role of the GI Microbiome

Until now, most of the clinical evaluation of NDFCs has occurred through one-sided studies that solely assess either the GI microbiome or the host while completely overlooking the other[74,234]. Clinical research is needed that assesses the effect of NDFCs in rigorously designed randomized controlled trials with relevant clinical endpoints and a parallel characterization of the

role of the GI microbiota in these effects. Doing so would require close collaborations between nutritionally and microbiologically focused research groups to facilitate truly interdisciplinary research.

Despite clear evidence of the benefits of NDFCs from epidemiological studies, results from human intervention studies remain inconsistent[222]. These inconsistencies could stem from a variety of reasons. Most of the DF assessed in epidemiological studies is derived from whole foods (fruits, vegetables, and whole grains), in which the NDFCs are consumed intact within a food matrix that also includes components such as phytochemicals and bioactive lipids[235]. These bioactive compounds are likely to act synergistically within the food matrix[236,237], and once purified to be used as a supplement, the health effects of the NDFC might be lost or reduced[238]. However, purified DFs and prebiotics, as well as their fermentation products (SCFAs), have been repeatedly shown to be beneficial in mouse models[221]. The variability in human intervention studies could arise from both the highly inter-individualized nature of the human GI microbiome[67,69,73] and the variability in the host's metabolic response to NDFCs[10,53]. These two factors are inherently higher in humans, as mice colonies are often composed of inbred mice housed in a highly standardized environment and are fed homogenous diets. Given that the GI microbiotas in human subjects differ in the degree by which they are able to utilize specific NDFCs[89,90], inter-individual variation is likely to be more pronounced in studies using a single purified substrate instead of a mixture of substrates or a whole food containing multiple DF chemistries. In this respect, studies should characterize the chemical structure of the NDFCs to establish structure-function relationships between NDFC-chemistry and GI microbiome gene content, because this could be used to personalize approaches[36].

What is needed are human intervention studies with clinical endpoints that compare single NDFCs and their mixtures to those effects observed from whole foods, while also including a multi-omics approach for the analysis of the GI microbiome. Metagenomic analyses can identify specific shifts in GI microbiome composition and structure (*e.g.* diversity) that correlate with health outcomes. Such shifts, if they exist, would suggest that health benefits are due to selective changes of the GI microbiota in accordance to the original prebiotic concept[46]. However, such studies should also include predictive modeling to determine how inter-individual differences in microbiome composition and functional capacity impact clinical outcomes. These studies could provide an explanation for the high variation observed in intervention studies with

NDFCs and would establish a basis for personalized NDFC applications. Pairing this approach with a metabolomic analysis can further identify the compounds that are associated with health outcomes, which could range from microbial metabolites that originate from the fermentation of NDFCs to phytochemicals and their metabolic derivatives. Research has shown that phytochemicals present in fruits, vegetables, whole grains, and many DF extracts are also metabolically transformed by the GI microbiota and absorbed by the host, correlating with health benefits[239].

Considering that the human-microbiome symbiosis evolved with a supply of NDCs beyond 100 g/day, future human intervention studies should clearly be performed with more physiologically relevant doses[29]. Supplementation of 10 to 15 grams in intervention studies ensures participants meet current DF recommendations of around 30 g/day[12], but this intake is still far below that of our ancestors[27,29], potentially hindering the opportunity to detect evolutionary routed interrelationships between NDFCs, the GI microbiota, and health. Although experiments in nonindustrialized populations are clearly confounded by the possibility that specific DF-degrading bacteria have been lost[4,6], human studies that used NDC doses greater than 50 g/day did detect health benefits through the assessment of CNCD markers. For instance, by switching African Americans over to a more traditional South African diet that consisted of 55 g/day of NDCs, markers of colon cancer were improved in only 2 weeks[11]. Furthermore, following a 2-week dietary intervention that resembled an ancestral diet, with around 143 g/day of NDCs provided as green leafy vegetables, fruit, and nuts, a 25% reduction in total cholesterol was observed, which is a response comparable to cholesterol-lowering medications[240,241]. These studies clearly provide a rationale for the use of higher doses of DF in clinical research.

The approach described in this paragraph would ultimately help to identify putative mechanisms by which NDFCs exert their health effects, which would allow the formation of hypotheses that would inform the design of clinical studies and assist in the development of dietary strategies and targeted applications of NDFCs to improve human health.

1.2.6.2 Elucidating the Exact Mechanisms

Although well-conducted human studies with clinical endpoints would be sufficient to establish the efficiency of NDFCs and obtain health claims for DFs or prebiotics[44,242], it is important to point out that such research would only establish correlations and not causation. Correlations can be misleading because directionality cannot be established, especially since host

parameters altered through NDFCs (*e.g.* inflammation, metabolic outcomes) can in themselves have an effect on the GI microbiome[243,244]. In addition, intake of RS has clearly established that the health effects of a NDFC can be completely independent of the GI microbiota, even though clear correlations between diet-induced shifts in the microbiome and host markers exist[245].

In this respect, it is important to consider that human studies have unavoidable limitations when it comes to establishing mechanisms, because the health effects of NDFCs can be completely microbiome-independent. For example, NDCs increase fecal bulk and decrease colonic transit time[246], which in turn influences GI microbiota composition[247]. Ingestion of viscous NDCs may also increase the viscosity of digesta, interrupting the rate of nutrient absorption, and ultimately promoting an improvement in clinical markers, especially post-prandial glycemic response[248,249]. Furthermore, NDCs are able to sequester compounds such as sterols, bile acids, and carcinogens[43,250,251], inhibiting their absorbance and enhancing their excretion. Modulation of the bile acid pool alone may have systemic implications on glucose and lipid metabolism, as well as systemic inflammation through action on the farnesoid X receptor and the GPR TGR5[252]. These microbiota-independent mechanisms can be difficult to distinguish from microbiota-dependent mechanisms, especially because they still might lead to strong correlations between diet-induced shifts in microbiome composition and host markers[245].

Elucidation of the role of the GI microbiome in the health effects of NDFCs and the underlying mechanisms will require innovate experimental approaches such as utilizing animal studies in parallel with human intervention studies to directly test the role of the GI microbiota in health outcomes[10,221]. Although establishing the contribution of the GI microbiota in the health outcomes of an NDFC would in theory be required to establish its prebiotic action, such studies would be extremely difficult and costly, and probably an unrealistic demand for the purpose of defining if a NDFC qualifies as a prebiotic. Therefore, despite the limitations discussed above, the establishment of correlations between microbiome features and health benefits of a NDFC should probably be considered sufficient from a practical standpoint to establish which NDFCs constitute prebiotics[44]. Establishing the causal role of the GI microbiota and the underlying mechanisms would remain essential information for the development of improved nutritional strategies. Only an in-depth mechanistic understanding will allow for the selection of NDFCs, or mixtures thereof, to systematically target specific features of the GI microbiome (*i.e.* specific

taxa, diversity, metabolites) with the goal of correcting both immunometabolic abnormalities and dysbiotic features that underlie CNCDs.

1.2.7 Conclusion

The human diet has clearly changed over the last millennia, resulting in diminished potential to support our GI microbial community with growth substrates due to the dramatic reduction in the intake of NDFCs to a mere fraction of what was present in the diet of our ancestors[29]. Evidence points to this 'fiber gap' as being one prominent driving force behind the increased prevalence of CNCDs[12,28]. Although increased consumption of NDFC-rich whole foods such as fruits, vegetables, and whole grains is preferable from a nutritional perspective, efforts to increase their consumption have been ineffective to date, because DF levels remain low despite substantial efforts from nutritional agencies[37]. Humans resist long-term changes in their dietary habits[253], illustrating a need for NDFC sources that can readily enrich the standard western diet[27]. A broad array of NDFCs, which have vast potential to enhance the food supply, already exist on the market; however, product development and consumer research, in combination with clinical research, is needed to determine practical and cost-effective means by which these NDFCs can be incorporated into the food supply[27].

We know that there is a high degree of inter-individual variation in GI microbial response to NDFCs[67,69,73], and we have the toolset available to determine to what degree this individuality affects health outcomes in clinical studies[53]. This essential knowledge would ultimately support the development of a framework by which interventions with NDFCs could be personalized. This assures tremendous potential for growth within this field, promising exciting new developments as the focus of human nutrition shifts toward targeted nourishment of our symbiotic microbial communities as a way of preventing and treating CNCDs through supplementation with NDFCs.

1.3 References

1. Logan AC, Jacka FN, Prescott SL. Immune-Microbiota Interactions: Dysbiosis as a Global Health Issue. *Curr Allergy Asthma Rep.* 2016;16(2):13.
2. Blaser MJ, Falkow S. What are the consequences of the disappearing human microbiota? *Nat Rev Microbiol.* 2009;7(12):887-94.
3. Segata N. Gut Microbiome: Westernization and the Disappearance of Intestinal Diversity. *Curr Biol.* 2015;25(14):R611-R13.

4. Sonnenburg ED, Smits SA, Tikhonov M, Higginbottom SK, Wingreen NS, Sonnenburg JL. Diet-induced extinctions in the gut microbiota compound over generations. *Nature.* 2016;529(7585):212-15.
5. Jew S, AbuMweis SS, Jones PJ. Evolution of the Human Diet: Linking Our Ancestral Diet to Modern Functional Foods as a Means of Chronic Disease Prevention. *J Med Food.* 2009;12(5):925-34.
6. Sonnenburg ED, Sonnenburg JL. Starving our microbial self: the deleterious consequences of a diet deficient in microbiota-accessible carbohydrates. *Cell Metab.* 2014;20(5):779-86.
7. Tap J, Furet J-P, Bensaada M*, et al.* Gut microbiota richness promotes its stability upon increased dietary fibre intake in healthy adults. *Environ Microbiol.* 2015;17(12):4954-64.
8. Martínez I, Lattimer JM, Hubach KL*, et al.* Gut microbiome composition is linked to whole grain-induced immunological improvements. *ISME J.* 2013;7(2):269-80.
9. Martínez I, Stegen JC, Maldonado-Gómez MX*, et al.* The gut microbiota of rural papua new guineans: composition, diversity patterns, and ecological processes. *Cell Rep.* 2015;11(4):527-38.
10. Kovatcheva-Datchary P, Nilsson A, Akrami R*, et al.* Dietary Fiber-Induced Improvement in Glucose Metabolism Is Associated with Increased Abundance of *Prevotella*. *Cell Metab.* 2015;22(6):971-82.
11. O'Keefe SJD, Li JV, Lahti L*, et al.* Fat, fibre and cancer risk in African Americans and rural Africans. *Nat Commun.* 2015;6:6342.
12. Jones JM. CODEX-aligned dietary fiber definitions help to bridge the 'fiber gap'. *Nutr J.* 2014;13:34.
13. Fungwe TV, Bente L, Hiza H. The Food Supply and Dietary Fiber: Its Availability and Effect on Health. Center for Nutrition Policy and Promotion. November, 2007.
14. Storey M, Anderson P. Income and race/ethnicity influence dietary fiber intake and vegetable consumption. *Nutr Res.* 2014;34(10):844-50.
15. Abdullah MMH, Gyles CL, Marinangeli CPF, Carlberg JG, Jones PJH. Cost-of-illness analysis reveals potential healthcare savings with reductions in type 2 diabetes and cardiovascular disease following recommended intakes of dietary fiber in Canada. *Front Pharmacol.* 2015;6:167.
16. Walter J, Ley RE. The human gut microbiome: ecology and recent evolutionary changes. *Annu Rev Microbiol.* 2011;65:411-29.
17. Schroeder BO, Bäckhed F. Signals from the gut microbiota to distant organs in physiology and disease. *Nat Med.* 2016;22(10):1079-89.
18. Walker AW, Lawley TD. Therapeutic modulation of intestinal dysbiosis. *Pharmacol Res.* 2013;69(1):75-86.
19. Brahe LK, Astrup A, Larsen LH. Can We Prevent Obesity-Related Metabolic Diseases by Dietary Modulation of the Gut Microbiota? *Adv Nutr.* 2016;7(1):90-101.
20. Olle B. Medicines from microbiota. *Nat Biotechnol.* 2013;31(4):309–15.
21. David LA, Maurice CF, Carmody RN*, et al.* Diet rapidly and reproducibly alters the human gut microbiome. *Nature.* 2014;505(7484):559-63.
22. Wu GD, Chen J, Hoffmann C*, et al.* Linking long-term dietary patterns with gut microbial enterotypes. *Science.* 2011;334(6052):105-08.

23. Wu GD, Compher C, Chen EZ, *et al.* Comparative metabolomics in vegans and omnivores reveal constraints on diet-dependent gut microbiota metabolite production. *Gut.* 2016;65(1):63-72.
24. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr.* 1995;125(6):1401-12.
25. Bach J-F. The effect of infections on susceptibility to autoimmune and allergic diseases. *N Engl J Med.* 2002;347(12):911-20.
26. Bickler SW, DeMaio A. Western diseases: current concepts and implications for pediatric surgery research and practice. *Pediatr Surg Int.* 2008;24(3):251-5.
27. Deehan EC, Walter J. The Fiber Gap and the Disappearing Gut Microbiome: Implications for Human Nutrition. *Trends Endocrinol Metab.* 2016;27(5):239-42.
28. Burkitt DP, Walker ARP, Painter NS. Dietary Fiber and Disease. *JAMA.* 1974;229(8):1068-74.
29. Eaton S, Eaton SI, Konner M. Paleolithic nutrition revisited: a twelve-year retrospective on its nature and implications. *Eur J Clin Nutr.* 1997;51(4):207-16.
30. Burkitt DP. Some diseases characteristic of modern Western civilization. *BMJ.* 1973;1(5848):274-78.
31. Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. *Cell.* 2016;165(6):1332-45.
32. Yatsunenko T, Rey FE, Manary MJ, *et al.* Human gut microbiome viewed across age and geography. *Nature.* 2012;486(7402):222-27.
33. Clemente JC, Pehrsson EC, Blaser MJ, *et al.* The microbiome of uncontacted Amerindians. *Sci Adv.* 2015;1(3):e1500183.
34. Schnorr SL, Candela M, Rampelli S, *et al.* Gut microbiome of the Hadza hunter-gatherers. *Nat Commun.* 2014;5:3654.
35. De Filippo C, Cavalieri D, Di Paola M, *et al.* Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci USA.* 2010;107(33):14691-96.
36. Hamaker BR, Tuncil YE. A perspective on the complexity of dietary fiber structures and their potential effect on the gut microbiota. *J Mol Biol.* 2014;426(23):3838-50.
37. King DE, Mainous AGr, Lambourne CA. Trends in dietary fiber intake in the United States, 1999-2008. *J Acad Nutr Diet.* 2012;112(5):642-68.
38. Hill DR, Newburg DS. Clinical applications of bioactive milk components. *Nutr Rev.* 2015;73(7):463-76.
39. Butto LF, Haller D. Functional relevance of microbiome signatures: The correlation era requires tools for consolidation. *J Allergy Clin Immunol.* 2017;139(4):1092-98.
40. Yang L, Lu X, Nossa CW, Francois F, Peek RM, Pei Z. Inflammation and intestinal metaplasia of the distal esophagus are associated with alterations in the microbiome. *Gastroenterology.* 2009;137(2):588-97.
41. Wu T, Zhang Z, Liu B, *et al.* Gut microbiota dysbiosis and bacterial community assembly associated with cholesterol gallstones in large-scale study. *BMC Genomics.* 2013;14:669.
42. Dahl WJ, Stewart ML. Position of the Academy of Nutrition and Dietetics: Health Implications of Dietary Fiber. *J Acad Nutr Diet.* 2015;115(11):1861-70.
43. Anderson JW, Baird P, Davis RHJ, *et al.* Health benefits of dietary fiber. *Nutr Rev.* 2009;67(4):188-205.

44. Bindels LB, Delzenne NM, Cani PD, Walter J. Towards a more comprehensive concept for prebiotics. *Nat Rev Gastroenterol Hepatol.* 2015;12(5):303-10.
45. Gibson GR, Hutkins R, Sanders ME*, et al.* Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol.* 2017;14(8):491-502.
46. Gibson GR, Scott KP, Rastall RA*, et al.* Dietary prebiotics: current status and new definition. *Food Sci Tech Bull Funct Foods.* 2010;7(1):1-19.
47. Roberfroid M. Prebiotics: the concept revisited. *J Nutr.* 2007;137(3 Suppl 2):830S-37S.
48. Katsnelson A. Core Concept: Prebiotics gain prominence but remain poorly defined. *Proc Natl Acad Sci USA.* 2016;113(50):14168-69.
49. Verspreet J, Damen B, Broekaert WF, Verbeke K, Delcour JA, Courtin CM. A Critical Look at Prebiotics Within the Dietary Fiber Concept. *Annu Rev Food Sci Technol.* 2016;7:167-90.
50. Shanahan F. Fiber man meets microbial man. *Am J Clin Nutr.* 2015;101(1):1-2.
51. Louis P, Flint HJ, Michel C. How to Manipulate the Microbiota: Prebiotics. *Adv Exp Med Biol.* 2016;902:119-42.
52. Quevrain E, Maubert M-A, Michon C*, et al.* Identification of an anti-inflammatory protein from *Faecalibacterium prausnitzii*, a commensal bacterium deficient in Crohn's disease. *Gut.* 2015;65(3):415-25.
53. Zeevi D, Korem T, Zmora N*, et al.* Personalized Nutrition by Prediction of Glycemic Responses. *Cell.* 2015;163(5):1079-94.
54. Everard A, Belzer C, Geurts L*, et al.* Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. *Proc Natl Acad Sci USA.* 2013;110(22):9066-71.
55. Atarashi K, Tanoue T, Oshima K*, et al.* Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature.* 2013;500(7461):232-36.
56. Round JL, Lee SM, Li J*, et al.* The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science.* 2011;332(6032):974-77.
57. Le Chatelier E, Nielsen T, Qin J*, et al.* Richness of human gut microbiome correlates with metabolic markers. *Nature.* 2013;500(7464):541-46.
58. Cotillard A, Kennedy SP, Kong LC*, et al.* Dietary intervention impact on gut microbial gene richness. *Nature.* 2013;500(7464):585-88.
59. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. *Nature.* 2012;489(7415):220-30.
60. LeBlanc JG, Chain F, Martin R, Bermudez-Humaran LG, Courau S, Langella P. Beneficial effects on host energy metabolism of short-chain fatty acids and vitamins produced by commensal and probiotic bacteria. *Microb Cell Fact.* 2017;16(1):79.
61. Canfora EE, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity. *Nat Rev Endocrinol.* 2015;11(10):577-91.
62. Zheng J, Ruan L, Sun M, Gänzle M. A Genomic View of Lactobacilli and Pediococci Demonstrates that Phylogeny Matches Ecology and Physiology. *Appl Environ Microbiol.* 2015;81(20):7233-43.
63. Flint HJ, Duncan SH, Scott KP, Louis P. Links between diet, gut microbiota composition and gut metabolism. *Proc Nutr Soc.* 2015;74(1):13-22.
64. Klijn A, Mercenier A, Arigoni F. Lessons from the genomes of bifidobacteria. *FEMS Microbiol Rev.* 2005;29(3):491-509.

65. Makras L, Falony G, Van der Meulen R, De Vuyst L. Production of organic acids from fermentation of mannitol, fructooligosaccharides and inulin by a cholesterol removing Lactobacillus acidophilus strain. *J Appl Microbiol.* 2006;100(6):1388-89.
66. Dewulf EM, Cani PD, Claus SP*, et al.* Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. *Gut.* 2013;62(8):1112-21.
67. Venkataraman A, Sieber JR, Schmidt AW, Waldron C, Theis KR, Schmidt TM. Variable responses of human microbiomes to dietary supplementation with resistant starch. *Microbiome.* 2016;4(1):33.
68. Walker AW, Ince J, Duncan SH*, et al.* Dominant and diet-responsive groups of bacteria within the human colonic microbiota. *ISME J.* 2011;5(2):220-30.
69. Martínez I, Kim J, Duffy PR, Schlegel VL, Walter J. Resistant starches types 2 and 4 have differential effects on the composition of the fecal microbiota in human subjects. *PLoS One.* 2010;5(11):e15046.
70. El Kaoutari A, Armougom F, Gordon JI, Raoult D, Henrissat B. The abundance and variety of carbohydrate-active enzymes in the human gut microbiota. *Nat Rev Microbiol.* 2013;11(7):497-504.
71. Flint HJ, Bayer EA, Rincon MT, Lamed R, White BA. Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. *Nat Rev Microbiol.* 2008;6(2):121-31.
72. Chung WSF, Walker AW, Louis P*, et al.* Modulation of the human gut microbiota by dietary fibres occurs at the species level. *BMC Biology.* 2016;14(1):3.
73. Davis LMG, Martínez I, Walter J, Goin C, Hutkins RW. Barcoded pyrosequencing reveals that consumption of galactooligosaccharides results in a highly specific bifidogenic response in humans. *PLoS One.* 2011;6(9):e25200.
74. Sawicki CM, Livingston KA, Obin M, Roberts SB, Chung M, McKeown NM. Dietary Fiber and the Human Gut Microbiota: Application of Evidence Mapping Methodology. *Nutrients.* 2017;9(2):125.
75. Walker AW, Duncan SH, McWilliam Leitch EC, Child MW, Flint HJ. pH and peptide supply can radically alter bacterial populations and short-chain fatty acid ratios within microbial communities from the human colon. *Appl Environ Microbiol.* 2005;71(7):3692-700.
76. Duncan SH, Louis P, Thomson JM, Flint HJ. The role of pH in determining the species composition of the human colonic microbiota. *Environ Microbiol.* 2009;11(8):2112-22.
77. Tannock GW, Lawley B, Munro K*, et al.* RNA-stable-isotope probing shows utilization of carbon from inulin by specific bacterial populations in the rat large bowel. *Appl Environ Microbiol.* 2014;80(7):2240-47.
78. Holscher HD. Dietary fiber and prebiotics and the gastrointestinal microbiota. *Gut Microbes.* 2017;8(2):172-84.
79. Slavin JL. Fiber and prebiotics: mechanisms and health benefits. *Nutrients.* 2013;5(4):1417-35.
80. Hipsley EH. Dietary “Fibre” and Pregnancy Toxaemia. *BMJ.* 1953;2(4833):420–22.
81. Trowell HC. Definition of dietary fiber. *Lancet.* 1974;1(7856):503.
82. Joint FAO/WHO Food Standards Programme. Secretariat of the CODEX Alimentarius Commission: CODEX Alimentarius (CODEX) Guidelines on Nutrition Labeling CAC/GL 2-1985 as last amended 2010. Rome, Italy: FAO. 2010.

83. Food and Drug Administration H. Food Labeling: Revision of the Nutrition and Supplement Facts Labels. Maryland, United States.May 27,, 2016. RIN 0910-AF22.
84. Fuller S, Beck E, Salman H, Tapsell L. New Horizons for the Study of Dietary Fiber and Health: A Review. *Plant Foods Hum Nutr.* 2016;71(1):1-12.
85. Tungland BC, Meyer D. Nondigestible Oligo- and Polysaccharides (Dietary Fiber): Their Physiology and Role in Human Health and Food. *Compr Rev Food Sci Food Saf.* 2002;1(3):90-109.
86. Raigond P, Ezekiel R, Raigond B. Resistant starch in food: a review. *J Sci Food Agr.* 2015;95(10):1968-78.
87. Mudgil D, Barak S. Composition, properties and health benefits of indigestible carbohydrate polymers as dietary fiber: a review. *Int J Biol Macromol.* 2013;61:1-6.
88. Guillona F, Champ M. Structural and physical properties of dietary fibres, and consequences of processing on human physiology. *Food Res Int.* 2000;33(3-4):233–45.
89. Vrieze A, Van Nood E, Holleman F*, et al.* Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology.* 2012;143(4):913-16 e7.
90. Hehemann J-H, Correc G, Barbeyron T, Helbert W, Czjzek M, Michel G. Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota. *Nature.* 2010;464(7290):908-12.
91. Chassard C, Delmas E, Robert C, Bernalier-Donadille A. The cellulose-degrading microbial community of the human gut varies according to the presence or absence of methanogens. *FEMS Microbiol Ecol.* 2010;74(1):205-13.
92. Walter J. Murine Gut Microbiota—Diet Trumps Genes. *Cell Host Microbe.* 2015;17(1):3-5.
93. Kovatcheva-Datchary P, Egert M, Maathuis A*, et al.* Linking phylogenetic identities of bacteria to starch fermentation in an *in vitro* model of the large intestine by RNA-based stable isotope probing. *Environ Microbiol.* 2009;11(4):914-26.
94. Leitch ECM, Walker AW, Duncan SH, Holtrop G, Flint HJ. Selective colonization of insoluble substrates by human faecal bacteria. *Environ Microbiol.* 2007;9(3):667-79.
95. Rakoff-Nahoum S, Coyne MJ, Comstock LE. An ecological network of polysaccharide utilization among human intestinal symbionts. *Curr Biol.* 2014;24(1):40-49.
96. Flint HJ, Scott KP, Duncan SH, Louis P, Forano E. Microbial degradation of complex carbohydrates in the gut. *Gut Microbes.* 2012;3(4):289-306.
97. Belenguer A, Duncan SH, Calder AG*, et al.* Two routes of metabolic cross-feeding between *Bifidobacterium adolescentis* and butyrate-producing anaerobes from the human gut. *Appl Environ Microbiol.* 2006;72(5):3593-99.
98. Coyte KZ, Schluter J, Foster KR. The ecology of the microbiome: Networks, competition, and stability. *Science.* 2015;350(6261):663-66.
99. Koropatkin NM, Cameron EA, Martens EC. How glycan metabolism shapes the human gut microbiota. *Nat Rev Microbiol.* 2012;10(5):323-35.
100. Rey FE, Faith JJ, Bain J*, et al.* Dissecting the in vivo metabolic potential of two human gut acetogens. *J Biol Chem.* 2010;285(29):22082-90.
101. Fischbach MA, Sonnenburg JL. Eating for two: how metabolism establishes interspecies interactions in the gut. *Cell Host Microbe.* 2011;10(4):336-47.
102. Barcenilla A, Pryde SE, Martin JC*, et al.* Phylogenetic relationships of butyrate-producing bacteria from the human gut. *Appl Environ Microbiol.* 2000;66(4):1654-61.

103. Chassard C, Bernalier-Donadille A. H2 and acetate transfers during xylan fermentation between a butyrate-producing xylanolytic species and hydrogenotrophic microorganisms from the human gut. *FEMS Microbiol Lett.* 2006;254(1):116-22.
104. Duncan SH, Louis P, Flint HJ. Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. *Appl Environ Microbiol.* 2004;70(10):5810-17.
105. Lozupone CA, Hamady M, Cantarel BL*, et al.* The convergence of carbohydrate active gene repertoires in human gut microbes. *Proc Natl Acad Sci USA.* 2008;105(39):15076-81.
106. Vandeputte D, Falony G, Vieira-Silva S*, et al.* Prebiotic inulin-type fructans induce specific changes in the human gut microbiota. *Gut.* 2017;66(11):1968-74.
107. Heiman ML, Greenway FL. A healthy gastrointestinal microbiome is dependent on dietary diversity. *Mol Metab.* 2016;5(5):317-20.
108. Clayton JB, Vangay P, Huang H*, et al.* Captivity humanizes the primate microbiome. *Proc Natl Acad Sci USA.* 2016;113(37):10376-81.
109. Kong LC, Holmes BA, Cotillard A*, et al.* Dietary patterns differently associate with inflammation and gut microbiota in overweight and obese subjects. *PLoS One.* 2014;9(10):e109434.
110. Upadhyaya B, McCormack L, Fardin-Kia AR*, et al.* Impact of dietary resistant starch type 4 on human gut microbiota and immunometabolic functions. *Sci Rep.* 2016;6:28797.
111. West NP, Christophersen CT, Pyne DB*, et al.* Butyrylated starch increases colonic butyrate concentration but has limited effects on immunity in healthy physically active individuals. *Exerc Immunol Rev.* 2013;19:102-19.
112. Hooda S, Boler BMV, Serao MCR*, et al.* 454 pyrosequencing reveals a shift in fecal microbiota of healthy adult men consuming polydextrose or soluble corn fiber. *J Nutr.* 2012;142(7):1259-65.
113. Wong JM, de Souza R, Kendall CW, Emam A, Jenkins DJ. Colonic health: fermentation and short chain fatty acids. *J Clin Gastroenterol.* 2006;40(3):235-43.
114. Cummings JH, Pomare E, Branch W, Naylor C, Macfarlane GT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut.* 1987;28(10):1221-27.
115. Boets E, Gomand SV, Deroover L*, et al.* Systemic availability and metabolism of colonic-derived short-chain fatty acids in healthy subjects - a stable isotope study. *J Physiol.* 2017;595(2):541-55.
116. Yang J, Martínez I, Walter J, Keshavarzian A, Rose DJ. *In vitro* characterization of the impact of selected dietary fibers on fecal microbiota composition and short chain fatty acid production. *Anaerobe.* 2013;23:74-81.
117. Yang J, Rose DJ. Long-term dietary pattern of fecal donor correlates with butyrate production and markers of protein fermentation during in vitro fecal fermentation. *Nutr Res.* 2014;34(9):749-59.
118. Roediger W. Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. *Gut.* 1980;21(9):793-98.
119. Cummings JH, Macfarlane GT. The control and consequences of bacterial fermentation in the human colon. *J Appl Bacteriol.* 1991;70(6):443-59.
120. Duncan SH, Belenguer A, Holtrop G, Johnstone AM, Flint HJ, Lobley GE. Reduced dietary intake of carbohydrates by obese subjects results in decreased concentrations of

butyrate and butyrate-producing bacteria in feces. *Appl Environ Microbiol.* 2007;73(4):1073-78.

121. Russell WR, Gratz SW, Duncan SH*, et al.* High-protein, reduced-carbohydrate weight-loss diets promote metabolite profiles likely to be detrimental to colonic health. *Am J Clin Nutr.* 2011;93(5):1062-72.
122. Windey K, De Preter V, Verbeke K. Relevance of protein fermentation to gut health. *Mol Nutr Food Res.* 2012;56(1):184-96.
123. Sonnenburg JL, Xu J, Leip DD*, et al.* Glycan foraging *in vivo* by an intestine-adapted bacterial symbiont. *Science.* 2005;307(5717):1955-59.
124. Earle KA, Billings G, Sigal M*, et al.* Quantitative Imaging of Gut Microbiota Spatial Organization. *Cell Host Microbe.* 2015;18(4):478-88.
125. Desai Mahesh S, Seekatz Anna M, Koropatkin Nicole M*, et al.* A Dietary Fiber-Deprived Gut Microbiota Degrades the Colonic Mucus Barrier and Enhances Pathogen Susceptibility. *Cell.* 2016;167(5):1339-53.e21.
126. Lambeau KV, McRorie JWJ. Fiber supplements and clinically proven health benefits: How to recognize and recommend an effective fiber therapy. *J Am Acad Nurse Pract.* 2017;29(4):216-23.
127. Schwiertz A, Taras D, Schäfer K*, et al.* Microbiota and SCFA in Lean and Overweight Healthy Subjects. *Obesity.* 2010;18(1):190-95.
128. Belcheva A, Irrazabal T, Robertson SJ*, et al.* Gut microbial metabolism drives transformation of MSH2-deficient colon epithelial cells. *Cell.* 2014;158(2):288-99.
129. Lupton JR. Microbial degradation products influence colon cancer risk: the butyrate controversy. *J Nutr.* 2004;134(2):479-82.
130. Du H, van der A DL, Boshuizen HC*, et al.* Dietary fiber and subsequent changes in body weight and waist circumference in European men and women. *Am J Clin Nutr.* 2010;91(2):329-36.
131. Ben Q, Sun Y, Chai R, Qian A, Xu B, Yuan Y. Dietary fiber intake reduces risk for colorectal adenoma: a meta-analysis. *Gastroenterology.* 2014;146(3):689-99 e6.
132. Kunzmann AT, Coleman HG, Huang W-Y, Kitahara CM, Cantwell MM, Berndt SI. Dietary fiber intake and risk of colorectal cancer and incident and recurrent adenoma in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. *Am J Clin Nutr.* 2015;102(4):881-90.
133. Tan J, McKenzie C, Potamitis M, Thorburn AN, Mackay CR, Macia L. The role of short-chain fatty acids in health and disease. *Adv Immunol.* 2014;121:91-119.
134. Tazoe H, Otomo Y, Karaki S-i*, et al.* Expression of short-chain fatty acid receptor GPR41 in the human colon. *Biomedical Research.* 2009;30(3):149-56.
135. Tazoe H, Otomo Y, Kaji I, Tanaka R, Karaki S-i, Kuwahara A. Roles of short-chain fatty acids receptors, GPR41 and GPR43 on colonic functions. *J Physiol Pharmacol.* 2008;59(Suppl 2):251-62.
136. Brown AJ, Goldsworthy SM, Barnes AA*, et al.* The Orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *J Biol Chem.* 2003;278(13):11312-19.
137. Le Poul E, Loison C, Struyf S*, et al.* Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. *J Biol Chem.* 2003;278(28):25481-89.

138. Thangaraju M, Cresci GA, Liu K, *et al.* GPR109A is a G-protein-coupled receptor for the bacterial fermentation product butyrate and functions as a tumor suppressor in colon. *Cancer Research.* 2009;69(7):2826-32.
139. Wanders D, Graff EC, Judd RL. Effects of high fat diet on GPR109A and GPR81 gene expression. *Biochem Biophys Res Commun.* 2012;425(2):278-83.
140. Cresci GA, Thangaraju M, Mellinger JD, Liu K, Ganapathy V. Colonic gene expression in conventional and germ-free mice with a focus on the butyrate receptor GPR109A and the butyrate transporter SLC5A8. *J Gastrointest Surg.* 2010;14(3):449-61.
141. Blundell JE, De Graaf C, Hulshof T, *et al.* Appetite control: methodological aspects of the evaluation of foods. *Obes Rev.* 2010;11(3):251-70.
142. Samuel BS, Shaito A, Motoike T, *et al.* Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. *Proc Natl Acad Sci USA.* 2008;105(43):16767-72.
143. Tolhurst G, Heffron H, Lam YS, *et al.* Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. *Diabetes.* 2012;61(2):364-71.
144. Savage A, Adrian TE, Carolan G, Chatterjee V, Bloom S. Effects of peptide YY (PYY) on mouth to caecum intestinal transit time and on the rate of gastric emptying in healthy volunteers. *Gut.* 1987;28(2):166-70.
145. Batterham RL, Cowley MA, Small CJ, *et al.* Gut hormone PYY(3-36) physiologically inhibits food intake. *Nature.* 2002;418(6898):650-54.
146. Wei Y, Mojsov S. Tissue-specific expression of the human receptor for glucagon-like peptide-I: brain, heart and pancreatic forms have the same deduced amino acid sequences. *FEBS Lett.* 1995;358(3):219-24.
147. Merchenthaler I, Lane M, Shughrue P. Distribution of pre-pro-glucagon and glucagon-like peptide-1 receptor messenger RNAs in the rat central nervous system. *J Comp Neurol.* 1999;403(2):261-80.
148. Schjoldager B, Mortensen P, Christiansen J, Ørskov C, Holst JJ. GLP-1 (glucagon-like peptide 1) and truncated GLP-1, fragments of human proglucagon, inhibit gastric acid secretion in humans. *Dig Dis Sci.* 1989;34(5):703-08.
149. Näslund E, Bogefors J, Skogar S, *et al.* GLP-1 slows solid gastric emptying and inhibits insulin, glucagon, and PYY release in humans. *Am J Physiol.* 1999;277(3 Pt 2):R910-R16.
150. Xiong Y, Miyamoto N, Shibata K, *et al.* Short-chain fatty acids stimulate leptin production in adipocytes through the G protein-coupled receptor GPR41. *Proc Natl Acad Sci USA.* 2004;101(4):1045-50.
151. Zaibi MS, Stocker CJ, O'Dowd J, *et al.* Roles of GPR41 and GPR43 in leptin secretory responses of murine adipocytes to short chain fatty acids. *FEBS Lett.* 2010;584(11):2381-86.
152. Frost G, Sleeth ML, Sahuri-Arisoylu M, *et al.* The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nat Commun.* 2014;5:3611.
153. De Vadder F, Kovatcheva-Datchary P, Goncalves D, *et al.* Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. *Cell.* 2014;156(1-2):84-96.
154. Delaere F, Duchampt A, Mounien L, *et al.* The role of sodium-coupled glucose co-transporter 3 in the satiety effect of portal glucose sensing. *Mol Metab.* 2013;2(1):47-53.

155. Kimura I, Inoue D, Maeda T, *et al.* Short-chain fatty acids and ketones directly regulate sympathetic nervous system via G protein-coupled receptor 41 (GPR41). *Proc Natl Acad Sci USA.* 2011;108(19):8030-35.
156. Gao Z, Yin J, Zhang J, *et al.* Butyrate improves insulin sensitivity and increases energy expenditure in mice. *Diabetes.* 2009;58(7):1509-17.
157. Freitag J, Berod L, Kamradt T, Sparwasser T. Immunometabolism and autoimmunity. *Immunol Cell Biol.* 2016;94(10):925-34.
158. Hajer GR, van Haeften TW, Visseren FLJ. Adipose tissue dysfunction in obesity, diabetes, and vascular diseases. *Eur Heart J.* 2008;29(24):2959-71.
159. Beserra BTS, Fernandes R, do Rosario VA, Mocellin MC, Kuntz MGF, Trindade EBSM. A systematic review and meta-analysis of the prebiotics and synbiotics effects on glycaemia, insulin concentrations and lipid parameters in adult patients with overweight or obesity. *Clin Nutr.* 2015;34(5):845-58.
160. Whitehead A, Beck EJ, Tosh S, Wolever TMS. Cholesterol-lowering effects of oat β-glucan: a meta-analysis of randomized controlled trials. *Am J Clin Nutr.* 2014;100(6):1413-21.
161. Kellow NJ, Coughlan MT, Reid CM. Metabolic benefits of dietary prebiotics in human subjects: a systematic review of randomised controlled trials. *Br J Nutr.* 2014;111(7):1147-61.
162. Ning H, Van Horn L, Shay CM, Lloyd-Jones DM. Associations of dietary fiber intake with long-term predicted cardiovascular disease risk and C-reactive protein levels (from the National Health and Nutrition Examination Survey Data [2005-2010]). *Am J Cardiol.* 2014;113(2):287-91.
163. Yao B, Fang H, Xu W, *et al.* Dietary fiber intake and risk of type 2 diabetes: a dose-response analysis of prospective studies. *Eur J Epidemiol.* 2014;29(2):79-88.
164. Kreymann B, Ghatei MA, Williams G, Bloom SR. Glucagon-Like Peptide-1 7-36: A Physiological Incretin in Man. *Lancet.* 1987;330(8571):1300-04.
165. Komatsu R, Matsuyama T, Namba M, *et al.* Glucagonostatic and insulinotropic action of glucagonlike peptide I-(7-36)-amide. *Diabetes.* 1989;38(7):902-05.
166. Farilla L, Bulotta A, Hirshberg B, *et al.* Glucagon-like peptide 1 inhibits cell apoptosis and improves glucose responsiveness of freshly isolated human islets. *Endocrinology.* 2003;144(12):5149-58.
167. Holz GGI, Kühtreiber WM, Habener JF. Pancreatic beta-cells are rendered glucose-competent by the insulinotropic hormone glucagon-like peptide-1(7-37). *Nature.* 1993;361(6410):362-65.
168. Pingitore A, Chambers ES, Hill T, *et al.* The diet-derived short chain fatty acid propionate improves beta-cell function in humans and stimulates insulin secretion from human islets in vitro. *Diabetes Obes Metab.* 2017;19(2):257-65.
169. Roder PV, Wu B, Liu Y, Han W. Pancreatic regulation of glucose homeostasis. *Exp Mol Med.* 2016;48:e219.
170. Clore JN, Stillman J, Sugerman H. Glucose-6-phosphatase flux in vitro is increased in type 2 diabetes. *Diabetes.* 2000;49(6):969-74.
171. Magnusson I, Rothman DL, Katz LD, Shulman RG, Shulman GI. Increased rate of gluconeogenesis in type II diabetes mellitus. A 13C nuclear magnetic resonance study. *J Clin Invest.* 1992;90(4):1323-27.

172. den Besten G, Bleeker A, Gerding A*, et al.* Short-Chain Fatty Acids Protect Against High-Fat Diet–Induced Obesity via a PPARγ-Dependent Switch From Lipogenesis to Fat Oxidation. *Diabetes.* 2015;64(7):2398-408.
173. Wolever TM, Spadafora P, Eshuis H. Interaction between colonic acetate and propionate in humans. *Am J Clin Nutr.* 1991;53(3):681-87.
174. Ge H, Li X, Weiszmann J*, et al.* Activation of G protein-coupled receptor 43 in adipocytes leads to inhibition of lipolysis and suppression of plasma free fatty acids. *Endocrinology.* 2008;149(9):4519-26.
175. Al-Lahham Sa, Roelofsen H, Rezaee F*, et al.* Propionic acid affects immune status and metabolism in adipose tissue from overweight subjects. *Eur J Clin Invest.* 2012;42(4):357-64.
176. Gregor MF, Hotamışlıgil GS. Inflammatory mechanisms in obesity. *Annu Rev Immunol.* 2011;29:415-45.
177. Jiao J, Xu J-Y, Zhang W, Han S, Qin L-Q. Effect of dietary fiber on circulating C-reactive protein in overweight and obese adults: a meta-analysis of randomized controlled trials. *Int J Food Sci Nutr.* 2015;66(1):114-19.
178. North CJ, Venter CS, Jerling JC. The effects of dietary fibre on C-reactive protein, an inflammation marker predicting cardiovascular disease. *Eur J Clin Nutr.* 2009;63(8):921-33.
179. Piya MK, Harte AL, McTernan PG. Metabolic endotoxaemia: is it more than just a gut feeling? *Curr Opin Lipidol.* 2013;24(1):78-85.
180. Lassenius MI, Pietiläinen KH, Kaartinen K*, et al.* Bacterial endotoxin activity in human serum is associated with dyslipidemia, insulin resistance, obesity, and chronic inflammation. *Diabetes Care.* 2011;34(8):1809-15.
181. Hawkesworth S, Moore SE, Fulford AJ*, et al.* Evidence for metabolic endotoxemia in obese and diabetic Gambian women. *Nutr Diabetes.* 2013;3:e83.
182. Amar J, Burcelin R, Ruidavets JB*, et al.* Energy intake is associated with endotoxemia in apparently healthy men. *Am J Clin Nutr.* 2008;87(5):1219-23.
183. Cani PD, Amar J, Iglesias MA*, et al.* Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes.* 2007;56(7):1761-72.
184. Everard A, Lazarevic V, Derrien M*, et al.* Responses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic obese and diet-induced leptin-resistant mice. *Diabetes.* 2011;60(11):2775-86.
185. Neyrinck AM, Possemiers S, Druart C*, et al.* Prebiotic Effects of Wheat Arabinoxylan Related to the Increase in *Bifidobacteria*, *Roseburia* and *Bacteroides*/*Prevotella* in Diet-Induced Obese Mice. *PLoS One.* 2011;6(6):e20944.
186. Cani PD, Possemiers S, Van de Wiele T*, et al.* Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut.* 2009;58(8):1091-103.
187. Kelly CJ, Zheng L, Campbell EL*, et al.* Crosstalk between Microbiota-Derived Short-Chain Fatty Acids and Intestinal Epithelial HIF Augments Tissue Barrier Function. *Cell Host Microbe.* 2015;17(5):662-71.
188. Saeedi BJ, Kao DJ, Kitzenberg DA*, et al.* HIF-dependent regulation of claudin-1 is central to intestinal epithelial tight junction integrity. *Mol Biol Cell.* 2015;26(12):2252-62.

189. Peng L, He Z, Chen W, Holzman IR, Lin J. Effects of butyrate on intestinal barrier function in a Caco-2 cell monolayer model of intestinal barrier. *Pediatr Res.* 2007;61(1):37-41.
190. Chen H, Wang W, Degroote J*, et al.* Arabinoxylan in wheat is more responsible than cellulose for promoting intestinal barrier function in weaned male piglets. *J Nutr.* 2015;145(1):51-58.
191. Gaudier E, Jarry A, Blottière HM*, et al.* Butyrate specifically modulates MUC gene expression in intestinal epithelial goblet cells deprived of glucose. *Am J Physiol Gastrointest Liver Physiol.* 2004;287(6):G1168-G74.
192. Burger-van Paassen N, Vincent A, Puiman PJ*, et al.* The regulation of intestinal mucin MUC2 expression by short-chain fatty acids: implications for epithelial protection. *Biochem J.* 2009;420(2):211-19.
193. Singh N, Gurav A, Sivaprakasam S*, et al.* Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. *Immunity.* 2014;40(1):128-39.
194. Nowarski R, Jackson R, Gagliani N*, et al.* Epithelial IL-18 Equilibrium Controls Barrier Function in Colitis. *Cell.* 2015;163(6):1444-56.
195. Cipolletta D. Adipose tissue-resident regulatory T cells: phenotypic specialization, functions and therapeutic potential. *Immunology.* 2014;142(4):517-25.
196. Zeng H, Chi H. Metabolic control of regulatory T cell development and function. *Trends Immunol.* 2015;36(1):3-12.
197. Smith PM, Howitt MR, Panikov N*, et al.* The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science.* 2013;341(6145):569-73.
198. Arpaia N, Campbell C, Fan X*, et al.* Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature.* 2013;504(7480):451-55.
199. Furusawa Y, Obata Y, Fukuda S*, et al.* Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature.* 2013;504(7480):446-50.
200. Park J, Kim M, Kang SG*, et al.* Short-chain fatty acids induce both effector and regulatory T cells by suppression of histone deacetylases and regulation of the mTOR-S6K pathway. *Mucosal Immunol.* 2015;8(1):80-93.
201. Schwarz A, Bruhs A, Schwarz T. The Short-Chain Fatty Acid Sodium Butyrate Functions as a Regulator of the Skin Immune System. *J Invest Dermatol.* 2017;137(4):855-64.
202. Trompette A, Gollwitzer ES, Yadava K*, et al.* Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat Med.* 2014;20(2):159-66.
203. Chang PV, Hao L, Offermanns S, Medzhitov R. The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition. *Proc Natl Acad Sci USA.* 2014;111(6):2247-52.
204. Kamp ME, Shim R, Nicholls AJ*, et al.* G Protein-Coupled Receptor 43 Modulates Neutrophil Recruitment during Acute Inflammation. *PLoS One.* 2016;11(9):e0163750.
205. Vinolo MAR, Rodrigues HG, Hatanaka E, Sato FT, Sampaio SC, Curi R. Suppressive effect of short-chain fatty acids on production of proinflammatory mediators by neutrophils. *J Nutr Biochem.* 2011;22(9):849-55.
206. Maslowski KM, Vieira AT, Ng A*, et al.* Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature.* 2009;461(7268):1282-86.

207. Wu W, Sun M, Chen F, *et al.* Microbiota metabolite short-chain fatty acid acetate promotes intestinal IgA response to microbiota which is mediated by GPR43. *Mucosal Immunol.* 2016;10(4):946-56.
208. Kim M, Qie Y, Park J, Kim CH. Gut Microbial Metabolites Fuel Host Antibody Responses. *Cell Host Microbe.* 2016;20(2):202-14.
209. Sun J, Furio L, Mecheri R, *et al.* Pancreatic β-Cells Limit Autoimmune Diabetes via an Immunoregulatory Antimicrobial Peptide Expressed under the Influence of the Gut Microbiota. *Immunity.* 2015;43(2):304-17.
210. Cani PD, Neyrinck AM, Fava F, *et al.* Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. *Diabetologia.* 2007;50(11):2374-83.
211. He B, Nohara K, Ajami NJ, *et al.* Transmissible microbial and metabolomic remodeling by soluble dietary fiber improves metabolic homeostasis. *Sci Rep.* 2015;5:10604.
212. Priyadarshini M, Villa SR, Fuller M, *et al.* An Acetate-Specific GPCR, FFAR2, Regulates Insulin Secretion. *Mol Endocrinol.* 2015;29(7):1055-66.
213. Psichas A, Sleeth ML, Murphy KG, *et al.* The short chain fatty acid propionate stimulates GLP-1 and PYY secretion via free fatty acid receptor 2 in rodents. *Int J Obes.* 2015;39(3):424-29.
214. Brooks L, Viardot A, Tsakmaki A, *et al.* Fermentable carbohydrate stimulates FFAR2-dependent colonic PYY cell expansion to increase satiety. *Mol Metab.* 2017;6(1):48-60.
215. Natarajan N, Hori D, Flavahan S, *et al.* Microbial short chain fatty acid metabolites lower blood pressure via endothelial G-protein coupled receptor 41. *Physiol Genomics.* 2016;48(11):826-34.
216. Macia L, Tan J, Vieira AT, *et al.* Metabolite-sensing receptors GPR43 and GPR109A facilitate dietary fibre-induced gut homeostasis through regulation of the inflammasome. *Nat Commun.* 2015;6:6734.
217. Coates ME. Gnotobiotic animals in research: their uses and limitations. *Lab Anim.* 1975;9(4):275-82.
218. Belkaid Y, Hand T. Role of the Microbiota in Immunity and Inflammation. *Cell.* 2014;157(1):121–41.
219. Bäckhed F, Manchester JK, Semenkovich CF, Gordon JI. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proc Natl Acad Sci USA.* 2007;104(3):979-84.
220. Schwabe RF, Jobin C. The microbiome and cancer. *Nat Rev Cancer.* 2013;13(11):800-12.
221. Sonnenburg JL, Bäckhed F. Diet-microbiota interactions as moderators of human metabolism. *Nature.* 2016;535(7610):56-64.
222. Buyken AE, Goletzke J, Joslowski G, *et al.* Association between carbohydrate quality and inflammatory markers: systematic review of observational and interventional studies. *Am J Clin Nutr.* 2014;99(4):813-33.
223. Food and Drug Administration H. Subpart E-Specific Requirements for Health Claims. Maryland, United States. April 1, 2016.
224. EFSA Panel on Dietetic Products Nutrition and Allergies. Scientific Opinion on the substantiation of a health claim related to "native chicory inulin" and maintenance of normal defecation by increasing stool frequency pursuant to Article 13.5 of Regulation (EC) No 1924/2006. *EFSA J.* 2015;13(1):3951.

225. Lambert JE, Parnell JA, Tunnicliffe JM, Han J, Sturzenegger T, Reimer RA. Consuming yellow pea fiber reduces voluntary energy intake and body fat in overweight/obese adults in a 12-week randomized controlled trial. *Clin Nutr.* 2017;36(1):126-33.
226. Vanegas SM, Meydani M, Barnett JB*, et al.* Substituting whole grains for refined grains in a 6-wk randomized trial has a modest effect on gut microbiota and immune and inflammatory markers of healthy adults. *Am J Clin Nutr.* 2017;105(3):635-50.
227. Clarke ST, Green-Johnson JM, Brooks SPJ*, et al.* β2-1 Fructan supplementation alters host immune responses in a manner consistent with increased exposure to microbial components: results from a double-blinded, randomised, cross-over study in healthy adults. *Br J Nutr.* 2016;115(10):1748-59.
228. Collado Yurrita L, San Mauro Martin I, Ciudad-Cabanas MJ, Calle-Puron ME, Hernandez Cabria M. Effectiveness of inulin intake on indicators of chronic constipation; a meta-analysis of controlled randomized clinical trials. *Nutr Hosp.* 2014;30(2):244-52.
229. Liu F, Prabhakar M, Ju J, Long H, Zhou H-W. Effect of inulin-type fructans on blood lipid profile and glucose level: a systematic review and meta-analysis of randomized controlled trials. *Eur J Clin Nutr.* 2017;71(1):9-20.
230. Brighenti F. Dietary Fructans and Serum Triacylglycerols: A Meta-Analysis of Randomized Controlled Trials. *J Nutr.* 2007;137(11 Suppl):2552S-56S.
231. Fechner A, Kiehntopf M, Jahreis G. The formation of short-chain fatty acids is positively associated with the blood lipid-lowering effect of lupin kernel fiber in moderately hypercholesterolemic adults. *J Nutr.* 2014;144(5):599-607.
232. Freeland KR, Wolever TMS. Acute effects of intravenous and rectal acetate on glucagon-like peptide-1, peptide YY, ghrelin, adiponectin and tumour necrosis factor-alpha. *Br J Nutr.* 2010;103(3):460-66.
233. van der Beek CM, Canfora EE, Lenaerts K*, et al.* Distal, not proximal, colonic acetate infusions promote fat oxidation and improve metabolic markers in overweight/obese men. *Clin Sci.* 2016;130(22):2073-82.
234. Livingston KA, Chung M, Sawicki CM*, et al.* Development of a Publicly Available, Comprehensive Database of Fiber and Health Outcomes: Rationale and Methods. *PLoS One.* 2016;11(6):e0156961.
235. Saura-Calixto F. Dietary fiber as a carrier of dietary antioxidants: an essential physiological function. *J Agric Food Chem.* 2011;59(1):43-49.
236. Vitaglione P, Mennella I, Ferracane R*, et al.* Whole-grain wheat consumption reduces inflammation in a randomized controlled trial on overweight and obese subjects with unhealthy dietary and lifestyle behaviors: role of polyphenols bound to cereal dietary fiber. *Am J Clin Nutr.* 2015;101(2):251-61.
237. Quiros-Sauceda A, Palafox-Carlos H, Sayago-Ayerdi S*, et al.* Dietary fiber and phenolic compounds as functional ingredients: interaction and possible effect after ingestion. *Food Funct.* 2014;5(6):1063-72.
238. Jacobs DR, Tapsell LC. Food, Not Nutrients, Is the Fundamental Unit in Nutrition. *Nutr Rev.* 2007;65(10):439-50.
239. Cardona F, Andres-Lacueva C, Tulipani S, Tinahones FJ, Queipo-Ortuno MI. Benefits of polyphenols on gut microbiota and implications in human health. *J Nutr Biochem.* 2013;24(8):1415-22.
240. Jenkins DJ, Kendall CW, Popovich DG*, et al.* Effect of a very-high-fiber vegetable, fruit, and nut diet on serum lipids and colonic function. *Metabolism.* 2001;50(4):494-503.

241. Kendall CW, Esfahani A, Jenkins DJ. The link between dietary fibre and human health. *Food Hydrocoll.* 2010;24(1):42-48.
242. Delcour JA, Aman P, Courtin CM, Hamaker BR, Verbeke K. Prebiotics, Fermentable Dietary Fiber, and Health Claims. *Adv Nutr.* 2016;7(1):1-4.
243. Martínez I, Perdicaro DJ, Brown AW*, et al.* Diet-induced alterations of host cholesterol metabolism are likely to affect the gut microbiota composition in hamsters. *Appl Environ Microbiol.* 2013;79(2):516-24.
244. Carding S, Verbeke K, Vipond DT, Corfe BM, Owen LJ. Dysbiosis of the gut microbiota in disease. *Microb Ecol Health Dis.* 2015;26:26191.
245. Bindels LB, Segura Munoz RR, Gomes-Neto JC*, et al.* Resistant starch can improve insulin sensitivity independently of the gut microbiota. *Microbiome.* 2017;5(1):12.
246. Burkitt DP, Walker ARP, Painter NS. Effect of dietary fibre on stools and the transit-times, and its role in the causation of disease. *Lancet.* 1972;2(7792):1408-12.
247. Falony G, Joossens M, Vieira-Silva S*, et al.* Population-level analysis of gut microbiome variation. *Science.* 2016;352(6285):560-64.
248. Cameron-Smith D, Collier G, O'Dea K. Effect of soluble dietary fibre on the viscosity of gastrointestinal contents and the acute glycaemic response in the rat. *Br J Nutr.* 1994;71(4):563-71.
249. Dikeman CL, Murphy MR, Fahey GCJ. Dietary fibers affect viscosity of solutions and simulated human gastric and small intestinal digesta. *J Nutr.* 2006;136(4):913-19.
250. Ferguson LR, Harris PJ. Adsorption of Carcinogens by Dietary Fiber. In: Cho SS, Dreher ML, eds. *Hand-book of Dietary Fiber*. New York: Marcel Dekker; 2001:207–18.
251. Gunness P, Gidley MJ. Mechanisms underlying the cholesterol-lowering properties of soluble dietary fibre polysaccharides. *Food Funct.* 2010;1(2):149-55.
252. Li T, Chiang JYL. Bile acid signaling in metabolic disease and drug therapy. *Pharmacol Rev.* 2014;66(4):948-83.
253. Shepherd R. Resistance to changes in diet. *Proc Nutr Soc.* 2002;61(2):267-72.

CHAPTER 2: Rationale, Objectives, and Hypotheses

2.1 Rationale and Overall Research Goal

Obesity and associated comorbidities, such as cardiovascular disease and type II diabetes, have reached epidemic proportions world-wide[254,255], with the global economic burden of obesity alone estimated at over a trillion dollars annually[256]. Although an overly simplistic model of energy homeostasis, excess body weight generally results from an imbalance between calories consumed and energy expended[257]. This imbalance is partially facilitated by diets high in refined, energy-dense foods but low in non-digestible carbohydrates known as dietary fibers (DF)[258], which are frequently consumed by individuals in socioeconomically developed societies. Obesity is further characterized by a state of metabolically driven chronic low-grade systemic inflammation, for which growing evidence implicates dysfunctional immunometabolism in the pathogenesis of several obesity-associated comorbidities[259,260]. Therefore, successful therapeutic strategies for overcoming these chronic diseases would ideally impact energy homeostasis, promote satiety, and induce beneficial immunometabolic effects.

Virtually all nutritional policies advocate for the consumption of nutrient-dense plant-based diets rich in fruits, vegetables, whole grains, legumes, nuts, and seeds for the treatment and prevention of obesity and associated comorbidities[42,261-263]. Contained within the matrixes of plant-foods are a diverse array of health-promoting bioactive compounds such as vitamins, minerals, phytochemicals, and DFs[264,265], with DF being particularly well-recognized as an important component of a healthy diet[42,266]. Findings from large-scale observational studies provide consistent evidence that increased DF consumption, when provided by plant-foods, reduces the risk of chronic disease[267-269] and all-cause mortality[270,271]. Mechanistic studies have further established processes by which DFs alter energy homeostasis, promote satiety, and induce immunometabolic effects[84,272,273]. Although achieving recommended amounts of DF with plant-foods is certainly the most advantageous for chronic disease prevention, efforts to increase consumption have not significantly changed consumer behavior[274,275]. Therefore affluent societies suffer from a 'fiber gap'[12] where average intakes of DF remain at only half of what is recommended[37,275], a factor that has potentially contributed to the obesity epidemic[27,275]. In **Chapter 1.1**, a conceptual framework was set forth to close the fiber gap, proposing that purified DFs could be synthesized or isolated from plant-foods and incorporated into regularly consumed

foods such as white flour-rich products[13] in order to reach recommended amounts[27,276,277]. Indeed, a variety of purified DFs are already commercially available with physicochemical attributes that allow for their application in refined foods without affecting product quality[27,278].

One emerging mechanism by which DFs exert physiologic effects relevant to obesity involves the favorable modulation of the microbial community that inhabits our gastrointestinal tract[266,272]. The composition of this community, termed the gut microbiota, and particularly its metabolic functions have been linked to the pathophysiology of obesity and associated comorbidities[279], and are therefore potential therapeutic targets. As several purified DFs have established microbiota-accessible carbohydrates[27], their use in foods or as supplements could alter the gut microbiota in ways that may confer health benefits in humans[273,276]. For instance, purified DFs have been shown to induce a general shift in microbial metabolism towards beneficial short-chain fatty acids (SCFAs) and away from the production of *N*-nitroso compounds, sulfides, and other detrimental metabolites generated during the degradation of host mucins and dietary proteins[121,125,280-282]. Purified DFs also display substantial structural differences that dictate which microbes are involved in their degradation and direct SCFA production[116,283-285], which opens exciting opportunities for precision microbiota manipulation to maintain health or treat chronic disease[36,286,287]. Overall, the partial replacement of readily digestible ingredients with purified DFs would have tremendous potential for closing the fiber gap and perhaps regressing the prevalence of obesity and its associated comorbidities in part by remodeling the gut microbiota. In **Chapter 1.2**, the role of DF in modulating the gut microbiota to improve human health was discussed, including effects on the composition and metabolic functions of the gut microbiota and the mechanisms by which DFs promote health. Ecological concepts important for both the interpretation and prediction of gut microbial responses to DF, such as functional redundancy, inter-species interactions, and inter-personal differences, were also discussed, along with current promises and challenges for the field.

While prospects for the health promotion of purified DFs appear promising, their applications in human dietary intervention trials have yielded conflicting results with extensive between-study heterogeneity[84,222,277]. For this reason, disagreements remain as to whether DF can exert reliable health effects when a simple reductionist approach is applied with purified DFs[288-291]. As cautioned by Mozaffarian and colleagues, simple reductionist approaches to signal nutrients such as DF may actually facilitate uncertainties and led to misleading concepts, since

nutrients are not consumed in isolation but instead as part of foods and dietary patterns[292]. Therefore, intact plant-food matrices[293,294], and the synergistic interactions between phytochemicals and DFs contained within said matrices[295,296], might be needed to achieve more consistent health benefits linked to DF. It is also feasible that DFs are not the primary health-promoting component of plant-foods[264,265,291,297], or that physiological effects of DF are diminished or even lost once isolated from food matrices[267,298,299]. In addition, higher amounts of DF closer to the diet of our ancestors (*i.e.* >50 g/day) may be required for reliable and sustained health outcomes and for physiologically relevant changes to gut microbiota configurations[273,300-303]. However, the exact efficacious dose of DF required for measurable effects in humans, and if such doses are beyond the tolerance of modern humans, remains unknown[300].

To ascertain whether purified DFs hold merit for the promotion of human health and treatment of chronic disease, several important questions remain to be answered. (i) Can DFs exert reliable physiological benefits in humans when a reductionist approach is used with different purified DFs ingredients? If so, what are the efficacious doses, and are there physicochemical properties that should be considered to achieve specific health outcomes? (ii) Would daily consumption of efficacious doses be tolerable, and would modern humans adapt to these doses? (iii) How do different purified DFs affect the composition and metabolic activity of the gut microbiota and to what degree are effects individualized? Can individualized responses of health-relevant SCFAs be predicted by features of the gut microbial community despite ecological constraints, and if so, what are the best predicting factors? (iv) Can discrete DF structures at specific doses be used to induce predictable changes to the gut microbiota that are relevant for health despite ecological constraints of the gut microbiota? If so, what are the efficacious doses required to maximize these effects?

The goals of the research discussed in this PhD dissertation were to address these open questions, to explore the potential of purified DFs to beneficially modulate the gut microbiota and improve human health through more targeted approaches, and to overall contribute to the conceptual framework presented in **Chapter 1.1.** These goals were achieved by (i) systematically reviewing the application of purified DFs in human intervention trials and (ii) characterizing in humans the effects of high-dose supplementation with distinct DF structures on gut microbial community composition, the output of health-relevant SCFAs, and gastrointestinal symptoms.

2.2 Research Objectives, Specific Aims, and Hypotheses

2.2.1 Objective 1: To summarize the immunometabolic effects of purified DFs through a systematic review of human intervention trials that used isolated and synthetic DFs and assess immunometabolic risk markers of disease.

Specific Aims:

i. To evaluate the effect of isolated and synthetic DF supplementation on well-established risk markers of metabolic disease related to glycemia, systemic inflammation, and lipidemia in intervention studies in healthy and/or at-risk adults.

ii. To determine whether reported effects differ based on DF dose, DF physicochemical properties, intervention duration, and the placebo used.

This objective was investigated in **Chapter 3**.

2.2.2 Objective 2: To explore in humans the effect of long-chain, corn bran arabinoxylan (AX) at high daily doses on fecal microbiota composition and SCFAs, as compared to microcrystalline cellulose (MCC), and to integrate findings using an ecological framework.

Specific Aims:

i. To conduct a six-week, parallel-arm, exploratory randomized controlled trial in individuals with overweight and obesity and to characterize the short- and long-term effects (*i.e.* weeks 1 and 6) of high-dose AX supplementation on fecal microbiota composition and SCFA concentrations relative to MCC (non-fermentable control).

ii. To systematically assess whether baseline microbiota composition, fiber-induced compositional shifts, or baseline diet history explain SCFA responses observed among individuals.

Hypothesis:

Supplementation with AX and MCC would show structure-dependent effects on fecal microbiota composition and SCFA concentrations, with compositional shifts further linking to the output of fecal SCFAs.

This objective was investigated in **Chapter 4**. The effects of AX and MCC supplementation on perceived satiety and surrogate endpoints, and whether the effects of DF were predictable by

features of the fecal microbiota or related molecular markers, were further investigated in **Appendix A**.

2.2.3 Objective 3: To evaluate the gastrointestinal tolerance of fermentable AX at efficacious amounts, when compared to non-fermentable MCC, and to systematically investigate links to the fecal microbiota and diet.

Specific Aims:

i. To extend the exploratory randomized controlled trial conducted to achieve Objective 2 in order to evaluate the severity of gastrointestinal symptoms during high-dose AX supplementation relative to MCC and to determine the degree to which humans adapt to tolerate AX.
ii. To systematically assess whether individual differences in AX tolerance associate with fecal microbiota composition, pH, SCFAs, or diet history.

Hypothesis:

The severity of gastrointestinal symptoms reported during AX supplementation would be individualized with detectable links to baseline diet history and AX-induces changes in fecal microbiota composition and/or SCFA concentrations.

This objective was investigated in **Chapter 5**.

2.2.4 Objective 4: To determine if specific doses of discrete DF structure could be used in humans to direct changes in fecal microbiota composition and its output of beneficial SCFAs.

Specific Aims:

i. To perform a randomized, placebo-controlled, double-blind, parallel-four-arm, dose-response study and to compare in humans the effects and dose-response relationships (at doses up to 50 g/day) of three chemically modified resistant starches (RS4s) with discrete structures on fecal microbiota composition, SCFA profiles, and perceived gastrointestinal tolerance.
ii. To assess whether substrate-specific effects on fecal microbiota composition associate with fecal SCFA responses.

Hypothesis:

Small discrete differences in the chemical structure of RS4s would direct changes in fecal microbiota composition and its SCFA output.

This objective was investigated in **Chapter 6**.

2.3 References

12. Jones JM. CODEX-aligned dietary fiber definitions help to bridge the 'fiber gap'. *Nutr J.* 2014;13:34.
13. Fungwe TV, Bente L, Hiza H. The Food Supply and Dietary Fiber: Its Availability and Effect on Health. Center for Nutrition Policy and Promotion. November, 2007.
27. Deehan EC, Walter J. The Fiber Gap and the Disappearing Gut Microbiome: Implications for Human Nutrition. *Trends Endocrinol Metab.* 2016;27(5):239-42.
36. Hamaker BR, Tuncil YE. A perspective on the complexity of dietary fiber structures and their potential effect on the gut microbiota. *J Mol Biol.* 2014;426(23):3838-50.
37. King DE, Mainous AGr, Lambourne CA. Trends in dietary fiber intake in the United States, 1999-2008. *J Acad Nutr Diet.* 2012;112(5):642-68.
42. Dahl WJ, Stewart ML. Position of the Academy of Nutrition and Dietetics: Health Implications of Dietary Fiber. *J Acad Nutr Diet.* 2015;115(11):1861-70.
84. Fuller S, Beck E, Salman H, Tapsell L. New Horizons for the Study of Dietary Fiber and Health: A Review. *Plant Foods Hum Nutr.* 2016;71(1):1-12.
116. Yang J, Martínez I, Walter J, Keshavarzian A, Rose DJ. *In vitro* characterization of the impact of selected dietary fibers on fecal microbiota composition and short chain fatty acid production. *Anaerobe.* 2013;23:74-81.
121. Russell WR, Gratz SW, Duncan SH*, et al.* High-protein, reduced-carbohydrate weight-loss diets promote metabolite profiles likely to be detrimental to colonic health. *Am J Clin Nutr.* 2011;93(5):1062-72.
125. Desai Mahesh S, Seekatz Anna M, Koropatkin Nicole M*, et al.* A Dietary Fiber-Deprived Gut Microbiota Degrades the Colonic Mucus Barrier and Enhances Pathogen Susceptibility. *Cell.* 2016;167(5):1339-53.e21.
222. Buyken AE, Goletzke J, Joslowski G*, et al.* Association between carbohydrate quality and inflammatory markers: systematic review of observational and interventional studies. *Am J Clin Nutr.* 2014;99(4):813-33.
254. Ng M, Fleming T, Robinson M*, et al.* Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet.* 2014;384(9945):766-81.
255. NCD Risk Factor Collaboration (NCD-RisC). Worldwide Trends in Diabetes Since 1980: A Pooled Analysis of 751 Population-Based Studies With 4.4 Million Participants *Lancet.* 2016;387(10027):1513-30.
256. Tremmel M, Gerdtham U-G, Nilsson PM, Saha S. Economic Burden of Obesity: A Systematic Literature Review. *Int J Environ Res Public Health.* 2017;14(4):435.
257. Fock KM, Khoo J. Diet and exercise in management of obesity and overweight. *J Gastroenterol Hepatol.* 2013;28(S4):59-63.

258. Hall KD, Ayuketah A, Brychta R, *et al.* Ultra-Processed Diets Cause Excess Calorie Intake and Weight Gain: An Inpatient Randomized Controlled Trial of Ad Libitum Food Intake. *Cell Metab.* 2019;30(1):67-77.e3.
259. Hotamışlıgil GS. Foundations of Immunometabolism and Implications for Metabolic Health and Disease. *Immunity.* 2017;47(3):406-20.
260. Saltiel AR, Olefsky JM. Inflammatory mechanisms linking obesity and metabolic disease. *J Clin Invest.* 2017;127(1):1-4.
261. United States Department of Health and Human Services, United States Department of Agriculture. Dietary guidelines for Americans 2015-2020. December, 2015. Available from: https://health.gov/dietaryguidelines/2015/resources/2015-2020_Dietary_Guidelines.pdf.
262. Health Canada. Canada's Dietary Guidelines for Health Professionals and Policy Makers. January, 2019. Available from: https://food-guide.canada.ca/static/assets/pdf/CDG-EN-2018.pdf.
263. Bermudez OI, Dwyer JT, Yu W, Tolstoi LG. Dietary Guidelines around the World: An Update. In: Berdanier CD, Dwyer JT, Feldman EB, eds. *Handbook of Nutrition and Food*. 2nd ed. Boca Raton (USA): CRC Press; 2007:429-49.
264. Rose DJ. Impact of whole grains on the gut microbiota: the next frontier for oats? *Br J Nutr.* 2014;112(Suppl 2):S44-S49.
265. Abuajah CI, Ogbonna AC, Osuji CM. Functional components and medicinal properties of food: a review. *J Food Sci Technol.* 2015;52(5):2522-29.
266. Makki K, Deehan EC, Walter J, Bäckhed F. The Impact of Dietary Fiber on Gut Microbiota in Host Health and Disease. *Cell Host Microbe.* 2018;23(6):705-15.
267. Reynolds A, Mann J, Cummings J, Winter N, Mete E, Te Morenga L. Carbohydrate quality and human health: a series of systematic reviews and meta-analyses. *Lancet.* 2019;393(10170):434-45.
268. AlEssa HB, Bhupathiraju SN, Malik VS, *et al.* Carbohydrate quality and quantity and risk of type 2 diabetes in US women. *Am J Clin Nutr.* 2015;102(6):1543-53.
269. Veronese N, Solmi M, Caruso MG, *et al.* Dietary fiber and health outcomes: an umbrella review of systematic reviews and meta-analyses. *Am J Clin Nutr.* 2018;107(3):436–44.
270. Yang Y, Zhao L-G, Wu Q-J, Ma X, Xiang Y-B. Association between dietary fiber and lower risk of all-cause mortality: a meta-analysis of cohort studies. *Am J Epidemiol.* 2015;181(2):83-91.
271. Kim Y, Je Y. Dietary fiber intake and total mortality: a meta-analysis of prospective cohort studies. *Am J Epidemiol.* 2014;180(6):565-73.
272. Kaczmarczyk MM, Miller MJ, Freund GG. The health benefits of dietary fiber: beyond the usual suspects of type 2 diabetes mellitus, cardiovascular disease and colon cancer. *Metabolism.* 2012;61(8):1058-66.
273. Deehan EC, Duar RM, Armet AM, Perez-Muñoz ME, Jin M, Walter J. Modulation of the Gastrointestinal Microbiome with Nondigestible Fermentable Carbohydrates To Improve Human Health. *Microbiol Spectr.* 2017;5(5).
274. Mobley AR, Jones JM, Rodriguez J, Slavin JL, Zelman KM. Identifying practical solutions to meet America's fiber needs: proceedings from the Food & Fiber Summit. *Nutrients.* 2014;6(7):2540-51.

275. Clemens R, Kranz S, Mobley AR, *et al.* Filling America's fiber intake gap: summary of a roundtable to probe realistic solutions with a focus on grain-based foods. *J Nutr.* 2012;142(7):1390S-401S.
276. Sonnenburg ED, Sonnenburg JL. The ancestral and industrialized gut microbiota and implications for human health. *Nat Rev Microbiol.* 2019;17(6):383-90.
277. Thompson SV, Hannon BA, An R, Holscher HD. Effects of isolated soluble fiber supplementation on body weight, glycemia, and insulinemia in adults with overweight and obesity: a systematic review and meta-analysis of randomized controlled trials. *Am J Clin Nutr.* 2017;106(6):1514-28.
278. Sharma SK, Bansal S, Mangal M, Dixit AK, Gupta RK, Mangal AK. Utilization of Food Processing By-products as Dietary, Functional and Novel Fibre: A Review. *Crit Rev Food Sci Nutr.* 2016;56(10):1647-61.
279. Meijnikman AS, Gerdes VE, Nieuwdorp M, Herrema H. Evaluating Causality of Gut Microbiota in Obesity and Diabetes in Humans. *Endocr Rev.* 2018;39(2):133-53.
280. Zhao L, Zhang F, Ding X, *et al.* Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes. *Science.* 2018;359(6380):1151-56.
281. Birkett A, Muir J, Phillips J, Jones G, O'Dea K. Resistant starch lowers fecal concentrations of ammonia and phenols in humans. *Am J Clin Nutr.* 1996;63(5):766-72.
282. Boler BMV, Serao MCR, Bauer LL, *et al.* Digestive physiological outcomes related to polydextrose and soluble maize fibre consumption by healthy adult men. *Br J Nutr.* 2011;106(12):1864-71.
283. Reichardt N, Vollmer M, Holtrop G, *et al.* Specific substrate-driven changes in human faecal microbiota composition contrast with functional redundancy in short-chain fatty acid production. *ISME J.* 2018;12(2):610-22.
284. Alexander C, Swanson KS, Fahey GC, Garleb KA. Perspective: Physiologic Importance of Short-Chain Fatty Acids from Nondigestible Carbohydrate Fermentation. *Adv Nutr.* 2019;10(4):576-89.
285. Tuncil YE, Thakkar RD, Arioglu-Tuncil S, Hamaker BR, Lindemann SR. Subtle Variations in Dietary-Fiber Fine Structure Differentially Influence the Composition and Metabolic Function of Gut Microbiota. *mSphere.* 2020;5(3):e00180-20.
286. Cantu-Jungles TM, Hamaker BR. New View on Dietary Fiber Selection for Predictable Shifts in Gut Microbiota. *mBio.* 2020;11(1):e02179-19.
287. Hamaker BR, Cantu-Jungles TM. Discrete Fiber Structures Dictate Human Gut Bacteria Outcomes. *Trends Endocrinol Metab.* 2020;epub ahead of print.
288. Coffin CS, Shaffer EA. The hot air and cold facts of dietary fibre. *Can J Gastroenterol.* 2006;20(4):255-56.
289. Papas MA, Giovannucci E, Platz EA. Fiber from Fruit and Colorectal Neoplasia. *Cancer Epidemiol Biomarkers Prev.* 2004;13(8):1267-70.
290. Dwyer JT, Ausman LM. Fiber: unanswered questions. *J Natl Cancer Inst.* 1992;84(24):1851-53.
291. Fardet A. New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre? *Nutr Res Rev.* 2010;23(1):65-134.
292. Mozaffarian D, Rosenberg I, Uauy R. History of modern nutrition science-implications for current research, dietary guidelines, and food policy. *BMJ.* 2018;361:k2392.

293. Aberg S, Mann J, Neumann S, Ross AB, Reynolds AN. Whole-Grain Processing and Glycemic Control in Type 2 Diabetes: A Randomized Crossover Trial. *Diabetes Care.* 2020.
294. Jenkins DJ, Wesson V, Wolever TM*, et al.* Wholemeal versus wholegrain breads: proportion of whole or cracked grain and the glycaemic response. *BMJ.* 1988;297(6654):958-60.
295. Kohn JB. Is Dietary Fiber Considered an Essential Nutrient? *J Acad Nutr Diet.* 2016;116(2):360.
296. Raninen K, Lappi J, Mykkanen H, Poutanen K. Dietary fiber type reflects physiological functionality: comparison of grain fiber, inulin, and polydextrose. *Nutr Rev.* 2011;69(1):9-21.
297. Jones JM. Dietary fibre's co-passengers: is it the fibre or the co-passengers? In: van der Kamp JW, Jones JM, McCleary BV, Topping DL, eds. *Dietary fibre: new frontiers for food and health*. Wageningen, Netherlands: Wageningen Academic Publishers; 2010:365-78.
298. Poutanen K. Effect of Processing on the Properties of Dietary Fibre. In: McCleary BV, Prosky L, eds. *Advanced Dietary Fibre Technology*. Oxford, UK: Blackwell Science Ltd.; 2001:277-82.
299. Grundy MM-L, Fardet A, Tosh SM, Rich GT, Wilde PJ. Processing of oat: the impact on oat's cholesterol lowering effect. *Food Funct.* 2018;9(3):1328-43.
300. Tannock GW, Liu Y. Guided dietary fibre intake as a means of directing short-chain fatty acid production by the gut microbiota. *J Roy Soc New Zeal.* 2019.
301. O'Grady J, O'Connor EM, Shanahan F. Review article: dietary fibre in the era of microbiome science. *Aliment Pharmacol Ther.* 2019;49(5):506-15.
302. O'Keefe SJ. The association between dietary fibre deficiency and high-income lifestyle-associated diseases: Burkitt's hypothesis revisited. *Lancet Gastroenterol Hepatol.* 2019;4(12):984-96.
303. O'Keefe SJD. The Need to Reassess Dietary Fiber Requirements in Healthy and Critically Ill Patients. *Gastroenterol Clin North Am.* 2018;47(1):219-29.

CHAPTER 3: The Effect of Isolated and Synthetic Dietary Fibers on Markers of Metabolic Diseases in Human Intervention Studies: A Systematic Review

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3.1 Introduction

Obesity and its associated comorbidities, such as type-2 diabetes and cardiovascular disease, have reached epidemic proportions in countries that have adopted a western diet[304]. This diet is typically low in fruits, vegetables, whole grains, legumes, nuts, and seeds, all of which are the only food sources of dietary fiber (DF)[12]. DF is defined as non-digestible carbohydrate polymers that either occur naturally in food (intrinsic and intact DFs), are isolated from food (by physical, enzymatic, or chemical means) or are chemically synthesized, the latter two requiring evidence to support their physiological benefit to health[12]. DF is considered by most health professionals and regulatory agencies to be an integral component of a healthy diet, being implicated in the reduction of chronic diseases[42,242]. Observational studies support the health benefits of high DF intake[267], such as a reduction in low-grade systemic inflammation[222], obesity[130], metabolic syndrome[305,306], type II diabetes[163,307], and cardiovascular disease[269,308], as well as all-cause mortality[270]. In addition, a substantial body of research in animal disease models demonstrated health-promoting effects of isolated and synthetic DFs and established mechanisms by which pathologies are prevented[272,273]. DF sequesters bile acids and cholesterol in the small intestine and promotes their excretion[251], while viscous DFs impede the absorption of glucose and lipids[309]. Further, fermentation of DF by the gut microbiota leads to the production of short-chain fatty acids, which have a wide variety of immunological, metabolic, and hormonal effects, such as promoting satiety, reducing inflammation, and improving glucose and lipid metabolism[31,266].

The average intake of DF remains decidedly inadequate in affluent societies despite considerable efforts from policy makers and health professionals to increase consumption of whole foods[37]. A more promising strategy to enhance DF consumption, and thereby overall health, could potentially be achieved by enriching the food supply with isolated or synthetic DFs[27,277]. However, despite the convincing effects of DF found in observational[267,269] and animal studies[272,273], results of human intervention trials with isolated and synthetic DFs are inconsistent[84]. Buyken and colleagues systematically reviewed observational studies that assessed the relationship between DF intake and systemic inflammation, and compared these findings to results of intervention studies that provided DF supplements[222]. Only a single intervention study out of 11 reported a significant reduction in C-reactive protein (CRP) levels, while 13 out of 16 observational studies reported a significant inverse association between systemic inflammation and DF intake. Further, Thompson *et al*. reported improvements in body weight and glycemia from isolated soluble DF in overweight and obese populations in a systematic review and meta-analysis of intervention studies, but the authors recommended caution in the interpretation of these findings due to significant between-study heterogeneity[277]. These findings raise important questions: why do observational studies more consistently show beneficial effects of increased DF intake as compared to intervention trials, and are isolated and synthetic DFs a viable alternative to whole foods rich in intrinsic and intact DFs?

The objective of **Chapter 3** was to evaluate the effect of isolated and synthetic DF supplementation on well-established risk markers of metabolic disease related to glycemia, systemic inflammation, and lipidemia in intervention studies in healthy and/or at-risk adults. We further determined whether reported effects differed based on DF dose, DF physicochemical properties, intervention duration, and the placebo used. Given the extensive heterogeneity in the study designs used in the included publications (*e.g.* single-arm and multi-arm interventions), a meta-analysis was not conducted. Instead, the results were summarized to provide recommendations for the design of future interventions aimed at elucidating the role of DF supplementation in the prevention of chronic disease.

3.2 Methods

In order to achieve a comprehensive overview of the available literature on isolated and synthetic DF, randomized placebo-controlled trials, pilot studies, and single-arm interventions

trials that assessed a wide range of immunometabolic markers were included. This resulted in extensive heterogeneity in the designs of the studies found and, consequently, a meta-analysis was not completed. This systematic review was conducted based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement[310], as well as the *Cochrane Handbook for Systematic Reviews and Interventions* guidelines[311]. Although the study was not registered with PROSPERO, no deviations from the original protocol were made.

3.2.1 Study Selection Criteria

Since the objective of this review was to assess the ability of DF supplementation to reduce disease risk in both healthy adults and those at risk for metabolic diseases (*e.g.* hypercholesterolemia and prediabetes), we focused on well-established markers of pathophysiologies of obesity and its comorbidities, such as type-2 diabetes and cardiovascular disease. These immunometabolic markers were grouped under the following themes: (1) dysglycemia and insulin resistance [fasting glucose, insulin, homeostatic model assessment of insulin resistance (HOMA-IR), and postprandial glucose and insulin responses to an oral glucose tolerance test, described as glucose area under the curve (AUC) and insulin AUC], (2) systemic inflammation [fasting CRP and interleukin 6 (IL-6)], and (3) dyslipidemia [fasting total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG)]. Human intervention studies with a range of experimental designs (*e.g.* randomized placebo-controlled studies, single-arm studies, and pilot studies) published in English that assessed the effect DF supplements with at least 50% DF content per gram dry weight on the markers of interest were included.

The gold standard for investigating the effectiveness of an intervention is a randomized, double-blinded, placebo-controlled trial. The placebo should be as similar as possible to the treatment in appearance but physiologically and functionally inert; therefore, having no effect on the primary outcomes[312]. This is often difficult to achieve in nutrition research[313], as the placebo may adversely affect the assessed outcomes, which is the case for placebos composed of digestible carbohydrates since these have been shown to affect immunometabolic markers negatively[314,315]. Therefore, studies that did not report baseline data for each intervention arm, or if such data could not be obtained from the authors upon request, were excluded as these studies did not allow for the effects of the placebo to be quantified.

The selection criteria are summarized in **Table 3.1**. Studies with multiple treatment arms were included if at least one arm met inclusion criteria. Intervention arms that tested DF together with calorie restriction protocols, medications, probiotics, or in the context of whole-food treatments were excluded, as these interventions may have had DF-independent effects on the immunometabolic markers assessed.

Table 3.1. Predefined inclusion and exclusion criteria

INCLUSION DF Intervention Studies:	**EXCLUSION** DF Intervention Studies:
1) Published in English between January 1990 and December 2018.	1) Population with a history of type II diabetes, cardiovascular, renal, hepatic, or acute inflammatory diseases.
2) Assessed the metabolic effect of DF by comparing it to a control arm or baseline measurements.	2) Combined other interventions (*i.e.* weight loss, low-fat diet, medications).
3) Measured fasting glucose, insulin, HOMA-IR, CRP, IL-6, LDL-C, HDL-C, TC, TG, or postprandial glucose or insulin AUC after an OGTT.	3) DF source is of low purity, as in <50% total DF based on dry weight.
4) Normoweight, overweight, and/or obese adult population (mean age: 18 - 64 y).	4) Insufficient data available on DF used, including source, purity, and/or dose.
5) Intervention duration ≥2 wks.	5) DF effect data is insufficient or missing for either the baseline or the control arm.

AUC, area under the curve; CRP, C-reactive protein; DF, dietary fiber; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; IL, interleukin; LDL-C, low-density lipoprotein cholesterol; OGTT, oral glucose tolerance test; TC, total cholesterol; TG, triglycerides

3.2.2 Search Strategy

The databases PubMed, MEDLINE, CINAHL Plus with Full Text, Scopus, and Google Scholar were used to conduct a systematic literature search, as outlined in **Figure 3.1**. The selected search terms were as follows: (“dietary fibre” OR “dietary fiber” OR prebiotic) AND (“insulin resistance” OR “insulin sensitivity” OR "glucose metabolism"; inflammation OR CRP; “blood lipids” OR lipoproteins OR cholesterol). Two individuals (AMA and ECD) completed the initial searches independently in May 2016, screening studies published from January 1990 onward, and all disagreements were resolved through discussions. Identified publications were in- or excluded based on their title and/or abstract using selection criteria, and duplicate publications were removed. The remaining publications were reviewed in full and were assessed

against the selection criteria to determine eligibility. The reference lists of eligible articles were screened for additional publications not identified in the initial searches. These publications were also reviewed in full and assessed using the same selection criteria. An updated search was conducted in December 2018 to include studies published since May 2016, and the same search strategy was used.

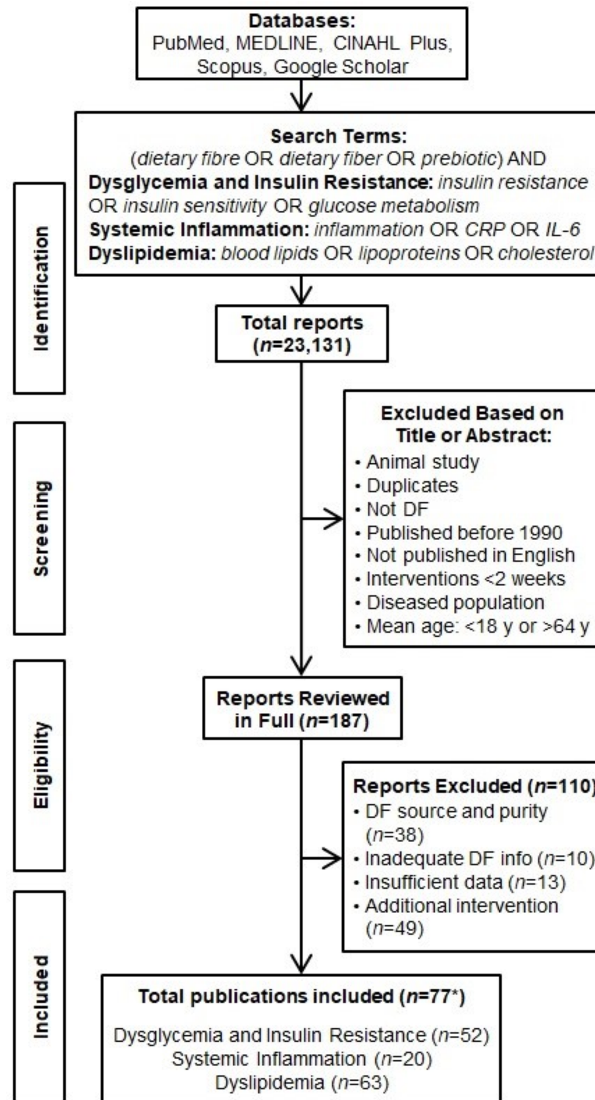

Figure 3.1. PRISMA flow diagram of article search and selection process. The literature search was first conducted during May 2016, and included all studies published from January 1990 onward. An updated literature search was conducted in December 2018. *Includes two papers that analyzed the same subject population, but performed different analyses.

3.2.3 Data Extraction

One author (AMA) extracted the data, which included information on the study population (mean age, mean BMI, population characteristics), the intervention (study design, duration), the DF supplement (type, dose, purity), the placebo (type, dose), and whether the DF had a statistically significant effect on the immunometabolic markers when compared to baseline values and/or the placebo. For studies that did not report the actual amount of DF administered, the exact dose of DF was determined using the DF content of the supplement reported online. To ensure accuracy, two individuals not involved in the initial data extraction (ECD and JVT) reviewed the data and any discrepancies were resolved through discussions.

3.2.4 Risk of Bias Quality Assessment

Methodological quality was assessed for each publication according to the Cochrane Collaboration's Risk of Bias assessment tool[311]. Two individuals (AMA and ECD) completed this assessment independently and all discrepancies were resolved through discussions.

3.2.5 Data Stratification

Since different DF types and doses were often compared in the same study, we considered individual treatment arms within studies as separate interventions. In order to synthesize the findings from each study, the effect of DF within each intervention arm was categorized based on either a statistically significant difference relative to baseline or against placebo ($\alpha=0.05$, $p<0.05$).

Interventions were stratified based on DF dose, DF physicochemical properties, intervention duration, and the placebo used in order to characterize how these variables influenced the effects of DF supplementation. Three categories were used for DF dose: ≤10.0 grams, 10.1-20.0 grams, and ≥20.1 grams of DF. DFs were categorized based on their described physicochemical properties as insoluble and nonviscous (consisting only of resistant starches; RSs), soluble and nonviscous (includes DFs that show a low increase in viscosity when dissolved), viscous soluble, and mixed plant cell wall (MPCW) DFs (henceforth referred to as RS, nonviscous, viscous, and MPCW, respectively) using information provided in the publications themselves or studies that characterized similar DF products; for example, corn bran hemicellulose[316] and rhubarb stalk DF[317]. MPCW, as defined by the FDA, refers to an isolated DF ingredient that is comprised of two or more plant cell wall DF-types and, thus, potentially two or more of the described physicochemical properties (*i.e.* insoluble, nonviscous, and

viscous), along with other compounds, like minerals or phytochemicals, depending on the isolation method[318]. If an intervention arm contained a mixture of nonviscous and viscous DF-types (*e.g.* acacia gum and isolated pectin), then it was categorized as viscous. We focused the characterization of DF physicochemical properties solely on solubility and viscosity, as these characteristics have been attributed to health benefits of DF[309]. Fermentability, which is also a key physicochemical property of DF, was not assessed, as it was not studied in most publications and remains poorly described for many DFs[319]. Intervention arms were also stratified into one of three categories based on their intervention duration: 2-4 weeks, 5-12 weeks, and ≥13 weeks. Finally, placebos were stratified into one of three categories. The first was 'digestible carbohydrates', which were supplemented in powder form (*e.g.* maltodextrin that participants mixed into food/drink) or were added to either juice or a sugar-sweetened beverage (*e.g.* fruit juice with maltodextrin versus fruit juice with DF). The second was 'vehicle', which were the food matrices used in the intervention arm without the isolated DF (*e.g.* bread rolls, bagels). The third was 'inert', which were either non-digestible carbohydrates, such as microcrystalline cellulose, or drinks that were either unsweetened or contained artificial sweeteners, both of which would resist digestion and absorption and likely have minimal to no effect on the assessed markers. Although artificial sweeteners have been shown to influence glucose metabolism indirectly by acting on the gut microbiota[320], their effects on the reviewed immunometabolic markers are controversial[321].

We conducted a separate analysis with placebo-controlled interventions that reported significant effects to the immunometabolic markers assessed to determine to what degree the placebo accounted for the effect of DF on these markers. If raw data were not reported for either the intervention or placebo arms (*i.e.* presented only in figures), then these studies were excluded from this analysis as we could not quantify the effect of the placebo. For each intervention arm included in this analysis, the change relative to baseline in the intervention arm was subtracted from that in the placebo arm to obtain the total difference in effect between the arms (ΔP-ΔDF). The change relative to baseline in the intervention and placebo arms were then both divided by ΔP-ΔDF and multiplied by 100 ($i.e. \frac{\text{either } \Delta\text{DF or } \Delta\text{P}}{(\Delta\text{P}-\Delta\text{DF})} x\ 100$) to calculate the percent of the effect attributable to the DF and to the placebo, respectively. No effect (0%) was attributable to the placebo if there was no change in the placebo relative to baseline and there was a significant

difference in the DF group when compared to the placebo, or if a significant effect was only reported in the DF group when compared to baseline (and not to placebo).

3.3 Results

3.3.1 Selection of Interventions

A PRISMA flow chart of the overall search strategy and results is depicted in **Figure 3.1**. A total number of 23,131 publications were identified, with 187 remaining after duplicates were removed and titles/abstracts were screened. After full text review, 110 additional papers were removed based on inclusion/exclusion criteria. These included 12 publications describing placebo-controlled studies that reported DF effects relative to placebo without reporting baseline data, and this information could not be obtained from the authors[322-331]. These publications were removed since a digestible carbohydrate was used as the placebo, and it was not possible to assess the degree to which an adverse effect of the placebo contributed to significant differences.

Ultimately, 77 publications were included that described 106 intervention arms that supplemented isolated or synthetic DFs (>50% DF on a dry weight basis). Of the 77 publications, 54 described randomized controlled trials[225,231,332-382], of which 14 had more than one DF intervention arm[359-363,365-371]. There were four publications where a standardized background diet was consumed in both arms with and without the DF[231,372,373,379], three publications where the control arm received no dietary intervention[375-377], and one publication that used inulin as a nonviscous control[371]. Two publications were controlled but not randomized[383,384]. Further, 20 studies had no control arm. Of these, 14 publications used a single-arm study design[385-398], while the remaining six publications employed either a parallel-arm or crossover study design to compare different DF doses[399] or types[400,401], or to compare a DF to an intervention not included in this review (such as a probiotic[402], a high-DF diet[403], or a lower purity DF[404]). Finally, one publication was comprised of four individual sub-studies: three were randomized placebo-controlled trials and one was a randomized crossover study without a placebo[405]. Of the 77 eligible publications, 52 assessed markers of dysglycemia and/or insulin resistance, 20 assessed markers of systemic inflammation, and 63 assessed markers of dyslipidemia. Information on intervention arms that reported a significant effect is provided in **Table S.3.1**, with information on all reviewed intervention arms provided in **Tables S.3.2-S.3.4**.

3.3.2 Influence of DF on Immunometabolic Markers

There were substantial differences in the efficacy of DF supplementation in improving immunometabolic markers. While 36-49% of the treatment arms reported a significant reduction to insulin AUC, HOMA-IR, TC, and LDL-C, less than 20% of interventions led to improvements in CRP, IL-6, glucose, insulin, glucose AUC, HDL-C, and TG (**Figure 3.2**, **Table S.3.1**). Similar findings were obtained when only higher quality studies (*i.e.* those without a 'high' risk of bias) (**Figure 3.3.A**) or those whose study population had risk factors for metabolic diseases (*e.g.* overweight/obese, hypercholesterolemic, and/or prediabetic subjects) (**Figure 3.3.B**) were included. Since only one intervention arm out of eleven reported a significant decrease to IL-6, we did not include this marker in the stratification analyses described below.

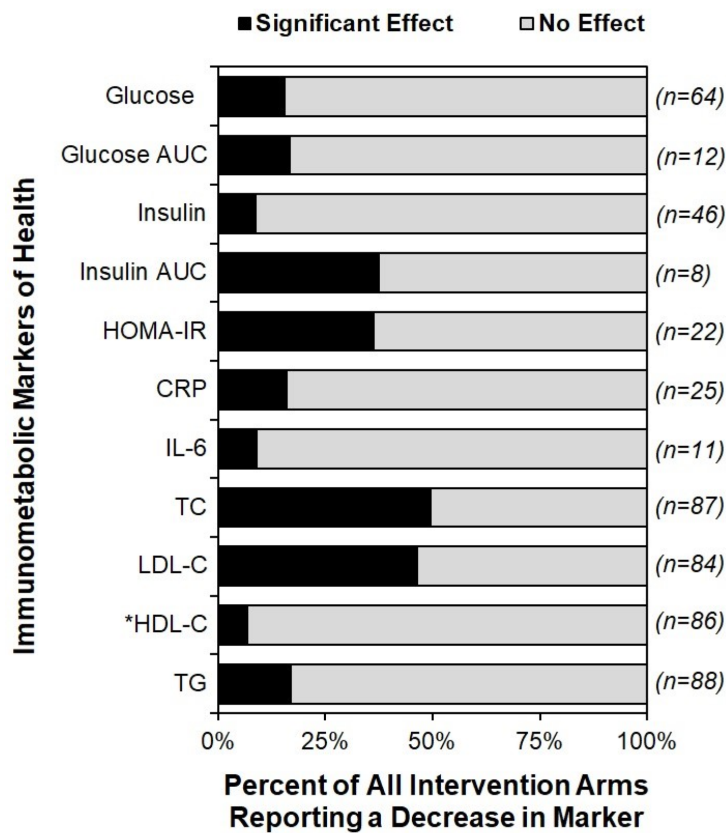

Figure 3.2. Reported effects of DF supplementation on immunometabolic markers in healthy adults. Intervention arms were considered to have a significant decrease in the assessed marker relative to baseline and/or placebo if the reported *p* value was <0.05. Data are reported as a percentage of all intervention arms. * Considered to have a significant increase in HDL-C rather than decrease. AUC, area under the curve; CRP, C-reactive protein; DF, dietary fiber; HOMA-IR, homeostatic model assessment of insulin resistance; HDL-C, high-density lipoprotein cholesterol; IL, interleukin; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.

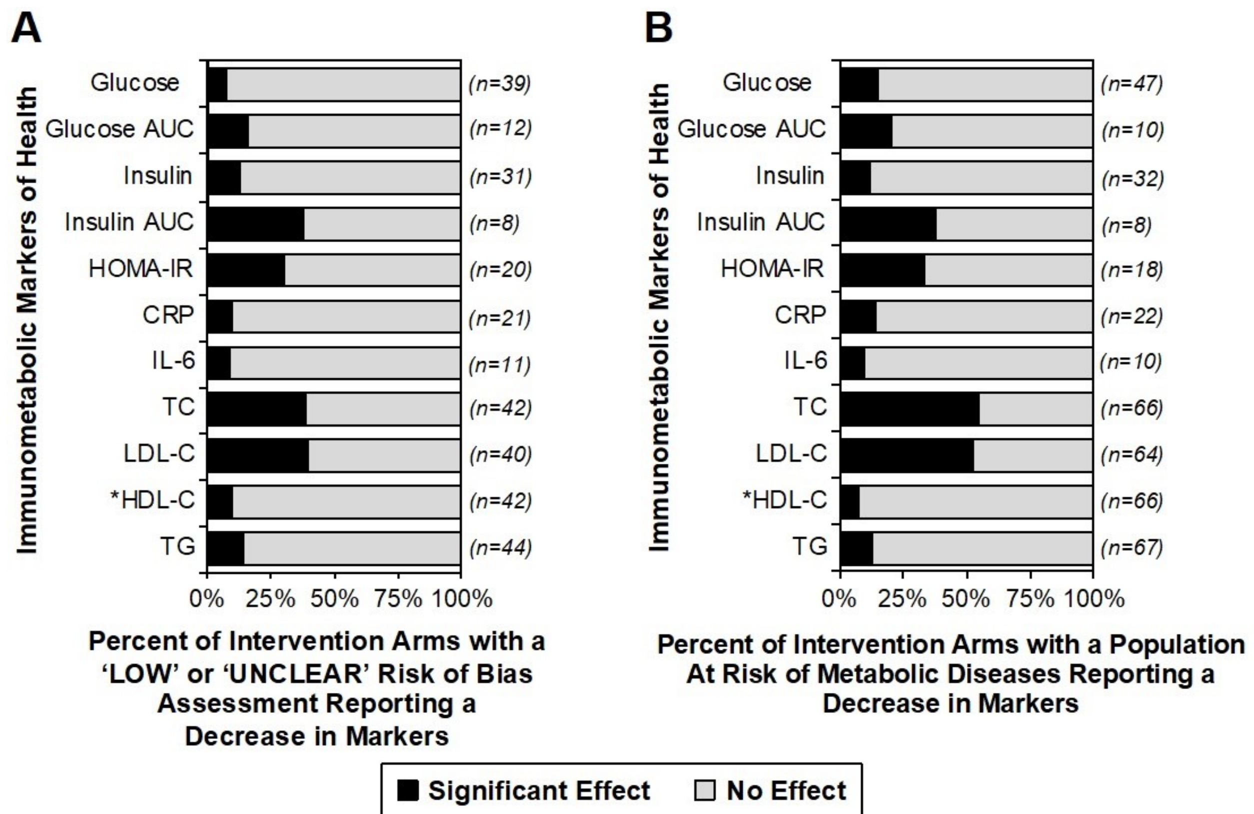

Figure 3.3. Reported effects of DF supplementation on immunometabolic markers in (A) higher quality publications (*i.e.* publications assigned an 'Unclear' or 'Low' Risk of Bias), and (B) at-risk populations with a dysregulated immunometabolic state (*i.e.* prediabetes, hypercholesterolemia, obesity). Intervention arms were considered to have a significant decrease in the assessed marker relative to baseline and/or placebo if the reported *p* value was <0.05. Data are reported as a percentage of all intervention arms. * Considered to have a significant increase in HDL-C rather than decrease. AUC, area under the curve; CRP, C-reactive protein; DF, dietary fiber; HOMA-IR, homeostatic model assessment of insulin resistance; HDL-C, high-density lipoprotein cholesterol; IL, interleukin; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.

3.3.3 Effect of DF Stratified by Dose

Out of the 106 interventions, 43 (41%) supplemented ≤10 g/d of DF, which was almost twice as many as those that supplemented ≥20.1 g/d (23%, 24 arms). There were only three studies that supplemented >30 g/d of DF: 32 g/d of lime-treated maize husk DF was supplemented for six weeks[388], 45 g/d of RS type-II was supplemented for 12 weeks[358], and 55 g/d of oligofructose was supplemented in a five-week dose-escalation study[390].

Stratification of the intervention arms by dose revealed that some markers were more likely to be improved after high doses of DF were supplemented. The influence of dose was most pronounced for CRP, where 40% (2 arms) of the interventions that provided ≥20.1 g/d DF

showed a significant reduction, while only 10% (2 arms) below this dose reported a significant effect (**Figure 3.4**). For psyllium specifically, there were four intervention arms that supplemented between 6 and 30 g/d, and only 30 g/d significantly reduced CRP[403]. An influence of dose was also observed for TC and LDL-C, where 63% and 56% of intervention arms (12 and 10 arms, respectively) providing ≥20.1 g/d DF showed significant improvements, while only 44% and 40% of intervention arms (16 and 14 arms, respectively) that supplemented ≤10 g/d DF showed benefits (**Figure 3.4**). Higher DF doses also appeared to improve TG (**Figure 3.4**), with 27% of interventions that supplemented ≥20.1 g/d DF reporting a significant reduction compared to 14% of interventions that used lower doses. Overall, the findings suggest that higher doses of DF are more likely to improve several immunometabolic markers. However, studies that supplement high DF doses are severely underrepresented in the literature, and more research, especially dose-response studies, is needed to draw concrete conclusions.

3.3.4 Effect of DF Stratified by Physicochemical Properties

The studies included in this review used a wide range of DF-types with different physicochemical properties that can influence their physiological effects[406]. Of these studies, 40% and 32% (42 and 34 arms) supplemented with viscous or nonviscous DFs, respectively. Of the remaining intervention arms, 22% (23 arms) supplemented MPCW DFs, while 7% (7 arms) supplemented an RS. Due to the small number of intervention arms, RSs were not included in the final stratification analyses, but were reported in **Tables S.3.2, S.3.3, and S.3.4**.

Stratification of the findings by DF physicochemical properties suggested that they are relevant depending on the markers assessed. While only 24% and 26% of intervention arms that supplemented nonviscous DFs reported a benefit for TC and LDL-C, respectively, 60% and 59% of studies with viscous DFs reported a significant effect (**Figure 3.4**). Psyllium[336,342], β-glucan[332,382], and konjac or guar gums[333,380,386,392,404] were especially effective. In addition, MPCW DF-types were equally as effective as viscous DFs (**Figure 3.4**). Further, 32% of MPCW DF intervention arms reported a significant reduction to TG, while only 12% of viscous and 15% of nonviscous DFs reported the same. The importance of DF physicochemical properties on the other immunometabolic markers was not conclusive due to the small number of studies.

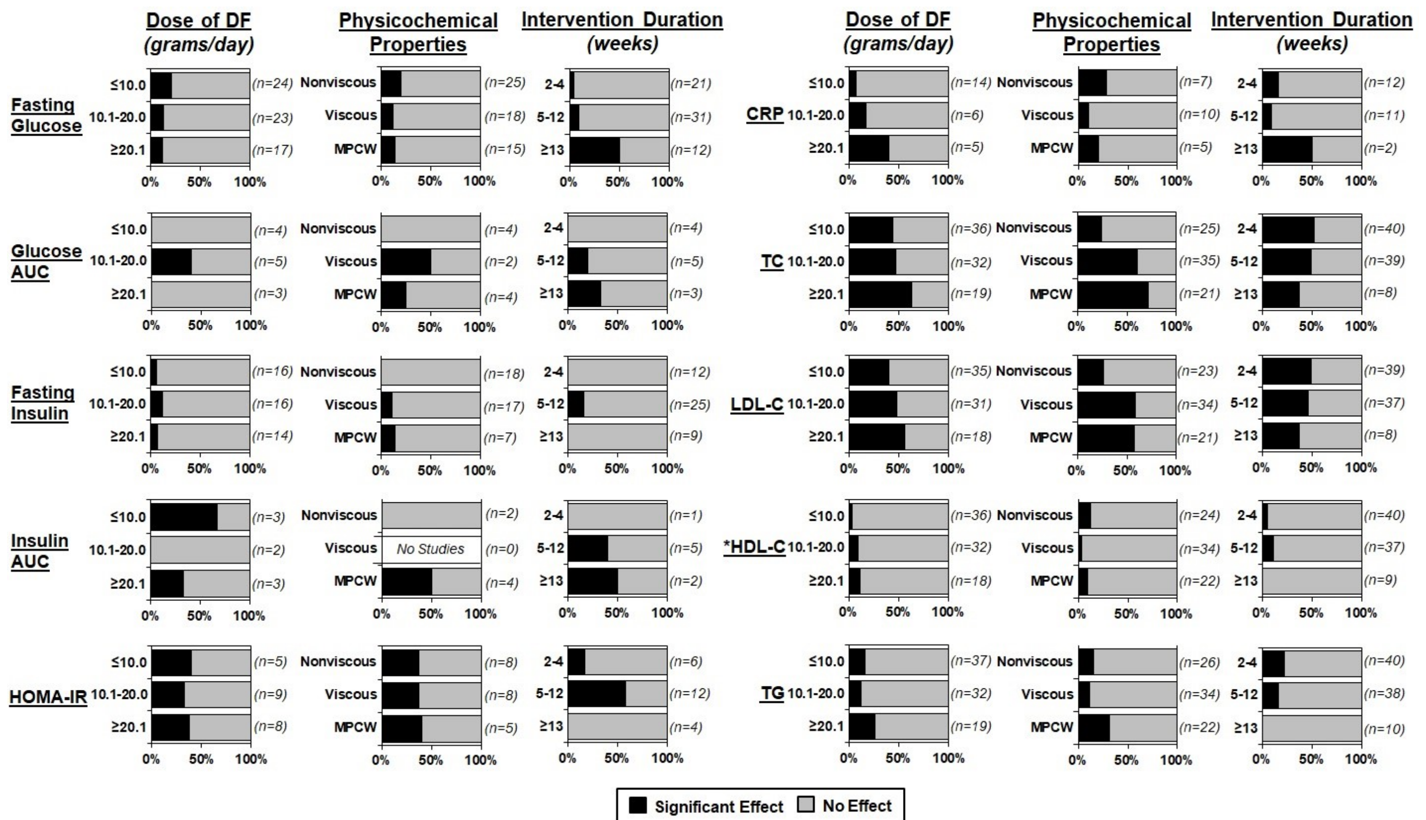

Figure 3.4. Reported effects of DF supplementation on immunometabolic markers in healthy adults when stratified by DF dose, DF physicochemical properties, and intervention duration. Intervention arms were considered to have a significant effect on the assessed marker relative to baseline and/or placebo if the reported p value was <0.05. Data are reported as a percentage of all intervention arms that assessed these markers. DFs were categorized as soluble with minimal viscosity, soluble with high viscosity, and mixed plant cell wall DFs (nonviscous, viscous, and MPCW, respectively) using information provided in the publications themselves or studies that characterized similar DF products. Seven interventions supplemented a resistant starch, but only two reported significant effects to the markers: 25 g/d reduced insulin, insulin AUC, and HOMA-IR[351], and 24 g/d reduced glucose, TC, and LDL-C[374]. * Considered to have a significant increase in HDL-C rather than decrease. AUC, area under the curve; CRP, C-reactive protein; DF, dietary fiber; HOMA-IR, homeostatic model assessment of insulin resistance; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.

3.3.5 Effect of DF Stratified by Intervention Duration

The majority of studies employed short durations of DF supplementation, with almost half (47%, 50 arms) of the interventions being 2-4 weeks, and only 11% (12 arms) being ≥13 weeks. There was only one study that supplemented DF for a year, which was a single-arm pilot study[385], and three studies that supplemented DF for six months[350,360,389].

Stratification of trials by intervention duration revealed that some markers were more likely to improve after a longer administration of DF. Of the interventions that were ≥13 weeks, 50% (6 arms) showed a significant reduction in fasting glucose, while only 8% (4 arms) reported a significant effect when the duration was ≤12 weeks (**Figure 3.4**). Similar findings were observed for CRP (**Figure 3.4**). Consuming DF for 2-4 weeks did not result in any improvements in glucose AUC, insulin, or insulin AUC (**Figure 3.4**). In contrast, short interventions were just as efficacious as longer interventions for reducing TC and LDL-C (**Figure 3.4**). These results suggest that while shorter intervention studies are sufficient for improving cholesterol metabolism, longer DF exposure may be necessary for improvements in markers of dysglycemia and systemic inflammation. However, interpretation of these findings warrants caution as very few intervention arms supplemented DF for ≥13 weeks. Furthermore, out of the ≥13-week intervention studies reporting a significant reduction to glucose or CRP, only one was assessed to have a 'low' risk of bias (**Tables S.3.2 and S.3.3**).

3.3.6 The Confounding Effect of the Placebo in DF Interventions

Of the 50 placebo-controlled studies included, 54% (27 studies) used digestible carbohydrates as placebos that were either provided alone (*e.g.* corn starch, maltodextrin) or within a food matrix containing mainly simple carbohydrates (*e.g.* juice). An additional 36% (18 studies) of studies provided the placebo as a digestible food vehicle without the DF (*e.g.* bagel, bread rolls), while only 10% (10 studies) used an inert, calorie-free placebo (*e.g.* cellulose, artificial sweeteners).

Of the 50 placebo-controlled studies, 28 saw benefits as a result of DF supplementation and reported raw baseline and post-intervention data for both the intervention and placebo arms. An analysis of these intervention arms revealed that less than 15% of the improvements in glucose, TC, and LDL-C could be attributed to the placebo, indicating that these findings were not confounded (**Figure 3.5**). In contrast, for IL-6, glucose AUC, and insulin AUC, more than half of the detected improvements (71%, 74%, and 51%, respectively) were attributable to the placebo exerting a detrimental effect on the marker. One can, therefore, conclude that the placebo types used were not inert and confounded the true effect of the DF supplement. These findings have implications for the design of placebo-controlled studies assessing the immunometabolic effects of DFs and their use in nutritional interventions.

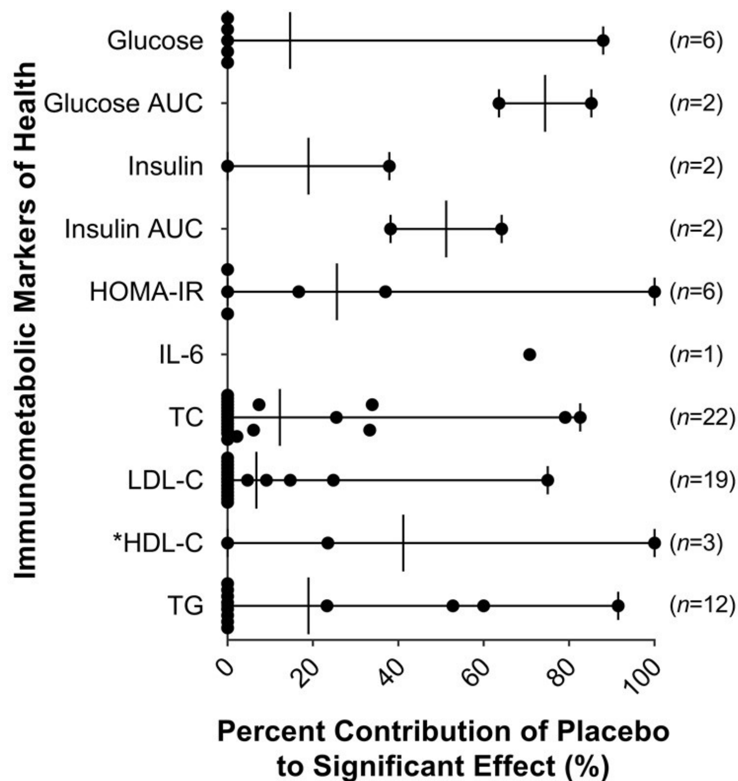

Figure 3.5. Contribution of the placebo to the perceived effect of DF on immunometabolic markers. The change relative to baseline was calculated and subtracted from the change reported by the placebo (*i.e.* ΔP-ΔDF). The change relative to baseline in each intervention arm and placebo were then both divided by this value and multiplied by 100 ($i.e. \frac{\text{either } \Delta DF \text{ or } \Delta P}{(\Delta P - \Delta DF)} x\ 100$) to calculate the percent of the effect attributable to DF supplementation and to placebo, respectively. * Considered to have a significant increase in HDL-C rather than decrease. AUC, area under the curve; CRP, C-reactive protein; DF, dietary fiber; HOMA-IR, homeostatic model assessment of insulin resistance; HDL-C, high-density lipoprotein cholesterol; IL, interleukin; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.

3.4 Discussion

The results from this systematic review revealed that the efficacy of isolated and synthetic DFs depends markedly on the immunometabolic end-points assessed. While HOMA-IR, insulin AUC, TC, and LDL-C improved in around half of the interventions, CRP, IL-6, glucose, glucose AUC, insulin, HDL-C, and TG did not show any effect in more than 80% of the interventions. These results are in agreement with other systematic reviews that reported reductions in LDL-C[159,160,407-412], TC[159,160,407,408], and HOMA-IR[413] from DF supplementation, while no effects were observed for CRP and IL-6[222], fasting glucose and insulin[159], and HDL-C

and TG[160,161,407,408]. In contrast to these findings, two meta-analyses have reported significant reductions to fasting glucose[277,413] and insulin[277] from viscous DF supplementation. However, these meta-analyses included intervention studies that assessed the effect of DF relative to a placebo, which may explain the discrepancy to our findings, especially given the potential confounding adverse effect of some placebos on these markers. Overall, findings herein demonstrate that the effects of isolated and synthetic DFs as hitherto used in intervention studies are at best, inconsistent and, at worst, negligible, mainly improving TC and LDL-C levels and insulin resistance, but not markers of dysglycemia and systemic inflammation.

These results are in agreement with a large body of research showing that the effects of DF supplementation are much less consistent than those reported in observational studies[84,222,414]. This poses the important question of what causes this discrepancy. Physiological effects of DF may diminish or even be lost once they are isolated from the food matrix[267,298,299,415]. This concern is reflected in the FDA's regulatory definition of DF, which considers the physiological benefits of intrinsic and intact DFs in plants as established, while requiring experimental demonstration of the same for isolated and synthetic non-digestible carbohydrates[12,318]. It has been suggested that the benefits detected in observational studies may not be derived from DF, but rather from other food constituents (*e.g.* micronutrients) and bioactive compounds (*e.g.* phytochemicals) present in plants[235,297,416]. However, the findings of this systematic review do clearly support the ability of isolated and synthetic DFs to improve cholesterol levels and insulin resistance. Although there is indeed little effect on markers of dysglycemia and inflammation, our stratification analyses on DF dose, DF physicochemical properties, and intervention duration suggested that most studies do not utilize DF supplements to their fullest potential.

In terms of dose, it was found that interventions that supplied ≥20.1 g/d of DF resulted in a higher proportion of interventions that resulted in significant improvements for several markers, including cholesterol levels and CRP. Although based on a small number of interventions, these findings are consistent with other studies. Supplementation of an oat bran, rye bran, and sugar beet fiber mixture at a dose of 48 g/d of DF significantly reduced CRP, while a dose of 30 g/d was ineffective[417]. In addition, a diet composed of green leafy vegetables, fruit, and nuts providing 143 g/d of DF significantly reduced LDL-C compared to a low-fat therapeutic diet[240]. Further, a recent series of meta-analyses reported that daily consumption of 25-29 g of DF generated the greatest benefits on a range of clinical outcomes when compared to lower

doses, and dose-response curves suggested that additional benefits would result from even higher intakes[267], a conclusion echoed in the Institute of Medicine's Dietary Reference Intakes[418].

Considering the importance of DF physicochemical properties, it was found that viscous DFs were especially effective at reducing cholesterol levels. Viscous DFs can decrease cholesterol levels by binding bile acids that have been secreted into the small intestine, enhancing their excretion. This leads to an increase in bile acid synthesis, which lowers blood cholesterol levels[251]. Interestingly, these findings show that MPCW DFs improve cholesterol levels as consistently as viscous DFs. The mechanisms behind the cholesterol-lowering effect of MPCW DFs are not as well understood as for viscous DFs. However, these MPCW DFs can contain insoluble hemicelluloses, as well as lignin and phytochemicals, all of which have been shown to bind bile acids and cholesterol and increase their excretion[419-422].

In regards to intervention duration, it is conceivable that DF-induced bile acid excretion would not require extensive time to reduce blood cholesterol levels, since inhibiting the reabsorption of cholesterol has a direct effect on metabolic processes and outcomes[251,423]. This could explain why short intervention durations were sufficient to improve cholesterol markers. In contrast, improvements to dysglycemia and inflammation appeared to require longer study durations. Although the mechanisms by which benefits in these markers arise are not completely understood, they likely require physiological statuses of responsible tissues and cells (*e.g.* β-cells, adipocytes, hepatocytes, myocytes, and various immune cell types) to change[259], which would require more time.

Analyses in **Chapter 3** also revealed that more than half of the placebo-controlled trials used digestible carbohydrates as a placebo, which have well-documented detrimental effects on immunometabolic markers[314,315]. A majority of the apparent beneficial effects of DF on IL-6, glucose AUC, and insulin AUC were indeed attributable to the placebo not being inert rather than the DF itself, which was recognized in one of the reviewed publications[346]. These findings imply that DFs may not directly benefit dysglycemia or systemic inflammation per se, but instead have no detrimental effect in contrast to digestible carbohydrates. Similar observations have also been made in intervention studies with whole grains[424,425] and a prebiotic in children[426], where benefits were driven primarily by the digestible carbohydrate controls having a detrimental effect, especially concerning inflammation. Given the difficulty of selecting a

placebo for nutritional studies[313], it is imperative to compare treatments not only to the placebo, but also to the baseline in order to assess for the potential confounding effects of the placebo.

Findings discussed herein provide some important insights on the role of DF in human health. The influence of DF dose, DF physicochemical properties, and intervention duration, as well as the confounding effects of placebos, which are all insufficiently considered in research studies[309,319,427,428], provide a potential explanation for why intervention studies are more inconsistent when compared to observational studies. While the latter inherently assess the long-term consumption of food matrices comprised of complex mixtures of DFs, having a range of physicochemical properties at doses that reflect a high habitual intake (>20 g/d)[163,267,429], intervention studies typically assess the effect of one or two DF-types with a limited range of physicochemical properties at varying doses and often shorter durations. Using a limited number of DF-types likely contributes to the inter-individual variation in responses in intervention studies, as the ability of the gut microbiota to ferment specific DF chemistries into beneficial metabolites differs among individuals[117,430]. In addition, observational studies assess the effect of diets rich in whole foods relative to diets high in refined foods, and findings could, at least in part, be driven by the detrimental effect of these foods. Therefore, despite the high proportion of intervention trials with no significant effect, DF might still be an active constituent of whole foods, but its effects might be reduced or lost due to between-study and inter-subject heterogeneities[277], and how DF supplements are applied.

In this respect, findings in **Chapter 3** of this dissertation suggest that DF supplementation could potentially be improved by implementing a more targeted and specific application. Most intervention studies in the literature supplement DFs at doses that are insufficient for reliable benefits[266,302,303] and are also too low from an evolutionary perspective, given that humans evolved consuming a diet that contained over 100 g/d of DF[5,27,240]. Further, physicochemical properties of DFs are often not considered or insufficiently chemically characterized, thus limiting the ability to draw clear conclusions across the full breadth of DF-types[319,427,428]. Third, mixtures of DF-types that mimic variation in the diet may overcome inter-individualized physiological responses through a diversified effect on the gut microbiota. Finally, isolated and synthetic DFs may be most beneficial, especially for dysglycemia and systemic inflammation, when they replace digestible carbohydrates in foods rather than being provided as a supplement in addition to the habitual diet. Based on the findings of this systematic review (**Figure 3.4 and**

Table S.3.1), recommendations were compiled on how future research should consider applying DFs in a more targeted and marker-specific way (**Table 3.2**). Several of these recommendations are, however, based on a limited number of studies. Well-controlled human trials with longer durations are needed that assess the effects of well-characterized DFs at relevant doses (and optimally also assess dose responses) on specific clinical outcomes predictive of health to substantiate whether a targeted approach improves the efficacy of DF supplementation[303,427,431,432].

A diet rich in plant-based whole foods is encouraged by dietary guidelines[261,262,433] and likely the best choice for optimal health[267,434]. However, society-wide consumption of DF-rich whole foods remains insufficient despite substantial efforts by educators and authorities, resulting in a 'fiber gap'[12,27,275]. DF supplements or foods enriched with DF have been proposed as alternatives to whole foods[27,277], but the health benefits of these diet items have been questioned[267]. The findings from this systematic review suggest that supplementation of isolated and synthetic DFs, as currently practiced, is likely a viable strategy to target cholesterol levels and insulin resistance, but not markers of dysglycemia and inflammation. Benefits in the latter might be achievable if specific DFs, or mixtures thereof, are supplemented for longer durations and at higher doses, especially when they replace digestible carbohydrates.

Table 3.2. Summary of findings and conclusions for a more targeted use of DF supplements

Immunometabolic marker	DF Dose	DF Physicochemical Properties	Intervention Duration	Placebo	Conclusions
Dysglycemia & Insulin Resistance					
Glucose	No effect of dose detected.	No clear pattern.	Longer durations of ≥13 wks resulted in an effect more often[346,360,375,376,393].	Placebo appeared not to confound findings.	Supplement DF for longer durations (≥13 wks).
Glucose AUC	Insufficient information available.	Insufficient information available.	Interventions ≤4 wks showed no effect.	Placebo had a strong confounding effect[225,346]. Inert compounds should be used.	Very limited information available, but interventions may need to be ≥5 wks. Given the negative effect of digestible CHO on hyperglycemia, benefits could be achieved by their replacement with DF.
Insulin	Little effect at any dose.	Little effect of any DF type.	Little effect with any intervention duration.	Insufficient data provided to determine the effect of the placebo.	DF supplementation, as currently used, does not appear to influence fasting insulin.
Insulin AUC	Insufficient information available.	Insufficient information available.	Insufficient information available.	Placebo had a strong confounding effect[349,351].	Very limited information available. Given the negative effect of digestible CHO on hyperinsulinemia, benefits could be achieved by their replacement with DF.
HOMA-IR	No effect of dose detected.	No difference in DF type detected.	Durations between 5-12 wks resulted in an effect more often[336,337,343,369,377].	Placebo appeared not to confound findings.	Supplement DF for ≥5 wks.
Inflammation					
CRP	Little effect at doses ≤20g/d; however, there was evidence of a dose response. Higher DF dose interventions resulted in an effect more often[231,403].	Insufficient information available.	Little effect for durations <13 wks. Studies of ≥13 wks administration showed an effect more often[385].	No placebo-controlled interventions included in this review showed an effect. However, digestible CHOs have been shown to induce inflammation[346,424]; therefore, inert compounds should be used.	Supplement higher doses of DF (>20g/d) for longer durations (≥13 wks). Given the pro-inflammatory effect of digestible CHO, benefits could be achieved by their replacement with DF.
IL-6	Insufficient information available.	Insufficient information available.	Insufficient information available.	Placebo had a detrimental effect that confounded the true effect of DF supplementation[346]. Inert compounds should be used.	Overall, insufficient information is available to make recommendations. Further research needs to be conducted assessing the effect of replacing digestible CHO with DFs.

(Continued)

Table 3.2. *Continued*

Immunometabolic marker	DF Dose	DF Physicochemical Properties	Intervention Duration	Placebo	Conclusions
Dyslipidemia					
TC	Lower doses were sufficient to reduce this marker, but doses ≥20.1g/d resulted in an effect more often [231,359,374,379,388].	Viscous and mixed plant cell wall DF-types resulted in an effect more often [231,332,341,363,387].	Short intervention durations (2-4 wks) were sufficient to reduce this marker [231,341,362,397,404].	Placebo had little effect; overall, did not confound findings.	Supplement higher doses of DF (≥20.1g/d) for 2-4 wks. Use viscous or mixed plant cell wall DFs.
LDL-C	Lower doses were sufficient to reduce this marker, but doses ≥20.1g/d resulted in an effect more often [231,340,342,379,396].	Viscous and mixed plant cell wall DF-types resulted in an effect more often [333,336,354,367,405].	Short intervention durations (2-4 wks) were sufficient to reduce this marker [341,362,380,386,404].	Placebo had little effect; overall, did not confound findings.	Supplement higher doses of DF (≥20.1g/d) for 2-4 wks. Use viscous or mixed plant cell wall DFs.
HDL-C	Little effect at any dose.	Little effect of any DF type.	Little effect at any intervention duration.	Insufficient information available.	DF supplementation does not appear to increase HDL-C.
TG	Little effect at any dose, but doses ≥20.1g/d resulted in an effect more often[231,343,359,396].	Little effect of any DF type, but mixed plant cell wall DFs resulted in an effect more often [231,341,359,373,396].	Little effect with any intervention duration.	Insufficient data provided to determine the effect of the placebo.	DF supplementation appears to affect TG minimally as currently used, but higher doses of mixed plant cell wall DFs may improve results.

AUC, area under the curve; CHO, carbohydrates; CRP, C-reactive protein; DF, dietary fiber; IL, interleukin; HOMA-IR, homeostatic model assessment for insulin resistance; HDL-C, high density lipoprotein cholesterol; IR, insulin resistance; LDL-C, low density lipoprotein cholesterol; TG, triglycerides; TC, total cholesterol

3.5 References

5. Jew S, AbuMweis SS, Jones PJ. Evolution of the Human Diet: Linking Our Ancestral Diet to Modern Functional Foods as a Means of Chronic Disease Prevention. *J Med Food.* 2009;12(5):925-34.
12. Jones JM. CODEX-aligned dietary fiber definitions help to bridge the 'fiber gap'. *Nutr J.* 2014;13:34.
27. Deehan EC, Walter J. The Fiber Gap and the Disappearing Gut Microbiome: Implications for Human Nutrition. *Trends Endocrinol Metab.* 2016;27(5):239-42.
31. Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. *Cell.* 2016;165(6):1332-45.
37. King DE, Mainous AGr, Lambourne CA. Trends in dietary fiber intake in the United States, 1999-2008. *J Acad Nutr Diet.* 2012;112(5):642-68.
42. Dahl WJ, Stewart ML. Position of the Academy of Nutrition and Dietetics: Health Implications of Dietary Fiber. *J Acad Nutr Diet.* 2015;115(11):1861-70.
84. Fuller S, Beck E, Salman H, Tapsell L. New Horizons for the Study of Dietary Fiber and Health: A Review. *Plant Foods Hum Nutr.* 2016;71(1):1-12.

117. Yang J, Rose DJ. Long-term dietary pattern of fecal donor correlates with butyrate production and markers of protein fermentation during in vitro fecal fermentation. *Nutr Res.* 2014;34(9):749-59.
130. Du H, van der A DL, Boshuizen HC*, et al.* Dietary fiber and subsequent changes in body weight and waist circumference in European men and women. *Am J Clin Nutr.* 2010;91(2):329-36.
159. Beserra BTS, Fernandes R, do Rosario VA, Mocellin MC, Kuntz MGF, Trindade EBSM. A systematic review and meta-analysis of the prebiotics and synbiotics effects on glycaemia, insulin concentrations and lipid parameters in adult patients with overweight or obesity. *Clin Nutr.* 2015;34(5):845-58.
160. Whitehead A, Beck EJ, Tosh S, Wolever TMS. Cholesterol-lowering effects of oat β-glucan: a meta-analysis of randomized controlled trials. *Am J Clin Nutr.* 2014;100(6):1413-21.
161. Kellow NJ, Coughlan MT, Reid CM. Metabolic benefits of dietary prebiotics in human subjects: a systematic review of randomised controlled trials. *Br J Nutr.* 2014;111(7):1147-61.
163. Yao B, Fang H, Xu W*, et al.* Dietary fiber intake and risk of type 2 diabetes: a dose-response analysis of prospective studies. *Eur J Epidemiol.* 2014;29(2):79-88.
222. Buyken AE, Goletzke J, Joslowski G*, et al.* Association between carbohydrate quality and inflammatory markers: systematic review of observational and interventional studies. *Am J Clin Nutr.* 2014;99(4):813-33.
225. Lambert JE, Parnell JA, Tunnicliffe JM, Han J, Sturzenegger T, Reimer RA. Consuming yellow pea fiber reduces voluntary energy intake and body fat in overweight/obese adults in a 12-week randomized controlled trial. *Clin Nutr.* 2017;36(1):126-33.
231. Fechner A, Kiehntopf M, Jahreis G. The formation of short-chain fatty acids is positively associated with the blood lipid-lowering effect of lupin kernel fiber in moderately hypercholesterolemic adults. *J Nutr.* 2014;144(5):599-607.
235. Saura-Calixto F. Dietary fiber as a carrier of dietary antioxidants: an essential physiological function. *J Agric Food Chem.* 2011;59(1):43-49.
240. Jenkins DJ, Kendall CW, Popovich DG*, et al.* Effect of a very-high-fiber vegetable, fruit, and nut diet on serum lipids and colonic function. *Metabolism.* 2001;50(4):494-503.
242. Delcour JA, Aman P, Courtin CM, Hamaker BR, Verbeke K. Prebiotics, Fermentable Dietary Fiber, and Health Claims. *Adv Nutr.* 2016;7(1):1-4.
251. Gunness P, Gidley MJ. Mechanisms underlying the cholesterol-lowering properties of soluble dietary fibre polysaccharides. *Food Funct.* 2010;1(2):149-55.
259. Hotamışlıgil GS. Foundations of Immunometabolism and Implications for Metabolic Health and Disease. *Immunity.* 2017;47(3):406-20.
261. United States Department of Health and Human Services, United States Department of Agriculture. Dietary guidelines for Americans 2015-2020. December, 2015. Available from: https://health.gov/dietaryguidelines/2015/resources/2015-2020_Dietary_Guidelines.pdf.
262. Health Canada. Canada's Dietary Guidelines for Health Professionals and Policy Makers. January, 2019. Available from: https://food-guide.canada.ca/static/assets/pdf/CDG-EN-2018.pdf.
266. Makki K, Deehan EC, Walter J, Bäckhed F. The Impact of Dietary Fiber on Gut Microbiota in Host Health and Disease. *Cell Host Microbe.* 2018;23(6):705-15.

267. Reynolds A, Mann J, Cummings J, Winter N, Mete E, Te Morenga L. Carbohydrate quality and human health: a series of systematic reviews and meta-analyses. *Lancet.* 2019;393(10170):434-45.
269. Veronese N, Solmi M, Caruso MG*, et al.* Dietary fiber and health outcomes: an umbrella review of systematic reviews and meta-analyses. *Am J Clin Nutr.* 2018;107(3):436–44.
270. Yang Y, Zhao L-G, Wu Q-J, Ma X, Xiang Y-B. Association between dietary fiber and lower risk of all-cause mortality: a meta-analysis of cohort studies. *Am J Epidemiol.* 2015;181(2):83-91.
272. Kaczmarczyk MM, Miller MJ, Freund GG. The health benefits of dietary fiber: beyond the usual suspects of type 2 diabetes mellitus, cardiovascular disease and colon cancer. *Metabolism.* 2012;61(8):1058-66.
273. Deehan EC, Duar RM, Armet AM, Perez-Muñoz ME, Jin M, Walter J. Modulation of the Gastrointestinal Microbiome with Nondigestible Fermentable Carbohydrates To Improve Human Health. *Microbiol Spectr.* 2017;5(5).
275. Clemens R, Kranz S, Mobley AR*, et al.* Filling America's fiber intake gap: summary of a roundtable to probe realistic solutions with a focus on grain-based foods. *J Nutr.* 2012;142(7):1390S-401S.
277. Thompson SV, Hannon BA, An R, Holscher HD. Effects of isolated soluble fiber supplementation on body weight, glycemia, and insulinemia in adults with overweight and obesity: a systematic review and meta-analysis of randomized controlled trials. *Am J Clin Nutr.* 2017;106(6):1514-28.
297. Jones JM. Dietary fibre's co-passengers: is it the fibre or the co-passengers? In: van der Kamp JW, Jones JM, McCleary BV, Topping DL, eds. *Dietary fibre: new frontiers for food and health*. Wageningen, Netherlands: Wageningen Academic Publishers; 2010:365-78.
298. Poutanen K. Effect of Processing on the Properties of Dietary Fibre. In: McCleary BV, Prosky L, eds. *Advanced Dietary Fibre Technology*. Oxford, UK: Blackwell Science Ltd.; 2001:277-82.
299. Grundy MM-L, Fardet A, Tosh SM, Rich GT, Wilde PJ. Processing of oat: the impact on oat's cholesterol lowering effect. *Food Funct.* 2018;9(3):1328-43.
302. O'Keefe SJ. The association between dietary fibre deficiency and high-income lifestyle-associated diseases: Burkitt's hypothesis revisited. *Lancet Gastroenterol Hepatol.* 2019;4(12):984-96.
303. O'Keefe SJD. The Need to Reassess Dietary Fiber Requirements in Healthy and Critically Ill Patients. *Gastroenterol Clin North Am.* 2018;47(1):219-29.
304. Ludwig DS, Hu FB, Tappy L, Brand-Miller J. Dietary carbohydrates: role of quality and quantity in chronic disease. *BMJ.* 2018;361:k2340.
305. Chen J-P, Chen G-C, Wang X-P, Qin L, Bai Y. Dietary Fiber and Metabolic Syndrome: A Meta-Analysis and Review of Related Mechanisms. *Nutrients.* 2018;10(1):24.
306. Wei B, Liu Y, Lin X, Fang Y, Cui J, Wan J. Dietary fiber intake and risk of metabolic syndrome: A meta-analysis of observational studies. *Clin Nutr.* 2018;37(6 Pt A):1935-42.
307. InterAct Consortium. Dietary fibre and incidence of type 2 diabetes in eight European countries: the EPIC-InterAct Study and a meta-analysis of prospective studies. *Diabetologia.* 2015;58(7):1394-408.
308. Pereira MA, O'Reilly E, Augustsson K*, et al.* Dietary fiber and risk of coronary heart disease: a pooled analysis of cohort studies. *Arch Intern Med.* 2004;164(4):370-76.

309. McRorie JWJ, McKeown NM. Understanding the Physics of Functional Fibers in the Gastrointestinal Tract: An Evidence-Based Approach to Resolving Enduring Misconceptions about Insoluble and Soluble Fiber. *J Acad Nutr Diet.* 2017;117(2):251-64.
310. Moher D, Shamseer L, Clarke M*, et al.* Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev.* 2015;4:1.
311. Higgins JP, Green S, (editors). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. *The Cochrane Collaboration.* 2011: Available from: www.handbook.cochrane.org.
312. de Craen AJ, Kaptchuk TJ, Tijssen JG, Kleijnen J. Placebos and placebo effects in medicine: historical overview. *J R Soc Med.* 1999;92(10):511-5.
313. Staudacher HM, Irving PM, Lomer MCE, Whelan K. The challenges of control groups, placebos and blinding in clinical trials of dietary interventions. *Proc Nutr Soc.* 2017;76(3):203-12.
314. Jenkins DJ, Kendall CW, Augustin LS*, et al.* Glycemic index: overview of implications in health and disease. *Am J Clin Nutr.* 2002;76(1):266S-73S.
315. Ludwig DS. The glycemic index: physiological mechanisms relating to obesity, diabetes, and cardiovascular disease. *JAMA.* 2002;287(18):2414-23.
316. Sugawara M, Suzuki T, Totsuka A, Takeuchi M, Ueki K. Composition of Corn Hull Dietary Fiber. *Starch/Stärke.* 1994;46(9):335-37.
317. Goel V, Cheema SK, Agellon LB, Ooraikul B, McBurney MI, Basu TK. *In vitro* binding of bile salt to rhubarb stalk powder. *Nutr Res.* 1998;18(5):893-903.
318. Office of Nutrition and Food Labeling. The Declaration of Certain Isolated or Synthetic Non-Digestible Carbohydrates as Dietary Fiber on Nutrition and Supplement Facts Labels: Guidance for Industry. Maryland, United States: Center for Food Safety and Applied Nutrition, Food and Drug Administration. June, 2018. Docket No: FDA-2018-D-1323.
319. Poutanen KS, Fiszman S, Marsaux CFM, Pentikainen SP, Steinert RE, Mela DJ. Recommendations for characterization and reporting of dietary fibers in nutrition research. *Am J Clin Nutr.* 2018;108(3):437-44.
320. Suez J, Korem T, Zeevi D*, et al.* Artificial sweeteners induce glucose intolerance by altering the gut microbiota. *Nature.* 2014;514(7521):181-86.
321. Ruiz-Ojeda FJ, Plaza-Díaz J, Sáez-Lara MJ, Gil A. Effects of Sweeteners on the Gut Microbiota: A Review of Experimental Studies and Clinical Trials. *Adv Nutr.* 2019;10(suppl_1):S31-S48.
322. Heijnen M-L, van Amelsvoort JM, Deurenberg P, Beynen AC. Neither raw nor retrograded resistant starch lowers fasting serum cholesterol concentrations in healthy normolipidemic subjects. *Am J Clin Nutr.* 1996;64(3):312-18.
323. Landin K, Holm G, Tengborn L, Smith U. Guar gum improves insulin sensitivity, blood lipids, blood pressure, and fibrinolysis in healthy men. *Am J Clin Nutr.* 1992;56(6):1061-65.
324. Effertz M, Denman P, Slavin JL. The effect of soy polysaccharide on body weight, serum lipids, blood glucose, and fecal parameters in moderately obese adults. *Nutr Res.* 1991;11(8):849-59.
325. Pasman WJ, Wils D, Saniez M-HH, Kardinaal AF. Long-term gastrointestinal tolerance of NUTRIOSE® FB in healthy men. *Eur J Clin Nutr.* 2006;60(8):1024-34.

326. Gower BA, Bergman R, Stefanovski D*, et al.* Baseline insulin sensitivity affects response to high-amylose maize resistant starch in women: a randomized, controlled trial. *Nutr Metab.* 2016;13:2.
327. Morel FB, Dai Q, Ni J, Thomas D, Parnet P, Fanca-Berthon P. α-Galacto-oligosaccharides Dose-Dependently Reduce Appetite and Decrease Inflammation in Overweight Adults. *J Nutr.* 2015;145(9):2052-59.
328. Robertson MD, Wright JW, Loizon E*, et al.* Insulin-sensitizing effects on muscle and adipose tissue after dietary fiber intake in men and women with metabolic syndrome. *J Clin Endocrinol Metab.* 2012;97(9):3326-32.
329. Cherbut C, Aube A, Mekki N, Dubois C, Lairon D, Barry J. Digestive and metabolic effects of potato and maize fibres in human subjects. *Br J Nutr.* 1997;77(1):33-46.
330. Robertson MD, Bickerton AS, Dennis AL, Vidal H, Frayn KN. Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism. *Am J Clin Nutr.* 2005;82(3):559-67.
331. Bodinham CL, Smith L, Wright J, Frost GS, Robertson MD. Dietary fibre improves first-phase insulin secretion in overweight individuals. *PLoS One.* 2012;7(7):e40834.
332. Braaten J, Wood P, Scott F*, et al.* Oat β-glucan reduces blood cholesterol concentration in hypercholesterolemic subjects. *Eur J Clin Nutr.* 1994;48(7):465-74.
333. Arvill A, Bodin L. Effect of short-term ingestion of konjac glucomannan on serum cholesterol in healthy men. *Am J Clin Nutr.* 1995;61(3):585-89.
334. Kohl A, Gogebakan O, Möhlig M*, et al.* Increased interleukin-10 but unchanged insulin sensitivity after 4 weeks of (1, 3)(1, 6)-β-glycan consumption in overweight humans. *Nutr Res.* 2009;29(4):248-54.
335. Cloetens L, Broekaert WF, Delaedt Y*, et al.* Tolerance of arabinoxylan-oligosaccharides and their prebiotic activity in healthy subjects: a randomised, placebo-controlled cross-over study. *Br J Nutr.* 2010;103(5):703-13.
336. Solà R, Bruckert E, Valls R-M*, et al.* Soluble fibre (*Plantago ovata* husk) reduces plasma low-density lipoprotein (LDL) cholesterol, triglycerides, insulin, oxidised LDL and systolic blood pressure in hypercholesterolaemic patients: A randomised trial. *Atherosclerosis.* 2010;211(2):630-37.
337. Russo F, Riezzo G, Chiloiro M*, et al.* Metabolic effects of a diet with inulin-enriched pasta in healthy young volunteers. *Curr Pharm Des.* 2010;16(7):825-31.
338. Pouteau E, Ferchaud-Roucher V, Zair Y*, et al.* Acetogenic fibers reduce fasting glucose turnover but not peripheral insulin resistance in metabolic syndrome patients. *Clin Nutr.* 2010;29(6):801-07.
339. Reimer RA, Pelletier X, Carabin IG*, et al.* Increased plasma PYY levels following supplementation with the functional fiber PolyGlycopleX in healthy adults. *Eur J Clin Nutr.* 2010;64(10):1186-91.
340. Li S, Guerin-Deremaux L, Pochat M, Wils D, Reifer C, Miller LE. NUTRIOSE dietary fiber supplementation improves insulin resistance and determinants of metabolic syndrome in overweight men: a double-blind, randomized, placebo-controlled study. *Appl Physiol Nutr Metab.* 2010;35(6):773-82.
341. Ruiz-Roso B, Quintela JC, de la Fuente E, Haya J, Pérez-Olleros L. Insoluble carob fiber rich in polyphenols lowers total and LDL cholesterol in hypercholesterolemic sujects. *Plant Foods Hum Nutr.* 2010;65(1):50-56.

342. Pal S, Khossousi A, Binns C, Dhaliwal S, Ellis V. The effect of a fibre supplement compared to a healthy diet on body composition, lipids, glucose, insulin and other metabolic syndrome risk factors in overweight and obese individuals. *Br J Nutr.* 2011;105(1):90-100.
343. Hashizume C, Kishimoto Y, Kanahori S, Yamamoto T, Okuma K, Yamamoto K. Improvement effect of resistant maltodextrin in humans with metabolic syndrome by continuous administration. *J Nutr Sci Vitaminol.* 2012;58(6):423-30.
344. Jackson K, Taylor G, Clohessy A, Williams C. The effect of the daily intake of inulin on fasting lipid, insulin and glucose concentrations in middle-aged men and women. *Br J Nutr.* 1999;82(1):23-30.
345. de Luis DA, de la Fuente B, Izaola O, Aller R, Gutiérrez S, Morillo M. Double blind randomized clinical trial controlled by placebo with a FOS enriched cookie on saciety and cardiovascular risk factors in obese patients. *Nutr Hosp.* 2013;28(1):78-85.
346. Reimer RA, Yamaguchi H, Eller LK*, et al.* Changes in visceral adiposity and serum cholesterol with a novel viscous polysaccharide in Japanese adults with abdominal obesity. *Obesity.* 2013;21(9):E379-E87.
347. Childs CE, Roytio H, Alhoniemi E*, et al.* Xylo-oligosaccharides alone or in synbiotic combination with *Bifidobacterium animalis* subsp. *lactis* induce bifidogenesis and modulate markers of immune function in healthy adults: a double-blind, placebo-controlled, randomised, factorial cross-over study. *Br J Nutr.* 2014;111(11):1945-56.
348. Tripkovic L, Muirhead N, Hart K, Frost GS, Lodge J. The effects of a diet rich in inulin or wheat fibre on markers of cardiovascular disease in overweight male subjects. *J Hum Nutr Diet.* 2015;28(5):476-85.
349. Brahe LK, Le Chatelier E, Prifti E*, et al.* Dietary modulation of the gut microbiota--a randomised controlled trial in obese postmenopausal women. *Br J Nutr.* 2015;114(3):406-17.
350. Stenman LK, Lehtinen MJ, Meland N*, et al.* Probiotic With or Without Fiber Controls Body Fat Mass, Associated With Serum Zonulin, in Overweight and Obese Adults-Randomized Controlled Trial. *EBioMedicine.* 2016;13:190-200.
351. Dainty SA, Klingel SL, Pilkey SE*, et al.* Resistant Starch Bagels Reduce Fasting and Postprandial Insulin in Adults at Risk of Type 2 Diabetes. *J Nutr.* 2016;146(11):2252-59.
352. Guess ND, Dornhorst A, Oliver N, Frost GS. A Randomised Crossover Trial: The Effect of Inulin on Glucose Homeostasis in Subtypes of Prediabetes. *Ann Nutr Metab.* 2016;68(1):26-34.
353. Canfora EE, van der Beek CM, Hermes GDA*, et al.* Supplementation of Diet With Galacto-oligosaccharides Increases *Bifidobacteria*, but Not Insulin Sensitivity, in Obese Prediabetic Individuals. *Gastroenterology.* 2017;153(1):87-97.e3.
354. Zunft H, Luder W, Harde A*, et al.* Carob pulp preparation rich in insoluble fibre lowers total and LDL cholesterol in hypercholesterolemic patients. *Eur J Nutr.* 2003;42(5):235-42.
355. Parnell JA, Klancic T, Reimer RA. Oligofructose decreases serum lipopolysaccharide and plasminogen activator inhibitor-1 in adults with overweight/obesity. *Obesity.* 2017;25(3):510-13.
356. Alfa MJ, Strang D, Tappia PS*, et al.* A Randomized Placebo Controlled Clinical Trial to Determine the Impact of Digestion Resistant Starch *MSPrebiotic*® on Glucose, Insulin, and Insulin Resistance in Elderly and Mid-Age Adults. *Front Med.* 2018;4:260.

357. Krumbeck JA, Rasmussen HE, Hutkins RW*, et al.* Probiotic *Bifidobacterium* strains and galactooligosaccharides improve intestinal barrier function in obese adults but show no synergism when used together as synbiotics. *Microbiome.* 2018;6(1):121.
358. Peterson CM, Beyl RA, Marlatt KL*, et al.* Effect of 12 wk of resistant starch supplementation on cardiometabolic risk factors in adults with prediabetes: a randomized controlled trial. *Am J Clin Nutr.* 2018;108(3):492-501.
359. Lampe J, Slavin JL, Baglien K, Thompson W, Duane W, Zavoral J. Serum lipid and fecal bile acid changes with cereal, vegetable, and sugar-beet fiber feeding. *Am J Clin Nutr.* 1991;53(5):1235-41.
360. Marett R, Slavin JL. No long-term benefits of supplementation with arabinogalactan on serum lipids and glucose. *J Am Diet Assoc.* 2004;104(4):636-39.
361. King DE, Mainous AGr, Egan BM, Woolson RF, Geesey ME. Effect of psyllium fiber supplementation on C-reactive protein: the trial to reduce inflammatory markers (TRIM). *Ann Fam Med.* 2008;6(2):100-06.
362. Maki KC, Carson ML, Kerr Anderson WH*, et al.* Lipid-altering effects of different formulations of hydroxypropylmethylcellulose. *J Clin Lipidol.* 2009;3(3):159-66.
363. Reppas C, Swidan S, Tobey S, Turowski M, Dressman J. Hydroxypropylmethylcellulose significantly lowers blood cholesterol in mildly hypercholesterolemic human subjects. *Eur J Clin Nutr.* 2009;63(1):71-77.
364. Giacco R, Clemente G, Luongo D*, et al.* Effects of short-chain fructo-oligosaccharides on glucose and lipid metabolism in mild hypercholesterolaemic individuals. *Clin Nutr.* 2004;23(3):331-40.
365. Maki KC, Pelkman CL, Finocchiaro ET*, et al.* Resistant starch from high-amylose maize increases insulin sensitivity in overweight and obese men. *J Nutr.* 2012;142(4):717-23.
366. François IEJA, Lescroart O, Veraverbeke WS*, et al.* Effects of a wheat bran extract containing arabinoxylan oligosaccharides on gastrointestinal health parameters in healthy adult human volunteers: a double-blind, randomised, placebo-controlled, cross-over trial. *Br J Nutr.* 2012;108(12):2229-42.
367. Gato N, Kadowaki A, Hashimoto N, Yokoyama S-i, Matsumoto K. Persimmon fruit tannin-rich fiber reduces cholesterol levels in humans. *Ann Nutr Metab.* 2013;62(1):1-6.
368. Savastano DM, Hodge RJ, Nunez DJ, Walker A, Kapikian R. Effect of two dietary fibers on satiety and glycemic parameters: a randomized, double-blind, placebo-controlled, exploratory study. *Nutr J.* 2014;13:45.
369. Pal S, Ho S, Gahler RJ, Wood S. Effect on Insulin, Glucose and Lipids in Overweight/Obese Australian Adults of 12 Months Consumption of Two Different Fibre Supplements in a Randomised Trial. *Nutrients.* 2017;9(2):91.
370. Salden BN, Troost FJ, Wilms E*, et al.* Reinforcement of intestinal epithelial barrier by arabinoxylans in overweight and obese subjects: A randomized controlled trial: Arabinoxylans in gut barrier. *Clin Nutr.* 2018;37(2):471-80.
371. Lyon M, Wood S, Pelletier X, Donazzolo Y, Gahler R, Bellisle F. Effects of a 3-month supplementation with a novel soluble highly viscous polysaccharide on anthropometry and blood lipids in nondieting overweight or obese adults. *J Hum Nutr Diet.* 2011;24(4):351-59.
372. Keogh GF, Cooper GJS, Mulvey TB*, et al.* Randomized controlled crossover study of the effect of a highly β-glucan-enriched barley on cardiovascular disease risk factors in mildly hypercholesterolemic men. *Am J Clin Nutr.* 2003;78(4):711-18.

373. Sandström B, Hansen LT, Sørensen A. Pea fiber lowers fasting and postprandial blood triglyceride concentrations in humans. *J Nutr.* 1994;124(12):2386-96.
374. Park OJ, Kang NE, Chang MJ, Kim WK. Resistant starch supplementation influences blood lipid concentrations and glucose control in overweight subjects. *J Nutr Sci Vitaminol.* 2004;50(2):93-99.
375. Pérez-Jiménez J, Serrano J, Tabernero M*, et al.* Effects of grape antioxidant dietary fiber in cardiovascular disease risk factors. *Nutrition.* 2008;24(7-8):646-53.
376. Urquiaga I, D'Acuña S, Pérez D*, et al.* Wine grape pomace flour improves blood pressure, fasting glucose and protein damage in humans: a randomized controlled trial. *Biol Res.* 2015;48:49.
377. Martínez-Maqueda D, Zapatera B, Gallego-Narbón A, Vaquero MP, Saura-Calixto F, Pérez-Jiménez J. A 6-week supplementation with grape pomace to subjects at cardiometabolic risk ameliorates insulin sensitivity, without affecting other metabolic syndrome markers. *Food Funct.* 2018;9(11):6010-19.
378. Jensen C, Spiller G, Gates J, Miller A, Whittam J. The effect of acacia gum and a water-soluble dietary fiber mixture on blood lipids in humans. *J Am Coll Nutr.* 1993;12(2):147-54.
379. Hall R, Johnson S, Baxter A, Ball M. Lupin kernel fibre-enriched foods beneficially modify serum lipids in men. *Eur J Clin Nutr.* 2005;59(3):325-33.
380. Yoshida M, Vanstone C, Parsons W, Zawistowski J, Jones P. Effect of plant sterols and glucomannan on lipids in individuals with and without type II diabetes. *Eur J Clin Nutr.* 2006;60(4):529-37.
381. Garcia AL, Steiniger J, Reich S-C*, et al.* Arabinoxylan fibre consumption improved glucose metabolism, but did not affect serum adipokines in subjects with impaired glucose tolerance. *Horm Metab Res.* 2006;38(11):761-66.
382. Queenan KM, Stewart ML, Smith KN, Thomas W, Fulcher RG, Slavin JL. Concentrated oat β-glucan, a fermentable fiber, lowers serum cholesterol in hypercholesterolemic adults in a randomized controlled trial. *Nutr J.* 2007;6:6.
383. Brighenti F, Casiraghi MC, Canzi E, Ferrari A. Effect of consumption of a ready-to-eat breakfast cereal containing inulin on the intestinal milieu and blood lipids in healthy male volunteers. *Eur J Clin Nutr.* 1999;53(9):726-33.
384. Urquiaga I, Troncoso D, Mackenna MJ*, et al.* The Consumption of Beef Burgers Prepared with Wine Grape Pomace Flour Improves Fasting Glucose, Plasma Antioxidant Levels, and Oxidative Damage Markers in Humans: A Controlled Trial. *Nutrients.* 2018;10(10).
385. Kapoor MP, Ishihara N, Okubo T. Soluble dietary fibre partially hydrolysed guar gum markedly impacts on postprandial hyperglycaemia, hyperlipidaemia and incretins metabolic hormones over time in healthy and glucose intolerant subjects. *J Funct Foods.* 2016;24:207-20.
386. Gallaher DD, Gallaher CM, Mahrt GJ*, et al.* A glucomannan and chitosan fiber supplement decreases plasma cholesterol and increases cholesterol excretion in overweight normocholesterolemic humans. *J Am Coll Nutr.* 2002;21(5):428-33.
387. Ganji V, Kuo J. Serum lipid responses to psyllium fiber: differences between pre- and post-menopausal, hypercholesterolemic women. *Nutr J.* 2008;7:22.

388. Vidal-Quintanar RL, Mendívil RL, Peña M, Fernandez ML. Lime-treated maize husks lower plasma LDL-cholesterol levels in normal and hypercholesterolaemic adult men from northern Mexico. *Br J Nutr.* 1999;81(4):281-88.
389. Hanai H, Ikuma M, Sato Y*, et al.* Long-term effects of water-soluble corn bran hemicellulose on glucose tolerance in obese and non-obese patients: improved insulin sensitivity and glucose metabolism in obese subjects. *Biosci Biotechnol Biochem.* 1997;61(8):1358-61.
390. Pedersen C, Lefevre S, Peters V*, et al.* Gut hormone release and appetite regulation in healthy non-obese participants following oligofructose intake. A dose-escalation study. *Appetite.* 2013;66:44-53.
391. Glover DA, Ushida K, Phillips AO, Riley SG. Acacia(sen) SUPERGUM™ (Gum arabic): An evaluation of potential health benefits in human subjects. *Food Hydrocoll.* 2009;23(8):2410-15.
392. Kawatra A, Kapoor A, Seghal S. Hypocholesterolemic effect of guargum in overweight adults. *Plant Foods Hum Nutr.* 1991;41(3):241-45.
393. Nasir O, Babiker S, Salim A-MM. Protective effect of gum arabic supplementation for type 2 diabetes mellitus and its complications. *Int J Multidiscip Curr Res.* 2016;4:288-94.
394. Sundell I, Rånby M. Oat husk fiber decreases plasminogen activator inhibitor type 1 activity. *Haemostasis.* 1993;23(1):45-50.
395. Zunft H, Luder W, Harde A, Haber B, Graubaum H, Gruenwald J. Carob pulp preparation for treatment of hypercholesterolemia. *Adv Ther.* 2001;18(5):230-36.
396. Cossack Z, Musaiger AO. Effect on lipid metabolism of beet fibre in desert nomads with low habitual fibre intake. *Eur J Clin Nutr.* 1991;45(2):105-10.
397. Goel V, Ooraikul B, Basu T. Cholesterol lowering effects of rhubarb stalk fiber in hypercholesterolemic men. *J Am Coll Nutr.* 1997;16(6):600-04.
398. Nicolosi R, Bell S, Bistrian B, Greenberg I, Forse R, Blackburn G. Plasma lipid changes after supplementation with β-glucan fiber from yeast. *Am J Clin Nutr.* 1999;70(2):208-12.
399. Robinson RR, Feirtag J, Slavin JL. Effects of dietary arabinogalactan on gastrointestinal and blood parameters in healthy human subjects. *J Am Coll Nutr.* 2001;20(4):279-85.
400. Smith KN, M QK, Thomas W, Fulcher RG, Slavin JL. Physiological effects of concentrated barley β-glucan in mildly hypercholesterolemic adults. *J Am Coll Nutr.* 2008;27(3):434-40.
401. Liu F, Li P, Chen M*, et al.* Fructooligosaccharide (FOS) and Galactooligosaccharide (GOS) Increase *Bifidobacterium* but Reduce Butyrate Producing Bacteria with Adverse Glycemic Metabolism in healthy young population. *Sci Rep.* 2017;7(1):11789.
402. Worthley DL, Le Leu RK, Whitehall VL*, et al.* A human, double-blind, placebo-controlled, crossover trial of prebiotic, probiotic, and synbiotic supplementation: effects on luminal, inflammatory, epigenetic, and epithelial biomarkers of colorectal cancer. *Am J Clin Nutr.* 2009;90(3):578-86.
403. King DE, Egan BM, Woolson RF, Mainous AG, Al-Solaiman Y, Jesri A. Effect of a high-fiber diet vs a fiber-supplemented diet on C-reactive protein level. *Arch Intern Med.* 2007;167(5):502-06.
404. Spiller G, Farquhar J, Gates J, Nichols S. Guar gum and plasma cholesterol. Effect of guar gum and an oat fiber source on plasma lipoproteins and cholesterol in hypercholesterolemic adults. *Arterioscler Thromb.* 1991;11(5):1204-08.

405. Haskell WL, Spiller GA, Jensen CD, Ellis BK, Gates JE. Role of water-soluble dietary fiber in the management of elevated plasma cholesterol in healthy subjects. *Am J Cardiol.* 1992;69(5):433-39.
406. Blackwood A, Salter J, Dettmar P, Chaplin M. Dietary fibre, physicochemical properties and their relationship to health. *J R Soc Promot Health.* 2000;120(4):242-47.
407. Hartley L, May MD, Loveman E, Colquitt JL, Rees K. Dietary fibre for the primary prevention of cardiovascular disease. *Cochrane Database Syst Rev.* 2016(1):CD011472.
408. Onakpoya IJ, Heneghan CJ. Effect of the novel functional fibre, polyglycoplex (PGX), on body weight and metabolic parameters: A systematic review of randomized clinical trials. *Clin Nutr.* 2015;34(6):1109-14.
409. Ho HV, Sievenpiper JL, Zurbau A*, et al.* The effect of oat β-glucan on LDL-cholesterol, non-HDL-cholesterol and apoB for CVD risk reduction: a systematic review and meta-analysis of randomised-controlled trials. *Br J Nutr.* 2016;116(8):1369-82.
410. Ho HVT, Jovanovski E, Zurbau A*, et al.* A systematic review and meta-analysis of randomized controlled trials of the effect of konjac glucomannan, a viscous soluble fiber, on LDL cholesterol and the new lipid targets non-HDL cholesterol and apolipoprotein B. *Am J Clin Nutr.* 2017;105(5):1239-47.
411. Jovanovski E, Yashpal S, Komishon A*, et al.* Effect of psyllium (*Plantago ovata*) fiber on LDL cholesterol and alternative lipid targets, non-HDL cholesterol and apolipoprotein B: a systematic review and meta-analysis of randomized controlled trials. *Am J Clin Nutr.* 2018;108(5):922-32.
412. Ho HV, Sievenpiper JL, Zurbau A*, et al.* A systematic review and meta-analysis of randomized controlled trials of the effect of barley β-glucan on LDL-C, non-HDL-C and apoB for cardiovascular disease risk reduction. *Eur J Clin Nutr.* 2016;70(11):1239-45.
413. Jovanovski E, Khayyat R, Zurbau A*, et al.* Should Viscous Fiber Supplements Be Considered in Diabetes Control? Results From a Systematic Review and Meta-analysis of Randomized Controlled Trials. *Diabetes Care.* 2019;42(5):755-66.
414. Smith CE, Tucker KL. Health benefits of cereal fibre: a review of clinical trials. *Nutr Res Rev.* 2011;24(1):118-31.
415. Bourquin LD, Titgemeyer EC, Garleb KA, Fahey GCJ. Short-chain fatty acid production and fiber degradation by human colonic bacteria: effects of substrate and cell wall fractionation procedures. *J Nutr.* 1992;122(7):1508-20.
416. van Dam R, Seidell J. Carbohydrate intake and obesity. *Eur J Clin Nutr.* 2007;61 Suppl 1:S75-S99.
417. Johansson-Persson A, Ulmius M, Cloetens L, Karhu T, Herzig K-H, Önning G. A high intake of dietary fiber influences C-reactive protein and fibrinogen, but not glucose and lipid metabolism, in mildly hypercholesterolemic subjects. *Eur J Nutr.* 2014;53(1):39-48.
418. National Research Council. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients). Washington, D.C.: The National Academies Press; 2005.
419. Chou S-Y, Chien P-J, Chau C-F. Particle size reduction effectively enhances the cholesterol-lowering activities of carrot insoluble fiber and cellulose. *J Agric Food Chem.* 2008;56(22):10994-98.
420. Chau C-F, Huang Y-L. Effects of the insoluble fiber derived from *Passiflora edulis* seed on plasma and hepatic lipids and fecal output. *Mol Nutr Food Res.* 2005;49(8):786-90.

421. Hamauzu Y, Mizuno Y. Non-extractable procyanidins and lignin are important factors in the bile acid binding and radical scavenging properties of cell wall material in some fruits. *Plant Foods Hum Nutr.* 2011;66(1):70-77.
422. Ngamukote S, Makynen K, Thilawech T, Adisakwattana S. Cholesterol-lowering activity of the major polyphenols in grape seed. *Molecules.* 2011;16(6):5054-61.
423. Ellegård L, Andersson H. Oat bran rapidly increases bile acid excretion and bile acid synthesis: an ileostomy study. *Eur J Clin Nutr.* 2007;61(8):938-45.
424. Roager HM, Vogt JK, Kristensen M*, et al.* Whole grain-rich diet reduces body weight and systemic low-grade inflammation without inducing major changes of the gut microbiome: a randomised cross-over trial. *Gut.* 2019;68(1):83-93.
425. Hajihashemi P, Azadbakht L, Hashemipor M, Kelishadi R, Esmaillzadeh A. Whole-grain intake favorably affects markers of systemic inflammation in obese children: a randomized controlled crossover clinical trial. *Mol Nutr Food Res.* 2014;58(6):1301-08.
426. Nicolucci AC, Hume MP, Martinez I, Mayengbam S, Walter J, Reimer RA. Prebiotic Reduces Body Fat and Alters Intestinal Microbiota in Children With Overweight or Obesity. *Gastroenterology.* 2017;153:711–22.
427. Capuano E. The behavior of dietary fiber in the gastrointestinal tract determines its physiological effect. *Crit Rev Food Sci Nutr.* 2017;57(16):3543-64.
428. Gemen R, de Vries JF, Slavin JL. Relationship between molecular structure of cereal dietary fiber and health effects: focus on glucose/insulin response and gut health. *Nutr Rev.* 2011;69(1):22-33.
429. Kim Y, Je Y. Dietary fibre intake and mortality from cardiovascular disease and all cancers: A meta-analysis of prospective cohort studies. *Arch Cardiovasc Dis.* 2016;109(1):39-54.
430. Ze X, Duncan SH, Louis P, Flint HJ. *Ruminococcus bromii* is a keystone species for the degradation of resistant starch in the human colon. *ISME J.* 2012;6(8):1535-43.
431. Leach JD. Evolutionary perspective on dietary intake of fibre and colorectal cancer. *Eur J Clin Nutr.* 2007;61(1):140-42.
432. Freeland KR, Wilson C, Wolever TMS. Adaptation of colonic fermentation and glucagon-like peptide-1 secretion with increased wheat fibre intake for 1 year in hyperinsulinaemic human subjects. *Br J Nutr.* 2010;103(1):82-90.
433. Harvard University. The Healthy Eating Plate. 2011. Available at: Available from: https://www.hsph.harvard.edu/nutritionsource/healthy-eating-plate/.
434. Willett WC, Skerrett PJ. Eat, drink, and be healthy: the Harvard Medical School guide to healthy eating. New York, New York: Free Press; 2017.

3.6 Supplementary Tables

Table S.3.1. Characteristics of studies reporting a significant effect of DF supplementation on immunometabolic markers in healthy adults

Reference	Study design	n	Study population characteristics	Age (years)[1]	Duration (wks)	Control	Fiber type	Dose (g/d)[2]	Fiber properties	Marker(s) Affected
Cossack, 1991	Single arm	10	Hypercholesterolemic	53 ± 8	5	None	Sugar beet fiber	26.6	MPCW	↓ LDL-C, TC, TG
Kawatra, 1991	Single arm	20	Overweight	*30-50*	6	None	Guar gum	12.0	Viscous	↓ LDL-C, TC
Lampe, 1991	R, crossover (1.43 wks)[3]									
	Fiber	32	Healthy	27.1 ± 2.1	3	No DF	Mixed vegetable fiber	10.0	MPCW	↓ TG
	Fiber	34					Mixed vegetable fiber	30.0	MPCW	↓ TC, TG
	Fiber	15					Sugar beet fiber	30.0	MPCW	↓ LDL-C, TC, TG
Spiller, 1991	Crossover (0 wks)	13	Hypercholesterolemic	62 ± 3.0	3	None	Guar gum	11.0	Viscous	↓ LDL-C, TC
Haskell, 1992	R, crossover (0 wks)	14	Hypercholesterolemic	52.5 ± 10.8	4	None	Guar gum	10.0	Viscous	↓ LDL-C, TC
							WSDF mixture	15.0	Viscous	↓ LDL-C, TC
	R, DB, parallel									
	Fiber	12	Hypercholesterolemic	56.3 ± 9.5	4	No DF	WSDF mixture	5.0	Viscous	↔
	Fiber	13		56.3 ± 9.5			WSDF mixture	10.0	Viscous	↔
	Fiber	12		56.3 ± 9.5			WSDF mixture	15.0	Viscous	↓ LDL-C, TC
	Control	11		56.3 ± 9.5						
Jensen, 1993	R, DB, parallel									
	Fiber	14	Hypercholesterolemic	56 ± 9	4	None	Acacia gum	15.0	Nonviscous	↔
	Fiber	15		52 ± 9	4	None	WSDF mixture	15.0	Viscous	↓ LDL-C, TC
Braaten, 1994	R, crossover (3 wks)									
	Males	9	Hypercholesterolemic	52 ± 2.6	4	Maltodextrin	β-glucan (oat)	5.8	Viscous	↓ LDL-C, TC, TG
	Females	10		56 ± 1.3						
Sandström, 1994	R, crossover (2 wks)	11	Healthy	23	2	No DF	Pea fiber	20.0	MPCW	↓TG
Arvill, 1995	R, DB, crossover (2 wks)	63	Hypercholesterolemic	47 ± 8.2	4	Corn starch	Konjac glucomannan	3.87	Viscous	↓ LDL-C, TC, TG
Goel, 1997	Single arm	10	Hypercholesterolemic	44 ± 2.9	4	None	Rhubarb stalk fiber	20.0	MPCW	↓ LDL-C, TC
Brighenti, 1999	Crossover (0 wks)	12	Healthy	23.3 ± 0.5	4	No DF	Inulin	9.0	Nonviscous	↓ TC, TG
Jackson, 1999	R, DB, parallel									
	Fiber	27	Hypercholesterolemic	52.6 ± 8.6	8	Maltodextrin	Inulin	10.0	Nonviscous	↓ TG
	Control	27		51.9 ± 10.5						
Nicolosi, 1999	Single-arm	15	Hypercholesterolemic, obese	51 ± 7	8	None	β-glucan (yeast)	15.0	Nonviscous	↓ LDL-C, TC ↑ HDL-C
Vidal-Quintanar, 1999	Single-arm (sub-group analysis)	12	Hypercholesterolemic	39.5 ± 8.8	6	None	LTMH fiber	32.0	MPCW	↓ LDL-C, TC
		11	Healthy	30.2 ± 7.6			LTMH fiber	32.0	MPCW	↓ LDL-C, TC
Zunft, 2001	Single arm	47	Hypercholesterolemic	54.8 ± 9.9	8	None	Carob fiber	15.0	MPCW	↓ LDL-C, TC
Gallaher, 2002	Single-arm	21	Overweight	28.9 ± 9.8	4	None	Chitosan, konjac	2.36	Viscous	↓ LDL-C, TC
Zunft, 2003	R, DB, parallel									
	Fiber	29	Hypercholesterolemic	55 ± 10	6	No DF	Carob fiber	15.0	MPCW	↓ LDL-C, TC
	Control	29		53.8 ± 12						
Marett, 2004	R, DB, parallel									
	Fiber	18	Healthy	29	26	Rice starch	Arabinogalactan (larch)	8.4	Nonviscous	↓ glucose

(Continued)

Table S.3.1. *Continued*

Reference	Study design	*n*	Study population characteristics	Age (years)[1]	Duration (wks)	Control	Fiber type	Dose (g/d)[2]	Fiber properties	Marker(s) Affected
	Fiber	19				Rice starch	Arabinogalactan (tamarack)	8.4	Nonviscous	↓ glucose
	Control	17								
Park, 2004	R, DB, parallel									
	Fiber	12	Overweight/obese	42.3 ± 3.1	3	Corn starch	RS type-III	24.0	Insoluble	↓ glucose, LDL-C, TC
	Control	13		43.6 ± 2.8						
Hall, 2005	R, SB, crossover (4 wks)	38	Healthy	41.0 ± 1.9	4	Low DF diet	Lupin kernel fiber	22.2[4]	MPCW	↓ LDL-C, TC
Garcia, 2006	R, SB, crossover (6 wks)	11	Overweight/obese, IGT	55.5 ± 6.2	6	No DF	Arabinoxylan (wheat)	12.2	Viscous	↓ glucose, TG
Yoshida, 2006	R, DB, crossover (4 wks)	16	Healthy	55.2 ± 6.9	3	No DF	Glucomannan	10.0	Viscous	↓ LDL-C, TC
King, 2007	R, crossover (3 wks)[3]	17	Obese, hypertensive	38.3 ± 1.2	3	DASH diet	Psyllium	30.0	Viscous	↓ CRP
		18	Lean, normotensive							
Queenan, 2007	R, DB, parallel									
	Fiber	35	Hypercholesterolemic	44.5 ± 2.2	6	Dextrose	β-glucan (oat)	6.0	Viscous	↓ LDL-C, TC
	Control	40		45.3 ± 2.0						
Ganji, 2008	Single-arm (sub-group analysis)		Hypercholesterolemic							
		8	Pre-menopausal	34.6 ± 11.5	6	None	Psyllium	12.9	Viscous	↔
		11	Post-menopausal	52.9 ± 2.8			Psyllium	12.9	Viscous	↓ TC
Pérez-Jiménez, 2008	R, parallel									
	Fiber	34	Hypercholesterolemic, healthy	35.5 ± 11.8	16	No treatment	Grape antioxidant dietary fiber	5.25	MPCW	↓ glucose, TC
	Control	9		34.6 ± 12.4						
Smith, 2008	R, DB, parallel									
	Fiber	45	Hypercholesterolemic	44.1 ± 13	6	None	LMW β-glucan (barley)	6.0	Nonviscous	↓ CRP, LDL-C
	Fiber	45		45.1 ± 14			HMW β-glucan (barley)	6.0	Viscous	↔
Maki, 2009	R, DB, parallel									
	Fiber	16	Hypercholesterolemic	58.9 ± 3.2	4	No DF	HPMC (high viscosity)	3.0	Viscous	↔
	Fiber	17		55.7 ± 3.1			HPMC (low viscosity)	5.0	Nonviscous	↓ LDL-C, TC
	Fiber	32		53.2 ± 2.8			HPMC (high viscosity)	5.0	Viscous	↓ LDL-C, TC
	Fiber	29		58.6 ± 1.9			HPMC (low viscosity)	10.0	Nonviscous	↓ LDL-C, TC
	Fiber	15		56.7 ± 2.1			HPMC (moderate viscosity)	10.0	Viscous	↓ LDL-C, TC
	Fiber	27		55.1 ± 1.9			HPMC (moderately high viscosity)	10.0	Viscous	↓ LDL-C, TC
	Control	29		57.3 ± 2.5						
Reppas, 2009	R, DB, parallel									
	Fiber	20	Hypercholesterolemic	41.6	6	No DF	HPMC (high viscosity)	5.0	Viscous	↓ LDL-C, TC
	Fiber	10					HPMC (high viscosity)	15.0	Viscous	↓ LDL-C, TC
	Control	10								
Li, 2010	R, DB, parallel									
	Fiber	60	Overweight	30.4 ± 4.3	12	Maltodextrin	NUTRIOSE	28.9	Nonviscous	↓ glucose, HOMA-IR, TC LDL-C, ↑ HDL-C
	Control	60		31.6 ± 4.1						

(Continued)

Table S.3.1. *Continued*

Reference	Study design	*n*	Study population characteristics	Age (years)[1]	Duration (wks)	Control	Fiber type	Dose (g/d)[2]	Fiber properties	Marker(s) Affected
Reimer, 2010	R, DB, parallel									
	Fiber	27	Healthy	32.3 ± 10.3	3	Skim milk powder	PolyGlycopleX	8.7	Viscous	↓ HOMA-IR
	Control	27		30.9 ± 10.8						
Ruiz-Roso, 2010	R, DB, parallel									
	Fiber	43	Hypercholesterolemic	42.9 ± 9.5	4	Dextrose	Carob fiber	8.0	MPCW	↓ LDL-C, TC, TG
	Control	45		44.1 ± 9.9						↑ HDL-C
Russo, 2010	R, DB, crossover (8 wks)	15	Healthy	18.8 ± 0.7	5	No DF	Inulin	11.0	Nonviscous	↓ glucose, HOMA-IR, TG ↑ HDL-C
Solà, 2010	R, DB, parallel									
	Fiber	101	Hypercholesterolemic	54.2 ± 9.9	8	MCC	Psyllium	11.5	Viscous	↓ insulin, HOMA-IR
	Control	108		55.5 ± 11.2						
Lyon, 2011	R, DB, parallel									
	Fiber (M)	15	Healthy	38.1 ± 7.2	15	Inulin (low viscous control)	PolyGlycopleX	13.1	Viscous	↔
	Fiber (F)	15		34.7 ± 10.4			PolyGlycopleX	13.1	Viscous	↓ LDL-C, TC
	Control (M)	13		38.8 ± 7.1						
	Control (F)	17		37.1 ± 10.8						
Pal, 2011	R, SB, parallel									
	Fiber	16	Overweight/obese	41.3 ± 2.3	12	Breadcrumbs	Psyllium	29.7	Viscous	↓ LDL-C, TC
	Control	15		44.8 ± 1.6						
Hashizume, 2012	R, DB, parallel									
	Fiber	15	Metabolic syndrome	60.1 ± 8.9	12	No DF	Resistant maltodextrin	27.0	Nonviscous	↓ glucose, HOMA-IR, TC, TG
	Control	15		61.2 ± 11.6						
Gato, 2013	R, DB, parallel									
	Fiber	13	Hypercholesterolemic	40.6 ± 1.9	12	No DF	Persimmon fiber	9.0	MPCW	↓ TC
	Fiber	13		36.4 ± 1.8			Persimmon fiber	15.0	MPCW	↓ LDL-C, TC
	Control	14		36.6 ± 1.8						
Reimer, 2013	R, DB, parallel									
	Fiber	28	Abdominal adiposity	*20-65*	14	Rice flour	PolyGlycopleX	13.1	Viscous	↓ IL-6, glucose, glucose AUC
	Control	28		*20-65*						
Fechner, 2014	R, DB, crossover (2 wks)	52	Hypercholesterolemic	46.9 ± 3.2	4	No DF	Lupin fiber	21.7	MPCW	↓ CRP, LDL-C, TC, TG
							Citrus fiber	23.1	MPCW	↓ LDL-C, TC
Brahe, 2015	R, SB, parallel									
	Fiber	19	Obese, post-menopausal	60.6 ± 6.4	6	No DF	Flaxseed mucilage	10.0	MPCW	↓ insulin AUC
	Control	20		58.5 ± 5.3						

(Continued)

Table S.3.1. *Continued*

Reference	Study design	*n*	Study population characteristics	Age (years)[1]	Duration (wks)	Control	Fiber type	Dose (g/d)[2]	Fiber properties	Marker(s) Affected
Urquiaga, 2015	R, parallel									
	Fiber	25	Metabolic syndrome	44.5 ± 9.3	16	No treatment	Wine grape pomace	10.0	MPCW	↓ glucose, insulin AUC
	Control	13		43.1 ± 8.4						
Dainty, 2016	R, DB, crossover (4 wks)	24	Overweight/obese	55.3 ± 1.6	8	No DF	RS type-II (Hi-maze)	25.0	Insoluble	↓ insulin, insulin AUC, HOMA-IR
Kapoor, 2016	Single arm, pilot	6	Healthy	46.3 ± 2.9	52	None	Partially hydrolyzed guar gum	14.4	Nonviscous	↓ CRP, LDL-C ↑ HDL-C
Nasir, 2016	Single arm	10	Prediabetic	*35-60*	16	None	Acacia gum	10.0	Nonviscous	↓ glucose
Lambert, 2017	R, DB, parallel									
	Fiber	22	Overweight/obese	44 ± 15	12	No DF	Yellow pea hull fiber	13.8	MPCW	↓ glucose AUC
	Control	22		44 ± 15						
Pal, 2017	R, DB, parallel									
	Fiber	39	Overweight/obese	49.9 ± 11.0	12	Rice flour	Psyllium	12.9	Viscous	↓ insulin, HOMA-IR ↑ HDL-C
	Fiber	43		47.9 ± 12.1			PolyGlycopleX	13.1	Viscous	
	Control	45		49.8 ± 11.8						
Martínez-Maqueda, 2018	R, crossover (4 wks)	49	Metabolic syndrome	42.6 ± 1.6	6	No treatment	Wine grape pomace	8.0	MPCW	↓ insulin, HOMA-IR
Urquiaga, 2018	Crossover, (4 wks)	27	Metabolic syndrome	43.6 ± 11.2	4	No DF	Wine grape pomace	3.5	MPCW	↓ HOMA-IR

↓, significant decrease; ↑, significant increase; ↔, no significant change; AUC, area under the curve; CRP, C-reactive protein; DB, double-blinded; HDL-C, high density lipoprotein cholesterol; HMW, high molecular weight; HOMA-IR, homeostatic model assessment for insulin resistance; HPMC, hydroxypropyl methylcellulose; IGT, impaired glucose tolerance; IL, interleukin; LTMH, lime-treated maize husk, LMW, low molecular weight; LDL-C, low density lipoprotein cholesterol; MCC, microcrystalline cellulose; MPCW, mixed plant cell wall DF type; No DF, the control was the same product received by the treatment group but without the dietary fiber added; R, randomized; RS, resistant starch; SB, single-blinded; TG, triglycerides; TC, total cholesterol; WSDF, water soluble dietary fiber mixture of psyllium, pectin, guar gum, and locust bean gum.

[1]Mean age is presented as they were in the original articles (as mean ± SD, mean ± SEM, or a range).

[2]Dietary fiber dose was corrected for the purity of the fiber.

[3]Washout period in weeks of crossover studies included in parentheses.

[4]Fiber dose was based on individual energy intake; mean of group is provided here.

Table S.3.2. Characteristics of human dietary fiber interventions assessing fasting and post-prandial markers of dysglycemia and insulin resistance[1]

Reference	Study design	*n*	Study population characteristics	Age (years)[2]	BMI (kg/m²)[2]	Duration (wks)	Control	Fiber type	Fiber dose (g/d)[3]	Fiber properties	Effect on Glucose	Effect on Glucose AUC	Effect on Insulin	Effect on Insulin AUC	Effect on HOMA-IR	Risk of Bias
Sundell, 1993	Single arm	11	Healthy	31.7 ± 6.4	*(69.0 kg)*	5/2[9]	None	Oat husk fiber	5/10[9]	MPCW	↑[6]	–	↔	–	–	H
Hanai, 1997	Single arm	20	Obese IGT	54.3 ± 4.3	≥ 26.4	26	None	Corn bran hemicellulose	10.0	Viscous	↔	–	↔	–	–	H
		8	Normoweight IGT	57.5 ± 8.1	20 - 24											
		10	Normoweight healthy	48.7 ± 5.9	20 - 24											
Jackson, 1999	R, DB, parallel															
	Fiber	27	Hypercholest-erolemic	52.6 ± 8.6	26.5 ± 3.6	8	Maltodextrin	Inulin	10.0	Nonviscous	↔	–	↔	–	–	U
	Control	27		51.9 ± 10.5	26.1 ± 2.8											
Vidal-Quintanar, 1999	Single arm (sub-group analysis)	12	Hypercholest-erolemic	39.5 ± 8.8	28.9 ± 4.7	6	None	Lime-treated maize husks	32.0	MPCW	↔	–	–	–	–	H
		11	Normal	30.2 ± 7.6	24.6 ± 2.8			Lime-treated maize husks	32.0	MPCW	↔	–	–	–	–	
Robinson, 2001	R, crossover (0 wks)[4]															
	Fiber	20	Healthy	–	–	3	None	AG (larch)	15.0	Nonviscous	↔	–	↔	–	–	H
	Fiber							AG (larch)	30.0	Nonviscous	↑[6]	–	↔	–	–	
Zunft, 2001	Single arm	47	Hypercholest-erolemic	54.8 ± 9.9	*(72.5 kg)*	8	None	Carob fiber	15.0	MPCW	↑[6]	–	–	–	–	H
Keogh, 2003	R, SB, crossover (4 wks)	18	Hypercholest-erolemic	38.8 ± 10.1	27.4 ± 4.6	4	Add glucose to low DF foods	β-glucan (barley)	9.9[8]	Viscous	↔	↔	–	–	–	U
Zunft, 2003	R, DB, parallel															
	Fiber	29	Hypercholest-erolemic	55 ± 10	25.4 ± 3.1	6	No DF	Carob fiber	15.0	MPCW	↔	–	↔	–	–	H
	Control	29		53.8 ± 12	25.8 ± 3.9											
Giacco, 2004	R, DB, crossover (0 wks)	30	Hypercholest-erolemic	45.5 ± 9.9	26.6 ± 2.2	8	Maltodextrin	Short-chain FOS	10.6	Nonviscous	↔	–	–	–	–	U
Marett, 2004	R, DB, parallel															
	Fiber	18	Healthy	29	–	26	Rice starch	AG (larch)	8.4	Nonviscous	↓[6]	–	↔	–	–	H
	Fiber	19						AG (tamarack)	8.4	Nonviscous	↓[6]	–	↔	–	–	
	Control	17														

(Continued)

Table S.3.2. *(Continued)*

Reference	Study design	*n*	Study population characteristics	Age (years)[2]	BMI (kg/m^2)[2]	Duration (wks)	Control	Fiber type	Fiber dose (g/d)[3]	Fiber properties	Effect on Glucose	Effect on Glucose AUC	Effect on Insulin	Effect on Insulin AUC	Effect on HOMA-IR	Risk of Bias
Park, 2004	R, DB, parallel															
	Fiber	12	Overweight and obese	42.3 ± 3.1	26.6 ± 0.7	3	Corn starch	RS type-III	24.0	Insoluble	↓[6]	−	↔	−	−	H
	Control	13		43.6 ± 2.8	27.9 ± 0.5											
Hall, 2005	R, SB, crossover (4 wks)	38	Healthy	41.0 ± 1.9	26.7 ± 0.5	4	Low DF diet	Lupin kernel fiber	22.2[8]	MPCW	↔	−	↔	−	↔	U
Garcia, 2006	R, SB, crossover (6 wks)	11	Overweight, obese, IGT	55.5 ± 6.2	30.1 ± 5.7	6	No DF	Arabinoxylan (wheat)	12.2	Viscous	↓[5]	−	↔	−	−	U
Queenan, 2007	R, DB, parallel															
	Fiber	35	Hypercholesterolemic	44.5 ± 2.2	*(81.8 kg)*	6	Dextrose	β-glucan (oat)	6.0	Viscous	↔	−	↔	−	−	H
	Control	40		45.3 ± 2.0	*(79.6 kg)*											
Pérez-Jiménez, 2008	R, parallel															
	Fiber	34	Hypercholesterolemic and healthy	35.5 ± 11.8	26.1 ± 4.7	16	No treatment	Grape antioxidant dietary fiber	5.25	MPCW	↓[6]	−	−	−	−	H
	Control	9		34.6 ± 12.4	22.7 ± 2.4											
Smith, 2008	R, DB, parallel															
	Fiber	45	Hypercholesterolemic	44.1 ± 13	26.0 ± 3.2	6	None	LMW β-glucan (barley)	6.0	Nonviscous	↔	−	↔	−	−	H
	Fiber	45		45.1 ± 14	26.7 ± 4.2			HMW β-glucan (barley)	6.0	Viscous	↔	−	↔	−	−	
Cloetens, 2009	R, crossover, (4 wks)	20	Healthy	24 ± 5	20.9 ± 2.3	3	Maltodextrin	AXOS	10.0	Nonviscous	↔	−	−	−	−	H
Parnell, 2009, 2017	R, DB, parallel															
	Fiber	21	Overweight and obese	41.9 ± 12.7	30.4 ± 3.4	12	Maltodextrin	Oligofructose	21.0	Nonviscous	↔	−	↔	−	−	U
	Control	18		38.6 ± 13.0	29.8 ± 4.0											
Li, 2010	R, DB, parallel															
	Fiber	60	Overweight	30.4 ± 4.3	24.5 ± 0.2	12	Maltodextrin	NUTRIOSE	28.9	Nonviscous	↔	−	↔	−	↓[7]	L
	Control	60		31.6 ± 4.1	24.5 ± 0.3											
Pouteau, 2010	R, DB, crossover (6 wks)	21	Metabolic syndrome	47 ± 12	33.4 ± 3.0	5	No DF	Acacia gum (80%), apple pectin (20%)	28.0	Viscous	↔	−	↔	−	↔	U
Reimer, 2010	R, DB, parallel															
	Fiber	27	Healthy	32.3 ± 10.3	22.7 ± 2.1	3	Skim milk powder	PGX	8.7	Viscous	↔	−	↔	−	↓[7]	U
	Control	27		30.9 ± 10.8	22.8 ± 2.4											

(Continued

Table S.3.2. *(Continued)*

Reference	Study design	*n*	Study population characteristics	Age (years)[2]	BMI (kg/m^2)[2]	Duration (wks)	Control	Fiber type	Fiber dose (g/d)[3]	Fiber properties	Effect on Glucose	Effect on Glucose AUC	Effect on Insulin	Effect on Insulin AUC	Effect on HOMA-IR	Risk of Bias
Ruiz-Roso, 2010	R, DB, parallel															
	Fiber	43	Hypercholest-erolemic	42.9 ± 9.5	25.7 ± 3.5	4	Dextrose	Carob fiber	8.0	MPCW	↔	–	–	–	–	L
	Control	45		44.1 ± 9.9	25.5 ± 3.8											
Russo, 2010	R, DB, crossover (8 wks)	15	Healthy	18.8 ± 0.7	22.8 ± 2.3	5	No DF	Inulin	11.0	Nonviscous	↓[6]	–	↔	–	↓[6]	H
Solà, 2010	R, DB, parallel															
	Fiber	101	Hypercholest-erolemic	54.2 ± 9.9	26.8 ± 3.7	8	MCC	Psyllium	11.5	Viscous	↔	–	↓[7]	–	↓[7]	L
	Control	108		55.5 ± 11.5	27.4 ± 3.5											
Lyon, 2011	R, DB, parallel															
	Fiber (M)	13	Healthy	38.1 ± 7.2	29.8 ± 1.2	15	Inulin (low viscous control)	PGX	13.1	Viscous	↔	–	↔	–	–	U
	Fiber (F)	17		34.7 ± 10.4	30.1 ± 2.5			PGX	13.1	Viscous	↔	–	↔	–	–	
	Control (M)	15		38.8 ± 7.1	30.0 ± 1.5											
	Control (F)	14		37.1 ± 10.8	31.7 ± 1.8											
Pal, 2011	R, SB, parallel															
	Fiber	16	Overweight and obese	41.3 ± 2.3	34.0 ± 0.9	12	Breadcrumbs	Psyllium	29.7	Viscous	↔	–	↔	–	–	U
	Control	15		44.8 ± 1.6	33.7 ± 1.0											
François, 2012	R, DB, crossover (2 wks)															
	Fiber	57	Healthy	40.7 ± 2.2	23.5 ± 0.4	3	No DF	AXOS	2.9	Nonviscous	↔	–	↔	–	–	L
	Fiber							AXOS	9.5	Nonviscous	↔	–	↔	–	–	
Hashizume, 2012	R, DB, parallel															
	Fiber	15	Metabolic syndrome	60.1 ± 8.9	28.1 ± 2.3	12	No DF	Resistant maltodextrin	27.0	Nonviscous	↓[6]	–	↔	–	↓[6,7]	H
	Control	15		61.2 ± 11.6	26.8 ± 2.9											
Maki, 2012	R, DB, crossover (3 wks)															
	Fiber	33	Overweight and obese	49.5 ± 1.6	30.6 ± 0.5	4	Digestible starch (Amioca)	RS type-II	15.0	Insoluble	↔	–	–	–	–	U
	Fiber							RS type-II (Hi-maize)	30.0	Insoluble	↔	–	–	–	–	
	Control															
de Luis, 2013	R, DB, parallel															
	Fiber	18	Obese	45.3 ± 16.1	35.9 ± 3.4	4	No DF	FOS	9.8	Nonviscous	↔	–	↔	–	↔	U
	Control	18		50.8 ± 16.2	39.2 ± 7.2											

(Continued)

Table S.3.2. *(Continued)*

Reference	Study design	*n*	Study population characteristics	Age (years)[2]	BMI (kg/m^2)[2]	Duration (wks)	Control	Fiber type	Fiber dose (g/d)[3]	Fiber properties	Effect on Glucose	Effect on Glucose AUC	Effect on Insulin	Effect on Insulin AUC	Effect on HOMA-IR	Risk of Bias
Gato, 2013	R, DB, parallel															
	Fiber	13	Hypercholest-erolemic	40.6 ± 1.9	*(61.4 kg)*	12	No DF	Persimmon fiber	9.0	MPCW	↔	–	–	–	–	H
	Fiber	13		36.4 ± 1.8	*(64.4 kg)*			Persimmon fiber	15.0	MPCW	↔	–	–	–	–	
	Control	14		36.6 ± 1.8	*(63.5 kg)*											
Pedersen, 2013	Single arm, pilot	10	Healthy	25.0 ± 1.2	21.6 ± 0.7	1/1/1/1/1	None	Oligofructose	15/25/35/45/55[9]	Nonviscous	↔	–	↔	–	–	H
Reimer, 2013	R, DB, parallel															
	Fiber	28	Abdominal adiposity	20 - 65	26.7 ± 0.2	14	Rice flour	PGX	13.1	Viscous	↓[5]	↓[5]	↔	–	↔	U
	Control	28		20 - 65	27.2 ± 0.3											
Savastano, 2014	R, DB, parallel															
	Fiber	30	Overweight and obese	43.4 ± 11.8	28.3 ± 2.6	3	Maltodextrin	Oligofructose and pectin	15.0	Viscous	↔	–	↔	–	↔	L
	Fiber	29		39.2 ± 12.7	29.3 ± 2.8				30.0	Viscous	↔	–	↔	–	↔	
	Control	29		42.3 ± 12.8	29.4 ± 2.7											
Brahe, 2015	R, SB, parallel															
	Fiber	19	Obese, post-meno	60.6 ± 6.4	35.2 ± 4.5	6	No DF	Flaxseed mucilage	10.0	MPCW	↔	↔	↔	↓[6]	↔	U
	Control	20		58.5 ± 5.3	34.3 ± 3.8											
Tripkovic, 2015	R, SB, crossover (4 wks)	10	Overweight and obese	39.8 ± 9.6	30.2 ± 3.0	4	No DF	Inulin	15.0	Nonviscous	↔	↔	↔	↔	↔	U
Urquiaga, 2015	R, parallel															
	Fiber	25	Metabolic syndrome	44.5 ± 9.3	29.1 ± 3.9	16	No treatment	Wine grape pomace	10.0	MPCW	↓[6]	↔	↔	↓[5]	↔	U
	Control	13		43.1 ± 8.4	27.9 ± 3.5											
Dainty, 2016	R, DB, crossover (4 wks)	24	Overweight and obese	55.3 ± 1.6	30.2 ± 0.5	8	No DF	RS type-II (Hi-maze)	25.0	Insoluble	↔	↔	↓[6,7]	↓[5]	↓[7]	L
Guess, 2016	R, DB, crossover (4 wks)	7	IFG	63.8 ± 2.5	31.1 ± 1.0	2/2/2[9]	Cellulose	Inulin	10/20/30[9]	Nonviscous	↔	↔	↔	↑[7]	↔	L
		11	IGT	62.3 ± 3.6	28.0 ± 0.8											
		16	IFG/IGT	60.7 ± 2.7	28.4 ± 2.2											
Kapoor, 2016	Single arm, pilot	6	Healthy	46.3 ± 2.9	25.3 ± 0.6	52	None	Partially hydrolyzed guar gum	14.4	Nonviscous	↔	–	–	–	–	H
		4	IGT		29.2 ± 2.5											
		2	Prediabetic		29.9 ± 8.3											

(Continued)

Table S.3.2. *(Continued)*

Reference	Study design	*n*	Study population characteristics	Age (years)[2]	BMI (kg/m^2)[2]	Duration (wks)	Control	Fiber type	Fiber dose (g/d)[3]	Fiber properties	Effect on Glucose	Effect on Glucose AUC	Effect on Insulin	Effect on Insulin AUC	Effect on HOMA -IR	Risk of Bias
Nasir, 2016	Single arm	10	Prediabetic	35 - 60	38.5 ± 2.4	16	None	Acacia gum	10.0	Nonviscous	↓[6]	–	–	–	–	H
Stenman, 2016	R, DB, parallel															
	Fiber	53	Overweight and obese	48.8 ± 10.5	31.2 ± 1.6	26	MCC	Polydextrose	10.8	Nonviscous	↔	–	↔	–	↔	L
	Control	56		49.9 ± 8.5	31.2 ± 2.2											
Canfora, 2017	R, DB, parallel															
	Fiber	21	Obese, prediabetic	59.2 ± 7.2	33.3 ± 3.7	12	Maltodextrin	GOS	15.0	Nonviscous	↔	–	↔	–	↔	U
	Control	23		58.4 ± 7.3	32.3 ± 3.5											
Lambert, 2017	R, DB, parallel															
	Fiber	22	Overweight and obese	44 ± 15	33.1 ± 1.3	12	No DF	Yellow pea hull fiber	13.8	MPCW	↔	↓[7]	↔	↔	–	L
	Control	22		44 ± 15	33.3 ± 1.3											
Liu, 2017	R, DB, crossover (4 wks)															
	Fiber	24	Healthy	21.9 ± 2.8	23.1 ± 3.3	2	None	FOS	16.0	Nonviscous	↔	↑[6]	–	–	–	U
	Fiber	25		22.1 ± 2.7	23.1 ± 3.3			GOS	16.0	Nonviscous	↑[6]	↔	–	–	–	
Pal, 2017	R, DB, parallel															
	Fiber	39	Overweight and obese	49.9 ± 11.0	31.7 ± 3.2	12	Rice flour	Psyllium	12.9	Viscous	↔	–	↓[5]	–	↓[5]	L
	Fiber	43		47.9 ± 12.1	33.3 ± 4.3			PGX	13.1	Viscous	↔	–	↔	–	↔	
	Control	45		49.8 ± 11.8	32.0 ± 4.2											
Alfa, 2018	R, SB, parallel															
	Fiber	21	Healthy	42	*(78.4 kg)*	12	Digestible starch (Amioca)	RS type-II (Potato)	30.0	Insoluble	↔	–	↔	–	↔	U
	Control	21														
Krumbeck, 2018	R, DB, parallel															
	Fiber	20	Obese	45.9 ± 9.6	36.8 ± 5.6	3	Lactose	GOS	5.0	Nonviscous	↔	–	–	–	–	H
	Control	17		43.9 ± 8.8	34.0 ± 4.5											
Martínez-Maqueda, 2018	R, crossover (4 wks)	49	Metabolic syndrome	42.6 ± 1.6	–	6	No treatment	Wine grape pomace	8.0	MPCW	↔	↔	↓[6]	↔	↓[6]	U
Peterson, 2018	R, DB, parallel															
	Fiber	29	Overweight and obese, prediabetic	54 ± 10	35.5 ± 4.4	12	Digestible starch (Amioca)	RS type-II (Hi-maize)	45.0	Insoluble	↔	↔	↔	↔	–	L
	Control	30		55 ± 10	35.7 ± 5.2											

(Continued)

Table S.3.2. *(Continued)*

Reference	Study design	*n*	Study population characteristics	Age (years)[2]	BMI (kg/m^2)[2]	Duration (wks)	Control	Fiber type	Fiber dose (g/d)[3]	Fiber properties	Effect on Glucose	Effect on Glucose AUC	Effect on Insulin	Effect on Insulin AUC	Effect on HOMA -IR	Risk of Bias
Salden, 2018	R, DB, parallel															
	Fiber	16	Overweight and obese	49 ± 17	30.2 ± 1.9	6	Maltodextrin	Arabinoxylan (wheat)	7.5	Viscous	↔	−	↔	−	−	U
	Fiber	17		47 ± 15	31.5 ± 2.2			Arabinoxylan (wheat)	15.0	Viscous	↔	−	↔	−	−	
	Control	14		49 ± 17	31.4 ± 3.1											
Urquiaga, 2018	Crossover, (4 wks)	27	Metabolic syndrome	43.6 ± 11.2	29.5 ± 3.7	4	No DF	Wine grape pomace	3.5	MPCW	↔	−	−	−	↓[6]	H

[1]↓, significant decrease; ↑, significant increase; ↔, no significant change; AG, arabinogalactan; AXOS, arabinoxylan-oligosaccharides; AUC, area under the curve analysis; DB, double blinded; FOS, fructooligosaccharides; GOS, galactooligosaccharides; HMW, high molecular weight; H, high risk of bias; HOMA-IR, homeostatic model assessment for insulin resistance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; L, low risk of bias; LMW, low molecular weight; MCC, microcrystalline cellulose; MetS, metabolic syndrome; MPCW, mixed plant cell wall DF type; No DF, the control was the same product received by the treatment group but without the dietary fiber added; PGX, PolyGlycopleX, proprietary fiber mixture of konjac, sodium alginate, and xanthan gum; post-meno, post-menopausal women; R, randomized; RS, resistant starch; SB, single-blinded; U, unclear risk of bias.

[2]Mean age and BMI are presented as they were in the original articles (as mean ± SD, mean ± SEM, or a range). If BMI was not reported, then either weight in kilograms (kg) or percentage of ideal body weight (IBW) was presented in parentheses and italicized.

[3]Dietary fiber dose was corrected for the purity of the fiber.

[4]Washout period in weeks of crossover studies included in parentheses.

[5]Significant result when treatment arm is compared to the control arm that had the opposite effect.

[6]Significant result when compared within the treatment arm to baseline data or change over time.

[7]Significant result when treatment arm is compared to the control arm.

[8]Fiber dose was based on individual energy intake; mean of group is provided here.

[9]Study design employed an escalating fiber dose. Values separated with a "/" denotes individual escalating fiber doses and their corresponding intervention duration.

Table S.3.3. Characteristics of human dietary fiber interventions assessing fasting markers of systemic inflammation[1]

Reference	Study design	*n*	Study population characteristics	Age (years)[2]	BMI (kg/m^2)[2]	Duration (wks)	Control	Fiber type	Fiber dose (g/d)[3]	Fiber Properties	Effect on CRP	Effect on IL-6	Risk of Bias
King, 2007	R, crossover (3 wks)[4]	17 18	Obese, hypertensive Lean, normotensive	38.3 ± 1.2	28.4 ± 1.1	3	DASH diet	Psyllium	30.0	Viscous	↓[6]	−	U
Queenan, 2007	R, DB, parallel												
	Fiber	35	Hypercholesterolemic	44.5 ± 2.2	*(81.8 kg)*	6	Dextrose	β-glucan (oat)	6.0	Viscous	↔	−	H
	Control	40		45.3 ± 2.0	*(79.6 kg)*								
King, 2008	R, parallel												
	Fiber	54	Overweight and obese with elevated CRP levels	50.6 ± 6.1	32.6 ± 5.6	12	No DF	Psyllium	6.0	Viscous	↔	↔	L
	Fiber	51		51.4 ± 6.4	33.8 ± 5.9			Psyllium	12.0		↔	↔	
	Control	57		49.4 ± 6.1	33.7 ± 6.6								
Smith, 2008	R, DB, parallel												
	Fiber	45	Hypercholesterolemic	44.1 ± 13	26.0 ± 3.2	6	None	LMW β-glucan (barley)	6.0	Nonviscous	↓[6]	−	H
	Fiber	45		45.1 ± 14	26.7 ± 4.2			HMW β-glucan (barley)	6.0	Viscous	↔	−	
Kohl, 2009	R, DB, crossover (4 wks)	12	Overweight with elevated CRP levels	49.7 ± 3.9	32.3 ± 1.0	4	Digestible starch (Amioca)	β-glucan (brewer's yeast)	1.3	Nonviscous	↔	↔	L
Worthley 2009	R, DB, crossover (0 wks)	18	Healthy	61.2 ± 8.4	−	4	None	RS type-II (Hi-maize)	12.5	Insoluble	↔	↔	U
Maki, 2009	R, DB, parallel												
	Fiber	16	Hypercholesterolemic	58.9 ± 3.2	28.9 ± 1.1	4	No DF	HPMC (high viscosity)	3.0	Viscous	↔	−	U
	Fiber	17		55.7 ± 3.1	28.9 ± 1.3			HPMC (low viscosity)	5.0	Nonviscous	↔	−	
	Fiber	32		53.2 ± 2.8	31.2 ± 1.3			HPMC (high viscosity)	5.0	Viscous	↔	−	
	Fiber	29		58.6 ± 1.9	28.9 ± 0.9			HPMC (low viscosity)	10.0	Nonviscous	↔	−	
	Fiber	15		56.7 ± 2.1	31.1 ± 2.1			HPMC (moderate viscosity)	10.0	Viscous	↔	−	
	Fiber	27		55.1 ± 1.9	27.7 ± 0.9			HPMC (moderately high viscosity)	10.0	Viscous	↔	−	
	Control	29		57.3 ± 2.5	27.4 ± 1.0								
Solà, 2010	R, DB, parallel												
	Fiber	101	Hypercholesterolemic	54.2 ± 9.9	26.8 ± 3.7	8	MCC	Psyllium	11.5	Viscous	↔	↔	L
	Control	108		55.5 ± 11.5	27.4 ± 3.5								
de Luis, 2013	R, DB, parallel												
	Fiber	18	Obese	45.3 ± 16.1	35.9 ± 3.4	4	No DF	FOS	9.8	Nonviscous	↔	−	U
	Control	18		50.8 ± 16.2	39.2 ± 7.2								

(Continued)

Table S.3.3. *Continued*

Reference	Study design	n	Study population characteristics	Age (years)[2]	BMI (kg/m²)[2]	Duration (wks)	Control	Fiber type	Fiber dose (g/d)[3]	Fiber Properties	Effect on CRP	Effect on IL-6	Risk of Bias
Reimer, 2013	R, DB, parallel												
	Fiber	28	Abdominal adiposity	20 - 65	26.7 ± 0.2	14	Rice flour	PGX	13.1	Viscous	–	↓[5]	U
	Control	28		20 - 65	27.2 ± 0.3								
Fechner, 2014	R, DB, crossover (2 wks)	52	Hypercholesterolemic	46.9 ± 3.2	26.5 ± 5.9	4	Low DF diet	Lupin fiber	21.7	MPCW	↓[6]	–	L
								Citrus fiber	23.1	MPCW	↔	–	
Brahe, 2015	R, SB, parallel												
	Fiber	19	Obese, post-meno	60.6 ± 6.4	35.2 ± 4.5	6	No DF	Flaxseed mucilage	10	MPCW	↔	↔	U
	Control	20		58.5 ± 5.3	34.3 ± 3.8								
Kapoor, 2016	Single arm, pilot	6	Healthy	46.3 ± 2.9	25.3 ± 0.6	52	None	Partially hydrolyzed guar gum	14.4	Nonviscous	↓[6]	–	H
Stenman, 2016	R, DB, parallel												
	Fiber	53	Overweight and obese	48.8 ± 10.5	31.2 ± 1.6	26	MCC	Polydextrose	10.8	Nonviscous	↔	↔	L
	Control	56		49.9 ± 8.5	31.2 ± 2.2								
Canfora, 2017	R, DB, parallel												
	Fiber	21	Obese, prediabetic	59.2 ± 7.2	33.3 ± 3.7	12	Maltodextrin	GOS	15.0	Nonviscous	–	↔	U
	Control	23		58.4 ± 7.3	32.3 ± 3.5								
Lambert, 2017	R, DB, parallel												
	Fiber	22	Overweight and obese	44 ± 15	33.1 ± 1.3	12	No DF	Yellow pea hull fiber	13.8	MPCW	↔	↔	L
	Control	22		44 ± 15	33.3 ± 1.3								
Parnell, 2017, 2009	R, DB, parallel												
	Fiber	20	Overweight and obese	41.9 ± 12.7	30.4 ± 3.4	12	Maltodextrin	Oligofructose	21.0	Nonviscous	–	↔	U
	Control	17		38.6 ± 13.0	29.8 ± 4.0								
Alfa, 2018	R, SB, parallel												
	Fiber	21	Healthy	42	*(78.4 kg)*	12	Digestible starch (Amioca)	RS type-II (Potato)	30.0	Insoluble	↔	–	U
	Control	21											
Martínez-Maqueda, 2018	R, crossover (4 wks)	49	Metabolic syndrome	42.6 ± 1.6	–	6	No treatment	Wine grape pomace	8.0	MPCW	↔	–	U

(Continued)

Table S.3.3. *Continued*

Reference	Study design	*n*	Study population characteristics	Age (years)[2]	BMI (kg/m^2)[2]	Duration (wks)	Control	Fiber type	Fiber dose (g/d)[3]	Fiber Properties	Effect on CRP	Effect on IL-6	Risk of Bias
Peterson, 2018	R, DB, parallel												
	Fiber	29	Overweight and obese, prediabetic	54 ± 10	35.5 ± 4.4	12	Digestible starch (Amioca)	RS type-II (Hi-maize)	45.0	Insoluble	↔	−	L
	Control	30		55 ± 10	35.7 ± 5.2								

[1]↓, significant decrease; ↑, significant increase; ↔, no significant change; CRP, C-reactive protein; DB, double-blinded; FOS, fructooligosaccharides; GOS, galactooligosaccharides; H, high risk of bias; HMW, high molecular weight; HPMC, hydroxypropyl methylcellulose; IL, interleukin; L, low risk of bias; LMW, low molecular weight; MCC, microcrystalline cellulose; MPCW, mixed plant cell wall DF type, No DF, the control was the same product received by the treatment group but without the dietary fiber added; PGX, PolyGlycopleX, proprietary fiber mixture of konjac, sodium alginate, and xanthan gum; post-meno, post-menopausal women; R, randomized; RS, resistant starch; U, unclear risk of bias.
[2]Mean age and BMI are presented as they were in the original articles (as mean ± SD, mean ± SEM, or as a range). If BMI was not reported, then either weight in kilograms (kg) or percentage of ideal body weight (IBW) was presented in parentheses and italicized.
[3]Dietary fiber dose was corrected for the purity of the fiber.
[4]Washout period (in weeks) of crossover studies included in parentheses.
[5]Significant result when treatment arm is compared to the control arm that had the opposite effect.
[6]Significant result when compared within the treatment arm to baseline data or change over time.
[7]Significant result when treatment arm is compared to the control arm.

Table S.3.4. Characteristics of human dietary fiber interventions assessing fasting markers of dyslipidemia[1]

Reference	Study design	n	Study population characteristics	Age (years)[2]	BMI (kg/m^2)[2]	Duration (wks)	Control	Fiber type	Fiber dose (g/d)[3]	Fiber properties	Effect on LDL-C	Effect on HDL-C	Effect on TC	Effect on TG	Risk of Bias
Cossack, 1991	Single arm	10	Hypercholest-erolemic	53 ± 8	*(89.9 kg)*	5	None	Sugar beet fiber	26.6	MPCW	↓[6]	↔	↓[6]	↓[6]	H
Kawatra, 1991	Single arm	20	Overweight	30 - 50	–	6	None	Guar gum	12.0	Viscous	↓[6]	↔	↓[6]	–	H
Lampe, 1991	R, crossover (1.43 wks)[4]														
	Fiber	32	Healthy	27.1 ± 2.1	22.7 ± 0.4	3	No DF	Mixed vegetable fiber	10.0	MPCW	↔	↔	↔	↓[6]	H
	Fiber	34						Mixed vegetable fiber	30.0	MPCW	↔	↔	↓[6]	↓[6]	
	Fiber	15						Sugar beet fiber	30.0	MPCW	↓[6]	↓[6]	↓[6]	↓[6]	
Spiller, 1991	Crossover (0 wks)	13	Hypercholest-erolemic	62 ± 3.0	*(70 kg)*	3	None	Guar gum	11.0	Viscous	↓[6]	↔	↓[6]	↔	H
Haskell, 1992	R, DB, parallel														
	Fiber	29	Hypercholest-erolemic	57.4 ± 10.2	*(71.6 kg)*	12	No DF	WSDF mixture	17.2	Viscous	↔	↔	↔	↔	H
	Control	29		57.4 ± 10.2	*(72.5 kg)*										
	R, DB, parallel														
	Fiber	20	Hypercholest-erolemic	56.4 ± 9.4	*(71.8 kg)*	4	No DF	Acacia gum	15.0	Nonviscous	↔	↔	↔	↔	H
	Control	20		56.4 ± 9.4	*(70.9 kg)*										
	R, crossover (0 wks)	14	Hypercholest-erolemic	52.5 ± 10.8	*(73.6 kg)*	4	None	Guar gum	10.0	Viscous	↓[6]	↓[6]	↓[6]	↔	H
					(74.0 kg)			WSDF mixture	15.0	Viscous	↓[6]	↓[6]	↓[6]	↔	
	R, DB, parallel														
	Fiber	12	Hypercholest-erolemic	56.3 ± 9.5	*(69.0 kg)*	4	No DF	WSDF mixture	5.0	Viscous	↔	↔	↔	↔	H
	Fiber	13		56.3 ± 9.5	*(72.7 kg)*			WSDF mixture	10.0	Viscous	↔	↓[5]	↔	↔	
	Fiber	12		56.3 ± 9.5	*(73.6 kg)*			WSDF mixture	15.0	Viscous	↓[7]	↔	↓[7]	↔	
	Control	11		56.3 ± 9.5	*(71.8 kg)*										
Jensen, 1993	R, DB, parallel														
	Fiber	14	Hypercholest-erolemic	56 ± 9	*(103% of IBW)*	4	None	Acacia gum	15.0	Nonviscous	↔	↔	↔	↔	U
	Fiber	15		52 ± 9	*(103% of IBW)*	4	None	WSDF mixture	15.0	Viscous	↓[6]	↔	↓[6]	↔	
Sundell, 1993	Single arm	11	Healthy	31.7 ± 6.4	*(69.0 kg)*	5/2[9]	None	Oat husk fiber	5/10[9]	MPCW	↔	↔	↔	↔	H
Braaten, 1994	R, crossover (3 wks)														
	Males	9	Hypercholest-erolemic	52 ± 2.6	26.0 ± 0.8	4	Maltodextrin	β-glucan (oat)	5.8	Viscous	↓[6,7]	↔	↓[6,7]	↓[6]	H
	Females	10		56 ± 1.3	26.3 ± 0.9										

(Continued)

Table S.3.4. *Continued*

Reference	Study design	*n*	Study population characteristics	Age (years)[2]	BMI (kg/m^2)[2]	Duration (wks)	Control	Fiber type	Fiber dose (g/d)[3]	Fiber properties	Effect on LDL-C	Effect on HDL-C	Effect on TC	Effect on TG	Risk of Bias
Sandström, 1994	R, crossover (2 wks)	11	Healthy	23	–	2	No DF	Pea fiber	20.0	MPCW	↔	↔	↔	↓[7]	H
Arvill, 1995	R, DB, crossover (2 wks)	63	Hypercholesterolemic	47 ± 8.2	*(90 kg)*	4	Corn starch	Konjac glucomannan	3.87	Viscous	↓[6]	↔	↓[6]	↓[5]	U
Goel, 1997	Single arm	10	Hypercholesterolemic	44 ± 2.9	27.9 ± 3.8	4	None	Rhubarb stalk fiber	20.0	MPCW	↓[6]	↔	↓[6]	↔	H
Brighenti, 1999	Crossover (0 wks)	12	Healthy	23.3 ± 0.5	25.7 ± 1.2	4	No DF	Inulin	9.0	Nonviscous	↔	↔	↓[6]	↓[6,7]	H
Jackson, 1999	R, DB, parallel														
	Fiber	27	Hypercholesterolemic	52.6 ± 8.6	26.5 ± 3.6	8	Maltodextrin	Inulin	10.0	Nonviscous	↔	↔	↔	↓[5]	U
	Control	27		51.9 ± 10.5	26.1 ± 2.8										
Nicolosi, 1999	Single-arm	15	Hypercholesterolemic, obese	51 ± 7	27.7 ± 5	8	None	β-glucan (yeast)	15.0	Nonviscous	↓[6]	↑[6]	↓[6]	–	H
Vidal-Quintanar, 1999	Single-arm (sub-group analysis)	12	Hypercholesterolemic	39.5 ± 8.8	28.9 ± 4.7	6	None	Lime-treated maize husks	32.0	MPCW	↓[6]	↔	↓[6]	↔	H
		11	Healthy	30.2 ± 7.6	24.6 ± 2.8			Lime-treated maize husks	32.0	MPCW	↓[6]	↔	↓[6]	↔	
Robinson, 2001	R, crossover (0 wks)														
	Fiber	20	Healthy	–	–	3	None	AG (larch)	15.0	Nonviscous	↔	↔	↔	↔	H
	Fiber							AG (larch)	30.0	Nonviscous	↔	↔	↔	↔	
Zunft, 2001	Single arm	47	Hypercholesterolemic	54.8 ± 9.9	*(72.5 kg)*	8	None	Carob fiber	15.0	MPCW	↓[6]	↔	↓[6]	↔	H
Gallaher, 2002	Single-arm	21	Overweight	28.9 ± 9.8	28.0 ± 4.6	4	None	Chitosan (50%), konjac glucomannan (50%)	2.36	Viscous	↓[6]	↓[6]	↓[6]	↔	H
Keogh, 2003	R, SB, crossover (4 wks)	18	Hypercholesterolemic	38.8 ± 10.1	27.4 ± 4.6	4	Add glucose to low DF foods	β-glucan (barley)	9.9[8]	Viscous	↔	↔	↔	↔	U
Zunft, 2003	R, DB, parallel														
	Fiber	29	Hypercholesterolemic	55 ± 10	25.4 ± 3.1	6	No DF	Carob fiber	15.0	MPCW	↓[7]	↔	↓[5]	↔	H
	Control	29		53.8 ± 12	25.8 ± 3.9										
Giacco, 2003	R, DB, crossover (0 wks)	30	Hypercholesterolemic	45.5 ± 9.9	26.6 ± 2.2	8	Maltodextrin	Short-chain FOS	10.6	Nonviscous	↔	↔	↔	↔	U

(Continued)

Table S.3.4. *Continued*

Reference	Study design	*n*	Study population characteristics	Age (years)[2]	BMI (kg/m^2)[2]	Duration (wks)	Control	Fiber type	Fiber dose (g/d)[3]	Fiber properties	Effect on LDL-C	Effect on HDL-C	Effect on TC	Effect on TG	Risk of Bias
Marett, 2004	R, DB, parallel														
	Fiber	18	Healthy	29	–	26	Rice starch	AG (larch)	8.4	Nonviscous	↔	↔	↔	↔	H
	Fiber	19						AG (tamarack)	8.4	Nonviscous	↔	↔	↔	↔	
	Control	17													
Park, 2004	R, DB, parallel														
	Fiber	12	Overweight and obese	42.3 ± 3.1	26.6 ± 0.7	3	Corn starch	RS type-III	24.0	Insoluble	↓[6]	↔	↓[6]	↔	H
	Control	13		43.6 ± 2.8	27.9 ± 0.5										
Hall, 2005	R, SB, crossover (4 wks)	38	Healthy	41.0 ± 1.9	26.7 ± 0.5	4	Low DF diet	Lupin kernel fiber	22.2[8]	MPCW	↓[6,7]	↔	↓[6,7]	↔	U
Garcia, 2006	R, SB, crossover (6 wks)	11	Overweight, obese, IGT	55.5 ± 6.2	30.1 ± 5.7	6	No DF	Arabinoxylan (wheat)	12.2	Viscous	–	–	↔	↓[5]	U
Yoshida, 2006	R, DB, crossover (4 wks)	16	Healthy	55.2 ± 6.9	27.7 ± 4.5	3	No DF	Glucomannan	10.0	Viscous	↓[7]	↔	↓[7]	↔	H
Queenan, 2007	R, DB, parallel														
	Fiber	35	Hypercholest-erolemic	44.5 ± 2.2	*(81.8 kg)*	6	Dextrose	β-glucan (oat)	6.0	Viscous	↓[6,7]	↔	↓[6]	↑[5]	H
	Control	40		45.3 ± 2.0	*(79.6 kg)*										
Ganji, 2008	Single-arm (sub-group analysis)		Hypercholest-erolemic,												
		8	Pre-meno	34.6 ± 11.5	24.2 ± 4.2	6	None	Psyllium	12.9	Viscous	↔	↔	↔	↔	H
		11	Post-meno	52.9 ± 2.8	22.6 ± 2.2			Psyllium	12.9	Viscous	↔	↓[6]	↓[6]	↔	
Pérez-Jiménez, 2008	R, parallel														
	Fiber	34	Hypercholest-erolemic and healthy	35.5 ± 11.8	26.1 ± 4.7	16	No treatment	Grape antioxidant dietary fiber	5.25	MPCW	↔	↔	↓[6]	↔	H
	Control	9		34.6 ± 12.4	22.7 ± 2.4										
Smith, 2008	R, DB, parallel														
	Fiber	45	Hypercholest-erolemic	44.1 ± 13	26.0 ± 3.2	6	None	LMW β-glucan (barley)	6.0	Nonviscous	↓[6]	↔	↔	↔	H
	Fiber	45		45.1 ± 14	26.7 ± 4.2			HMW β-glucan (barley)	6.0	Viscous	↔	↔	↔	↔	
Glover, 2009	Single arm	10	Healthy	39.7 ± 6.2	25.2 ± 2.4	12	None	Acacia gum	22.8	Nonviscous	–	–	↔	↔	H

(Continued)

Table S.3.4. *Continued*

Reference	Study design	*n*	Study population characteristics	Age (years)[2]	BMI (kg/m^2)[2]	Duration (wks)	Control	Fiber type	Fiber dose (g/d)[3]	Fiber properties	Effect on LDL-C	Effect on HDL-C	Effect on TC	Effect on TG	Risk of Bias
Maki, 2009	R, DB, parallel														
	Fiber	16	Hypercholest-erolemic	58.9 ± 3.2	28.9 ± 1.1	4	No DF	HPMC (high viscosity)	3.0	Viscous	↔	↔	↔	↔	U
	Fiber	17		55.7 ± 3.1	28.9 ± 1.3			HPMC (low viscosity)	5.0	Nonviscous	↓[6]	↓[6]	↓[6]	↔	
	Fiber	32		53.2 ± 2.8	31.2 ± 1.3			HPMC (high viscosity)	5.0	Viscous	↓[6]	↔	↓[6]	↔	
	Fiber	29		58.6 ± 1.9	28.9 ± 0.9			HPMC (low viscosity)	10.0	Nonviscous	↓[6]	↔	↓[6]	↑[6]	
	Fiber	15		56.7 ± 2.1	31.1 ± 2.1			HPMC (moderate viscosity)	10.0	Viscous	↓[6,7]	↔	↓[6,7]	↔	
	Fiber	27		55.1 ± 1.9	27.7 ± 0.9			HPMC (moderately high viscosity)	10.0	Viscous	↓[6,7]	↔	↓[6,7]	↔	
	Control	29		57.3 ± 2.5	27.4 ± 1.0										
Reppas, 2009	R, DB, parallel														
	Fiber	20	Hypercholest-erolemic	41.6	–	6	No DF	HPMC (high viscosity)	5.0	Viscous	↓[7]	↓[6]	↓[7]	↔	H
	Fiber	10						HPMC (high viscosity)	15.0	Viscous	↓[7]	↔	↓[7]	↔	
	Control	10													
Li, 2010	R, DB, parallel														
	Fiber	60	Overweight	30.4 ± 4.3	24.5 ± 0.2	12	Maltodextrin	NUTRIOSE	28.9	Nonviscous	↓[5]	↑[5]	↓[5]	↔	L
	Control	60		31.6 ± 4.1	24.5 ± 0.3										
Pouteau, 2010	R, DB, crossover (6 wks)	21	Metabolic syndrome	47 ± 12	33.4 ± 3.0	5	No DF	Acacia gum (80%), apple pectin (20%)	28.0	Viscous	↔	↔	↔	↔	U
Ruiz-Roso, 2010	R, DB, parallel														
	Fiber	43	Hypercholest-erolemic	42.9 ± 9.5	25.7 ± 3.5	4	Dextrose	Carob fiber	8.0	MPCW	↓[6,7]	↑[5]	↓[6,7]	↓[6,7]	L
	Control	45		44.1 ± 9.9	25.5 ± 3.8										
Russo, 2010	R, DB, crossover (8 wks)	15	Healthy	18.8 ± 0.7	22.8 ± 2.3	5	No DF	Inulin	11.0	Nonviscous	↔	↑[6]	↔	↓[6]	H
Solà, 2010	R, DB, parallel														
	Fiber	101	Hypercholest-erolemic	54.2 ± 9.9	26.8 ± 3.7	8	MCC	Psyllium	11.5	Viscous	↓[7]	↔	↓[7]	↓[7]	L
	Control	108		55.5 ± 11.5	27.4 ± 3.5										
Lyon, 2011	R, DB, parallel														
	Fiber (M)	15	Healthy	38.1 ± 7.2	29.8 ± 1.2	15	Inulin (low viscous control)	PGX	13.1	Viscous	↔	↔	↔	↔	U
	Fiber (F)	15		34.7 ± 10.4	30.1 ± 2.5			PGX	13.1	Viscous	↓[7]	↓[5]	↓[5]	↔	
	Control (M)	13		38.8 ± 7.1	30.0 ± 1.5										
	Control (F)	17		37.1 ± 10.8	31.7 ± 1.8										
Pal, 2011	R, SB, parallel														
	Fiber	16	Overweight and obese	41.3 ± 2.3	34.0 ± 0.9	12	Breadcrumbs	Psyllium	29.7	Viscous	↓[6,7]	↔	↓[6,7]	↔	U
	Control	15		44.8 ± 1.6	33.7 ± 1.0										

(Continued)

Table S.3.4. *Continued*

Reference	Study design	*n*	Study population characteristics	Age (years)[2]	BMI (kg/m^2)[2]	Duration (wks)	Control	Fiber type	Fiber dose (g/d)[3]	Fiber properties	Effect on LDL-C	Effect on HDL-C	Effect on TC	Effect on TG	Risk of Bias
François, 2012	R, DB, crossover (2 wks)														
	Fiber	57	Healthy	40.7 ± 2.2	23.5 ± 0.4	3	No DF	AXOS	2.9	Nonviscous	↔	↔	↔	↔	L
	Fiber							AXOS	9.5	Nonviscous	↔	↔	↔	↔	
Hashizume, 2012	R, DB, parallel														
	Fiber	15	Metabolic syndrome	60.1 ± 8.9	28.1 ± 2.3	12	No DF	Resistant maltodextrin	27.0	Nonviscous	↔	↔	↓[6]	↓[6,7]	H
	Control	15		61.2 ± 11.6	26.8 ± 2.9										
Maki, 2012	R, DB, crossover (3 wks)														
	Fiber	33	Overweight and obese	49.5 ± 1.6	30.6 ± 0.5	4	Digestible starch (Amioca)	RS type-II (Hi-maize)	15.0	Insoluble	↔	↔	↔	↔	U
	Fiber							RS type-II (Hi-maize)	30.0	Insoluble	↔	↔	↔	↔	
	Control														
de Luis, 2013	R, DB, parallel														
	Fiber	18	Obese	45.3 ± 16.1	35.9 ± 3.4	4	No DF	FOS	9.8	Nonviscous	↔	↔	↔	↔	U
	Control	18		50.8 ± 16.2	39.2 ± 7.2										
Gato, 2013	R, DB, parallel														
	Fiber	13	Hypercholesterolemic	40.6 ± 1.9	*(61.4 kg)*	12	No DF	Persimmon fiber	9.0	MPCW	↔	↔	↓[6]	↔	H
	Fiber	13		36.4 ± 1.8	*(64.4 kg)*			Persimmon fiber	15.0	MPCW	↓[6]	↔	↓[6]	↔	
	Control	14		36.6 ± 1.8	*(63.5 kg)*										
Reimer, 2013	R, DB, parallel														
	Fiber	28	Abdominal adiposity	20 - 65	26.7 ± 0.2	14	Rice flour	PGX	13.1	Viscous	↓[7]	↔	↓[7]	↔	U
	Control	28		20 - 65	27.2 ± 0.3										
Childs, 2014	R, DB, crossover (4 wks)	41	Healthy	43 ± 12	25 ± 3	3	Maltodextrin	XOS	8.0	Nonviscous	↔	↔	↔	↔	U
Fechner, 2014	R, DB, crossover (2 wks)	52	Hypercholesterolemic	46.9 ± 3.2	26.5 ± 5.9	4	No DF	Lupin fiber	21.7	MPCW	↓[6,7]	↔	↓[6,7]	↓[5]	L
								Citrus fiber	23.1	MPCW	↓[6]	↓[6]	↓[6]	↔	
Brahe, 2015	R, SB, parallel														
	Fiber	19	Obese, postmeno	60.6 ± 6.4	35.2 ± 4.5	6	No DF	Flaxseed mucilage	10.0	MPCW	↔	↔	↔	↔	U
	Control	20		58.5 ± 5.3	34.3 ± 3.8										
Tripkovic, 2015	R, SB, crossover (4 wks)	10	Overweight and obese	39.8 ± 9.6	30.2 ± 3.0	4	No DF	Inulin	15.0	Nonviscous	−	↔	↔	↔	U

(Continued)

Table S.3.4. *Continued*

Reference	Study design	*n*	Study population characteristics	Age (years)[2]	BMI (kg/m^2)[2]	Duration (wks)	Control	Fiber type	Fiber dose (g/d)[3]	Fiber properties	Effect on LDL-C	Effect on HDL-C	Effect on TC	Effect on TG	Risk of Bias
Urquiaga, 2015	R, parallel														
	Fiber	25	Metabolic syndrome	44.5 ± 9.3	29.1 ± 3.9	16	No treatment	Wine grape pomace flour	10.0	MPCW	–	↔	–	↔	U
	Control	13		43.1 ± 8.4	27.9 ± 3.5										
Dainty, 2016	R, DB, crossover (4 wks)	24	Overweight and obese	55.3 ± 1.6	30.2 ± 0.5	8	No DF	RS type-II (Hi-maize)	25.0	Insoluble	↔	↔	↔	↔	L
Kapoor, 2016	Single arm, pilot	6	Healthy	46.3 ± 2.9	25.3 ± 0.6	52	None	Partially hydrolyzed guar gum	14.4	Nonviscous	↓[6]	↑[6]	–	↔	H
Nasir, 2016	Single arm	10	Prediabetic	35 - 60	38.5 ± 2.4	16	None	Acacia gum	10.0	Nonviscous	–	–	↔	↔	H
Stenman, 2016	R, DB, parallel														
	Fiber	53	Overweight and obese	48.8 ± 10.5	31.2 ± 1.6	26	MCC	Polydextrose	10.8	Nonviscous	↔	↔	↔	↔	L
	Control	56		49.9 ± 8.5	31.2 ± 2.2										
Canfora, 2017	R, DB, parallel														
	Fiber	21	Obese, prediabetic	59.2 ± 7.2	33.3 ± 3.7	12	Maltodextrin	GOS	15.0	Nonviscous	–	–	–	↔	U
	Control	23		58.4 ± 7.3	32.3 ± 3.5										
Lambert, 2017	R, DB, parallel														
	Fiber	22	Overweight and obese	44 ± 15	33.1 ± 1.3	12	No DF	Yellow pea hull fiber	13.8	MPCW	↔	↔	↔	↔	L
	Control	22		44 ± 15	33.3 ± 1.3										
Pal, 2017	R, DB, parallel														
	Fiber	39	Overweight and obese	49.9 ± 11.0	31.7 ± 3.2	12	Rice flour	Psyllium	12.9	Viscous	↔	↔	↔	↔	L
	Fiber	43		47.9 ± 12.1	33.3 ± 4.3			PGX	13.1	Viscous	↔	↑[7]	↔	↔	
	Control	45		49.8 ± 11.8	32.0 ± 4.2										
Alfa, 2018	R, SB, parallel														
	Fiber	21	Healthy	42	*(78.4 kg)*	12	Digestible starch (Amioca)	RS type-II (Potato)	30.0	Insoluble	↔	↔	↔	↔	U
	Control	21													
Krumbeck, 2018	R, DB, parallel														
	Fiber	20	Obese	45.9 ± 9.6	36.8 ± 5.6	3	Lactose	GOS	5.0	Nonviscous	↔	↔	↔	↔	H
	Control	17		43.9 ± 8.8	34.0 ± 4.5										
Martínez-Maqueda, 2018	R, crossover (4 wks)	49	Metabolic syndrome	42.6 ± 1.6	–	6	No treatment	Wine grape pomace	8.0	MPCW	↔	↔	↔	↔	U

(Continued)

Table S.3.4. *Continued*

Reference	Study design	*n*	Study population characteristics	Age (years)[2]	BMI (kg/m^2)[2]	Duration (wks)	Control	Fiber type	Fiber dose (g/d)[3]	Fiber properties	Effect on LDL-C	Effect on HDL-C	Effect on TC	Effect on TG	Risk of Bias
Peterson, 2018	R, DB, parallel														
	Fiber	29	Overweight and obese, prediabetic	54 ± 10	35.5 ± 4.4	12	Digestible starch (Amioca)	RS type-II (Hi-maize)	45.0	Insoluble	↔	↔	↔	↔	L
	Control	30		55 ± 10	35.7 ± 5.2										
Salden, 2018	R, DB, parallel														
	Fiber	16	Overweight and obese	49 ± 17	30.2 ± 1.9	6	Maltodextrin	Arabinoxylan (wheat)	7.5	Viscous	↔	↔	↔	↔	U
	Fiber	17		47 ± 15	31.5 ± 2.2			Arabinoxylan (wheat)	15.0	Viscous	↔	↔	↔	↔	
	Control	14		49 ± 17	31.4 ± 3.1										

[1]↓, significant decrease; ↑, significant increase; ↔, no significant change; AG, arabinogalactan; AXOS, arabinoxylan-oligosaccharides; DB, double-blinded; FOS, fructooligosaccharides; GOS, galactooligosaccharides; H, high risk of bias; HDL-C, HDL cholesterol; HMW, high molecular weight; HPMC, hydroxypropyl methylcellulose; IBW, ideal body weight; IGT, impaired glucose tolerance; L, low risk of bias; LMW, low molecular weight; LDL-C, LDL cholesterol; MCC, microcrystalline cellulose; mixed vegetable fiber, fiber mixture of pea fiber (62%), soy polysaccharide (33%), and orange pectin (5%); MPCW, mixed plant cell wall DF type; No DF, the control was the same product received by the treatment group but without the dietary fiber added; pre-meno, pre-menopausal women; PGX, PolyGlycopleX, proprietary fiber mixture of konjac, sodium alginate, and xanthan gum; R, randomized; RS, resistant starch; SB, single-blinded; post-meno, post-menopausal women; TG, triglycerides; TC, total cholesterol; U, unclear risk of bias; WSDF, water soluble dietary fiber mixture of psyllium, pectin, guar gum, and locust bean gum; XOS, xylooligosaccharides.
[2]Mean age and BMI are presented as they were in the original articles (as mean ± SD, mean ± SEM, or a range). If BMI was not reported, then either weight in kilograms (kg) or percentage of ideal body weight (IBW) was presented in parentheses and italicized.
[3]Dietary fiber dose was corrected for the purity of the fiber.
[4]Washout period in weeks of crossover studies included in parentheses.
[5]Significant result when treatment arm is compared to the control arm that had the opposite effect.
[6]Significant result when compared within the treatment arm to baseline data or change over time.
[7]Significant result when treatment arm is compared to the control arm.
[8]Fiber dose was based on individual energy intake; mean of group is provided here.
[9]Study design employed an escalating fiber dose. Values separated with a "/" denotes individual escalating fiber doses and their corresponding intervention duration.

CHAPTER 4: Gut microbiota modulation with long-chain corn bran arabinoxylan in adults with overweight and obesity is linked to an individualized temporal increase in fecal propionate

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4.1 Introduction

Epidemiologic studies consistently associate dietary fiber (DF) consumption with a reduced incidence of obesity-associated pathologies[267,306]. In large-scale observational studies, whole grains and cereal-derived DFs (*e.g.* arabinoxylan [AX] and β-glucan) showed stronger associations with reduced risk of developing cardiovascular disease, type II diabetes, gastrointestinal cancers, and of all-cause mortality when compared to other DF sources[435,436]. A substantial body of animal research further consolidated the mechanisms by which DF reduces metabolic pathologies[273]. Despite these convincing associations, findings obtained from human dietary intervention trials aimed to improve metabolic risk markers by supplementing isolated DFs remain inconsistent[437], possibly due to an individualized clinical response[10,438].

Owing to their chemical structure, DFs resist digestion in the small intestine and reach the colon where they become substrates for the gut microbiota. The microbial fermentation of DF to short-chain fatty acids (SCFAs) has been implicated in the prevention of obesity-associated pathologies[61]. Propionate and butyrate are two SCFAs that are especially relevant, as they have been linked to beneficial immunological and metabolic effects[31]. Intervention studies with AX isolated from wheat endosperm, for instance, have demonstrated increased fecal concentrations of both butyrate and propionate[370]. DFs can further modulate gut microbiota composition in a structure-dependent way through the enrichment of bacterial taxa that utilize

the substrate and tolerate or benefit from the environmental changes caused by DF fermentation[273,439]. For example, dietary interventions with short-chain fractions of AX resulted in an enriched abundance of bacterial species that can either utilize AX oligosaccharides (AXOS) directly (*e.g. Bifidobacterium adolescentis* and *Bifidobacterium longum*) or benefit from metabolic by-products released during AXOS degradation (*e.g. Anaerobutyricum hallii* and *Faecalibacterium prausnitzii*)[440]. Although DF-induced alterations to the gut microbiota are significant, the effects are also highly individualized[438], and this variability might have clinical ramifications that could explain the individualized clinical responses[266].

To understand the individualized response of the gut microbiota to DF, an ecological perspective is required, as DF fermentation is determined by complex inter-species interactions between members of the gut microbiota[441]. The process is often based on a cross-feeding cascade, where primary degraders that access the DF provide break-down products (oligosaccharides, disaccharides, and monosaccharides) to other microbes, and metabolites that result from the fermentation of these products also serve as substrates[442]. Inter-individual variation in gut microbiota composition may result from the absence of 'keystone species' that initiate the degradation of recalcitrant DFs[430], differences in unrelated species with similar ecological functions that compete for the same substrate[105], or variation in strains of the same species that differ in their capacity to metabolize the substrate[443]. These compositional variations likely determine both the competitive and co-operative relationships between community members that form trophic networks, some of which organize into ecological 'guilds' that collaborate to degrade complex DFs[280]. Although inter-individual variation in the response of the gut microbiota to DF can influence metabolite outputs relevant to health (*i.e.* propionate or butyrate)[300], this topic, and the underlying ecological principles, have received little attention.

The objective of **Chapter 4** was to apply an ecological framework to characterize the compositional and metabolic responses of the human gut microbiota to a long-chain AX isolated from corn bran compared to a DF that is not fermented by the gut microbiota (microcrystalline cellulose, MCC). We further assessed whether nutritional and microbiota-related factors could explain the variable responses observed among individuals.

4.2 Methods

4.2.1 Subjects

Male and pre-menopausal, non-pregnant or lactating female subjects aged 19 to 50 years with overweight or class-I obesity (body mass index [BMI] 25.0 to 34.9 kg/m^2) and a stable body weight (±3% for ≥1 month) who were otherwise healthy 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 gastrointestinal disorders or surgeries; (2) history of diabetes mellitus; (3) chronic use of anti-hypertensive, lipid-lowering, anti-diabetic, anti-inflammatory, or laxative medications; (4) antibiotic use three months prior to 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.

4.2.2 Study Design

This six-week, parallel two-arm, exploratory randomized controlled trial (RCT) was prospectively registered on July 3, 2015 with ClinicalTrials.gov (NCT02322112) as part of a large parallel four-arm RCT that aimed to compare the effects of four structurally-distinct DFs (*i.e.* AX, acacia gum, resistant starch type-IV, and MCC) on the gut microbiota and human health, referred to as The Alberta FYBER (Feed Your gut Bacteria morE fibeR) Study (for original registration we refer to[444]). In response to requests by reviewers of a grant application, which advised against including a premarket DF ingredient in a larger human trial, the AX arm was separated from the original RCT on October 26, 2016 and data from the 15 subjects that completed the protocol were analyzed independently. All procedures involving human subjects and the separation of the arabinoxylan arm from the original RCT were approved by the Health Research Ethics Board of the University of Alberta (Approval Number: Pro00050274). Written informed consent was obtained from all study subjects prior to enrollment into the study. 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.

The study included five clinic visits (**Figure 4.1**). 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 completed 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 arm 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.

Thirty-eight subjects were enrolled in the study and instructed to consume their corresponding supplement for six weeks at a daily 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 for females and 17.5 g for males), as this was shown by pilot data to ease diet incorporation. After one week of treatment, subjects returned to provide a second fecal sample and to assess protocol adherence (visit 3), which was also assessed during their third week of treatment (visit 4). A final visit was required at endpoint (six weeks of treatment) to provide the third and final fecal sample and to assess overall protocol adherence (visit 5).

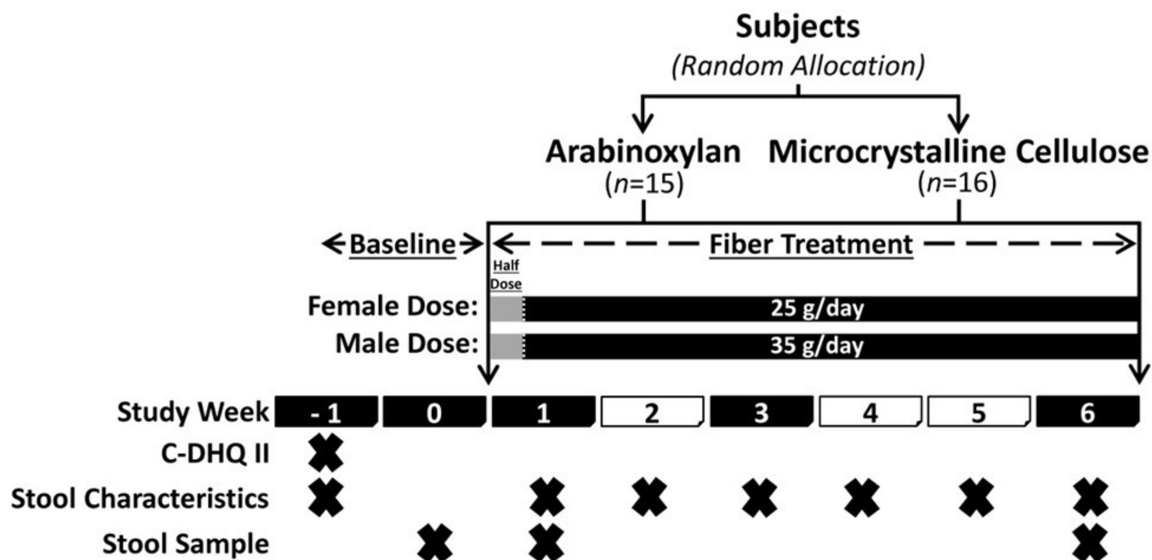

Figure 4.1. Study design. Shaded study week blocks indicate a scheduled clinic visit. The 'X' indicates the task was completed during the study week. C-DHQ II, Canadian diet history questionnaire II; stool characteristics, self-reported stool consistency and bowel movement frequency.

4.2.3 Treatments

The AX used in this study was BIO-FIBER GUM and was provided by Agrifiber Holdings LLC (Illinois, USA) as a single batch. The long-chain AX is an alkali-extract, soluble AX isolated from corn bran that contained 81.0 ± 1.3% AX. The AX was further analyzed for its monosaccharide composition by their trimethylsilyl derivatives using gas chromatography coupled with mass spectroscopy (models 7890A and 5975C inert MSD with a Triple Axis detector, Agilent Technologies Inc., California, USA) as previously described[445]. The results showed that the corn bran AX composes of 57.8% xylose and 32.5% arabinose (weight basis). As the backbone of AX is comprised of linear xylose with arabinose forming branching points, the arabinose-to-xylose ratio is often used to estimate AX branching density. The AX used here had an arabinose-to-xylose ratio of 0.56, which is similar to that reported for alkali extracted corn AXs[446-448]. The AX further contained 9.7% galactose, which is likely present in side chains as described for other corn AXs[447,448]. The relatively high arabinose-to-xylose ratio and abundant galactose collectively suggest that the corn bran AX is heavily branched with complex side chains, like the ones previously reported by Saulnier *et al.*,[448] Rose *et al.*,[446] and Rumpagaporn *et al.*[447]. The MCC used in this study was MICROCEL MC-12 and was provided by Blanver Farmoquimica LTDA (São Paulo, Brazil). The MCC is a large particle size (160-micron average), wood-derived cellulose DF processed with a dilute-acid to remove amorphous regions leaving only recalcitrant crystalline regions. The MCC was subjected to *in vitro* fecal fermentations to confirm resistance to microbial fermentation and, therefore, selected as a non-fermentable control.

Both DFs were administered as powdered supplements and incorporated daily into the subjects' preferred foods and drinks. The treatments were not identical in their appearance or physicochemical properties and, therefore, double-blinding was not possible. To achieve single-blinding, however, subjects were not informed of their DF treatment, and weekly doses were provided in sealed opaque bags that contained individually packaged, ready-to-use DF sachets. Subjects were instructed to return all provided sachets at their scheduled visits, where remaining DF was weighed to assess protocol adherence.

4.2.4 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 Canadian Diet History

Questionnaire II Past Month (C-DHQ II), a food frequency questionnaire adapted for the Canadian population from the validated US-DHQ II[449]. 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 2007 Food Guide serving-size-equivalents[450]. Prior to statistical analyses, C-DHQ II extracted data were assessed for extreme outliers using methods described by Kipnis and colleagues[451], and then calorie-adjusted using methods described by Willett and Stampfer[452].

Anthropometric measurements were also obtained at baseline and W6. 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 the National Institutes of Health guidelines. Body fat percentage was estimated by bioelectrical impedance analysis (Tanita TBF-300A Body Composition Analyzer, Illinois, USA) using a proprietary equation.

4.2.5 Assessment of Stool Consistency and Bowel Movement Frequency

Self-reported stool consistency and bowel movement frequency were obtained at baseline and then at the end of each intervention week using a 5-point hedonic scale. For consistency, the scale was anchored by "hard or fragmented" (0) and "runny or watery" (4) with a score of two indicating normal or "smooth, soft, and formed" stool. For frequency, the scale was anchored by "every third day or less often" (0) and "three times a day or more often" (4) with a score of two indicating "once a day". The area under the curve (AUC_{BL-W6}) was then calculated using the linear trapezoidal method.

4.2.6 Fecal Sample Collection and Processing

Fecal samples were collected at baseline, W1, and 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 four hours of defecation. Upon receipt, fecal samples were processed immediately in an anaerobic chamber (Bactron™, Shel Lab, Oregon, USA) with an environment consisting of 5% H_2, 5% CO_2, and 90% N_2. Raw fecal material was aliquoted for pH and moisture content measurements, and also diluted 1:10 in molecular grade phosphate-buffered saline for DNA extractions and 1:5 5% phosphoric acid for SCFA quantification. Aliquots were stored at -80°C and kept frozen until further processing.

4.2.7 Fecal pH, SCFA, and Moisture Content Quantification

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[69,453]. Quantification of fecal SCFAs was completed at the Agricultural, Food and Nutritional Science Chromatography Facility of the University of Alberta as previously described[454]. 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 relative percentage of each SCFA was determined by dividing these individual SCFAs by total SCFAs. Total branched short-chain fatty acid 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.

4.2.8 DNA Extraction, 16S Ribosomal RNA (rRNA) Gene Amplicons 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[455]. The V5-V6 regions of the 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[455]. Samples exceeding 16,000 reads were subsampled to 16,000 using USEARCH v8.1[456]. Removal of chimeric reads and clustering of operational taxonomic units (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[457]. OTUs were assigned taxonomy by using the Silva database (release 132[458]), and sequence identity at species level was confirmed using 16S rRNA gene databases on EzBioCloud[459], IMG/MER[460], and NCBI[461] platforms.

Prior to ordination and statistical analysis, OTU count data were converted into relative abundance and also centered log-ratio (CLR) transformed to correct for compositionality[462]. 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 downstream analyses, accounting for 88.1% of the approximately 1 million-curated reads. 16S rRNA gene amplicons sequencing data have been deposited in the NCBI Sequence Read Archive and are available for download under BioProject PRJNA564636.

4.2.9 Statistical Analysis

All univariate analyses were performed by GraphPad Prism (v8.0.1; www.graphpad.com), while multivariate and regression model analyses were performed using R (v3.5.3; www.r-project.org) unless otherwise stated. R scripts used in this study are available at GitHub (https://github.com/BioKhoi/Arabinoxylan_study_Microbiome_Journal). The statistical analyses conducted are discussed in detail in the sections below.

4.2.9.1 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[463] and ggplot2[464] packages). Differences in the communities of AX and MCC groups at specific time points were compared by permutational multivariate analysis of variance (PERMANOVA) using the Adonis function in vegan[463]. 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) dissimilarities. Differences in inter-subject diversity were determined within each treatment group relative to baseline using generalized estimated equation (GEE) models (geepack package[465]) followed by Bonferroni correction. Differences of intra-subject dissimilarity between AX and MCC 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[463].

4.2.9.2 Fecal Microbiome Composition and SCFA Analyses

Community membership of individual taxa was presented as relative abundance (mean ± SD), while CLR-transformed data were used for statistical analysis. Comparisons of phyla, families, genera, OTUs, and SCFAs between baseline and W6 were performed by Wilcoxon tests, while comparisons of shifts (*i.e.* ΔW6-baseline) between AX and MCC were performed by Mann-Whitney tests. P values were adjusted by Benjamini-Hochberg's false discovery rate (FDR) and considered statistically significant when q values were less than 0.15. Differences at W1 and W6 in the effects of DF on OTUs and SCFAs were determined using Friedman's test followed by Dunn's correction for multiple comparisons.

4.2.9.3 Co-Abundance Response Group (CARG) and Network Analyses

Potential syntrophic interactions between bacterial taxa in their response to AX were assessed using co-occurrence network analysis[466]. To determine groups of interacting OTUs in their response to AX (thus potential ecological guilds)[280], CARGs were determined from the top OTUs impacted by AX consumption (ΔW6-baseline unadjusted $p<0.1$; Wilcoxon test). Spearman's correlation analysis was performed between the CLR-transformed shifts (ΔW6-baseline) in these OTUs to construct a correlation matrix using Spearman's correlation coefficients, which was then converted into a distance matrix by (1 – correlation coefficients)[466]. Next, hierarchical clustering was performed on the distance matrix to build a tree using the complete-linkage clustering algorithm (ComplexHeatmap package[467]) where branch lengths reflect the degree of association between OTUs (*i.e.* shorter branches indicate that OTU responses to AX were more similar among individuals). Differences between distinct clusters of the Hierarchical tree, and thus individual CARGs, were determined by PERMANOVA using a cut-off of $p<0.05$[466]. In summary, OTUs within each CARG were observed to respond more similarly to AX when compared to OTUs within another CARG, and these responses showed significant clustering, which suggests enhanced co-operative relationships between taxa of the same CARG during AX degradation. 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, a Spearman's correlation network was calculated based on shifts in CLR-transformed abundance using

permutation tests (1000x) by CoNet[468] as previously described[469]. To focus on the most robust interactions, only OTUs with Spearman's rho values ≥0.5 or ≤-0.5 and FDR corrected $q<0.05$ were visualized in the network using Cytoscape (v3.61; www.cytoscape.org).

4.2.9.4 Differences in Bacterial Community Composition and Diet between W6-Propionate Responders and Nonresponders

To identify factors that contribute to the variation between W6-propionate responders and nonresponders, 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 microbiota and diet were visualized on principal component analysis (PCA) biplots using factoextra[470] and FactoMineR[471] packages.

4.2.9.5 Relationships between Bacterial Community and SCFA Responses with Microbiota and Diet Features

To explain the individualized response of the fecal microbiota to DF, multiple linear regression (MLR) analyses were employed using R. In order to perform the analysis, dimensionality of the microbiota and diet data were reduced by PCA into principal component 1 (PC1), PC2, and PC3, which represents the largest proportion of the inter-individual variability and captures the most information on microbiota and dietary variation. Microbiota 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[472]). Therefore, each MLR model presented only contained the top one or two predictors that explained the response variable the best. Dietary and microbiota-related predictors were treated separately in different models, and total grains, whole grains, and total DF intake were used as single dietary predictors. All models were adjusted by DF dose/sex 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, corrected Akaike information criterion (AICc) values were calculated using the AICcmodavg package[473]. AICc values were then converted to relative percentages by assigning the highest AICc value as 100%, and then remaining AICc values were calculated by $\frac{\text{AICc value}}{\text{Highest AICc value}} \times 100$. Thus, lower AICc values indicate higher quality models. Residuals for all linear regression models were plotted to check for homogeneity of variance and normality.

4.3 Results

4.3.1 Subject Characteristics and Protocol Adherence

To compare the effects of AX and MCC, we conducted a six-week, parallel two-arm, exploratory, randomized controlled trial in individuals with overweight and class-I obesity, where females received 25 g/d and males 35 g/d of either DF (**Figure 4.1**). Of the 38 subjects enrolled and randomized to an intervention arm, seven withdrew from the study (in the AX group, three experienced challenges consuming the supplement and one reported constipation; in the MCC group, two withdrew due to personal reasons and one due to constipation) and were, therefore, excluded from analyses (**Figure S.4.1**). Subjects that completed the study protocol (n=31) included 21 females and 10 males, aged 32.9 ± 8.5 years with a BMI of 28.7 ± 2.3 kg/m^2. No differences in age, sex, or BMI were detected between the intervention groups at baseline (**Table S.4.1**). Overall, protocol adherence, 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.

4.3.2 Effect on the Composition of the Fecal Microbiota

4.3.2.1 Fecal Microbiota Diversity

Non-metric multidimensional scaling 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 4.2.A**). 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 DF intervention (p=0.019). These changes occurred by AX inducing temporal shifts in fecal microbiota composition, determined as the average β-diversity between the individual's treatment and baseline samples, which were significantly larger when compared to the MCC group ($p \leq 0.015$ Mann–Whitney test; **Figure 4.2.B**). In addition, while MCC increased inter-individual differences (β-diversity between subjects; $p<0.001$, GEE model), AX reduced it (p=0.003, **Figure 4.2.C**).

Analysis of α-diversity showed that AX reduced fecal bacterial diversity (Shannon's index) (p=0.036, GEE model; **Figure 4.2.D**) but not richness (total OTUs) after six weeks of supplementation. Overall, these findings showed that while the non-fermentable MCC had no detectable effects on measures of bacterial diversity, AX altered the global bacterial community

within one week, inducing temporal shifts in composition and a reduction of both inter-individual variation and α-diversity.

4.3.2.2 Effect on the Relative Abundance of Bacterial Taxa and CARGs

Neither AX nor MCC altered microbiota composition at the phylum level. At lower taxonomic levels, changes in the relative abundance of two bacterial families were detected at six weeks of AX relative to baseline and MCC, namely an increase in *Bifidobacteriaceae* (q=0.04, Wilcoxon test; **Figure 4.2.E**, **Table S.4.2**) and a decrease in *Erysipelotrichaceae* (q=0.004). At the genus level, AX increased the genera *Bifidobacterium* and *Prevotella* when compared to both baseline and MCC, and enriched *Blautia* when compared to MCC. OTU level analysis revealed that 15 OTUs changed during AX treatment relative to baseline (henceforth referred to as 'significant OTUs'). In particular, 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* sp. (OTU11), *Clostridium leptum* (OTU46), *Mollicutes* (OTU32), and *Muribaculaceae* (OTU79) (q<0.15) became enriched, while OTUs related to *Ruminococcus bromii* (OTU5), *Eubacterium oxidoreducens* (OTU41), *Bacteroides uniformis* (OTU7), and *Faecalibacillus* spp. (OTU21) declined in relative abundance. Supplementation with MCC only increased the family *Lachnospiraceae* and the genus *Parasutterella* (q=0.117). Numerically, the dominant compositional effects of AX were, to a large degree, specific to *B. longum* (OTU4) and *P. copri* (OTU6), as these taxa increased in relative abundance by an average of 3.5% (46-fold) and 2.7% (4-fold), while other OTUs increased by ≤1.1%.

In an attempt to identify groups of co-operating species that could function as ecological guilds in the degradation of AX, we adapted a clustering approach conceptually similar to that described by Tong *et al*.[466]. Instead of absolute proportions of taxa, we used AX-induced shifts to identify clusters of species whose responses were inter-correlated. This analysis revealed a total of seven CARGs (**Figure 4.3.A**), five of which showed statistically significant responses to AX, while none responded to MCC (**Table S.4.2**). The CARG that showed the largest increase in relative abundance was CARG1 (p=0.0003, Wilcoxon test), which consisted of six out of the eleven OTUs that increased through AX (**Figure 4.3.B**). Among those six OTUs, *B. longum* (OTU4) exhibited the largest shift and showed significant connections to all but one member of CARG1 (r_s>0.5, q<0.05; Spearman's correlations using permutation tests), suggesting AX may

be degraded through co-operative interactions between these taxa. In CARG6, *P. copri* (OTU6) exhibited the largest response, but only showed one strong connection with another member of the CARG, *Bacteroides massiliensis* (OTU98; r_s=0.71, *q*=0.007), which suggests *P. copri* might act to a larger degree independently to degrade AX (**Figure 4.3.B**). The majority of taxa that decreased during AX consumption, particularly *B. uniformis* (OTU7), clustered within CARG7 and showed negative correlations with taxa of CARG1, CARG2, and CARG6, suggesting competitive or antagonistic interactions.

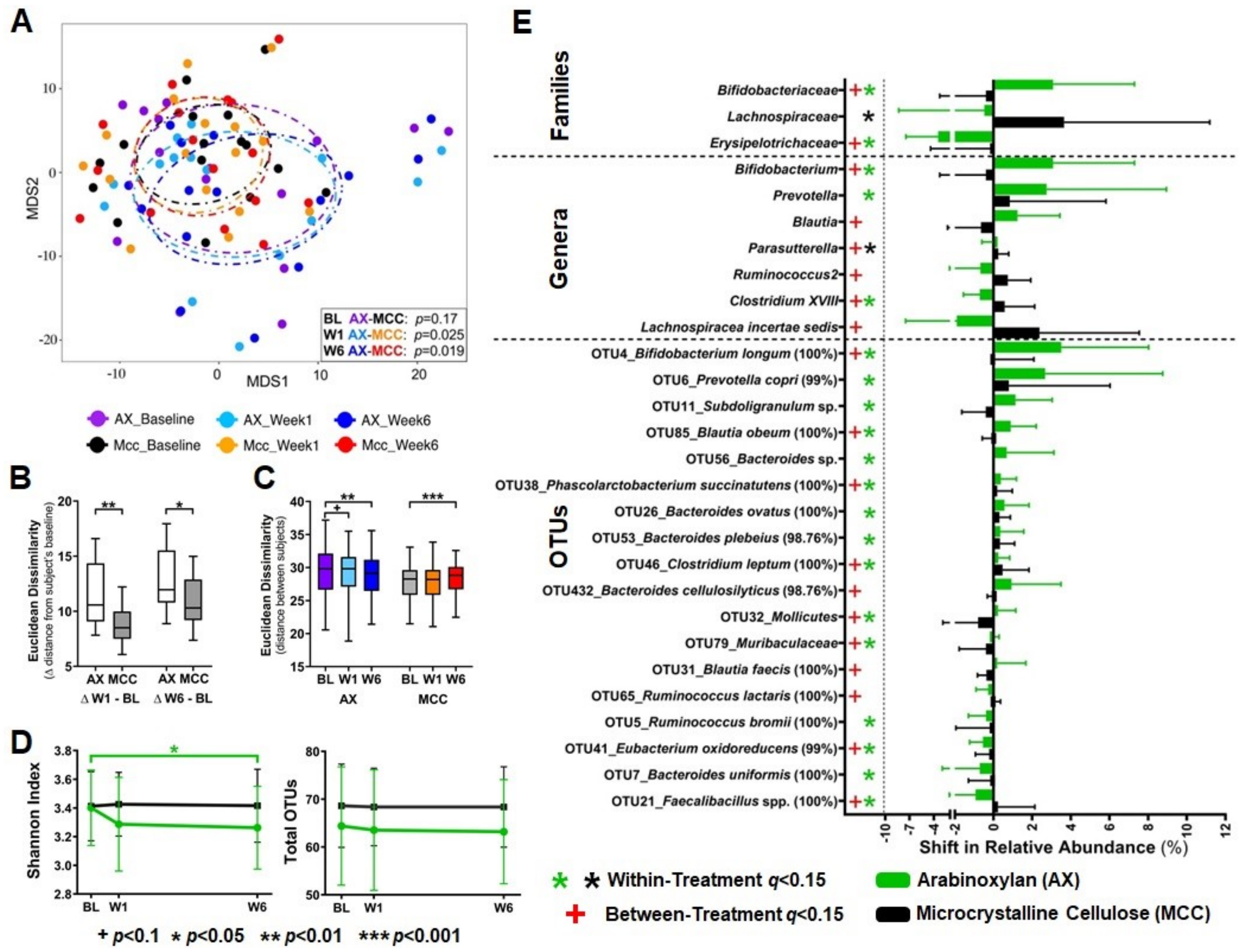

Figure 4.2. AX alters the global composition of fecal bacterial communities and induces distinct shifts in taxa. (A) Non-metric multidimensional scaling (NMDS) plot based on Euclidean distance metrics of AX and MCC groups at each time point (inter-subject β-diversity) showing changes in the distance between subjects over time. Euclidean distances **(B)** between fecal microbiotas of subjects at each study time point (inter-subject) and **(C)** between each subject's fecal microbiota at baseline and during W1 and W6 of treatment (intra-subject). **(D)** α-diversity (displayed as Shannon index and total OTUs) of the fecal microbiotas of subjects at each time point. **(E)** Absolute change (ΔW6–BL) in relative abundance of bacterial taxa affected

by the dietary intervention. Data analyzed using PERMANOVA for (A), GEE models (with Bonferroni correction) for **(B)** and **(D)**, and Mann-Whitney tests for **(C)**. For **(E)**, data were analyzed using either Wilcoxon tests to assess within-group changes relative to baseline, 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. AX, arabinoxylan; BL, baseline; MCC, microcrystalline cellulose; OTU, operational taxonomic unit; W1, week 1; W6, week 6.

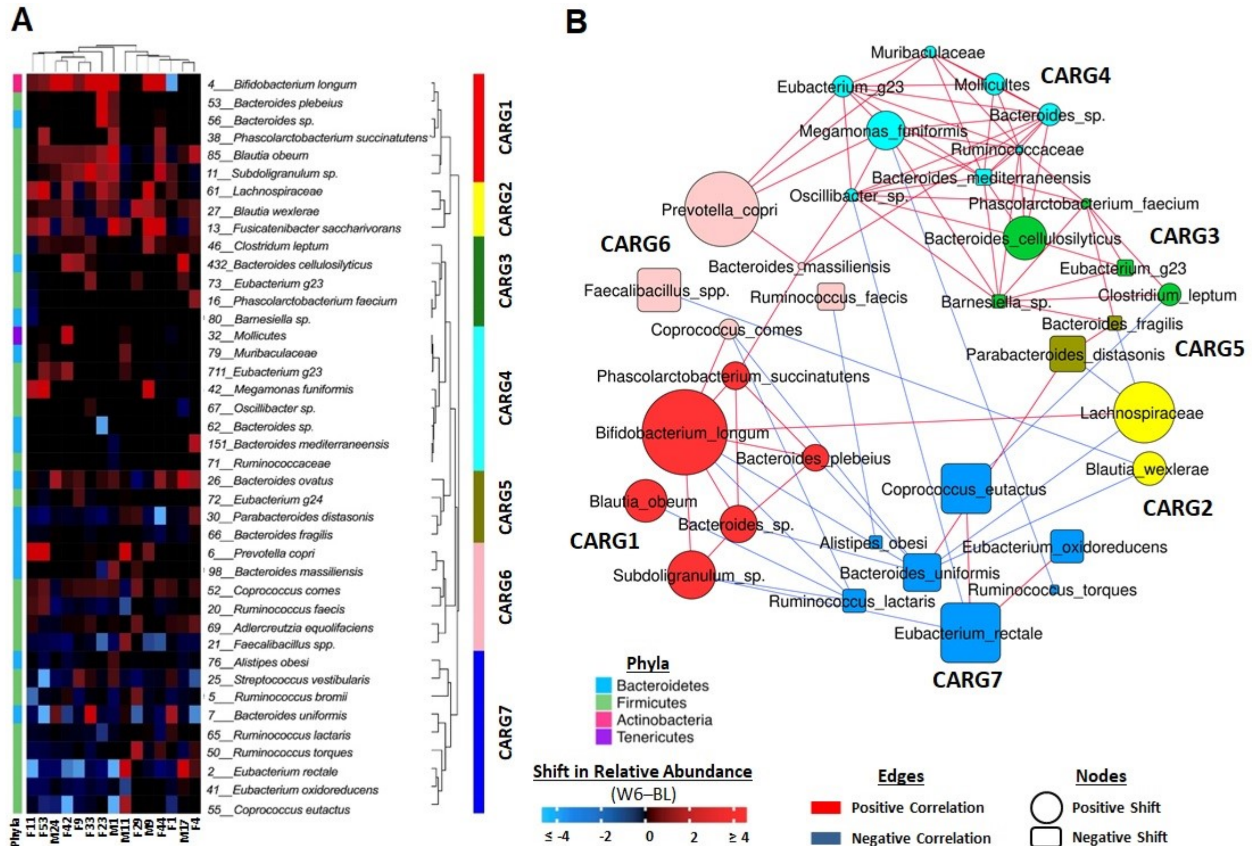

Figure 4.3. Identification of co-abundance response groups (CARGs) during AX supplementation. (A) Heatmap shows the change (ΔW6–BL) in relative abundance of 41 OTUs affected by AX (p<0.1, Wilcoxon test). The hierarchical dendrogram shows clustering of centered log-ratio (CLR) transformed OTUs (rows) 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 the direction of change (positive: circle; negative: square), and the color references the respective CARG to which it was clustered. Lines between nodes represent significant positive (red line) or negative (blue line) Spearman's correlations (r_s values ≥0.5 or ≤-0.5 and q values <0.05). AX, arabinoxylan; BL, baseline; OTU, operational taxonomic unit; W6, week 6.

4.3.2.3 Temporal Response of OTUs and CARGs

To determine if short- and long-term treatment with AX and MCC differed in their effects on the fecal microbiota, we compared shifts from baseline to week 1 (W1) with those from baseline to week 6 (W6); however, there were no detectable differences between the two time frames (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 detectable changes at six weeks (**Figure 4.4.A**). Considering these findings, analyses on compositional changes were performed with W6 data unless otherwise stated.

4.3.2.4 Inter-Individual Variation in Responses to AX

Bacterial shifts in response to AX and their magnitude were highly individualized (**Figure 4.4.B**). For instance, absolute increases in relative abundance ranging from 5% to 13% (2 to 429-fold change) were detected in seven subjects for the OTU classified as *B. longum* (OTU4), while other subjects showed either a much smaller increase, a decrease, or the species was undetectable. OTUs related to *B. obeum* (OTU85), *Subdoligranulum* sp. (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) responded in only four subjects, but effects were large, with the species expanding beyond 10% (2 to 7-fold change) of the total bacterial community in three subjects.

To determine drivers of these individualized responses, we used MLR analyses to test if responses in OTUs that showed, numerically, the largest shifts (*P. copri, B. longum, B. obeum,* and *Subdoligranulum* sp.) and in CARGs with significant responses (CARGs 1, 2, 3, 6, and 7) could be predicted by baseline diet or microbiota composition. Baseline microbiota (all OTUs and significant OTUs) and diet variables were first reduced in their dimensionality by PCA and then treated as predictors. This analysis revealed that individualized responses of bacterial taxa and CARGs to AX and MCC could not be predicted by baseline diet or microbiota composition (q>0.05; **Figure S.4.2**).

4.3.3 Effect on Stool Characteristics and Bowel Movements

While fecal moisture content was not changed by either DF (q>0.2, Wilcoxon test; **Table S.4.3**), subjects consuming AX reported softer stool consistencies when compared to subjects

consuming MCC (treatment effect p=0.049, GEE model; **Figure S.4.3.A**). Both AX and MCC led to an increase in bowel movements relative to baseline (p<0.05, GEE model; **Figure S.4.3.B**), with no difference detected between treatment groups (treatment effect p=0.8).

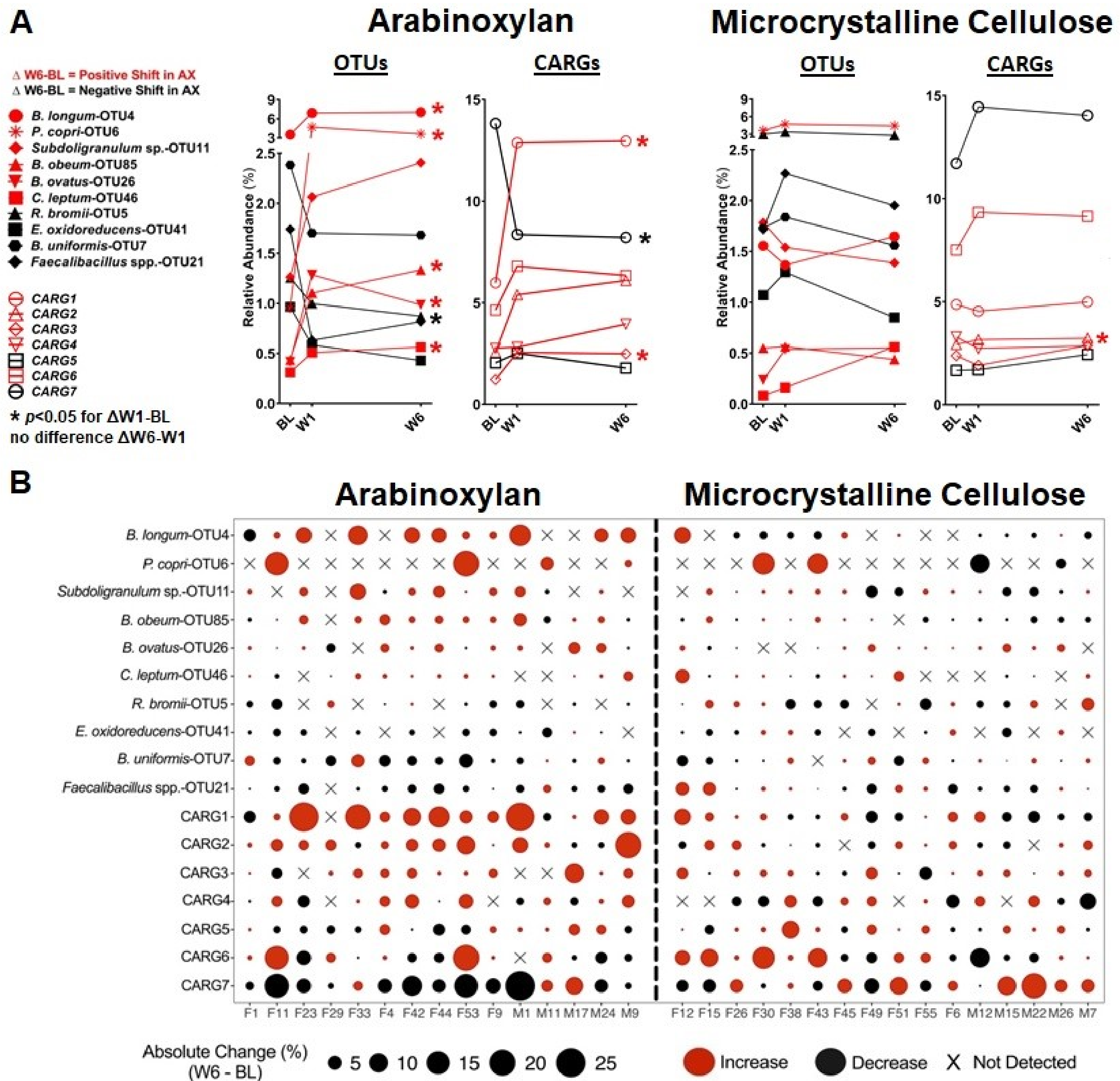

Figure 4.4. Temporal and individualized responses of the OTUs and CARGs affected by AX and MCC. (A) Plots show the temporal response of the ten most abundant OTUs (detected in >25% of subjects) and the seven CARGs. Centered log-ratio transformed data were analyzed by Friedman's test (with Dunn's correction) to assess within-group changes between time points (*i.e.* ΔW1–BL and ΔW6–W1). **(B)** Bubble plot shows individualized differences (ΔW6–BL) in relative proportions of the ten most abundant OTUs (percentage of total microbiota composition) and CARGs (sum of OTUs) detected after six weeks of AX and MCC supplementation. The size

of the bubble is proportional to the change in abundance relative to baseline, while the color of the bubble represents the direction of the change (red: increase; black: decrease). The 'X' indicates that the OTU was either undetected or the change was <0.02% relative abundance. AX, arabinoxylan; BL, baseline; CARG, co-abundance response group; MCC, microcrystalline cellulose; OTU, operational taxonomic unit; W1, week 1; W6, week 6.

4.3.3 Effect on Fecal pH and SCFAs

Fecal pH and SCFA concentrations did not change after six weeks of either DF treatment ($q>0.2$, Wilcoxon test; **Table S.4.3**). Considering that absolute concentrations of fecal SCFAs are affected by their absorption in the gut[474], we additionally assessed changes in the percentages of acetate, propionate, and butyrate relative to total SCFA concentrations at W6, which has been previously shown to vary little across colonic regions[114]. This analysis revealed an increase in the percentage of propionate produced through AX when compared to MCC ($q=0.07$, Mann–Whitney test) and a reduction in the percentage of butyrate relative to baseline ($q=0.13$, Wilcoxon test), although differences in butyrate were not detected when compared to MCC ($q=0.31$). Further investigation of the ratio between propionate and butyrate showed an increase in propionate relative to butyrate when compared to baseline ($q=0.06$) and MCC ($q=0.07$), suggesting AX supplementation directed the output of SCFAs in favor of propionate.

Characterization of the temporal response in the three primary SCFAs also showed an increase in fecal propionate concentrations by AX at W1 ($p=0.01$, Friedman's test) (**Figure 4.5.A**). Although propionate concentrations remained elevated at W6, this increase was not statistically significant when compared to baseline ($p=0.15$). This loss of significance was caused by an increase in the inter-individual variation at W6 (**Figure 4.5.B**). Visual evaluation of the individualized temporal response of propionate to AX revealed clear separation of subjects into two distinct patterns (**Figure 4.5.B**). 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 'W6-nonresponders' (Δ W6-W1<0). In general, W6-responders showed a higher output of propionate at W6 ($p=0.0045$, Friedman's test) but not at W1, while the opposite is seen in W6-nonresponders ($p=0.014$). The two groups differed by propionate concentrations at W6 ($p=0.012$, Mann–Whitney test).

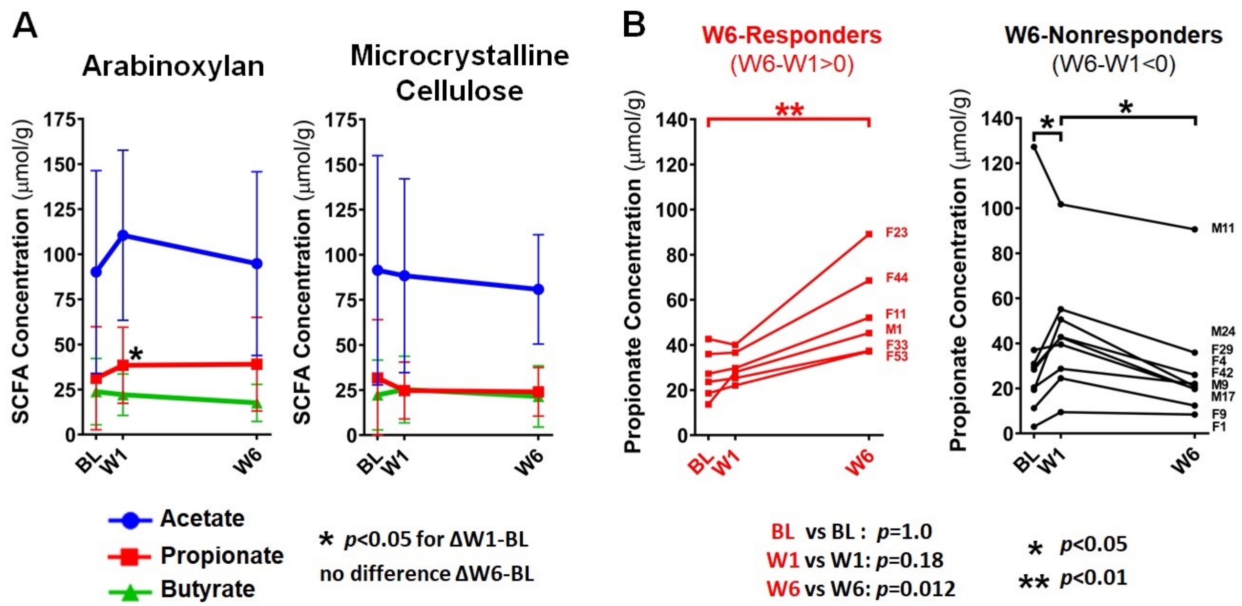

Figure 4.5. Temporal and individualized output of fecal SCFAs in response to AX and MCC supplementation. (A) Line plots show the temporal response of acetate, propionate, and butyrate; reported as mean ± SD. **(B)** Individualized temporal propionate response of W6-responders (red) and W6-nonresponder (black) (grouped based on ΔW6–W1). Data analyzed for **(A and B)** using Friedman's 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. AX, arabinoxylan; BL, baseline; CARG, co-abundance response group; MCC, microcrystalline cellulose; OTU, operational taxonomic unit; SCFA, short-chain fatty acid; W1, week 1; W6, week 6.

4.3.4 W6-Propionate Responders and Nonresponders Differ in their Microbiota Response to AX

Microbiota compositional (baseline and shifts) and diet data were ordinated using PCA, and then differences between W6-propionate responders and nonresponders were tested using PERMANOVA. This analysis revealed that the bacterial communities of W6-responders were indistinguishable from W6-nonresponders at baseline but differed in their response to AX (ΔW6-baseline; **Figure 4.6**). This was detected if the analysis was based on all OTUs (p=0.004), the 15 significant diet-responsive OTUs (p=0.025), or the seven CARGs (p=0.025). In contrast, neither baseline microbiota composition (**Figure 4.6)** nor dietary factors (**Figure S.4.4.A**) separated according to W6 response (p>0.1). In addition, comparing W6-responders and W6-nonresponders in terms of their baseline total grain, whole grain, and total DF consumption or their stool consistency and bowel movement frequency during treatment did not reveal any differences either (p>0.1, Mann–Whitney test) (**Figures S.4.3.C and S.4.4.B**). Together, these

findings indicate that the temporal response in fecal propionate concentrations is primarily associated with the shifts in the microbiota and not baseline microbiota composition or diet.

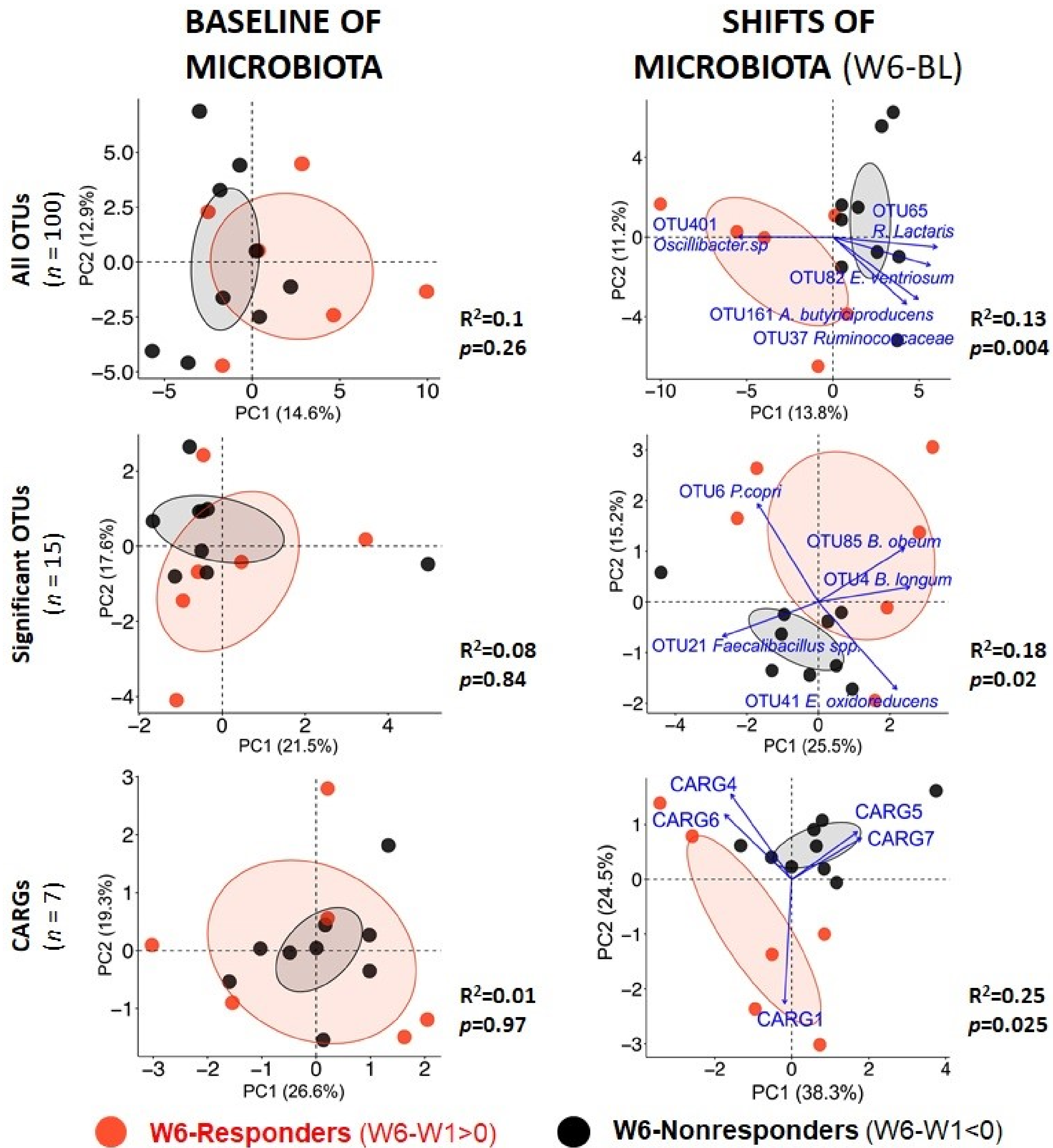

Figure 4.6. The individualized temporal propionate response to AX associates with compositional responses in the fecal microbiota. Principal component analysis plots based on Euclidean distance comparing the relative abundance of fecal microbiota, both at baseline and AX-induced shifts (ΔW6–baseline), between W6-responders (red) and W6-nonresponders (black). Microbiota variables (*i.e.* OTU or CARG) that contributed the most to inter-subject variation were shown as vectors on the plot when statistical significances were determined by PERMANOVA ($p<0.05$). AX, arabinoxylan; CARG, co-abundance response group; OTU, operational taxonomic unit; W1, week 1; W6, week 6.

4.3.5 Individualized SCFA Responses can be Predicted by Gut Microbiota Features

As with compositional responses, gut microbiota functional responses to DF interventions have been shown to be individualized[67,438,475], but what drives this variation is poorly understood. We applied MLR to determine whether fecal SCFA responses could be explained by stool consistency and bowel movement frequency, diet, or microbiota related factors, and then compared the quality of the models using AICc values (where lower values mean higher quality). These analyses revealed that the W6 SCFA response to AX could be predicted by the fecal microbiota (**Figures 4.7 and S.4.5**) but not by baseline diet, stool consistency, or bowel movement frequency reported during treatment (**Figures S.4.6.A and S.4.6.B**). The best models were achieved for propionate, especially when PCs generated from W6 shifts of all OTUs were used as predictors (**Figure 4.7.A, Table S.4.4**). Models were of lower quality when W6 shifts of significant OTUs, CARGs, PCs of CARGs, or single OTUs were used, suggesting that global community measures exhibited stronger linear relationships with the propionate response than single or groups of taxa. Although the models that used baseline and W1 shifts of OTUs as predictors were of lower quality than those based on W6 shifts, they are still valid, showing *q* values less than 0.05 after FDR correction. Linear relationships between propionate responses and significant predictors using baseline (PC1 of all OTUs) and shifts (CARG1) were further visualized using scatter plots (**Figure 4.7.B**), reaffirming the quality of the analysis, as a majority of subjects fall within the 95% confidence regions.

Significant models could also be designed for acetate and butyrate responses to AX (**Figure S.4.5**). Interestingly, in contrast to propionate, the best models to predict butyrate responses were achieved using shifts of a single OTU, *E. oxidoreducens* (OTU41), a known butyrate producer[476]. However, overall, the models for acetate and butyrate were of much lower quality than those for propionate. In summary, while individualized responses in SCFAs showed no association with diet, they could be predicted by microbiota shifts and baseline composition. In contrast to the analysis of the effects of AX, not one single MLR model was found to be significant for MCC, indicating that the statistical approach based on MLR models did not detect any associations independent of DF fermentation.

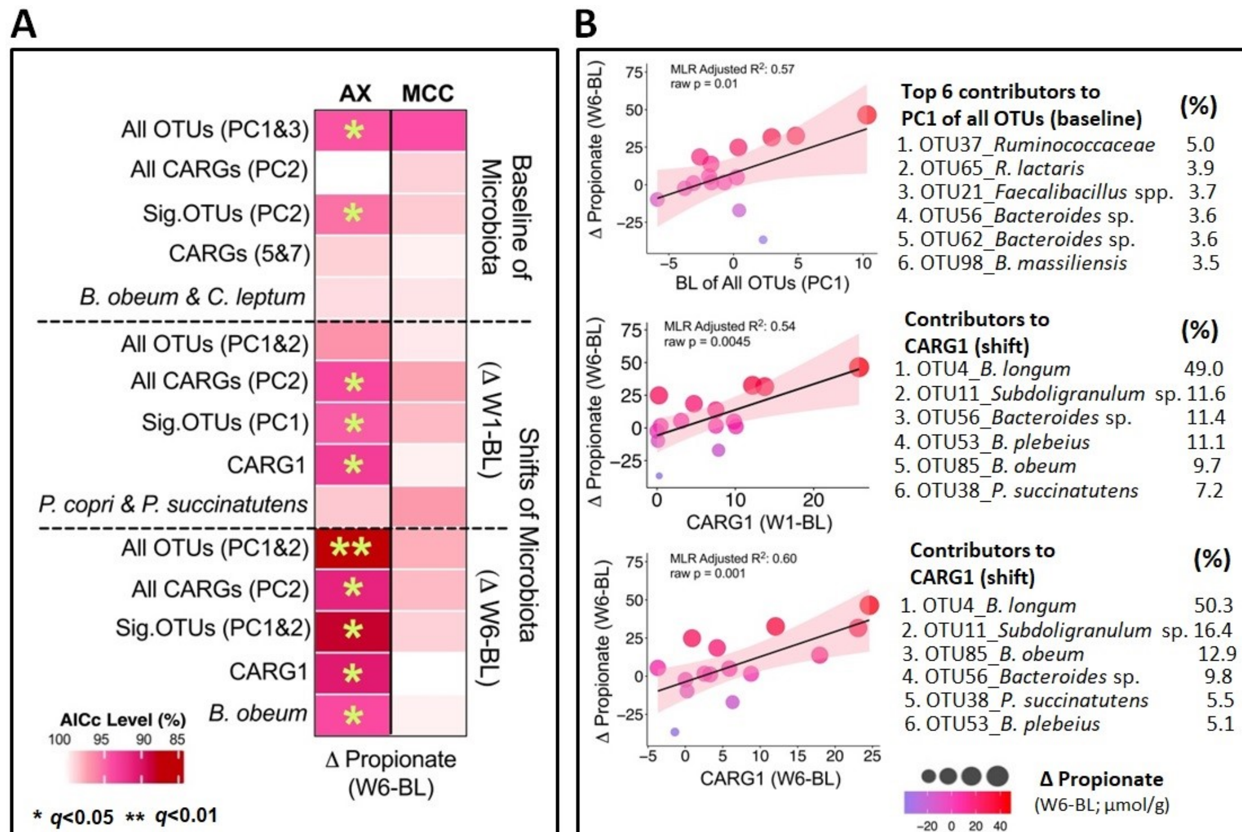

Figure 4.7. Individualized AX-induced propionate responses could be explained by baseline gut microbiota composition and microbiota shifts. (A) Heatmap shows the linear associations between the individualized propionate response (ΔW6–BL; dependent variable; columns) and microbiota profiles (BL, ΔW1–BL, ΔW6–BL; predictors; rows). Cells represent individual multiple linear regression models (with FDR correction) that assess whether the predictors explain the individualized propionate response. Multivariate microbiota data were simplified into principal component (PC) variables PC1, PC2, and PC3 prior to analysis. Each model contained the best one or two predictors of PCs, individual CARGs, or significant OTUs selected by stepwise regression. All models were adjusted by fiber dose/sex. Colors from white to red indicate relative AICc (corrected Akaike information criterion) values calculated by $\frac{AICc\ value}{Highest\ AICc\ value} x\ 100$. Lower AICc values (red) indicate higher quality models. **(B)** Scatter plots show the linear relationship between propionate responses (ΔW6–BL) and either the baseline contribution of all OTUs to PC1 or the shifts of CARG1. Color and size of each point indicate propionate response magnitude and the shaded area specifies the 95% confidence interval. The top six OTUs that contributed the most to either PC1 of all OTUs or CARG1 are further provided. AX, arabinoxylan; BL, baseline; CARG, co-abundance response group; MCC, microcrystalline cellulose; OTU, operational taxonomic unit; W1, week 1; W6, week 6.

4.3.6 Determining the Role of Bacterial Taxa in Propionate Response

MLR analyses were applied to determine connections between AX responding OTUs within CARGs 1 and 6, and fecal propionate concentrations (**Figure 4.8.A**). This analysis

revealed that shifts in *P. copri* (OTU6) did not predict propionate responses, while *B. longum* (OTU4) and correlated taxa in CARG1 showed stronger linear relationships. 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[477]. Such analysis provides a potential explanation for the metabolic interactions between proposed primary degraders, secondary fermenters, and metabolite utilizers that result in the promotion of propionate in response to AX (**Figure 4.8.B**).

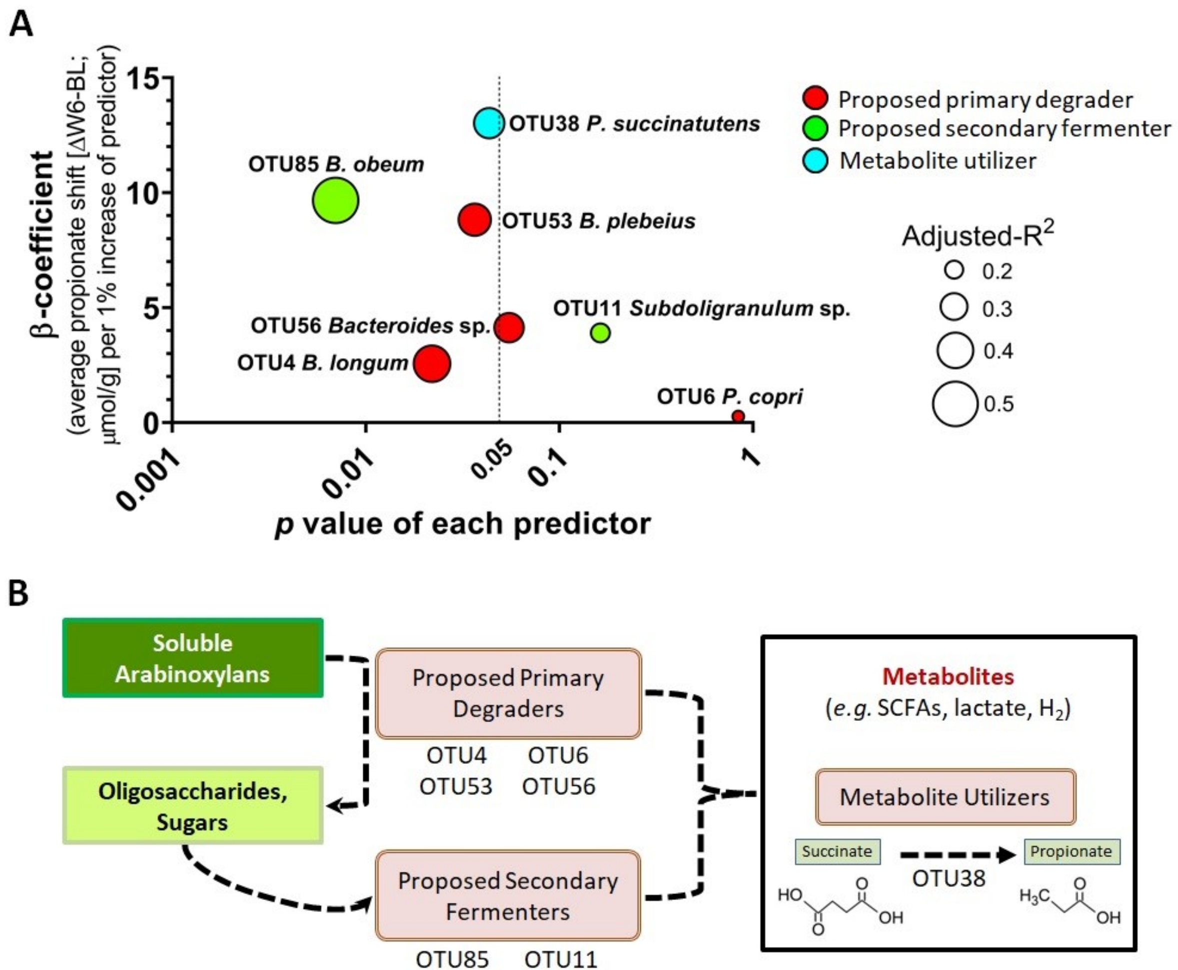

Figure 4.8. Relationship between propionate responses to AX and proposed primary degraders, secondary fermenters, and metabolite utilizers. (A) Individual multiple linear regression models determine OTU responses (ΔW6–BL) that predict the fecal propionate response (ΔW6–BL). Y-axis shows the β-coefficient for each predictor, as in the average propionate response when OTU relative abundance increases 1%. X-axis shows the *p* value for each predictor. All models were adjusted by fiber dose/sex, where bubble size represents the adjusted-R^2. **(B)** Proposed model of bacterial cross-feeding in the gut during degradation of complex, soluble AXs. AX, arabinoxylan; OTU, operational taxonomic unit.

4.4 Discussion

In this study, the impact of a six-week, high-dose corn bran AX supplementation on the composition and function of the fecal bacterial community was characterized 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, with propionate responses showing two distinct temporal patterns. Compositional responses to AX could not be predicted and functional responses were independent of stool consistency, bowel movement frequency, and baseline diet; however, baseline microbiota composition and especially the compositional shifts correlated with propionate responses. The non-fermentable MCC showed virtually no effect on gut microbiota composition or function.

An understanding of compositional and functional responses of the gut microbiota to changes in diet requires an ecological framework[441]. AX supplementation provides resources that can be used by microbes that possess the traits to either access the chemical structures directly or utilize public goods released during AX degradation[441]. In this study, the dominant effects of AX were directed toward two bacterial species, *B. longum* and *P. copri*, while nine additional OTUs showed smaller increases, including three *Bacteroides* species (*e.g. B. ovatus*, *B. plebeius*, and *Bacteroides* sp.). This high degree of specificity toward *B. longum* over other *Bifidobacterium* species is in agreement with other studies testing long-chain AXs[478-480] and genomic analyses that showed that genes encoding AX-degrading glycosidase (*e.g.* β-xylosidase and α-arabinofuranosidase) are conserved only among *B. longum* strains[481,482]. In contrast to the species-specific enrichment of *B. longum*, AX enriched several species within the phylum Bacteroidetes that possess the genetic and functional traits necessary for accessing AX[483-487]. Although AX utilization is not universally conserved among the genera *Bacteroides* and *Prevotella*, the species *P. copri*, *B. ovatus*, *B. cellulosilyticus*, and *B. plebeius* have been shown to be xylanolytic[483-485,487,488] and possess polysaccharide utilization loci that encode for the xylan utilization system[483,486,489], thus providing an explanation for their enrichments in our study.

Interestingly, there were several consistent effects observed between the corn bran AX used in this study and wheat bran extracted AXOS, such as increases in *Bifidobacterium longum*, *Prevotella copri*, *Bacteroides ovatus*, and *Blautia obeum* [440,490]. However, in contrast to corn-

bran AX, AXOS seem to have lower specificity and promote multiple species of *Bifidobacterium* and *Prevotella*, as well as several additional genera (*e.g. Eubacterium* and *Roseburia*). This difference in specificity is likely attributed to variations in their structural features. Specifically, 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[446-448]. To access and utilize such complex structures, bacteria require a more extensive repertoire of proteins and hydrolases relative to what is needed for AXOS utilization, which are generally simpler in structure[36]. For instance, *B. adolescentis* has been shown to utilize simple AXOS both in monoculture[491] and during co-culture with *B. ovatus*, but not during co-culture on corn bran AX[492].

Exploring the response of the bacterial community in the context of ecological guilds provides a more complete view of the interactions among the bacterial species in the degradation of AX. This analysis showed the strongest response in CARG1 and CARG2. The response within CARG1 is dominated by *B. longum*, which showed strong connections to four out of five members within CARG1 (*B. plebeius*, *Bacteroides* sp., *P. succinatutens*, and *Subdoligranulum* sp.) and one member in CARG2 (an unclassified *Lachnospiraceae*), suggesting syntrophic relationships. *B. longum* has been shown to be a primary degrader of AXs[481,482] that is able to cleave the complex AX structure by soluble AX-degrading glycosidase[493,494]. This degradation could release xylan and AXOS (or even xylose, arabinose, and galactose) to xylan-utilizing *Bacteroides* species like *B. plebeius*[485,486,489] and putative secondary fermenters like *B. obeum* and *Subdoligranulum* sp.[478,495,496] (**Figure 4.8.B**). 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 likely a primary degrader of AX[483,488], but showed only one strong correlation within CARG6, suggesting the bacterium behaves 'selfishly'. These findings suggest that no singular 'keystone species' initiates the degradation of AX, as it has been described for type-III resistant starches[430]. Most likely, several 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 microbiota metabolism and the increase in propionate. The specificity of long-chain AXs for propionate has been previously described[284,497] and is affiliated with a higher presence of arabinose side-chains[435,498]. Although *P. copri* is a primary degrader of AX, the species did not

predict propionate response herein, which is in accordance with previous suggestions that the bacterium acts selfishly[499] and does not produce propionate[477]. Metabolic interactions appear more relevant within CARG1. Although *B. longum* is numerically the dominant responder within this CARG, it does not produce propionate itself and is a poor predictor of propionate responses (**Figure 4.8.A**). However, the enrichment of *B. longum* is strongly linked to species that possess metabolic pathways for propionate production (*i.e. B. obeum*, *P. succinatutens*, *B. plebeius*, and *Bacteroides* sp.)[500,501], which are better predictors of propionate response. Although significant models were obtained with MLR using the single taxa of CARG1, the entire CARG1 was a better predictor of propionate shifts, indicating that groups of bacteria collaborate to produce propionate. Overall, the analyses on ecological guilds suggest co-operative 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.

Although significant effects of AX on microbiota composition and propionate production were detected, these effects displayed a high degree of individuality. In terms of taxa, this might be driven by the inter-individual differences in baseline microbiota composition and diet[438]. Although the responses of *P. copri* were strictly linked to the presence of the species at baseline, our MLR models showed no significant associations between baseline CLR-transformed abundances 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 microbiota exist but could not be detected with the small sample size of our study. MLR analyses further showed that baseline reported dietary history could not predict AX-induced shifts in bacterial taxa or CARGs. This might be reflective of the fact that diet is only one of many contributors to the variation of microbiomes[502,503], although we cannot exclude that our small sample size and limitations in self-reported food frequency questionnaire data contributed to the lack of signal[504]. Therefore, future studies on the individualized response to DF should be conducted with larger sample sizes, repeated dietary recalls or records, and whole metagenome sequencing to achieve higher resolution, strain-level distinctions that likely drive individuality.

Individuality was especially pronounced when looking at metabolite output. The MLR analyses revealed that shifts in propionate output (ΔW6-baseline) correlated with W6 shifts of

the microbiota, and to a lesser degree W1 shifts and baseline composition, but not diet, stool consistency, or bowel movement frequency. Shifts in CARGs provided a better prediction than individual taxa indicate the importance of ecological guilds in DF fermentation. However, models using the first two PCs generated from all OTUs, capturing 25% of the variance in the bacterial response, were of better quality than those using CARGs, demonstrating that propionate production is the result of a more complex trophic network that spans the wider bacterial community. This can potentially be explained by the well-recognized functional redundancy among distantly related members of the gut microbiota[477,505], and the stochastic nature by which they assemble into communities[16]. Although it is often assumed that functional redundancy results in gut microbiomes that are more similar between individuals on a functional level, findings on the propionate response to AX clearly show that differences among individuals exist in terms of how they ferment a DF. The hierarchy by which factors predict propionate response found in our MLR analysis (single taxa < CARGs < PCs) supports an ecological framework that considers microbiomes as complex communities of interacting members to interpret and predict functional outcomes of DF fermentation in future human intervention trials.

Although this study revealed ecological concepts to explain inter-individual variation in DF fermentation of the human gut microbiota, limitations must be acknowledged in our ability to identify relevant players within trophic networks and ecological guilds using sequencing data from a human intervention study. Analyses for the determination of CARGs were based on the correlation of compositional responses of the microbiota to AX. Although this analysis identified clusters of species with traits to utilize AX that are likely ecologically relevant, statistically significant correlations were also detected between CARGs, suggesting that trophic networks extend to the broader community. In addition, by being limited to correlations, this approach cannot identify causal links, and the focus on compositional shifts is unlikely to identify all members of trophic networks as not every species that contributes to the fermentation of a DF becomes enriched[273]. There are, therefore, limitations in our ability to identify all relevant primary degraders, secondary fermenters, and metabolite utilizers, and more sensitive approaches such as stable isotope probing[77] or bio-orthogonal non-canonical amino acid tagging[506] are required. Such studies could be complemented by co-culture experiments, such as those described by Ze *et al.*[430], to empirically test cross-feeding interactions and exert mechanisms by which gut bacteria collaborate to utilize specific DFs. The inclusion of such

mechanistic information on trophic networks would likely improve the quality of models that predict the fermentation of DF and its metabolic consequences.

From an applied perspective, the findings discussed herein have implications for the targeted use of AX to modulate the gut microbiota for improved health. Probiotic treatments with *B. longum* strains have been shown to be health-promoting in a variety of contexts[507], including gastrointestinal[508,509], immunological (*e.g.* anti-allergy and anti-inflammatory[510,511]), and psychological (*e.g.* depression and anxiety[512,513]) 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*. Although the role of *P. copri* in human health remains unclear, with potential deleterious effects reported (*e.g.* enhanced rheumatoid arthritis susceptibility[514]) that are likely dependent on strain-level differences, dietary environments, and host predisposition[443,515], this species was associated with improved glucose metabolism after whole grain barley treatment[10], and correlated with weight loss in volunteers that consumed diets high in whole grains[516,517]. *Prevotella* is a genus that has been consistently negatively associated with an industrialized lifestyle[9,34]. The reason for this reduction due to industrialization is unknown, but it has been speculated that reduced consumption of DF-rich foods is responsible[276]. 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 propionate administration has been shown to induce satiety[518], improve glucose homeostasis[519,520], and suppress pro-inflammatory interleukin-8 levels[519] in humans. Overall, findings in **Chapter 4** of this dissertation suggest that AX has prebiotic properties in that it promotes putatively health-related organisms and the production of propionate, making it a promising candidate for the prevention of obesity and associated pathologies, especially if its application is personalized.

4.5 Conclusions

The findings of this study are relevant as individualized responses of the gut microbiota to DF provide a potential explanation for their inconsistent clinical effects in human intervention studies[437]. 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.

This study further identified microbiota-related factors that can be used to predict AX-induced propionate responses. Although significant MLR models were developed based on baseline microbiota profiles, which has practical advantages in personalizing intervention studies by the prediction of responses pre-treatment, the best models were obtained with compositional shifts, especially when features of the broader community (*e.g.* PCs) were considered. This finding serves as a proof-of-principle for the value of an ecological approach toward predictions of metabolic effects of DF on the human gut microbiota. As the sample size of this exploratory study was too small to identify predictors that could be directly applied in independent studies, larger studies are needed to develop robust machine learning algorithms - ideally informed through an ecological framework - that identify the exact factors that predict microbiota responses to DF.

4.6 References

9. Martínez I, Stegen JC, Maldonado-Gómez MX*, et al.* The gut microbiota of rural papua new guineans: composition, diversity patterns, and ecological processes. *Cell Rep.* 2015;11(4):527-38.
10. Kovatcheva-Datchary P, Nilsson A, Akrami R*, et al.* Dietary Fiber-Induced Improvement in Glucose Metabolism Is Associated with Increased Abundance of *Prevotella*. *Cell Metab.* 2015;22(6):971-82.
16. Walter J, Ley RE. The human gut microbiome: ecology and recent evolutionary changes. *Annu Rev Microbiol.* 2011;65:411-29.
31. Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. *Cell.* 2016;165(6):1332-45.
34. Schnorr SL, Candela M, Rampelli S*, et al.* Gut microbiome of the Hadza hunter-gatherers. *Nat Commun.* 2014;5:3654.
36. Hamaker BR, Tuncil YE. A perspective on the complexity of dietary fiber structures and their potential effect on the gut microbiota. *J Mol Biol.* 2014;426(23):3838-50.
61. Canfora EE, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity. *Nat Rev Endocrinol.* 2015;11(10):577-91.
67. Venkataraman A, Sieber JR, Schmidt AW, Waldron C, Theis KR, Schmidt TM. Variable responses of human microbiomes to dietary supplementation with resistant starch. *Microbiome.* 2016;4(1):33.
69. Martínez I, Kim J, Duffy PR, Schlegel VL, Walter J. Resistant starches types 2 and 4 have differential effects on the composition of the fecal microbiota in human subjects. *PLoS One.* 2010;5(11):e15046.
77. Tannock GW, Lawley B, Munro K*, et al.* RNA-stable-isotope probing shows utilization of carbon from inulin by specific bacterial populations in the rat large bowel. *Appl Environ Microbiol.* 2014;80(7):2240-47.

105. Lozupone CA, Hamady M, Cantarel BL*, et al.* The convergence of carbohydrate active gene repertoires in human gut microbes. *Proc Natl Acad Sci USA.* 2008;105(39):15076-81.
114. Cummings JH, Pomare E, Branch W, Naylor C, Macfarlane GT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut.* 1987;28(10):1221-27.
266. Makki K, Deehan EC, Walter J, Bäckhed F. The Impact of Dietary Fiber on Gut Microbiota in Host Health and Disease. *Cell Host Microbe.* 2018;23(6):705-15.
267. Reynolds A, Mann J, Cummings J, Winter N, Mete E, Te Morenga L. Carbohydrate quality and human health: a series of systematic reviews and meta-analyses. *Lancet.* 2019;393(10170):434-45.
273. Deehan EC, Duar RM, Armet AM, Perez-Muñoz ME, Jin M, Walter J. Modulation of the Gastrointestinal Microbiome with Nondigestible Fermentable Carbohydrates To Improve Human Health. *Microbiol Spectr.* 2017;5(5).
276. Sonnenburg ED, Sonnenburg JL. The ancestral and industrialized gut microbiota and implications for human health. *Nat Rev Microbiol.* 2019;17(6):383-90.
280. Zhao L, Zhang F, Ding X*, et al.* Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes. *Science.* 2018;359(6380):1151-56.
284. Alexander C, Swanson KS, Fahey GC, Garleb KA. Perspective: Physiologic Importance of Short-Chain Fatty Acids from Nondigestible Carbohydrate Fermentation. *Adv Nutr.* 2019;10(4):576-89.
300. Tannock GW, Liu Y. Guided dietary fibre intake as a means of directing short-chain fatty acid production by the gut microbiota. *J Roy Soc New Zeal.* 2019.
306. Wei B, Liu Y, Lin X, Fang Y, Cui J, Wan J. Dietary fiber intake and risk of metabolic syndrome: A meta-analysis of observational studies. *Clin Nutr.* 2018;37(6 Pt A):1935-42.
370. Salden BN, Troost FJ, Wilms E*, et al.* Reinforcement of intestinal epithelial barrier by arabinoxylans in overweight and obese subjects: A randomized controlled trial: Arabinoxylans in gut barrier. *Clin Nutr.* 2018;37(2):471-80.
430. Ze X, Duncan SH, Louis P, Flint HJ. *Ruminococcus bromii* is a keystone species for the degradation of resistant starch in the human colon. *ISME J.* 2012;6(8):1535-43.
435. Broekaert WF, Courtin CM, Verbeke K, Van de Wiele T, Verstraete W, Delcour JA. Prebiotic and other health-related effects of cereal-derived arabinoxylans, arabinoxylan-oligosaccharides, and xylooligosaccharides. *Crit Rev Food Sci Nutr.* 2011;51(2):178-94.
436. Rimm EB, Ascherio A, Giovannucci E, Spiegelman D, Stampfer MJ, Willett WC. Vegetable, fruit, and cereal fiber intake and risk of coronary heart disease among men. *JAMA.* 1996;275(6):447-51.
437. Armet AM, Deehan EC, Thöne JV, Hewko SJ, Walter J. The Effect of Isolated and Synthetic Dietary Fibers on Markers of Metabolic Diseases in Human Intervention Studies: A Systematic Review. *Adv Nutr.* 2020;11(2):420–38.
438. Healey GR, Murphy R, Brough L, Butts CA, Coad J. Interindividual variability in gut microbiota and host response to dietary interventions. *Nutr Rev.* 2017;75(12):1059-80.
439. Deehan EC, Yang C, Perez-Muñoz ME*, et al.* Precision Microbiome Modulation with Discrete Dietary Fiber Structures Directs Short-Chain Fatty Acid Production. *Cell Host Microbe.* 2020;27(3):389-404.e6.
440. Kjølbæk L, Benítez-Páez A, Gómez Del Pulgar EM*, et al.* Arabinoxylan oligosaccharides and polyunsaturated fatty acid effects on gut microbiota and metabolic markers in

overweight individuals with signs of metabolic syndrome: A randomized cross-over trial. *Clin Nutr.* 2020;39(1):67-79.
441. Flint HJ, Duncan SH, Louis P. The impact of nutrition on intestinal bacterial communities. *Curr Opin Microbiol.* 2017;38:59-65.
442. Cockburn DW, Koropatkin NM. Polysaccharide Degradation by the Intestinal Microbiota and Its Influence on Human Health and Disease. *J Mol Biol.* 2016;428(16):3230-52.
443. De Filippis F, Pasolli E, Tett A*, et al.* Distinct Genetic and Functional Traits of Human Intestinal *Prevotella copri* Strains Are Associated with Different Habitual Diets. *Cell Host Microbe.* 2019;25(3):444-53.e3.
444. ClinicalTrials.gov. National Library of Medicine (US). Identifier NCT02322112, The Alberta FYBER (Feed Your gut Bacteria morE fibeR) Study. 2015, July 3. Available at: Retrieved April 25, 2020 from: https://clinicaltrials.gov/ct2/history/NCT02322112?V_2=View#StudyPageTop.
445. Tuncil YE, Nakatsu CH, Kazem AE*, et al.* Delayed utilization of some fast-fermenting soluble dietary fibers by human gut microbiota when presented in a mixture. *J Funct Foods.* 2017;32:347-57.
446. Rose DJ, Patterson JA, Hamaker BR. Structural differences among alkali-soluble arabinoxylans from maize (*Zea mays*), rice (*Oryza sativa*), and wheat (*Triticum aestivum*) brans influence human fecal fermentation profiles. *J Agric Food Chem.* 2010;58(1):493-99.
447. Rumpagaporn P, Reuhs BL, Kaur A, Patterson JA, Keshavarzian A, Hamaker BR. Structural features of soluble cereal arabinoxylan fibers associated with a slow rate of *in vitro* fermentation by human fecal microbiota. *Carbohydr Polym.* 2015;130:191-97.
448. Saulnier L, Vigouroux J, Thibault J-F. Isolation and partial characterization of feruloylated oligosaccharides from maize bran. *Carbohydr Res.* 1995;272(2):241-53.
449. Csizmadi I, Boucher BA, Lo Siou G*, et al.* Using national dietary intake data to evaluate and adapt the US Diet History Questionnaire: the stepwise tailoring of an FFQ for Canadian use. *Public Health Nutr.* 2016;19(18):3247-55.
450. McInerney M, Csizmadi I, Friedenreich CM*, et al.* Associations between the neighbourhood food environment, neighbourhood socioeconomic status, and diet quality: An observational study. *BMC Public Health.* 2016;16:984.
451. Kipnis V, Subar AF, Midthune D*, et al.* Structure of Dietary Measurement Error: Results of the OPEN Biomarker Study. *Am J Epidemiol.* 2003;158(1):14-21.
452. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. *Am J Epidemiol.* 1986;124(1):17-27.
453. Winter J, Young GP, Hu Y, Gratz SW, Conlon MA, Le Leu RK. Accumulation of promutagenic DNA adducts in the mouse distal colon after consumption of heme does not induce colonic neoplasms in the western diet model of spontaneous colorectal cancer. *Mol Nutr Food Res.* 2014;58(3):550-58.
454. Jin M, Kalainy S, Baskota N*, et al.* Faecal microbiota from patients with cirrhosis has a low capacity to ferment non-digestible carbohydrates into short-chain fatty acids. *Liver Int.* 2019;39(8):1437-47.
455. Krumbeck JA, Maldonado-Gomez MX, Martínez I*, et al. In vivo* selection to identify bacterial strains with enhanced ecological performance in synbiotic applications. *Appl Environ Microbiol.* 2015;81(7):2455-65.

456. Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics.* 2010;26(19):2460-61.
457. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol.* 2007;73(16):5261-67.
458. Quast C, Pruesse E, Yilmaz P*, et al.* The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 2013;41(Database issue):D590-D96.
459. Yoon S-H, Ha S-M, Kwon S*, et al.* Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol.* 2017;67(5):1613-17.
460. Markowitz VM, Chen I-MA, Palaniappan K*, et al.* IMG: the Integrated Microbial Genomes database and comparative analysis system. *Nucleic Acids Res.* 2012;40(D1):D115-D22.
461. Chen I-MA, Chu K, Palaniappan K*, et al.* IMG/M v.5.0: an integrated data management and comparative analysis system for microbial genomes and microbiomes. *Nucleic Acids Res.* 2019;47(D1):D666-D77.
462. Aitchison J. The Statistical Analysis of Compositional Data. *J R Statist Soc B.* 1982;44(2):139-60.
463. vegan: Community Ecology Package [computer program]. Version R-package version 2.5-52019.
464. Wickham H. ggplot2: Elegant Graphics for Data Analysis. 2 ed: Springer International Publishing; 2016.
465. Højsgaard S, Halekoh U, Yan J. The R Package geepack for Generalized Estimating Equations. *J Stat Softw.* 2005;15(2).
466. Tong X, Xu J, Lian F*, et al.* Structural Alteration of Gut Microbiota during the Amelioration of Human Type 2 Diabetes with Hyperlipidemia by Metformin and a Traditional Chinese Herbal Formula: a Multicenter, Randomized, Open Label Clinical Trial. *mBio.* 2018;9(3):e02392-17.
467. Gu Z, Eils R, Schlesner M. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics.* 2016;32(18):2847-28479.
468. Faust K, Raes J. CoNet app: inference of biological association networks using Cytoscape. *F1000Res.* 2016;5:1519.
469. Cai C, Zhang Z, Morales M, Wang Y, Khafipour E, Friel J. Feeding practice influences gut microbiome composition in very low birth weight preterm infants and the association with oxidative stress: A prospective cohort study. *Free Radic Biol Med.* 2019;142:146-54.
470. factoextra: Extract and Visualize the Results of Multivariate Data Analyses [computer program]. Version R-package version 1.0.52017.
471. Lê S, Josse J, Husson F. FactoMineR: An R package for multivariate analysis. *J Stat Softw.* 2008;25(1).
472. leaps: Regression Subset Selection [computer program]. Version R-package version 3.02017.
473. AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c) [computer program]. Version R-package versioin 2.2-22019.

474. Millet S, Van Oeckel MJ, Aluwe M, Delezie E, De Brabander DL. Prediction of *In Vivo* Short-Chain Fatty Acid Production in Hindgut Fermenting Mammals: Problems and Pitfalls. *Crit Rev Food Sci Nutr.* 2010;50(7):605-19.
475. McOrist AL, Miller RB, Bird AR*, et al.* Fecal butyrate levels vary widely among individuals but are usually increased by a diet high in resistant starch. *J Nutr.* 2011;141(5):883-89.
476. Krumholz LR, Bryant M. *Eubacterium oxidoreducens* sp. nov. requiring H_2 or formate to degrade gallate, pyrogallol, phloroglucinol and quercetin. *Arch Microbiol.* 1986;144(1):8-14.
477. Louis P, Flint HJ. Formation of propionate and butyrate by the human colonic microbiota. *Environ Microbiol.* 2017;19(1):29-41.
478. Van den Abbeele P, Gérard P, Rabot S*, et al.* Arabinoxylans and inulin differentially modulate the mucosal and luminal gut microbiota and mucin-degradation in humanized rats. *Environ Microbiol.* 2011;13(10):2667-80.
479. Van den Abbeele P, Venema K, Van de Wiele T, Verstraete W, Possemiers S. Different human gut models reveal the distinct fermentation patterns of Arabinoxylan versus inulin. *J Agric Food Chem.* 2013;61(41):9819-27.
480. Crittenden R, Karppinen S, Ojanen S*, et al. In vitro* fermentation of cereal dietary fibre carbohydrates by probiotic and intestinal bacteria. *J Sci Food Agr.* 2002;82(8):781-89.
481. Rivière A, Moens F, Selak M, Maes D, Weckx S, De Vuyst L. The ability of bifidobacteria to degrade arabinoxylan oligosaccharide constituents and derived oligosaccharides is strain dependent. *Appl Environ Microbiol.* 2014;80(1):204-17.
482. Komeno M, Hayamizu H, Fujita K, Ashida H. Two Novel α-l-Arabinofuranosidases from *Bifidobacterium longum* subsp. *longum* Belonging to Glycoside Hydrolase Family 43 Cooperatively Degrade Arabinan. *Appl Environ Microbiol.* 2019;85(6):e02582-18.
483. Fehlner-Peach H, Magnabosco C, Raghavan V*, et al.* Distinct Polysaccharide Utilization Profiles of Human Intestinal *Prevotella copri* Isolates. *Cell Host Microbe.* 2019;26(5):680-90 e5.
484. Tan H, Zhao J, Zhang H, Zhai Q, Chen W. Isolation of Low-Abundant Bacteroidales in the Human Intestine and the Analysis of Their Differential Utilization Based on Plant-Derived Polysaccharides. *Front Microbiol.* 2018;9:1319.
485. La Rosa SL, Kachrimanidou V, Buffetto F*, et al.* Wood-Derived Dietary Fibers Promote Beneficial Human Gut Microbiota. *mSphere.* 2019;4(1):e00554-18.
486. Zhang M, Chekan JR, Dodd D*, et al.* Xylan utilization in human gut commensal bacteria is orchestrated by unique modular organization of polysaccharide-degrading enzymes. *Proc Natl Acad Sci USA.* 2014;111(35):E3708-17.
487. Centanni M, Hutchison JC, Carnachan SM*, et al.* Differential growth of bowel commensal *Bacteroides* species on plant xylans of differing structural complexity. *Carbohydr Polym.* 2017;157:1374-82.
488. Pareek S, Kurakawa T, Das B*, et al.* Comparison of Japanese and Indian intestinal microbiota shows diet-dependent interaction between bacteria and fungi. *NPJ Biofilms Microbi.* 2019;5:37.
489. Dodd D, Mackie RI, Cann IK. Xylan degradation, a metabolic property shared by rumen and human colonic Bacteroidetes. *Mol Microbiol.* 2011;79(2):292-304.

490. Benítez-Páez A, Kjølbæk L, Gómez Del Pulgar EM*, et al.* A Multi-omics Approach to Unraveling the Microbiome-Mediated Effects of Arabinoxylan Oligosaccharides in Overweight Humans. *mSystems.* 2019;4(4):e00209-19.
491. Pastell H, Westermann P, Meyer AS, Tuomainen P, Tenkanen M. *In vitro* fermentation of arabinoxylan-derived carbohydrates by bifidobacteria and mixed fecal microbiota. *J Agric Food Chem.* 2009;57(18):8598-606.
492. Rogowski A, Briggs JA, Mortimer JC*, et al.* Glycan complexity dictates microbial resource allocation in the large intestine. *Nat Commun.* 2015;6:7481.
493. Lugli GA, Mancino W, Milani C*, et al.* Reconstruction of the Bifidobacterial Pan-Secretome Reveals the Network of Extracellular Interactions between Bifidobacteria and the Infant Gut. *Appl Environ Microbiol.* 2018;84(16):e00796-18.
494. Milani C, Lugli GA, Duranti S*, et al.* Bifidobacteria exhibit social behavior through carbohydrate resource sharing in the gut. *Sci Rep.* 2015;5:15782.
495. Holmstrøm K, Collins MD, Moller T, Falsen E, Lawson PA. *Subdoligranulum variabile* gen. nov., sp. nov. from human feces. *Anaerobe.* 2004;10(3):197-203.
496. Lawson PA, Finegold SM. Reclassification of *Ruminococcus obeum* as *Blautia obeum* comb. nov. *Int J Syst Evol Microbiol.* 2015;65(Pt 3):789-93.
497. Hopkins MJ, Englyst HN, Macfarlane S, Furrie E, Macfarlane GT, McBain AJ. Degradation of Cross-Linked and Non-Cross-Linked Arabinoxylans by the Intestinal Microbiota in Children. *Appl Environ Microbiol.* 2003;69(11):6354-60.
498. Rumpagaporn P, Reuhs BL, Cantu-Jungles TM*, et al.* Elevated propionate and butyrate in fecal ferments of hydrolysates generated by oxalic acid treatment of corn bran arabinoxylan. *Food Funct.* 2016;7(12):4935-43.
499. Chen T, Long W, Zhang C, Liu S, Zhao L, Hamaker BR. Fiber-utilizing capacity varies in *Prevotella*- versus *Bacteroides*-dominated gut microbiota. *Sci Rep.* 2017;7(1):2594.
500. Reichardt N, Duncan SH, Young P*, et al.* Phylogenetic distribution of three pathways for propionate production within the human gut microbiota. *ISME J.* 2014;8(6):1323-35.
501. Watanabe Y, Nagai F, Morotomi M. Characterization of *Phascolarctobacterium succinatutens* sp. nov., an asaccharolytic, succinate-utilizing bacterium isolated from human feces. *Appl Environ Microbiol.* 2012;78(2):511-18.
502. Rothschild D, Weissbrod O, Barkan E*, et al.* Environment dominates over host genetics in shaping human gut microbiota. *Nature.* 2018;555(7695):210-15.
503. Wang J, Thingholm LB, Skieceviciene J*, et al.* Genome-wide association analysis identifies variation in vitamin D receptor and other host factors influencing the gut microbiota. *Nat Genet.* 2016;48(11):1396-406.
504. Subar AF, Freedman LS, Tooze JA*, et al.* Addressing Current Criticism Regarding the Value of Self-Report Dietary Data. *J Nutr.* 2015;145(12):2639-45.
505. Louca S, Polz MF, Mazel F*, et al.* Function and functional redundancy in microbial systems. *Nat Ecol Evol.* 2018;2(6):936-43.
506. Hatzenpichler R, Scheller S, Tavormina PL, Babin BM, Tirrell DA, Orphan VJ. *In situ* visualization of newly synthesized proteins in environmental microbes using amino acid tagging and click chemistry. *Environ Microbiol.* 2014;16(8):2568-90.
507. Wong CB, Odamaki T, Xiao J-z. Beneficial effects of *Bifidobacterium longum* subsp. *longum* BB536 on human health: Modulation of gut microbiome as the principal action. *J Funct Foods.* 2019;54:506-19.

508. Colombel J, Cortot A, Neut C, Romond C. Yoghurt with *Bifidobacterium longum* Reduces Erythromycin-Induced Gastrointestinal Effects. *Lancet.* 1987;330(8549):43.
509. Tamaki H, Nakase H, Inoue S*, et al.* Efficacy of probiotic treatment with *Bifidobacterium longum* 536 for induction of remission in active ulcerative colitis: A randomized, double-blinded, placebo-controlled multicenter trial. *Dig Endosc.* 2016;28(1):67-74.
510. McCarville J, Dong J, Caminero A*, et al.* A Commensal *Bifidobacterium longum* Strain Prevents Gluten-Related Immunopathology in Mice through Expression of a Serine Protease Inhibitor. *Appl Environ Microbiol.* 2017;83(19):e01323-17.
511. Xiao J-Z, Kondo S, Yanagisawa N*, et al.* Probiotics in the treatment of Japanese cedar pollinosis: a double-blind placebo-controlled trial. *Clin Exp Allergy.* 2006;36(11):1425-35.
512. Pinto-Sanchez MI, Hall GB, Ghajar K*, et al.* Probiotic *Bifidobacterium longum* NCC3001 Reduces Depression Scores and Alters Brain Activity: A Pilot Study in Patients With Irritable Bowel Syndrome. *Gastroenterology.* 2017;153(2):448-59.e8.
513. Bercik P, Park AJ, Sinclair D*, et al.* The anxiolytic effect of *Bifidobacterium longum* NCC3001 involves vagal pathways for gut-brain communication. *Neurogastroenterol Motil.* 2011;23(12):1132-39.
514. Scher JU, Sczesnak A, Longman RS*, et al.* Expansion of intestinal *Prevotella copri* correlates with enhanced susceptibility to arthritis. *eLife.* 2013;2:e01202.
515. Cani PD. Human gut microbiome: hopes, threats and promises. *Gut.* 2018;67(9):1716-25.
516. Christensen L, Vuholm S, Roager HM*, et al. Prevotella* Abundance Predicts Weight Loss Success in Healthy, Overweight Adults Consuming a Whole-Grain Diet Ad Libitum: A Post Hoc Analysis of a 6-Wk Randomized Controlled Trial. *J Nutr.* 2019;149(12):2174-81.
517. Hjorth MF, Roager HM, Larsen TM*, et al.* Pre-treatment microbial *Prevotella*-to-*Bacteroides* ratio, determines body fat loss success during a 6-month randomized controlled diet intervention. *Int J Obes.* 2018;42(3):580-83.
518. Chambers ES, Viardot A, Psichas A*, et al.* Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults. *Gut.* 2015;64(11):1744-54.
519. Chambers ES, Byrne CS, Morrison DJ*, et al.* Dietary supplementation with inulin-propionate ester or inulin improves insulin sensitivity in adults with overweight and obesity with distinct effects on the gut microbiota, plasma metabolome and systemic inflammatory responses: a randomised cross-over trial. *Gut.* 2019;68(8):1430-38.
520. Venter C, Vorster H, Cummings JH. Effects of dietary propionate on carbohydrate and lipid metabolism in healthy volunteers. *Am J Gastroenterol.* 1990;85(5):549-53.

4.7 Supplementary Material

Table S.4.1. 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 (female/male)	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
Waist circumference (cm)	95.7 ± 8.8	92.9 ± 6.0	0.30
Percent body fat (%)	33.0 ± 9.3	32.0 ± 7.3	0.63
Females	36.4 ± 2.9	38.0 ± 6.1	0.20
Males	22.5 ± 3.6	23.0 ± 5.3	0.84
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 ± standard deviation 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.

Table S.4.2. Abundance of bacterial taxa and CARGs affected by the dietary interventions [1]

Taxonomic Group	Proportion of bacterial taxa expressed in relative abundance (Mean ± Standard Deviation)								
	Arabinoxylan (*n*=15)				Microcrystalline Cellulose (*n*=16)				Between *p* value (adj.)
	Baseline	Week 6	Within *p* value (adj.)	Δ Taxa	Baseline	Week 6	Within *p* value (adj.)	Δ Taxa	
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* sp.	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, *Faecalibacillus intestinalis/faecis*, 100%	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)**

Table S.4.2. *Continued*

Taxonomic Group	Arabinoxylan (*n*=15) Baseline	Week 6	Within *p* value (adj.)	Δ Taxa	Microcrystalline Cellulose (*n*=16) Baseline	Week 6	Within *p* value (adj.)	Δ Taxa	Between *p* value (adj.)
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)	0.3 ± 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 (Δ week 6-baseline) were determined by Wilcoxon tests, while between-group shifts (Δ arabinoxylan vs. Δ microcrystalline cellulose) were determined by Mann-Whitney 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.

Table S.4.3. Fecal pH, moisture content, and concentration and percentages of fecal SCFAs [1]

	Fecal pH, Moisture Content, and Short-Chain Fatty Acids (Mean ± Standard Deviation)								
	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(1.00)	-0.1 ± 0.6	6.8 ± 0.6	7.0 ± 0.6	0.18(0.76)	0.3 ± 0.8	0.095(0.31)
Fecal moisture content (%)	71.7 ± 7.3	72.4 ± 7.0	0.80(1.00)	0.7 ± 6.7	72.2 ± 8.7	69.9 ± 7.2	0.40(0.76)	-2.3 ± 11.2	0.52(0.70)
Fecal Concentration (µmol/g)									
Total SCFA	145.3 ± 99.0	151.4 ± 80.7	0.59(1.00)	6.2 ± 65.2	145.3 ± 111.9	126.2 ± 55.2	0.59(0.76)	-19.0 ± 96.9	0.56(0.70)
Acetate	90.2 ± 56.2	94.8 ± 50.9	0.99(1.00)	4.6 ± 37.0	91.4 ± 63.6	80.8 ± 30.3	0.32(0.76)	-10.6 ± 56.6	0.46(0.70)
Propionate	31.2 ± 28.6	39.0 ± 26.0	0.15(0.53)	7.8 ± 21.1	31.7 ± 32.4	24.0 ± 13.5	0.40(0.76)	-7.7 ± 24.8	0.11(0.31)
Butyrate	23.8 ± 18.4	17.6 ± 10.3	0.48(1.00)	-6.3 ± 16.7	22.3 ± 19.3	21.4 ± 16.9	0.93(0.94)	-0.8 ± 21.9	0.59(0.70)
Valerate	3.1 ± 1.6	3.1 ± 1.7	0.93(1.00)	-0.004 ± 1.1	3.5 ± 2.7	3.1 ± 1.6	0.59(0.76)	-0.3 ± 2.4	0.76(0.77)
Total BCFA	7.8 ± 3.6	8.4 ± 4.6	0.71(1.00)	0.6 ± 3.8	7.4 ± 3.6	6.4 ± 2.5	0.43(0.76)	-1.1 ± 4.8	0.39(0.70)
Isobutyrate	3.1 ± 1.1	3.4 ± 1.6	0.52(1.00)	0.3 ± 1.5	2.9 ± 1.3	2.6 ± 0.9	0.49(0.76)	-0.2 ± 1.8	0.26(0.61)
Isovalerate	4.7 ± 2.5	5.0 ± 3.0	0.97(1.00)	0.3 ± 2.4	4.6 ± 2.5	3.7 ± 1.7	0.29(0.76)	-0.8 ± 3.2	0.46(0.70)
Proportion of Total SCFA (%)									
Acetate	62.7 ± 6.4	62.7 ± 6.7	0.97(1.00)	-0.04 ± 6.9	66.1 ± 6.1	66.6 ± 9.2	0.78(0.92)	1.0 ± 8.3	0.66(0.72)
Propionate	20.7 ± 6.3	25.0 ± 6.8	0.04(0.19)	4.3 ± 7.8	18.9 ± 4.2	17.8 ± 5.6	0.23(0.76)	-1.4 ± 4.5	**0.01(0.07)**
Butyrate	16.6 ± 5.0	12.3 ± 5.2	**0.018(0.13)**	-4.3 ± 6.4	15.0 ± 4.1	15.9 ± 7.1	0.89(0.94)	0.4 ± 7.0	0.08(0.31)
Propionate-to-Butyrate Ratio	1.4 ± 0.5	3.3 ± 4.6	**0.004(0.06)**	2.0 ± 4.7	1.4 ± 0.6	1.4 ± 0.7	0.50(0.76)	-0.1 ± 0.6	**0.005(0.07)**

[1] Statistical significance of within-group shifts (Δ week 6-baseline) were determined by Wilcoxon tests, while between-group shifts (Δ arabinoxylan vs. Δ microcrystalline cellulose) were determined by Mann–Whitney tests. *p* values were adjusted by FDR, whereas FDR significance was set at q<0.15. Δ SCFA, absolute change from baseline to week 6. BCFAs, branched short-chain fatty acids; SCFAs, short-chain fatty acids.

Table S.4.4. MLR Analyses between arabinoxylan-induced fecal SCFA responses and bacterial features [1]

			Fecal SCFA Concentration Response (Δ week 6 - baseline; dependent variables)								
			Propionate			**Acetate**			**Butyrate**		
		Predictors	**β-Coef**	***p* value (adj.)**	**AICc**	**β-Coef**	***p* value (adj.)**	**AICc**	**β-Coef**	***p* value (adj.)**	**AICc**
Baseline Microbiota	All OTUs	PC1	2.86	**0.01(0.039)**							
		PC2			133.2	-4.15	0.03(0.056)	146.2	-1.21	0.05(0.07)	113.3
		PC3	2.77	0.03(0.06)							
	All CARGs	PC2	2.75	0.56(0.6)	141.1	-8.38	0.2 (0.24)	150	4.67	**0.02(0.048)**	111.3
	Sig. OTUs	PC1			134.5	-8.46	0.03(0.056)	150	-2.85	**0.02(0.049)**	115.6
		PC2	7.23	**0.02(0.049)**		2.30	0.58(0.64)		-0.80	0.52(0.55)	
	CARGs	CARG5	5.54	0.07(0.09)		10.16	**0.01(0.043)**				
		CARG6			139.0			144.2	-1.58	**0.014(0.043)**	110.3
		CARG7	1.43	0.03(0.056)							
	OTUs	OTU85 *B. obeum*	-21.86	0.03(0.056)							
		OTU46 *C. leptum*	-9.28	0.17(0.19)	139.5			147			110.7
		OTU41 *E. oxidoreducens*				-13.48	0.046(0.07)		-5.02	**0.016(0.047)**	
Shifts of Microbiota (Δ week 1 - baseline)	All OTUs	PC1	-2.66	0.03(0.06)							
		PC2	-2.48	0.07(0.09)	136.3			149.4			116.3
		PC3				3.35	0.14(0.17)		0.88	0.20(0.25)	
	All CARGs	PC1			132.13	-5.28	0.159(0.193)	152.9	-2.14	0.21(0.24)	120.62
		PC2	-9.57	**0.007(0.038)**		-7.22	0.33(0.375)		-0.038	0.98(0.98)	
	Sig. OTUs	PC1	-6.99	**0.011(0.039)**							
		PC2			133.2			144.4	2.67	0.08(0.10)	114.2
		PC3				12.00	**0.01(0.039)**				
	CARGs	CARG1	1.89	**0.0045(0.03)**	131.1			148.6			116.2
		CARG7				2.02	0.09(0.11)		0.482	0.23(0.25)	
	OTUs	OTU6 *P. copri*	-1.50	0.04(0.07)							
		OTU38 *P. succinatutens*	8.38	0.05(0.07)							
		OTU7 *B. uniformis*			138.67	10.32	**0.002(0.018)**	140.2			103.1
		OTU41 *E. oxidoreducens*							7.22	**0.0006(0.0097)**	
Shifts of Microbiota (Δ week 6 - baseline)	All OTUs	PC1	-4.23	**<0.0001(0.005)**							
		PC2	-1.86	0.046(0.07)	123.7	3.72	0.10(0.12)	148.7			118.0
		PC3							0.16	0.80(0.81)	
	All CARGs	PC2	-10.89	**0.001(0.014)**	128.95	-12.72	**0.018(0.048)**	144.95			108.98
		PC3							6.07	**0.007(0.038)**	
	Sig. OTUs	PC1	6.54	**0.0006(0.0097)**	125.0	8.05	**0.02(0.049)**	148.7			112.6
		PC2	6.40	**0.005(0.03)**		-3.92	0.35(0.38)		-3.14	0.039(0.067)	
	CARGs	CARG1	1.63	**0.0016(0.018)**		1.64	0.05(0.07)				
		CARG2			128.7	0.11	0.93(0.95)	151.6			109.4
		CARG3							2.13	**0.009(0.039)**	
	OTUs	OTU85 *B. obeum*	9.76	**0.007(0.038)**							
		OTU11 *Subdoligranulum* sp.			132.3	8.56	0.027(0.056)	145.8			108.4
		OTU41 *E. oxidoreducens*							8.48	**0.006(0.035)**	

[1] Each model contains the best one or two predictors of PCs, CARGs, or significant OTUs selected by stepwise regression. All models were adjusted by fiber dose/sex. Quality of each model was evaluated by corrected Akaike information criterion (AICc). adj., FDR adjusted *p* values; β-Coef: β-Coefficient; CARGs, co-abundance response groups; MLR, Multiple Linear Regression; PC, principle component; Sig. OTUs: significantly responding operational taxonomic units.

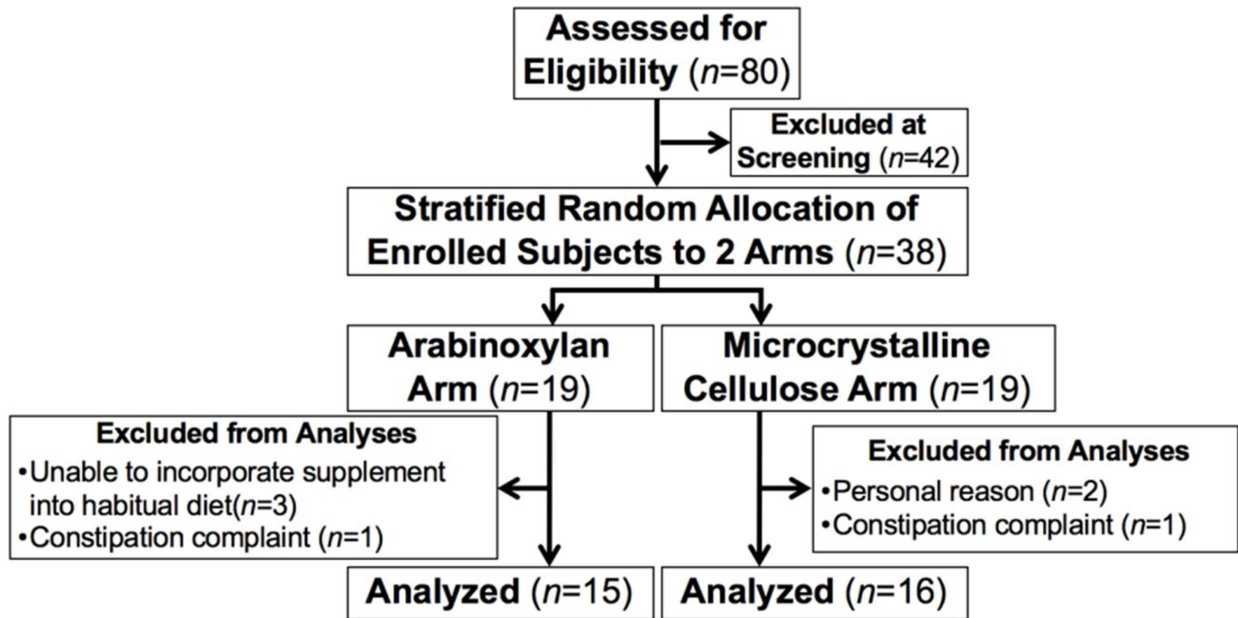

Figure S.4.1. Flow chart summarizing the flow of subjects through study.

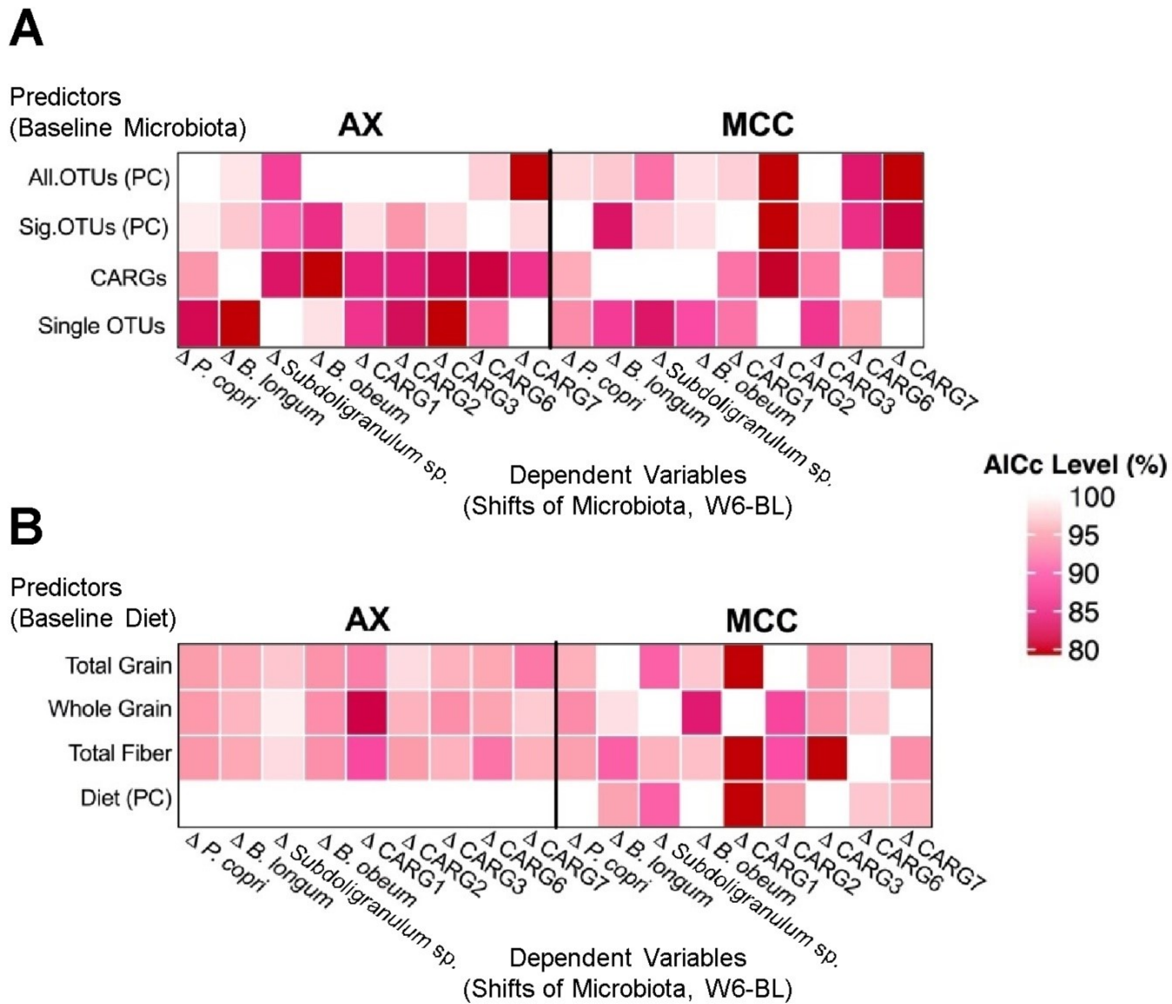

Figure S.4.2. Baseline fecal microbiota composition and diet showed no association with the individualized microbiota response to AX. (A) Heatmap shows the associations between microbiota compositional shifts (ΔW6–BL; dependent variables; columns) and baseline microbiota profiles (predictors; rows). **(B)** Heatmap shows the association between microbiota compositional shifts (ΔW6–BL; dependent variables; columns) and the baseline diet variables (predictors; rows). For both A and B, cells represent individual multiple linear regression models (with FDR correction) that assess whether the predictors explain the individualized compositional shifts. Multivariate microbiota and diet data were simplified into principal component (PC) variables PC1, PC2, and PC3 prior to analysis. Each model contained the best one or two predictors of PCs (microbiota and diet), individual CARGs, or significant OTUs (predictors selected by stepwise regression), or either total grains, whole grains, or total fiber alone. All models were adjusted by fiber dose/sex. Colors from white to red indicate relative AICc (corrected Akaike information criterion) values calculated by $\frac{AICc\ value}{Highest\ AICc\ value} x\ 100$. Lower AICc values (red) indicate higher quality models. AX; arabinoxylan; BL, baseline; CARG, co-abundance response group; MCC, microcrystalline cellulose; OTU, operational taxonomic unit; W1, week 1; W6, week 6.

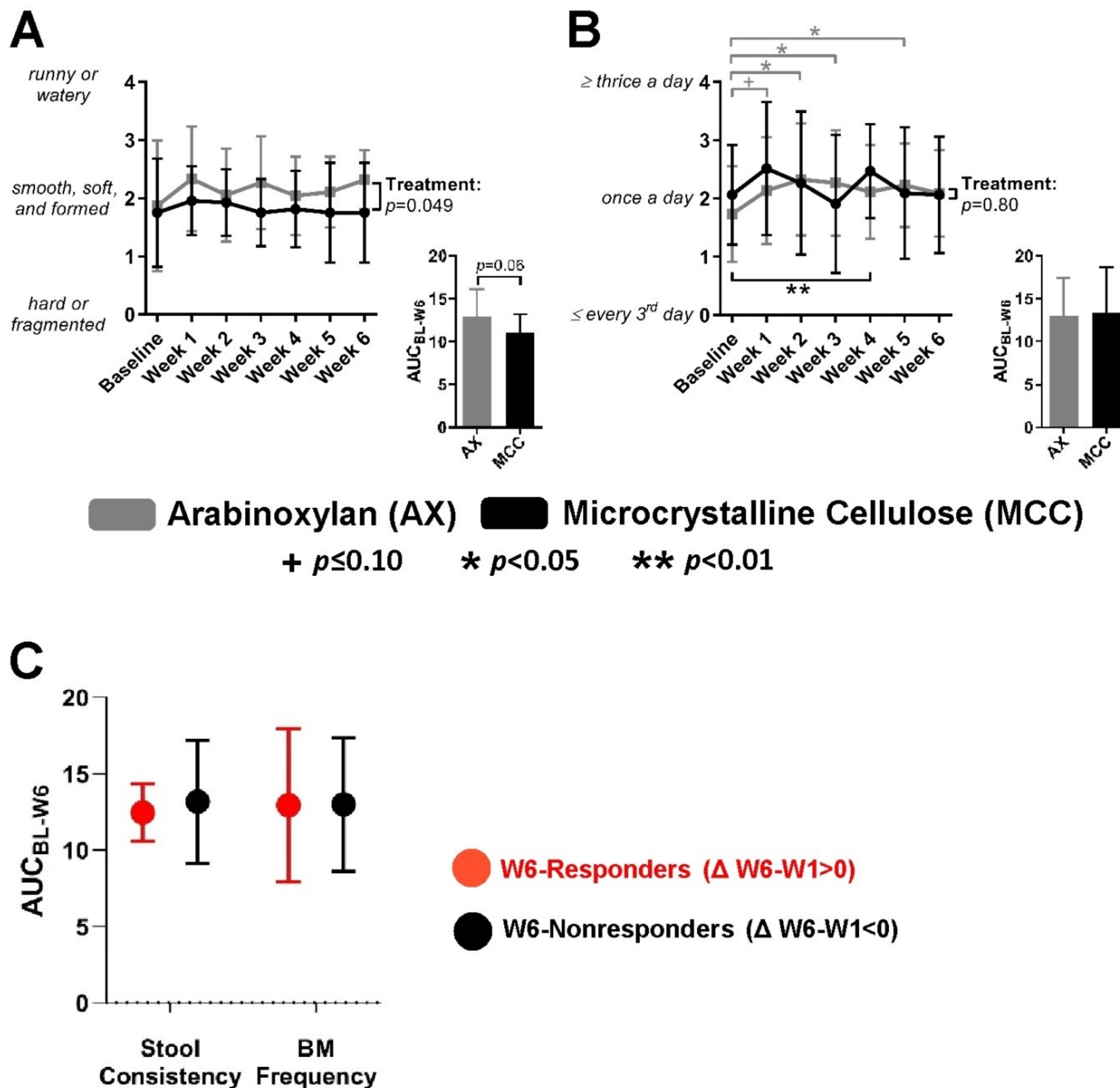

Figure S.4.3. Effects of AX and MCC on stool consistency and BM frequency. (A) Stool consistency and **(B)** BM frequency changes induced by fiber supplementation. For **(A and B)**, line graphs show weekly self-reported stool consistency and BM frequency ratings, respectively; reported as mean ± SD. For **(A and B)**, bar graphs (insets) show area under the curve values (AUC_{BL-W6}; mean ± SD). **(C)** Comparison between W6-responders (red) and W6-nonresponders (black) in stool consistency AUC_{BL-W6} and BM frequency AUC_{BL-W6}. Data analyzed for **(A and B)** by generalized estimating equation models and for **(A, B insets, and C)** by Mann-Whitney tests. BL, baseline; BM, bowel movement; MCC, microcrystalline cellulose; W1, week 1; W6, week 6.

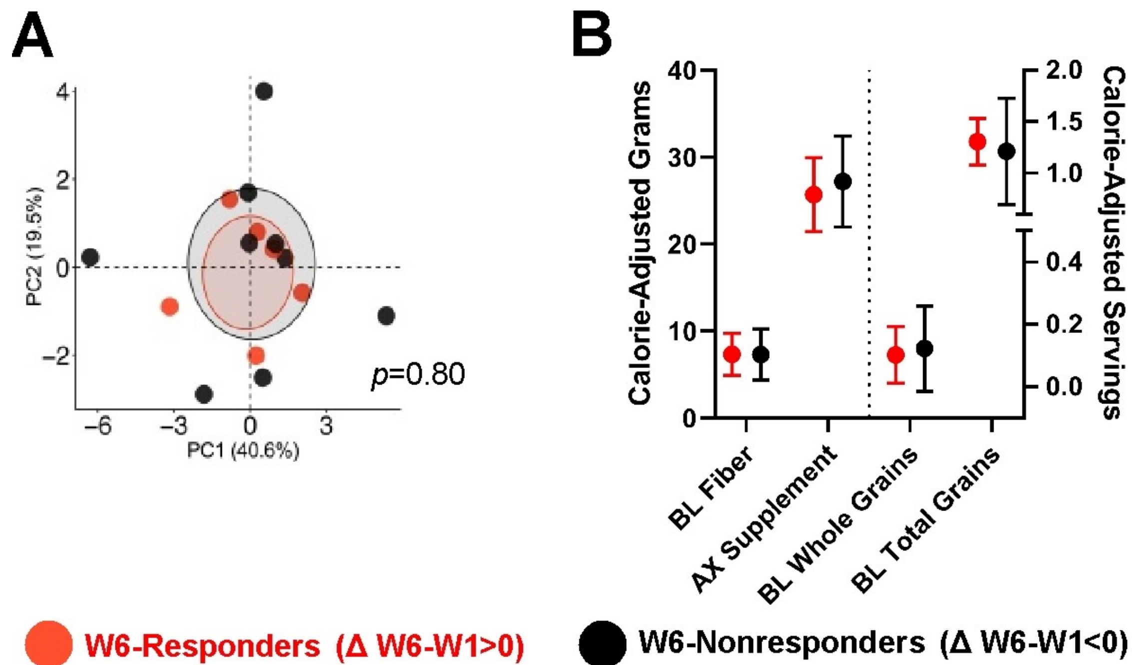

Figure S.4.4. Temporal propionate response to AX supplementation showed no association with baseline diet. (A) Principal component analysis plot based on Euclidean distance comparing the baseline, calorie-adjusted intake of Canada's 2007 Food Guide food group and macronutrient variables between W6-responders (red) and W6-nonresponders (black). Data were analyzed using PERMANOVA. **(B)** Comparison between W6-responders (red) and W6-nonresponders (black) in the calorie-adjusted intakes of single dietary factors (total grains, whole grains, total fiber, and AX supplement) performed using Mann-Whitney tests. AX; arabinoxylan; W1, week 1; W6, week 6.

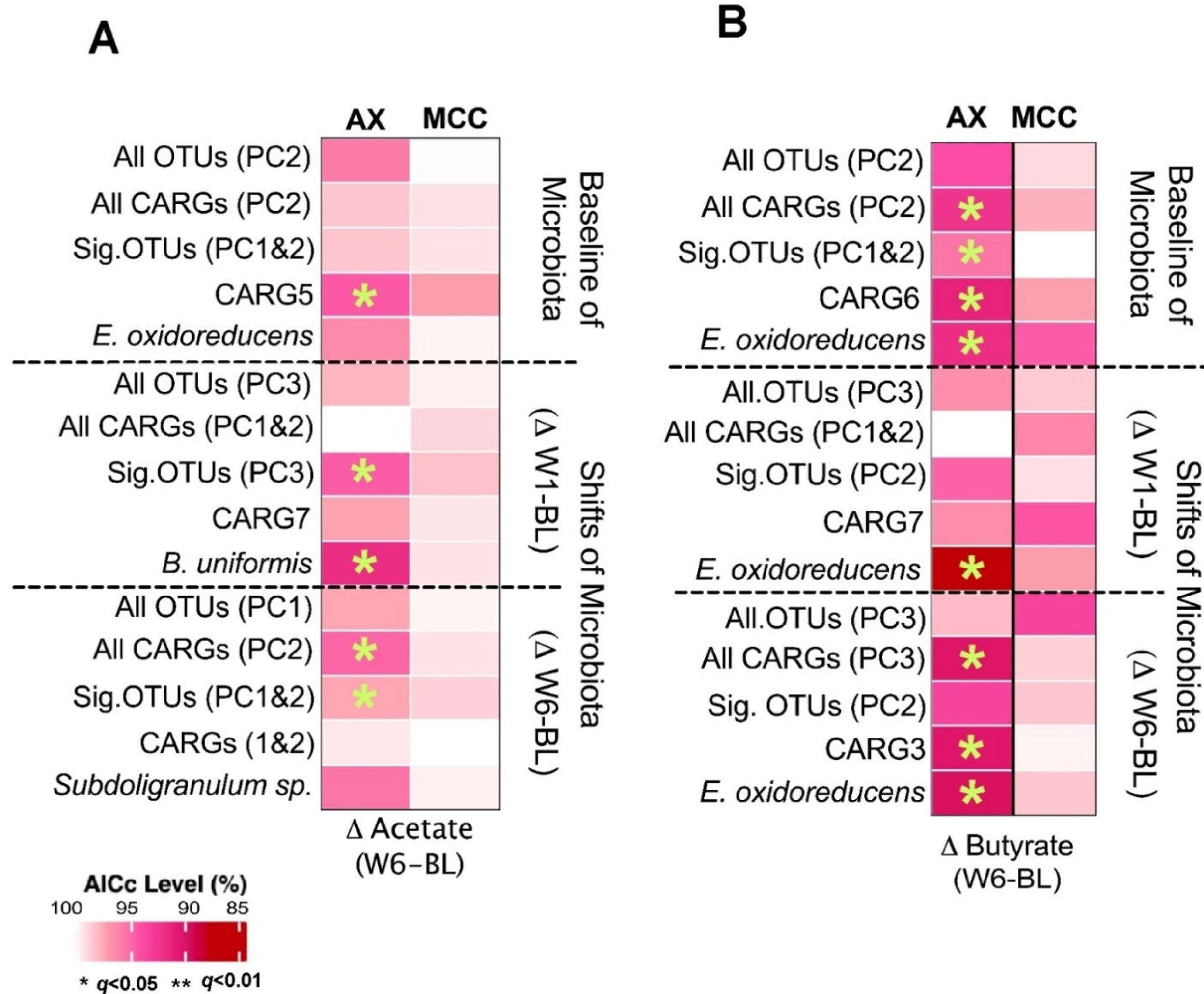

Figure S.4.5. Individualized acetate and butyrate response to arabinoxylan could be explained by baseline and shifts of the gut microbiota. Heatmap shows the associations between the individualized response of **(A)** acetate and **(B)** butyrate (ΔW6–BL; dependent variable; columns) and microbiota profiles (BL, ΔW1–BL, ΔW6–BL; predictors; rows). Cells represent individual multiple linear regression models (with FDR correction) that assess whether the predictors explain the individualized SCFA responses. Multivariate microbiota data were simplified into principal component (PC) variables PC1, PC2, and PC3 prior to analysis. Each model contained the best one or two predictors of PCs, individual CARGs, or significant OTUs selected by stepwise regression. All models were adjusted by fiber dose/sex. Colors from white to red indicate relative AICc (corrected Akaike information criterion) values calculated by $\frac{AICc\ value}{Highest\ AICc\ value} x\ 100$. Lower AICc values (red) indicate higher quality models. AX, arabinoxylan; BL, baseline; CARG, co-abundance response group; MCC, microcrystalline cellulose; OTU, operational taxonomic unit; W1, week 1; W6, week 6.

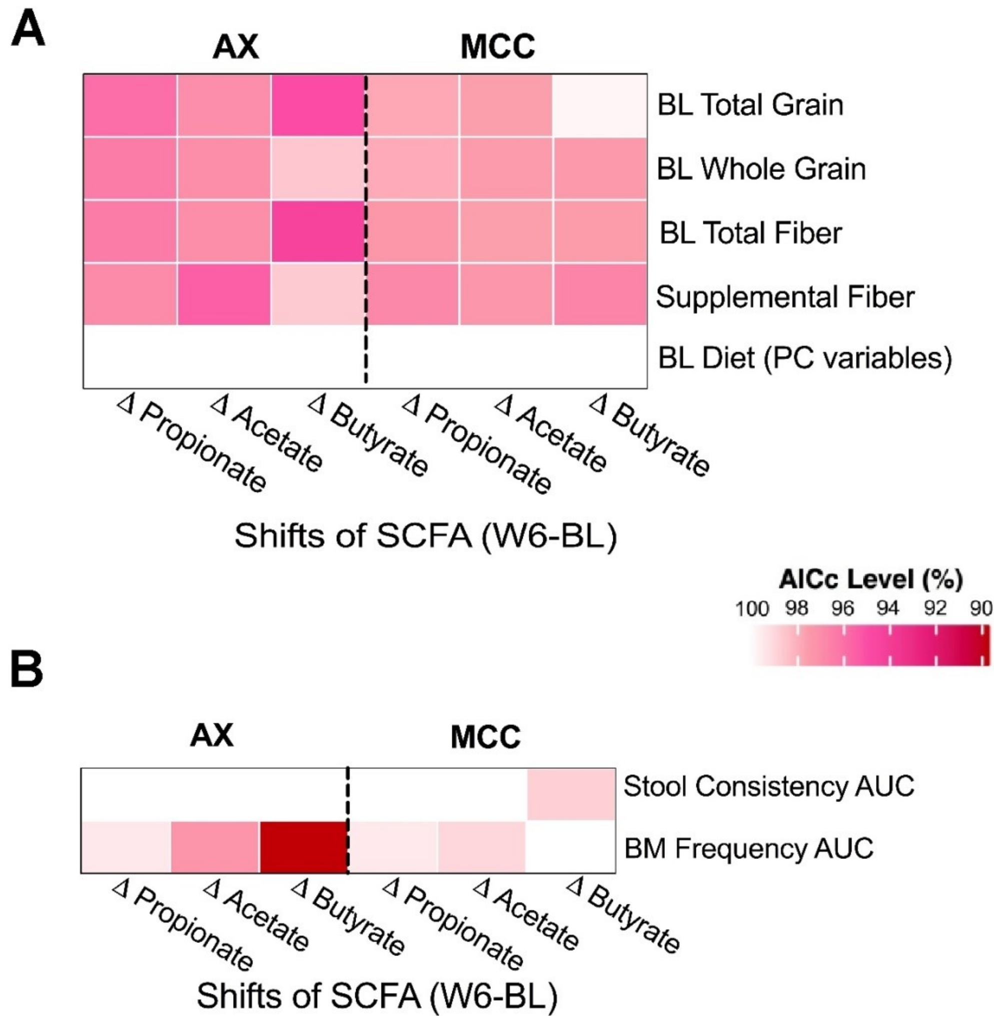

Figure S.4.6. Individualized SCFA response to AX could not be explained by baseline diet, stool consistency, or BM frequency during treatment. Heatmap shows the associations between the individualized SCFA response (acetate, propionate, butyrate; dependent variable; columns) and either **(A)** baseline diet or **(B)** stool consistency and BM frequency (predictors; rows). For A and B, 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 principal component (PC) variables PC1, PC2, and PC3 prior to analysis. Each model contained either the calorie-adjusted intakes of total grains, whole grains, total fiber, or total supplemental fiber; stool consistency or BM frequency; or the best one or two diet PCs as the predictors (PCs selected by stepwise regression). All models were adjusted by fiber dose/sex. Colors from white to red indicate relative AICc (corrected Akaike information criterion) values calculated by $\frac{AICc\ value}{Highest\ AICc\ value} x\ 100$. Lower AICc values (red) indicate higher quality models. AX, arabinoxylan; BL, baseline; BM, bowel movement; MCC, microcrystalline cellulose; SCFA, short-chain fatty acid; W6, week 6.

CHAPTER 5: Adaptation to tolerate high doses of arabinoxylan is linked to *Bifidobacterium longum* as a member of the human gut microbiota

5.1 Introduction

Dietary fiber (DF) is considered an important dietary component for the prevention of chronic diseases[267] and to ensure gut microbiome diversity and metabolic functionality[6,273]. Governmental and nutritional organizations encourage increased consumption of DF-rich whole foods to achieve recommended intakes of 25–38 g/day[12,275]. However, the average intake of DF in socioeconomically developed societies has remained at only half of what is recommended[275], resulting in a 'fiber gap'[12]. It has further been argued that the fiber gap might be even larger in light of the DF amounts consumed throughout human evolution, which likely impacted human physiology and its symbiotic inter-relationship with the gut microbiota[27,276,301]. In addition, suggestions have been made that higher doses of DF may be necessary for consistent health benefits[266,302], a notion supported by systematic reviews and meta-analyses[267,437]. DFs also offer exciting prospects for selective and targeted modulation of gut microbiota composition and metabolic functions relevant to health[36], but physiologically relevant changes to the gut microbiome may require higher doses[300,439]. Purified DFs can be used in foods or as supplements to reach appropriate levels of DF[27,276,277], but it remains unknown whether modern humans would tolerate DF amounts required for consistent health benefits and/or to induce physiologically relevant changes to the gut microbiota[300].

DFs remain largely intact until reaching the colon where they undergo differing degrees of fermentation by the microbiota, which results in the formation of short-chain fatty acids (SCFAs), lactic acid, and other organic acids that acidify the colonic environment[273], as well as gases such as H_2, CO_2, and CH_4[521]. Elevated colonic gas production leads to flatulence and also increased intestinal wall tension by raising intraluminal pressures, triggering the perception of bloating, abdominal discomfort, and related symptoms via colonic mechanoreceptor simulation[522,523]. Symptoms are dependent on DF molecular size and structure. Larger, more complex DF molecules, such as resistant starch, acacia gum, and long-chain arabinoxylans (AXs; a cereal derived DF[524]), have been shown *in vitro* to be fermented slower by fecal microbiota relative to inulin and resistant oligosaccharide molecules[447,525-527], which are tolerated less[528].

Accordingly, human trials have shown acceptable tolerance at supplementation doses around 40 g/day for resistant starch and acacia gum[439,529]. To my knowledge, the tolerance of long-chain AXs has not been assessed beyond 15 g/day[370], and limited knowledge exists on how gastrointestinal tolerance is linked to the gut microbiome.

Although symptoms as a result of DF fermentation are for the most part inevitable, limited evidence suggests that humans can, at least to some degree, 'adapt' to sustained DF consumption, a process proposed to involve the gut microbiota[530]. While symptom improvements are not typically investigated in DF intervention trials, previous studies supplementing with inulin[531], acacia gum[532], partially hydrolyzed guar gum[533], and NUTRIOSE® FB[325] have observed symptom adaptations within two to four weeks of treatment. In addition, Mego and colleagues have shown that self-reported flatulence and the number of gas evacuations decreased within two-weeks of galactooligosaccharide treatment[530]. These improvements stemmed predominantly from reductions in the volume of intestinal gas produced[534], with lower volumes of evacuated gas being associated with higher abundances of *Methanobrevibacter*, *Lachnospiraceae*, and *Clostridiaceae*[530]. However, the dose of this study was, with 2.8 g/day, lower than what might be required for physiological and maximum bifidogenic effects[73,535]. Therefore, whether humans can adapt to higher, more relevant supplementation doses and the factors that determine these responses (*e.g.* the gut microbiota), remains insufficiently understood.

In **Chapter 4 and Appendix A**, the effects of long-chain corn bran AX at daily doses of 25 and 35 grams (for women and men, respectively) on health[536] and the gut microbiota[537] were assessed using an exploratory randomized controlled trial (RCT) in individuals with overweight and obesity. These studies revealed that AX exerted global changes to fecal bacterial community composition; promoted a range of bacterial taxa such as *Bifidobacterium longum*, *Blautia obeum*, *Subdoligranulum* sp., and *Prevotella copri*; increased fecal propionate concentrations[537]; and improved perceived satiety and measures of glucose homeostasis[536]. In **Chapter 5**, this work was extended to evaluate the severity of gastrointestinal symptoms during high-dose AX supplementation, determined to what degree humans adapted to tolerate AX, and explored whether microbiota- and dietary-related factors associate with interpersonal differences in AX tolerance.

5.2 Methods

This six-week, parallel two-arm, exploratory RCT was registered with ClinicalTrials.gov, registry number NCT02322112, as part of a large four-arm RCT referred to as: The Alberta FYBER (Feed Your gut Bacteria morE fibeR) Study. The RCT aimed to compare the effects of an AX, acacia gum, resistant starch type-IV, and microcrystalline cellulose (MCC) on the gut microbiota and human health (original registration[444]). In response to requests by reviewers of a grant application, the AX arm was separated from the original RCT and data from the 15 protocol completers were analyzed independently. Study procedures were approved by the University of Alberta Health Research Ethics Board, identifier Pro00050274, with written informed consent obtained prior to participant enrollment (for study procedures refer to **Chapter 4**).

5.2.1 Study Design and Subjects

Thirty-eight volunteers with overweight or class-I obesity were enrolled in the study and instructed to supplement their diet, over six-weeks, with either AX or MCC at a daily dose of 25g (females) or 35g (males) (**Figure 5.1**). AX was BIO-FIBER GUM, a fermentable long-chain AX isolated from corn bran (Agrifiber Holdings LLC, Illinois, USA), while the non-fermentable control was MICROCEL MC-12, a large particle wood-derived MCC (Blanver Farmoquimica LTDA, São Paulo, Brazil)[537]. Thirty-one subjects aged 33 ± 9 years and body mass index 28.7 ± 2.3 kg/m^2 completed the intervention and were analyzed per-protocol, which consisted of 21 females and 10 males (AX arm: 10F and 5M; MCC arm: 11F and 5M; **Table S.5.1**). On average, the total intake of DF was increased during the intervention from 19 ± 5 and 21 ± 11 g/day to 40 ± 5 and 56 ± 10 g/day for females and males, respectively (assessed by two 24-hr recalls; **Appendix A**).

5.2.2 Assessment of Habitual Diet at Baseline

Diet history was assessed at baseline using the online one-month Canadian Diet History Questionnaire II (C-DHQ II), a food frequency questionnaire adapted for Canada[449]. C-DHQ II responses were analyses using Diet*Calc software (v1.5.0) and an updated C-DHQ II nutrient database, which included eight additional food group variables that align with Canada's 2007 Food Guide serving-size-equivalents[450]. Prior to statistical integration with gastrointestinal symptoms, C-DHQ II data were adjusted for total caloric intake[452].

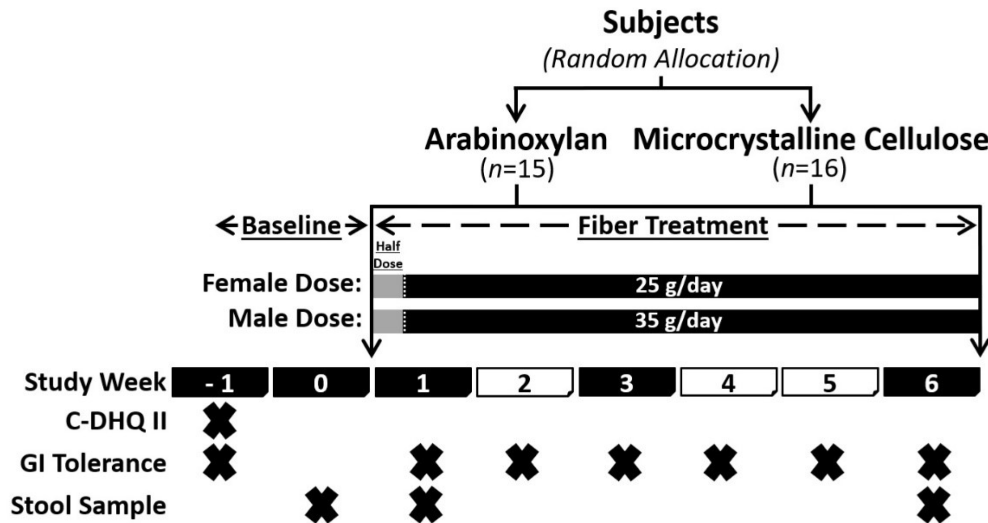

Figure 5.1. Study design. The 'X' indicates that the specific task was completed during the study week. C-DHQ II; Canadian Diet History Questionnaire II; GI, gastrointestinal.

5.2.3 Assessment of Perceived Gastrointestinal Tolerance

Participants reported gastrointestinal symptoms at baseline and then weekly during the intervention by completing a symptoms diary. At the end of each week, subjects rated their overall symptoms, flatulence, bloating, and stomach ache intensity using a scale from 0 (no symptoms) to 4 (severe symptoms)[69]. A composite symptom rating was calculated by summing flatulence, bloating, and stomach ache ratings, resulting in a possible range from 0 to 12 (higher ratings corresponded to less tolerance).

Two different approaches were used to quantify the severity of symptoms in response to AX and the degree of adaptation for each subject: absolute change (MAX) and area under the curve (AUC) (**Figure 5.2.A**). To calculate MAX severity, baseline values were subtracted from the highest reported rating during weeks 1 to 5 for each subject, where higher scores represent more intense symptoms. To calculate MAX adaptation, week 6 ratings were subtracted from the highest reported rating between weeks 1 and 5, where higher scores represent greater reductions in symptom intensity. For AUC analyses, $AUC_{severity}$ was calculated by computing the AUC from weeks 1 to 6, where higher scores mean more severe symptoms during the six-week intervention relative to baseline (**Figure 5.2.A**). $AUC_{adaptation}$ was calculated by determining the ratio between the AUC from weeks 1 to 3 divided by the AUC from weeks 4 to 6 ($AUC\ adaptation = \frac{AUC\ from\ weeks\ 1\ to\ 3}{AUC\ from\ weeks\ 4\ to\ 6}$), where higher scores equal better adaptation during the final three weeks of treatment.

5.2.4 Fecal Microbiota, pH, and SCFA Analyses

Findings from 16S rRNA gene amplicon profiling of fecal microbiota and the characterization of fecal pH and SCFAs have been discussed in **Chapter 4**. In **Appendix A**, we also determined which bacterial taxa were actively involved in AX degradation using an *ex vivo* approach based on bioorthogonal non-canonical amino acid-tagging (BONCAT). Briefly, this approach fluorescently labeled metabolically active bacteria during 6-hr anaerobic incubation with AX and *L*-azidohomoalanine, a marker of cellular activity. Then, active bacteria were isolated using fluorescence-activated cell-sorting. 16S rRNA gene amplicon sequencing and a bioinformatic analysis based on amplicon sequence variants (ASVs)[538] was used to determine which bacterial taxa utilized AX (see **Appendix A** for details). 16S rRNA gene amplicon data are available for download at the NCBI Sequence Read Archive under BioProjects: PRJNA564636 (fecal) and PRJNA630848 (*ex vivo*).

5.2.5 Statistical Analyses

Generalized estimated equation (GEE) models with Bonferroni post-hoc tests were applied to the gastrointestinal symptom and composite ratings to determine differences between-groups and within-group differences relative to baseline. Differences between AX and MCC for the calculated MAX and AUC severity scores were determined by Mann-Whitney tests. To test for adaption to AX and MCC, Bonferroni corrected Wilcoxon tests were applied to determine differences between the highest rating reported during weeks 1 to 5 and the week 6 rating, as well as the AUC of weeks 1 to 3 ratings and weeks 4 to 6 ratings.

To determine whether fecal microbiota composition, pH, SCFAs, or dietary intake correlated with symptom severity or adaptation, Spearman's correlations were applied. Significant associations were first identified by correlating flatulence, bloating, stomach ache, and composite severity scores with microbiota compositional variables measured in fecal samples during the intervention. As fecal samples were collected in weeks 1 and 6, symptoms during weeks 1 to 3 were hereby correlated with measurements in week 1 fecal samples, and symptoms during weeks 4 to 6 were correlated with measurements in week 6 fecal samples. For these analyses, α-diversity indices and all bacterial phyla, families, genera, and co-abundance response groups (CARGs; groups of inter-correlated operational taxonomic units [OTUs][537]) with average relative abundances above 0.15% (considering all fecal samples) were systematically assessed, while only OTUs significantly affected by AX (henceforth referred to as

'significant OTUs') were considered to reduce the chance of type I error from multiple comparisons (*i.e.* 15 OTUs instead of 100). Significant OTUs and CARGs were evaluated further to determine if their relative abundance at baseline correlated with MAX and AUC severity and adaptation scores. AX-induced shifts (week 6 – baseline) of significant OTUs, CARGs, pH, and SCFAs were correlated with severity and adaptation scores to assess whether microbial responses to AX relate to symptoms. Finally, to elucidate whether tolerance to AX is linked to bacterial taxa that are actively involved in the utilization of AX, correlations were assessed between severity and adaptation scores and the most abundant (average relative abundance >1.0%) bacterial ASVs within the active bacterial consortia as established by BONCAT.

Given the connections between habitual intake of animal- and plant-based diets and gut microbiome composition[539] and that AXs constitute the main non-cellulose DF in cereal grains[524], we further investigated whether calorie-adjusted intakes - during the month prior to treatment - of meat/meat alternatives (meat/alt; included eggs, legumes, nuts, and seeds), cholesterol (a nutrient found only in animal-based foods), whole grains, and DF, or the ratio between these food groups or nutrients, correlated with severity and adaptation scores. All statistical analyses were performed using GraphPad Prism v8.4.3, apart from GEE models, which were performed using R v3.5.3. Statistical significance was considered at $p<0.01$ for correlations with microbiota compositional data (to account for multiple comparisons), and at $p<0.05$ for the remaining analyses.

5.3 Results

5.3.1 Severity of Symptoms During AX and MCC Administration

Although AX was on average well tolerated (average scores were < 2 points), overall symptoms, flatulence, bloating, stomach ache, and composite ratings were significantly higher when compared to subjects consuming MCC (treatment effect $p<0.05$, GEE models; **Figures 5.2.B and S.5.1.A**). Considerable collinearity was detected between AX-induced symptoms; for instance, flatulence positively correlated with bloating ($r_s=0.55$, $p<0.0001$; **Figure S.5.2.A**). Comparison of MAX severity scores (*i.e.* highest symptom rating) between AX and MCC treatments showed that flatulence, bloating, and composite ratings increased during AX consumption as compared to MCC ($p<0.05$, Mann-Whitney tests; **Figures 5.2.C and S.5.1.B**). Accordingly, flatulence $AUC_{severity}$ scores (*i.e.* overall flatulence severity during the intervention)

were higher for AX relative to MCC (*p*=0.045), with differences in composite $AUC_{severity}$ also approaching statistical significance (*p*<0.1) (**Figure 5.2.C**).

5.3.2 Symptom Adaptation During AX Administration

Comparison of the most intense symptoms reported to symptoms at week 6 revealed that overall symptoms, flatulence, bloating, stomach ache, and composite ratings improved by the sixth week of AX consumption (*p*<0.05, Wilcoxon tests; **Figure 5.2.D**). Evaluating differences between weeks 1 to 3 and weeks 4 to 6 symptoms further indicated that flatulence and composite ratings began to improve after three weeks of AX (*p*<0.05; **Figure 5.2.D**). These findings suggest that although corn bran AX, at doses of 25 g/day and 35 g/day, led to moderate yet significantly increased symptoms and primarily flatulence, effects were temporary as most subjects 'adapted' to AX within six weeks of sustained consumption.

5.3.3 Inter-Individual Differences in Gastrointestinal Symptoms

Although significant increases in gastrointestinal symptoms were detected in the study cohort, symptom severity and the degree of adaptation were highly individualized (**Figures 5.3 and S.5.1.D**). For instance, MAX composite severity scores were increased by ≤ 2 out of 12 points for 40% of subjects, while 33% reported scores ≥ 4. For most individuals (80%), composite ratings reverted to baseline, as MAX composite severity and adaptation scores were equivalent (*i.e.* difference of ≤ 1 point). However, for the two subjects that reported the most intense symptoms, symptoms did not recover completely (*i.e.* F4 reduced from 10 to 6 points; M24 reduced from 9 to 6 points).

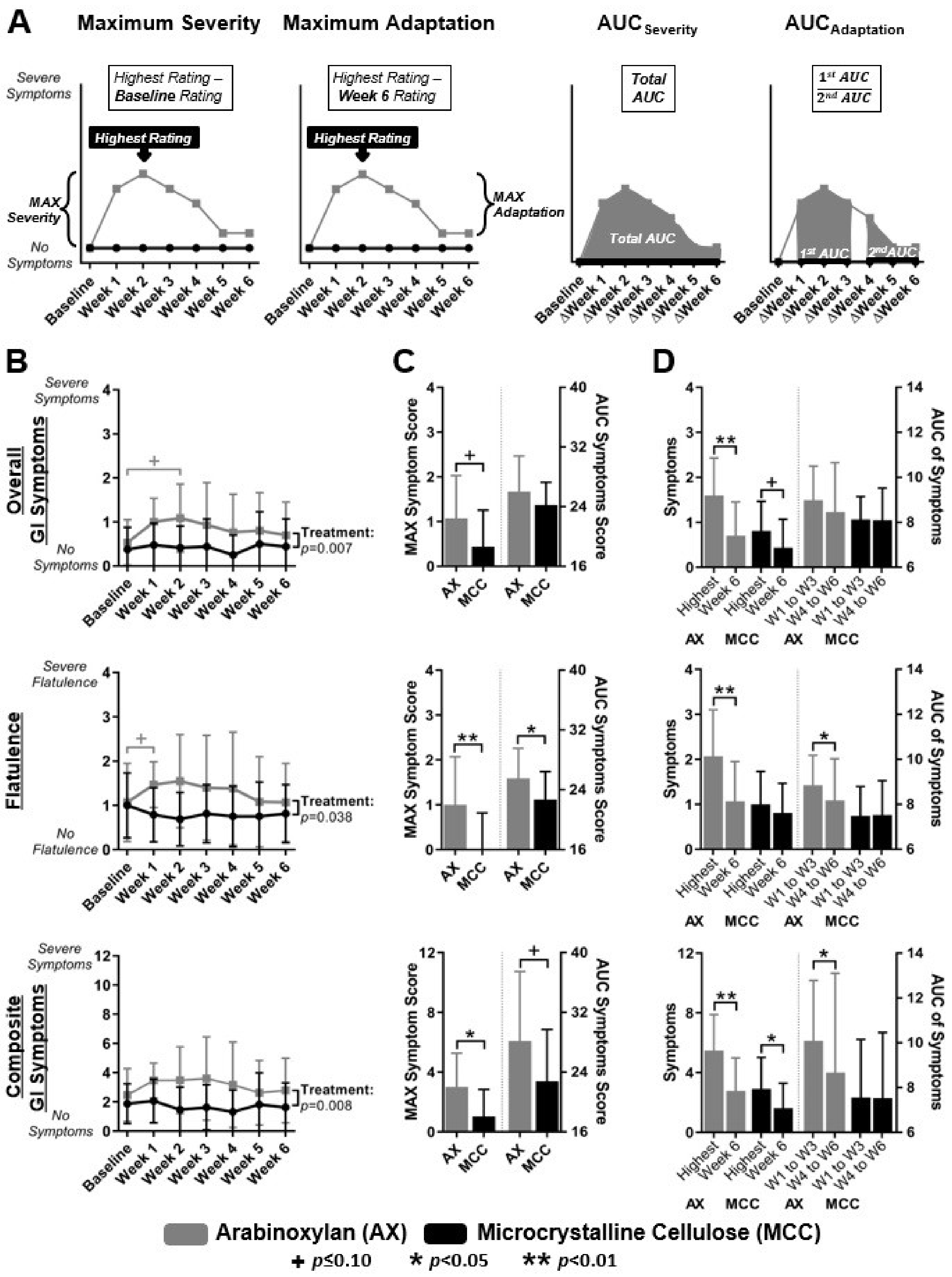

Figure 5.2. Symptoms in response to AX consumption and adaptation towards AX as compared to MCC consumption. *Legend continued on the next page.*

(A) Graphical representation of how severity and adaptation scores were determined. MAX severity and adaptation scores reflect the highest score between weeks 1 and 5 minus either baseline (severity) or week 6 (adaptation). $AUC_{severity}$ scores reflect the AUC from weeks 1 to 6, while $AUC_{adaptation}$ scores reflect the ratio between the AUC from weeks 1 to 3 and weeks 4 to 6. **(B)** Overall symptoms, flatulence, and composite symptoms (sum of flatulence, bloating, and stomach aches; see **Figure S.5.1** for bloating and stomach ache data) during AX and MCC consumption. **(C)** MAX and AUC symptom scores for AX and MCC. **(D)** Highest symptom during weeks 1 to 5 and week 6 symptoms for AX and MCC, as well as the AUC of symptoms from weeks 1 to 3 and weeks 4 to 6. Data in **(B)** were analyzed using GEE models with Bonferroni corrections, in **(C)** using Mann-Whitney tests, and in **(D)** using Wilcoxon tests with Bonferroni corrections. Data reported as mean ± SD. Statistical significance was considered at $p<0.05$. AUC, area under the curve; GI, gastrointestinal.

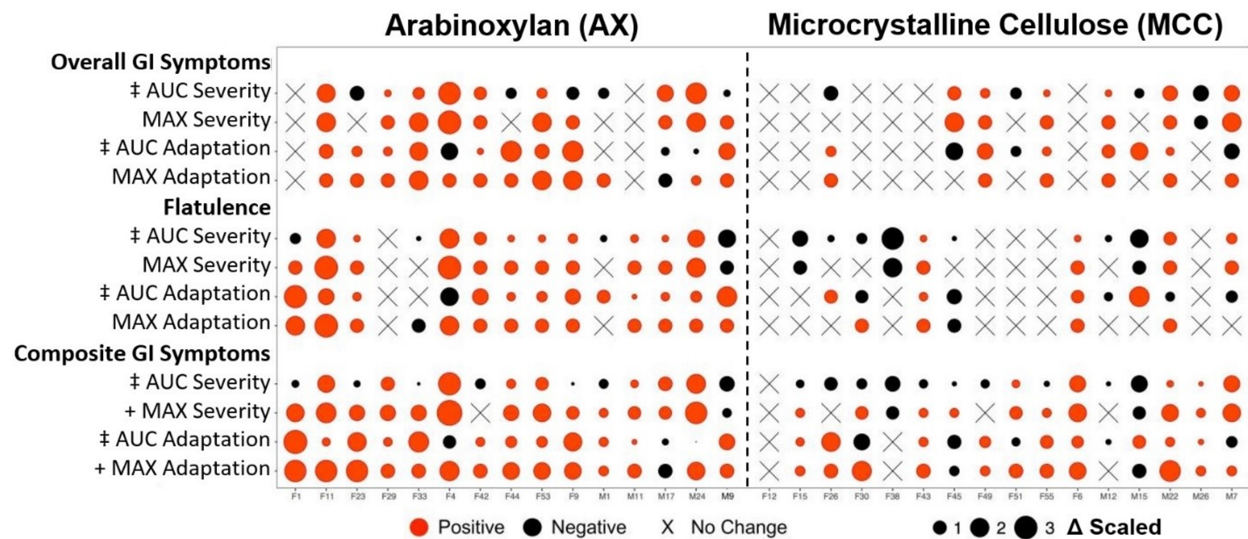

Figure 5.3. Bubble plots showing individualized symptom severity and adaptation scores during AX and MCC consumption. Red circles represent an increase, black circles represent a decrease, and 'X' represents no change in the feature during the intervention. The circle size is proportional to the scaled magnitude change relative to baseline or week 6. ‡ Feature scaled by ($AUC_{severity}$–24/SD) or ($AUC_{adaptation}$–1/SD), + Feature scaled by (MAX/SD). AUC, area under the curve; GI, gastrointestinal.

5.3.4 Links Between Symptom Severity and Fecal Microbiota Composition During AX Administration

Symptoms linked to increased DF consumption such as flatulence and bloating are likely the result of its fermentation by the gut microbiota[521], which shows substantial inter-individual variation[67,69,438]. We, therefore, performed a systematic analysis between the severity of gastrointestinal symptoms during AX and MCC supplementation and microbiota features, specifically α-diversity and the relative abundance of bacterial taxa at different taxonomic levels.

Spearman's correlation analyses also included CARGs, which are groups of inter-correlated OTUs[537], since bacteria collaborate during DF fermentation and engage in complex cross-feeding interactions within what can be considered ecological guilds[540]. As fecal samples were collected in weeks 1 and 6, severity scores from weeks 1 to 3 were correlated with measurements in week 1 samples, and severity scores from weeks 4 to 6 were correlated with measurements in week 6 samples.

The analysis showed that neither α-diversity, assessed by Shannon index, nor the total number of OTUs associated with symptom severity ratings for AX ($p>0.01$; **Figure 5.4.A**). Analysis at phylum-level revealed that during AX consumption, the abundance of Actinobacteria negatively correlated with composite ($r_s=-0.53$, $p=0.003$) and bloating ($r_s=-0.53$, $p=0.003$) severity, while Bacteroidetes positively correlated with bloating severity ($r_s=0.53$, $p=0.002$) (**Figure 5.4.A**). At lower taxonomic levels, the family *Bifidobacteriaceae* ($r_s=-0.52$, $p=0.003$; **Figure 5.4.A**) and genus *Bifidobacterium* ($r_s=-0.52$, $p=0.003$; **Figure 5.4.B**) negatively correlated with composite severity, while negative correlations with both bloating and stomach ache severity approached statistical significance ($p<0.05$). In contrast, positive correlations were detected between the family *Porphyromonadaceae* and composite ($r_s=0.52$, $p=0.003$) and bloating ($r_s=0.50$, $p=0.005$) severity; with *Odoribacter* and *Parabacteroides* genera also showing positive correlations with composite severity that approached statistical significance ($p<0.05$).

For OTUs and CARGs, analyses were focused on only AX-responsive OTUs (termed 'significant OTUs'[537]) to avoid type-1 error, while all seven CARGs were included. This analysis revealed that the relative abundance of *B. longum* (OTU4) during AX consumption negatively correlated with composite ($r_s=-0.48$, $p=0.007$) and bloating ($r_s=-0.54$, $p=0.002$) severity (**Figure 5.4.C**). The relative abundance of CARG1 - the CARG dominated by *B. longum* - was also shown to negatively correlate with composite severity ($r_s=-0.53$, $p=0.003$), while negative correlations for both bloating and stomach ache severity approached statistical significance ($p<0.05$). The above-mentioned correlations were not detected during MCC consumption ($p>0.1$; **Figures 5.4.A to 5.4.C**), which might indicate that these associations are related to symptoms induced by AX fermentation. Overall, these findings suggest that a higher abundance of *B. longum* during AX supplementation designates better gastrointestinal tolerance.

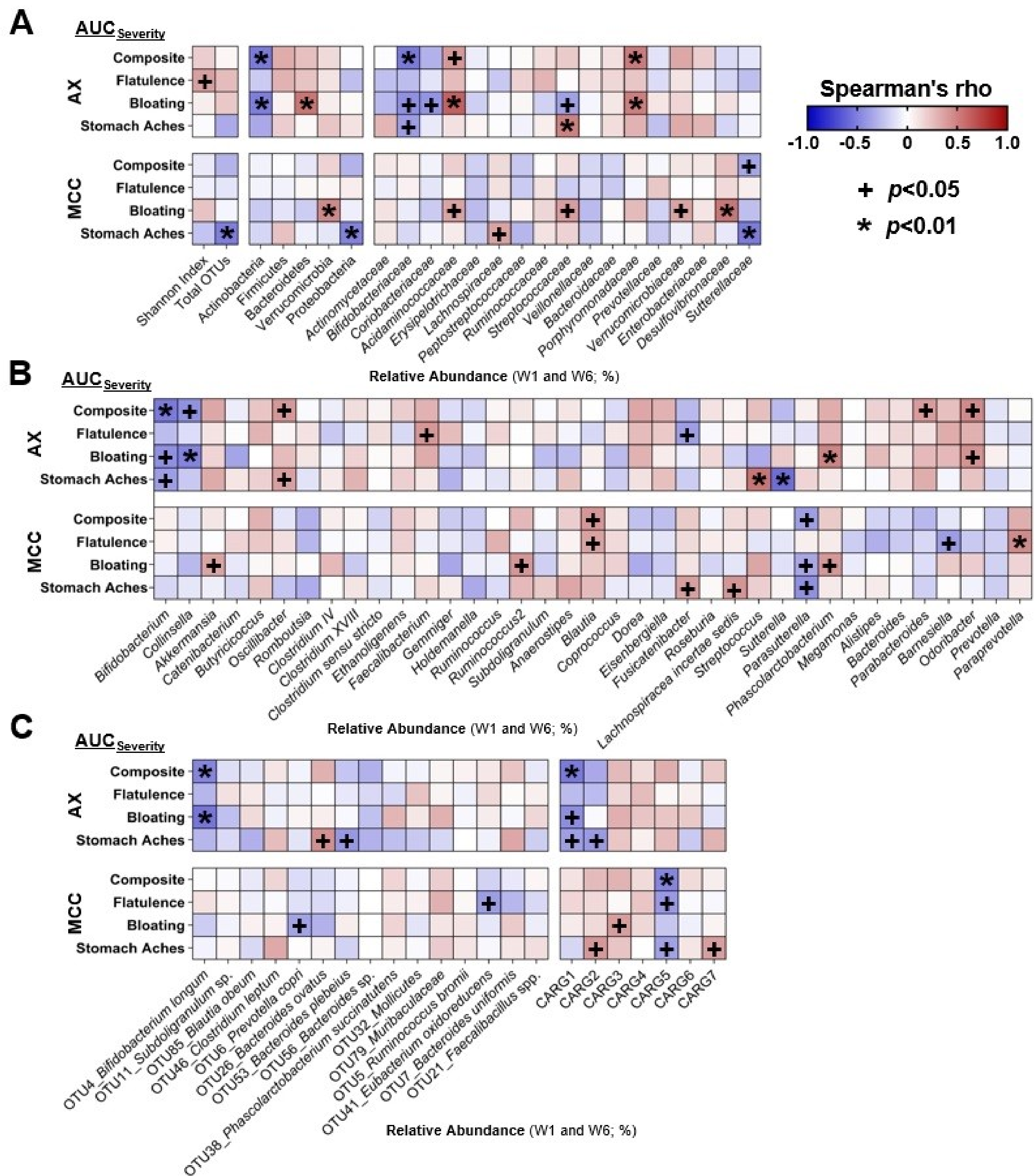

Figure 5.4. Associations between symptom severity and bacterial abundance during AX and MCC consumption. Heatmaps show Spearman's correlations between the composite, flatulence, bloating, and stomach ache AUC$_{severity}$ scores (segmented into weeks 1 to 3 and weeks 4 to 6) and the **(A)** α-diversity indices plus all bacterial phyla, families, **(B)** genera, and **(C)** CARGs with average relative abundances above 0.15%, and AX-responsive OTUs (weeks 1 and 6). Statistical significance was considered at *p*<0.01. AUC, area under the curve; AX, arabinoxylan; CARG, co-abundance response group; MCC, microcrystalline cellulose; OTU, operational taxonomic unit.

5.3.5 Severity and Adaptation Scores Correlated with Baseline Microbiota Composition, AX-Induced Shifts, and Bacterial Taxa Utilizing AX

To evaluate if the tolerance of AX is pre-determined by the baseline microbiota, we assessed whether severity and adaptation scores associated with relative pre-treatment abundances of the significant OTUs and CARGs. This analysis revealed that greater relative abundances of *B. longum* (OTU4; r_s=-0.67, p=0.007) and CARG1 (r_s=-0.67, p=0.008) prior to AX supplementation associated with lower bloating $AUC_{severity}$ scores (**Figure S.5.3**), with composite $AUC_{severity}$ also showing a tendency to be lower (p<0.05; **Figure 5.A**). Relative pre-treatment abundances of *B. longum* (OTU4; r_s=0.74, p=0.002) and CARG1 (r_s=0.77, p=0.001) were also positively associated with better composite $AUC_{adaptation}$ scores (**Figure 5.5.B**).

Next, we determined whether AX-induced shifts (week 6 – baseline) of significant OTUs and CARGs correlated with severity and adaptation scores. While associations were not detected for *B. longum* (OTU4) or CARG1 (p>0.05; **Figures 5.5.C and 5.5.D**), enrichment of *Subdoligranulum* sp. (OTU11) by AX was associated with higher composite $AUC_{adaptation}$ scores (r_s=0.68, p=0.007), with bloating and stomach ache $AUC_{adaptation}$ scores also showing a tendency to be higher (p<0.05) (**Figure S.5.4**).

Finally, to determine associations between taxa that are actively involved in the utilization of AX, severity and adaptation scores were correlated with the most abundant ASVs (average relative abundance >1.0%) identified by BONCAT. This analysis revealed that only the relative abundance of *B. longum* (ASVic9xvj), within the active consortia, positively associated with composite (r_s=0.69, p=0.006; **Figure 5.5.F**) and bloating (r_s=0.68, p=0.006; **Figure S.5.5**) $AUC_{adaptation}$ scores. Taken together, the above findings suggest that the adaptation to tolerate AX at high-doses is influenced more by the relative pre-treatment abundances of specific AX degrading microbes such as *B. longum*, than by AX-induced changes in community membership.

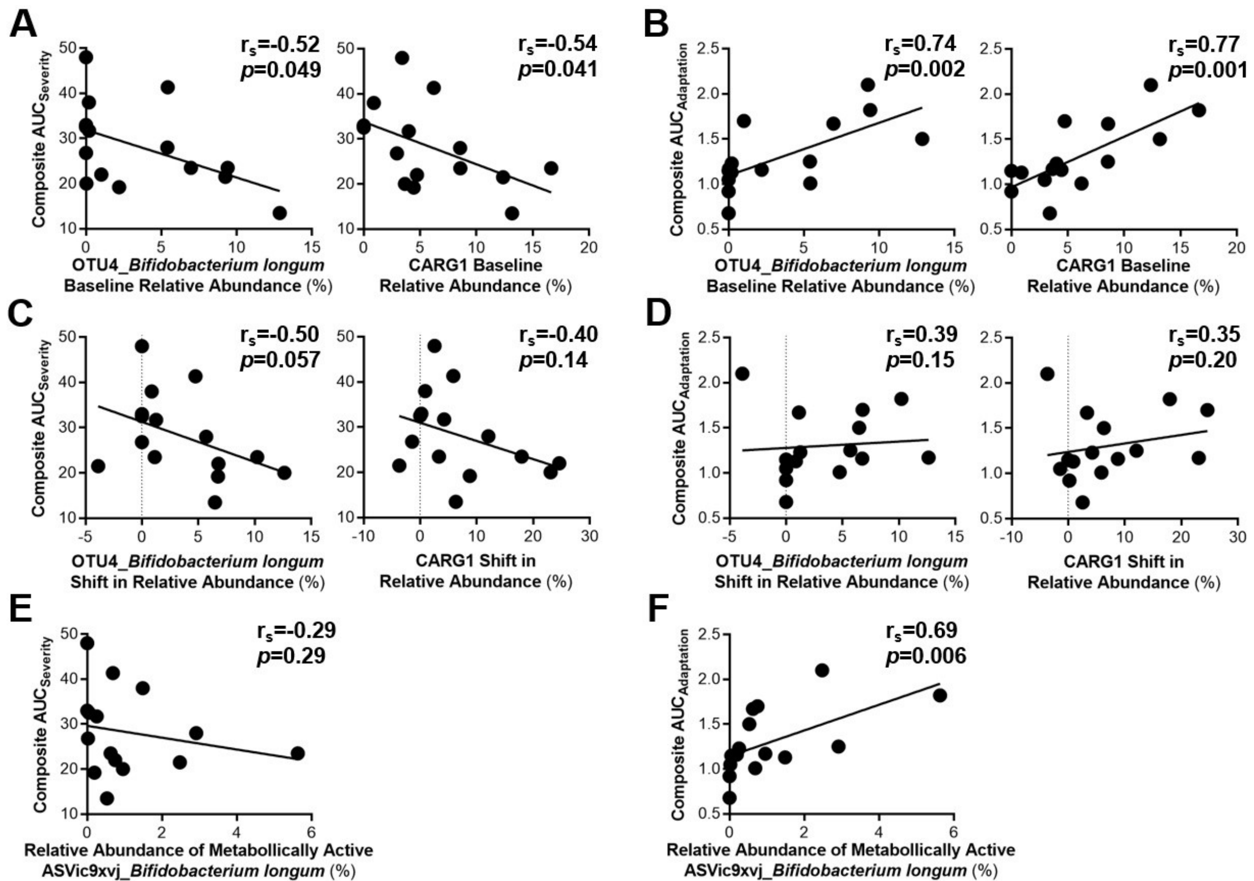

Figure 5.5. Improved composite scores during AX consumption were linked to the relative abundance of *Bifidobacterium longum*. Spearman's correlations between *B. longum*, CARG1, and AX-induced composite $AUC_{severity}$ (left) and $AUC_{adaptation}$ (right) scores. Correlations were performed on **(A and B)** baseline or **(C and D)** shifts in the relative fecal abundance of *B. longum* (OTU4) and CARG1, or **(E and F)** the relative *ex vivo* abundance of *B. longum* (ASVic9xvj). For correlations with flatulence, bloating, and stomach ache scores see **Figures S.5.3 to S.5.5**. The best-fitting line is the linear regression line. Statistical significance was considered at $p<0.01$. ASV, amplicon sequence variant; AUC, area under the curve; AX, arabinoxylan; CARG, co-abundance response group; OTU, operational taxonomic unit.

5.3.6 Tolerance to AX Relates to Changes in Fecal pH and SCFAs

In **Chapter 4**, AX was shown to increase fecal propionate (overall effect $p=0.015$, Friedman's test), while remaining SCFAs and fecal pH did not change (overall effect $p>0.1$)[537]. Given the substantial variation in these parameters, we asked whether inter-subject differences in severity and adaptation were linked to different shifts in fecal pH (week 6 – baseline). This analysis showed that fecal acidification was associated with both lower composite $AUC_{severity}$ for AX ($r_s=0.54$, $p=0.039$; **Figure 5.6.A**) and bloating $AUC_{severity}$ for MCC ($r_s=0.54$, $p=0.034$; **Figure 5.6.B**). Fecal acidification was also associated with greater abundances of *B. longum*

(OTU4) during AX consumption (r_s=-0.44, p=0.016; **Figure S.5.6.A**), a correlation that approached statistical significance for MCC (r_s=-0.30, p=0.093; **Figure S.5.6.B**).

Analysis of fecal SCFA shifts further revealed positive correlations between composite $AUC_{adaptation}$ scores and total SCFAs (r_s=0.55, p=0.036) and acetate (r_s=0.54, p=0.039) (**Figure 5.6.C**). Stomach ache $AUC_{adaptation}$ scores were also correlated positively with total SCFAs (r_s=0.60, p=0.021), acetate (r_s=0.59, p=0.022), and butyrate (r_s=0.57, p=0.027). Although no associations were detected between pH and SCFA shifts (p>0.1, data not shown), positive correlations that approached statistically significance were detected between acetate shifts and *B. longum* (OTU4) abundance during AX (r_s=0.33, p=0.072; **Figure S.5.6.A**) and MCC (r_s=0.30, p=0.097; **Figure S.5.6.B**) consumption. Overall, these findings suggest that the adaptation to tolerate AX is partially driven by inter-individual differences in microbial fermentation of AX and subsequent acidification of the colonic environment, primarily through acetate (the principal SCFA produced by bifidobacteria[541]).

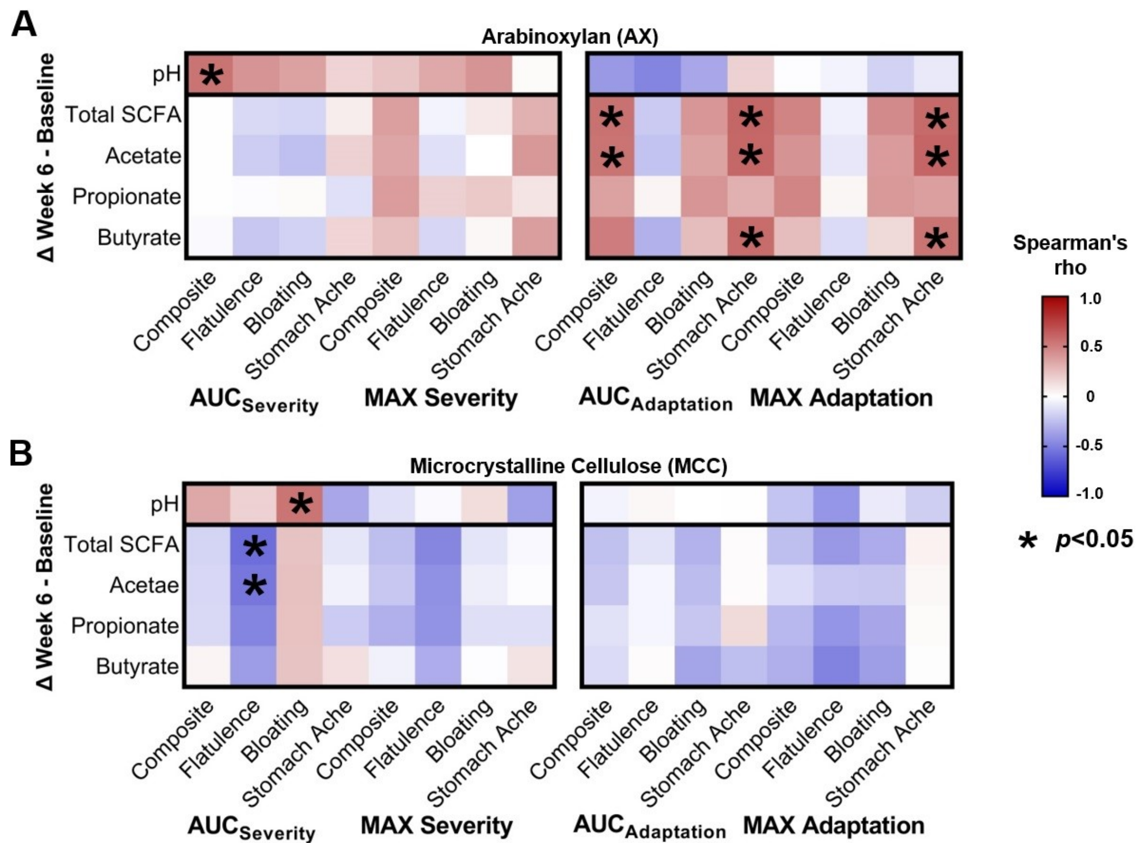

Figure 5.6. Shifts in fecal pH and SCFA concentrations correlated with the severity and adaptation of AX- and MCC-induced symptoms. *Legend continued on the next page.*

Heatmaps show Spearman's correlations between fecal pH and SCFA shifts (week 6 – baseline) and the MAX and AUC severity and adaptation scores for **(A)** AX and **(B)** MCC. Statistical significance was considered at $p<0.05$. AUC, area under the curve; AX, arabinoxylan; MCC, microcrystalline cellulose; SCFA, short-chain fatty acid.

5.3.7 Association Between Baseline Diet and Gastrointestinal Tolerance of AX and MCC

Previous research has shown that habitual intake of animal- vs. plant-based diets differentially affect gut microbiota composition and metabolic activity[21-23]. We, therefore, investigated whether pre-treatment, calorie-adjusted intake of meat/alt, whole grains (where AX is the dominant DF[524]), cholesterol (found only in animal-based foods), and DF, or the ratio between animal- and plant-based foods/nutrients were linked to symptom severity and adaptation.

This analysis revealed that, for AX, a higher proportion of meat/alt to whole grains in the subjects' pre-treatment diet correlated positively with composite ($r_s=0.68$; $p=0.007$) and flatulence ($r_s=0.70$; $p=0.005$) $AUC_{severity}$ scores, and negatively with composite $AUC_{adaptation}$ ($r_s=-0.54$; $p=0.042$) (**Figure 5.7.A**). The ratio of meat/alt to whole grains was further shown to positively correlate with MAX flatulence severity ($r_s=0.54$; $p=0.04$). In addition, higher cholesterol intake correlated positively with AX-induced bloating $AUC_{severity}$ ($r_s=0.63$; $p=0.013$), and negatively with composite ($r_s=-0.58$; $p=0.027$) and stomach ache ($r_s=-0.55$; $p=0.036$) $AUC_{adaptation}$ scores (**Figure 5.7.A**). In summary, habitual intake of more animal-based foods and less whole grains appears to elevate the perceived severity of gastrointestinal symptoms and lessen symptom improvements during AX supplementation.

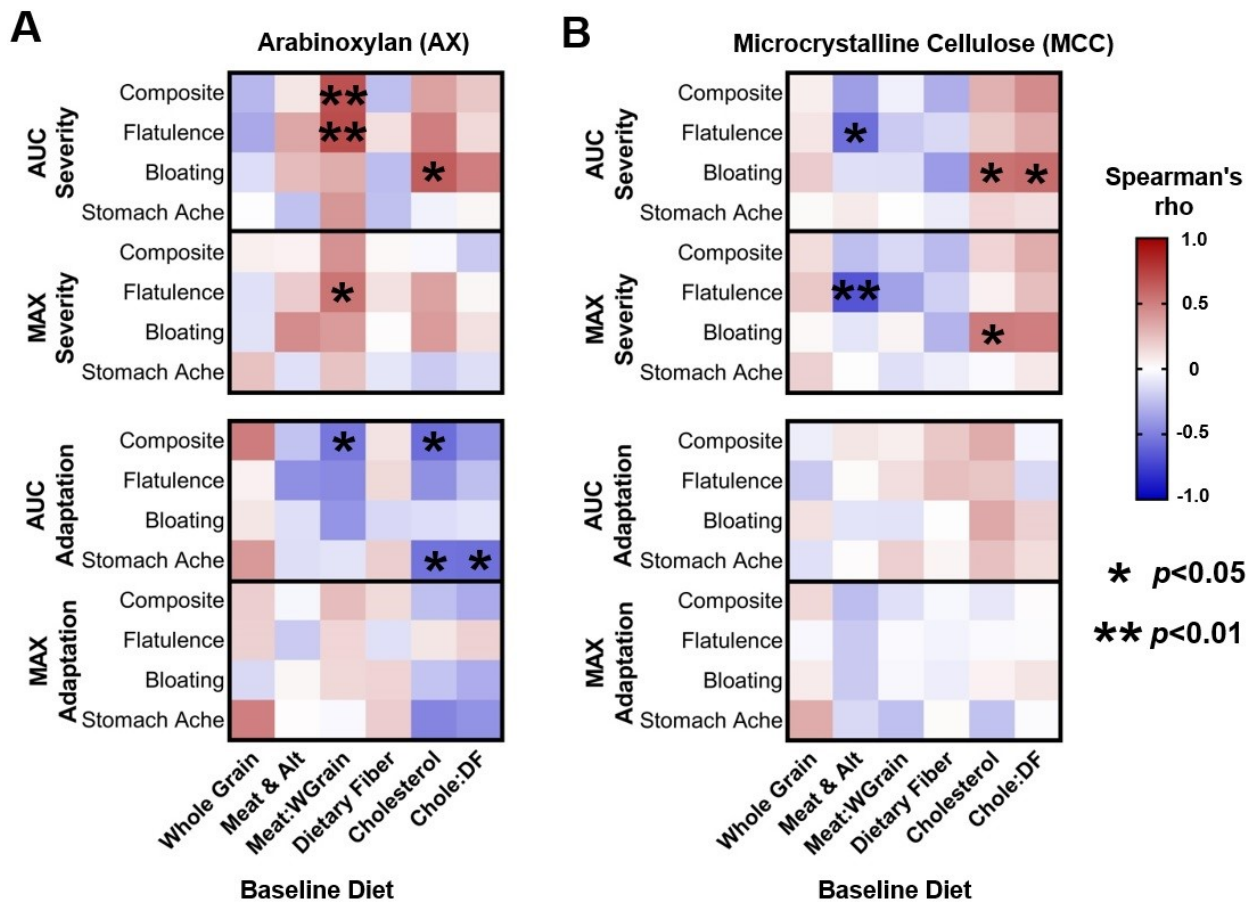

Figure 5.7. Baseline diet history correlated with symptom severity and adaptation during AX and MCC consumption. Heatmaps show Spearman's correlations between calorie-adjusted intakes of animal- and plant-based foods/nutrients at baseline, and the MAX and AUC severity and adaptation scores for **(A)** AX and **(B)** MCC. Statistical significance was considered at $p<0.05$. chole:DF, ratio of dietary cholesterol to dietary fiber; meat:wgrain, ratio of meat/meat alternatives to whole grains. AUC, area under the curve; AX, arabinoxylan; MCC, microcrystalline cellulose.

5.4 Discussion

This study showed that, confirming previous research with other fermentable DFs[522,528], consumption of corn bran AX at high doses of 25 g/day (females) or 35 g/day (males) intensified gastrointestinal symptoms. Even though AX induced symptoms within the first three weeks of supplementation, effects were transient, with ratings reverting almost completely back to baseline levels during weeks 4 to 6. This observation was the most important finding of our study as it indicates that humans can adapt to high amounts of AX within a relatively short time frame. While severity and adaptation responses were both subject-dependent, the strongest correlations detected were between adaptation scores and gut microbiota composition, fecal

acidification, and baseline diet. These findings provide information that can serve as a basis for the development of microbiome-targeted or dietary strategies aimed to attenuate DF-induced symptoms.

Fermentable DFs serve as substrates for the colonic microbiota, wherein microbial degradation of DF concurrently generates beneficial metabolites and gases (*e.g.* SCFAs and H_2, respectively)[273,521]. The latter cause flatulence and, upon colonic build-up, trigger bloating and stomach ache sensations[522]. Increased severity of gastrointestinal symptoms in our study, therefore, indicates that corn bran AX was fermented by the human gut microbiota, while MCC remained unfermented. However, it seems that there are marked differences among fermentable DFs. Comparison of our findings to published literature on inulin and resistant oligosaccharides suggests that the severity of symptoms induced by ~10 g/day of the latter are equivalent to that induced by 25–35 g/day of corn bran AX[528,535,542]. Higher tolerance of corn bran AX might arise from its complex molecular structure[537]. Accordingly, *in vitro* fecal fermentation studies have demonstrated lower gas production rates by corn bran AX relative to fructooligosaccharides and even AXs with simple molecular structures (*i.e.* sorghum and rice AX)[447,526]. Other molecularly complex DFs characterized by slow fermentation rates *in vitro* (*e.g.* resistant starch, acacia gum, and polydextrose[525,527,543]) have also shown similar degrees of tolerance at doses above 30 g/day[69,439,528,529]. Our findings, together with reports in the literature, suggest that increased structural complexity of DF promotes lower rates of fermentation, which permits colonic absorption and evacuation of gases without colonic build-up, leading to improved tolerance[522].

Previous studies have suggested that symptoms of DF consumption are predominantly influenced by the gut microbial community; affecting colonic gas production and the removal of H_2[530,534,544]. While associations were not detected with putative hydrogenotrophic bacterial taxa in our study (*i.e.* acetogens, methanogens, and sulfate-reducing bacteria), correlations were detected between *Bifidobacterium* abundance and severity and adaptation of both bloating and composite symptoms. These correlations are in agreement with previous findings from a longitudinal study in healthy individuals, where higher *Bifidobacterium* abundance was inversely associated with abdominal pain and intestinal discomfort[545], and with probiotic intervention trials where administration of *Bifidobacterium* strains reduced bloating in irritable bowel syndrome[546-548]. Although cause and effect relationships and mechanisms are not established in our study, several aspects of the metabolism of *Bifidobacterium* provide a potential explanation for reduced

gas production during AX fermentation. First, *Bifidobacterium* species are non-gas-producing[541]. Competition of *B. longum* for AX could therefore reduce net colonic gas production by other organisms. Second, *Bifidobacterium* produce lactate and acetate from carbohydrate fermentation[541], which acidify the colonic environment. In general, acidification has been shown to lower the rate and net production of H_2 during microbial fermentation of carbohydrates[549]. This mechanism is supported by the correlations between gastrointestinal tolerance to AX and shifts in fecal pH and acetate in our study. Interestingly, associations were also detected with CARG1, a co-abundance cluster that responded to AX and contained *B. longum* and *Subdoligranulum* sp. (which also produces lactate[495])[537], and the *ex vivo* activity of AX-utilizing *B. longum*. Overall, correlations detected in **Chapter 5** suggest that severity and adaptation of AX-induced symptoms are to some degree determined by the bacterial consortia involved in AX degradation and specifically the position of *B. longum* within the active consortia.

We can only speculate about the mechanisms by which the adaptation occurs. One possibility is that bacteria within CARG1, for instance, *B. longum*, adapt themselves to become more efficient at utilizing AX. In **Chapter 4**, we showed that, while AX-induced shifts in gut microbiota composition manifested within one week without further changes, adaptation in the production of propionate was detected in 40% of subjects and predicted by CARG1 shifts[537]. Therefore, it is feasible that within six-week, this bacterial consortium exhibits a functional adaptation towards reduced gas production during AX fermentation, improving symptoms perceived by the individual. However, other mechanisms are also possible; for instance, *B. longum* could mitigate the perception of visceral stimuli through upregulation of neurotransmitters, which would improve symptoms without altering gas production[550]. Therefore, future studies should apply more sophisticated techniques - such as ingestible gas-sensing capsules[521] - to evaluate adaptation.

Correlation analyses between gastrointestinal symptoms and diet indicate that habitual consumption of a diet higher in whole grains at the expense of animal-based foods enhances the tolerance of AX. Previous studies have suggested that long-term dietary habits play a role in shaping the composition and metabolic activity of the gut microbiota[22,23,539]. For instance, higher intakes of animal proteins and fats have been linked to greater relative abundances of the family *Porphyromonadaceae* and genera *Odoribacter*, *Parabacteroides*, and *Bacteroides*[22]. It is, therefore, reasonable to speculate that dietary patterns rich in whole grains (where AXs would be

abundant) over time select for microbes that more efficiently ferment these substrates, which might be linked to less H_2 production during AX fermentation. Diets rich in animal products, on the other hand, might select for microbes that are less AX adapted and produce more H_2. These speculations would align with the positive correlation detected between *Porphyromonadaceae* and composite $AUC_{severity}$, with correlations for *Odoribacter* and *Parabacteroides* approaching significance. Other groups have additionally reported positive associations between the genera *Parabacteroides* and *Bacteroides* and increased flatulence[551,552]. Overall, these findings suggest that habitual diet might be an important factor that shapes the metabolic capabilities of the gut microbiota through a long-term adaptive process, impacting its response and adaptability to dietary compounds such as AX.

The findings obtained in **Chapter 5** of this dissertation are important for several reasons. First, the study showed that humans are capable of adapting to DF doses that exert substantial changes to fecal bacterial community composition, increase propionate concentrations[537], and improve perceived satiety and insulin resistance[536]. Adaptation to efficacious amounts of DF provides an avenue by which physiologically relevant doses can be both achieved, through the application of purified DFs in foods or as supplements, and tolerated by humans, making closing the fiber gap feasible. Second, the links between symptom adaptation and the gut microbiota, specifically the abundance of *B. longum*, point towards the possibility of using *B. longum* strains well-adapted to competitively utilize AX as probiotics in order to enhance the tolerance of AX. Finally, the symptom findings during AX supplementation, as well as the detected associations between tolerance and diet history, overall suggest that functional characteristics of the gut microbiome are adaptable and can therefore be altered through selection, opening options to molt the gut microbiome. More broadly, the findings serve as a proof-of-principle for the rationale to develop nutritional strategies to enhance the gut microbiota's ability to improve food intolerance.

5.5 References

6. Sonnenburg ED, Sonnenburg JL. Starving our microbial self: the deleterious consequences of a diet deficient in microbiota-accessible carbohydrates. *Cell Metab.* 2014;20(5):779-86.
12. Jones JM. CODEX-aligned dietary fiber definitions help to bridge the 'fiber gap'. *Nutr J.* 2014;13:34.
21. David LA, Maurice CF, Carmody RN*, et al.* Diet rapidly and reproducibly alters the human gut microbiome. *Nature.* 2014;505(7484):559-63.

22. Wu GD, Chen J, Hoffmann C, *et al.* Linking long-term dietary patterns with gut microbial enterotypes. *Science.* 2011;334(6052):105-08.
23. Wu GD, Compher C, Chen EZ, *et al.* Comparative metabolomics in vegans and omnivores reveal constraints on diet-dependent gut microbiota metabolite production. *Gut.* 2016;65(1):63-72.
27. Deehan EC, Walter J. The Fiber Gap and the Disappearing Gut Microbiome: Implications for Human Nutrition. *Trends Endocrinol Metab.* 2016;27(5):239-42.
36. Hamaker BR, Tuncil YE. A perspective on the complexity of dietary fiber structures and their potential effect on the gut microbiota. *J Mol Biol.* 2014;426(23):3838-50.
67. Venkataraman A, Sieber JR, Schmidt AW, Waldron C, Theis KR, Schmidt TM. Variable responses of human microbiomes to dietary supplementation with resistant starch. *Microbiome.* 2016;4(1):33.
69. Martínez I, Kim J, Duffy PR, Schlegel VL, Walter J. Resistant starches types 2 and 4 have differential effects on the composition of the fecal microbiota in human subjects. *PLoS One.* 2010;5(11):e15046.
73. Davis LMG, Martínez I, Walter J, Goin C, Hutkins RW. Barcoded pyrosequencing reveals that consumption of galactooligosaccharides results in a highly specific bifidogenic response in humans. *PLoS One.* 2011;6(9):e25200.
266. Makki K, Deehan EC, Walter J, Bäckhed F. The Impact of Dietary Fiber on Gut Microbiota in Host Health and Disease. *Cell Host Microbe.* 2018;23(6):705-15.
267. Reynolds A, Mann J, Cummings J, Winter N, Mete E, Te Morenga L. Carbohydrate quality and human health: a series of systematic reviews and meta-analyses. *Lancet.* 2019;393(10170):434-45.
273. Deehan EC, Duar RM, Armet AM, Perez-Muñoz ME, Jin M, Walter J. Modulation of the Gastrointestinal Microbiome with Nondigestible Fermentable Carbohydrates To Improve Human Health. *Microbiol Spectr.* 2017;5(5).
275. Clemens R, Kranz S, Mobley AR, *et al.* Filling America's fiber intake gap: summary of a roundtable to probe realistic solutions with a focus on grain-based foods. *J Nutr.* 2012;142(7):1390S-401S.
276. Sonnenburg ED, Sonnenburg JL. The ancestral and industrialized gut microbiota and implications for human health. *Nat Rev Microbiol.* 2019;17(6):383-90.
277. Thompson SV, Hannon BA, An R, Holscher HD. Effects of isolated soluble fiber supplementation on body weight, glycemia, and insulinemia in adults with overweight and obesity: a systematic review and meta-analysis of randomized controlled trials. *Am J Clin Nutr.* 2017;106(6):1514-28.
300. Tannock GW, Liu Y. Guided dietary fibre intake as a means of directing short-chain fatty acid production by the gut microbiota. *J Roy Soc New Zeal.* 2019.
301. O'Grady J, O'Connor EM, Shanahan F. Review article: dietary fibre in the era of microbiome science. *Aliment Pharmacol Ther.* 2019;49(5):506-15.
302. O'Keefe SJ. The association between dietary fibre deficiency and high-income lifestyle-associated diseases: Burkitt's hypothesis revisited. *Lancet Gastroenterol Hepatol.* 2019;4(12):984-96.
325. Pasman WJ, Wils D, Saniez M-HH, Kardinaal AF. Long-term gastrointestinal tolerance of NUTRIOSE® FB in healthy men. *Eur J Clin Nutr.* 2006;60(8):1024-34.

370. Salden BN, Troost FJ, Wilms E, *et al.* Reinforcement of intestinal epithelial barrier by arabinoxylans in overweight and obese subjects: A randomized controlled trial: Arabinoxylans in gut barrier. *Clin Nutr.* 2018;37(2):471-80.
437. Armet AM, Deehan EC, Thöne JV, Hewko SJ, Walter J. The Effect of Isolated and Synthetic Dietary Fibers on Markers of Metabolic Diseases in Human Intervention Studies: A Systematic Review. *Adv Nutr.* 2020;11(2):420–38.
438. Healey GR, Murphy R, Brough L, Butts CA, Coad J. Interindividual variability in gut microbiota and host response to dietary interventions. *Nutr Rev.* 2017;75(12):1059-80.
439. Deehan EC, Yang C, Perez-Muñoz ME, *et al.* Precision Microbiome Modulation with Discrete Dietary Fiber Structures Directs Short-Chain Fatty Acid Production. *Cell Host Microbe.* 2020;27(3):389-404.e6.
444. ClinicalTrials.gov. National Library of Medicine (US). Identifier NCT02322112, The Alberta FYBER (Feed Your gut Bacteria morE fibeR) Study. 2015, July 3. Available at: Retrieved April 25, 2020 from: https://clinicaltrials.gov/ct2/history/NCT02322112?V_2=View#StudyPageTop.
447. Rumpagaporn P, Reuhs BL, Kaur A, Patterson JA, Keshavarzian A, Hamaker BR. Structural features of soluble cereal arabinoxylan fibers associated with a slow rate of *in vitro* fermentation by human fecal microbiota. *Carbohydr Polym.* 2015;130:191-97.
449. Csizmadi I, Boucher BA, Lo Siou G, *et al.* Using national dietary intake data to evaluate and adapt the US Diet History Questionnaire: the stepwise tailoring of an FFQ for Canadian use. *Public Health Nutr.* 2016;19(18):3247-55.
450. McInerney M, Csizmadi I, Friedenreich CM, *et al.* Associations between the neighbourhood food environment, neighbourhood socioeconomic status, and diet quality: An observational study. *BMC Public Health.* 2016;16:984.
452. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. *Am J Epidemiol.* 1986;124(1):17-27.
495. Holmstrøm K, Collins MD, Moller T, Falsen E, Lawson PA. *Subdoligranulum variabile* gen. nov., sp. nov. from human feces. *Anaerobe.* 2004;10(3):197-203.
521. Kalantar-Zadeh K, Berean KJ, Burgell RE, Muir JG, Gibson PR. Intestinal gases: influence on gut disorders and the role of dietary manipulations. *Nat Rev Gastroenterol Hepatol.* 2019;16(12):733-47.
522. Livesey G. Tolerance of low-digestible carbohydrates: a general view. *Br J Nutr.* 2001;85(Suppl 1):S7-S16.
523. Corsetti M, Gevers A-M, Caenepeel P, Tack J. The role of tension receptors in colonic mechanosensitivity in humans. *Gut.* 2004;53(12):1787-93.
524. Mendis M, Leclerc E, Simsek S. Arabinoxylans, gut microbiota and immunity. *Carbohydr Polym.* 2016;139:159-66.
525. Koecher KJ, Noack JA, Timm DA, Klosterbuer AS, Thomas W, Slavin JL. Estimation and interpretation of fermentation in the gut: coupling results from a 24 h batch *in vitro* system with fecal measurements from a human intervention feeding study using fructo-oligosaccharides, inulin, gum acacia, and pea fiber. *J Agric Food Chem.* 2014;62(6):1332-37.
526. Kaur A, Rose DJ, Rumpagaporn P, Patterson JA, Hamaker BR. *In vitro* batch fecal fermentation comparison of gas and short-chain fatty acid production using "slowly fermentable" dietary fibers. *J Food Sci.* 2011;76(5):H137-H42.

527. Erickson JM, Carlson JL, Stewart ML, Slavin JL. Fermentability of Novel Type-4 Resistant Starches in *In Vitro* System. *Foods.* 2018;7(2):E18.
528. Grabitske HA, Slavin JL. Gastrointestinal effects of low-digestible carbohydrates. *Crit Rev Food Sci Nutr.* 2009;49(4):327-60.
529. Cherbut C, Michel C, Raison V, Kravtchenko T, Severine M. Acacia Gum is a Bifidogenic Dietary Fibre with High Digestive Tolerance in Healthy Humans. *Microb Ecol Health Dis.* 2003;15(1):43-50.
530. Mego M, Manichanh C, Accarino A*, et al.* Metabolic adaptation of colonic microbiota to galactooligosaccharides: a proof-of-concept-study. *Aliment Pharmacol Ther.* 2017;45(5):670-80.
531. Kruse HP, Kleessen B, Blaut M. Effects of inulin on faecal bifidobacteria in human subjects. *Br J Nutr.* 1999;82(5):375-82.
532. Babiker R, Merghani TH, Elmusharaf K, Badi RM, Lang F, Saeed AM. Effects of Gum Arabic ingestion on body mass index and body fat percentage in healthy adult females: two-arm randomized, placebo controlled, double-blind trial. *Nutr J.* 2012;11:111.
533. Okubo T, Ishihara N, Takahashi H*, et al.* Effects of Partially Hydrolyzed Guar Gum Intake on Human Intestinal Microflora and Its Metabolism. *Biosci Biotechnol Biochem.* 1994;58(8):1364-69.
534. Mego M, Accarino A, Tzortzis G*, et al.* Colonic gas homeostasis: Mechanisms of adaptation following HOST-G904 galactooligosaccharide use in humans. *Neurogastroenterol Motil.* 2017;29(9):e13080.
535. Davis LMG, Martínez I, Walter J, Hutkins RW. A dose dependent impact of prebiotic galactooligosaccharides on the intestinal microbiota of healthy adults. *Int J Food Microbiol.* 2010;144(2):285-92.
536. Deehan EC, Zhang Z, Riva A*, et al.* Elucidation of the role of microbial fermentation in the physiological effects of dietary fiber in adults with overweight or obesity. *In Preparation*.
537. Nguyen NK, Deehan EC, Zhang Z*, et al.* Gut microbiota modulation with long-chain corn bran arabinoxylan in adults with overweight and obesity is linked to an individualized temporal increase in fecal propionate. *Microbiome.* 2020;8:118.
538. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods.* 2016;13(7):581-3.
539. Voreades N, Kozil A, Weir TL. Diet and the development of the human intestinal microbiome. *Front Microbiol.* 2014;5:494.
540. Lam YY, Zhang C, Zhao L. Causality in dietary interventions-building a case for gut microbiota. *Genome Med.* 2018;10(1):62.
541. Lee J-H, O'Sullivan DJ. Genomic Insights into Bifidobacteria. *Microbiol Mol Biol Rev.* 2010;74(3):378-416.
542. Holscher HD, Doligale JL, Bauer LL*, et al.* Gastrointestinal tolerance and utilization of agave inulin by healthy adults. *Food Funct.* 2014;5(6):1142-49.
543. Hernot DC, Boileau TW, Bauer LL*, et al. In vitro* fermentation profiles, gas production rates, and microbiota modulation as affected by certain fructans, galactooligosaccharides, and polydextrose. *J Agric Food Chem.* 2009;57(4):1354-61.
544. Smith NW, Shorten PR, Altermann EH, Roy NC, McNabb WC. Hydrogen cross-feeders of the human gastrointestinal tract. *Gut Microbes.* 2019;10(3):270-88.

545. Jalanka-Tuovinen J, Salonen A, Nikkila J*, et al.* Intestinal microbiota in healthy adults: temporal analysis reveals individual and common core and relation to intestinal symptoms. *PLoS One.* 2011;6(7):e23035.
546. Guyonnet D, Chassany O, Ducrotte P*, et al.* Effect of a fermented milk containing *Bifidobacterium animalis* DN-173 010 on the health-related quality of life and symptoms in irritable bowel syndrome in adults in primary care: a multicentre, randomized, double-blind, controlled trial. *Aliment Pharmacol Ther.* 2007;26(3):475-86.
547. Whorwell PJ, Altringer L, Morel J*, et al.* Efficacy of an encapsulated probiotic *Bifidobacterium infantis* 35624 in women with irritable bowel syndrome. *Am J Gastroenterol.* 2006;101(7):1581-90.
548. O'Mahony L, McCarthy J, Kelly P*, et al. Lactobacillus* and *Bifidobacterium* in Irritable Bowel Syndrome: Symptom Responses and Relationship to Cytokine Profiles. *Gastroenterology.* 2005;128(3):541-51.
549. Perman J, Modler S, Olson A. Role of pH in production of hydrogen from carbohydrates by colonic bacterial flora. Studies *in vivo* and *in vitro*. *J Clin Invest.* 1981;67(3):643-50.
550. Lomax AE, Pradhananga S, Sessenwein JL, O'Malley D. Bacterial modulation of visceral sensation: mediators and mechanisms. *Am J Physiol Gastrointest Liver Physiol.* 2019;317(3):G363-G72.
551. Hiel S, Bindels LB, Pachikian BD*, et al.* Effects of a diet based on inulin-rich vegetables on gut health and nutritional behavior in healthy humans. *Am J Clin Nutr.* 2019;109(6):1683-95.
552. Manichanh C, Eck A, Varela E*, et al.* Anal gas evacuation and colonic microbiota in patients with flatulence: effect of diet. *Gut.* 2014;63(3):401-08.

5.6 Supplementary Material

Table S.5.1. Subject characteristics at baseline

	AX Arm	MCC Arm	*p value*
Number	15	16	
Sex (female/male)	10/5	11/5	
Age (y)	33.7 ± 9.7	32.1 ± 7.4	0.91
Height (cm)	171.5 ± 8.4	168.8 ± 7.6	0.25
Weight (kg)	84.8 ± 12.3	81.9 ± 10.5	0.40
Body mass index (kg/m^2)	28.7 ± 2.7	28.7 ± 2.0	0.99
Waist circumference (cm)	95.7 ± 8.8	92.8 ± 6.2	0.30
Percent body fat (%)	33.0 ± 9.3	32.0 ± 7.3	0.63
Females	36.4 ± 2.9	38.0 ± 6.1	0.20
Males	22.5 ± 3.6	23.0 ± 5.3	0.84

Data presented as mean ± SD and analyzed by Mann-Whitney tests.

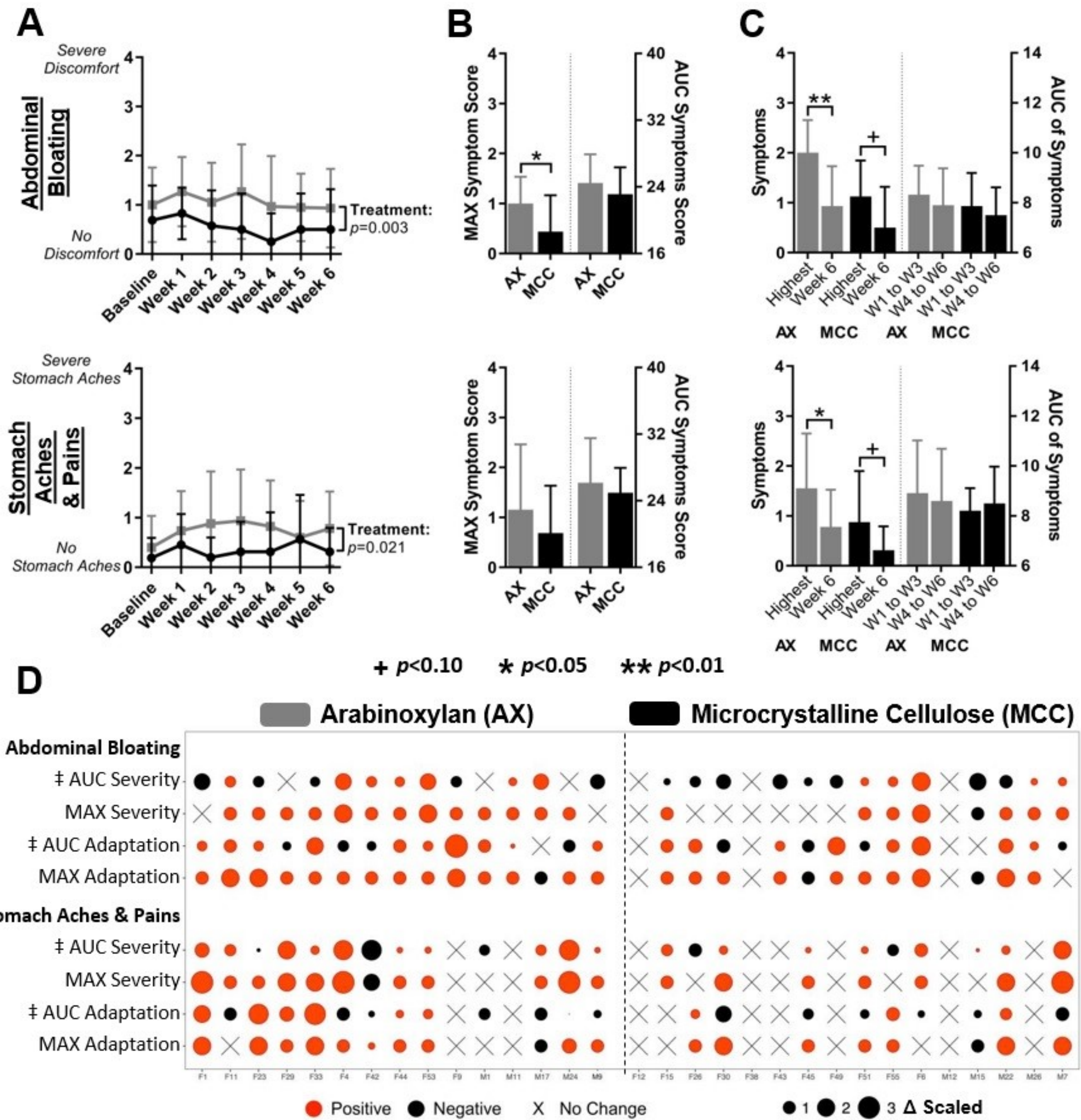

Figure S.5.1. Characterizing the severity and adaptation of bloating and stomach ache symptoms during AX and MCC consumption. **(A)** Bloating and stomach aches during AX and MCC consumption. **(B)** MAX and AUC symptom score for AX and MCC. **(D)** Highest symptom during weeks 1 to 5 and week 6 symptoms for AX and MCC, as well as the AUC of symptoms from weeks 1 to 3 and weeks 4 to 6. Data in **(A)** was analyzed using GEE models with Bonferroni corrections, in **(B)** using Mann-Whitney tests, and in **(C)** using Wilcoxon tests with Bonferroni corrections. Data reported as mean ± SD. Statistical significance was considered at *p*<0.05. **(D)** Bubble plots showing individualized bloating and stomach ache severity and adaptation scores for AX and MCC. Red circles represent an increase; black circles represent a decrease, and 'X' represents no change in the feature during the intervention. The circle size is proportional to the scaled magnitude change relative to baseline or week 6. ‡ Feature scaled by ($AUC_{severity}$–24/SD) or ($AUC_{adaptation}$–1/SD). AUC, area under the curve

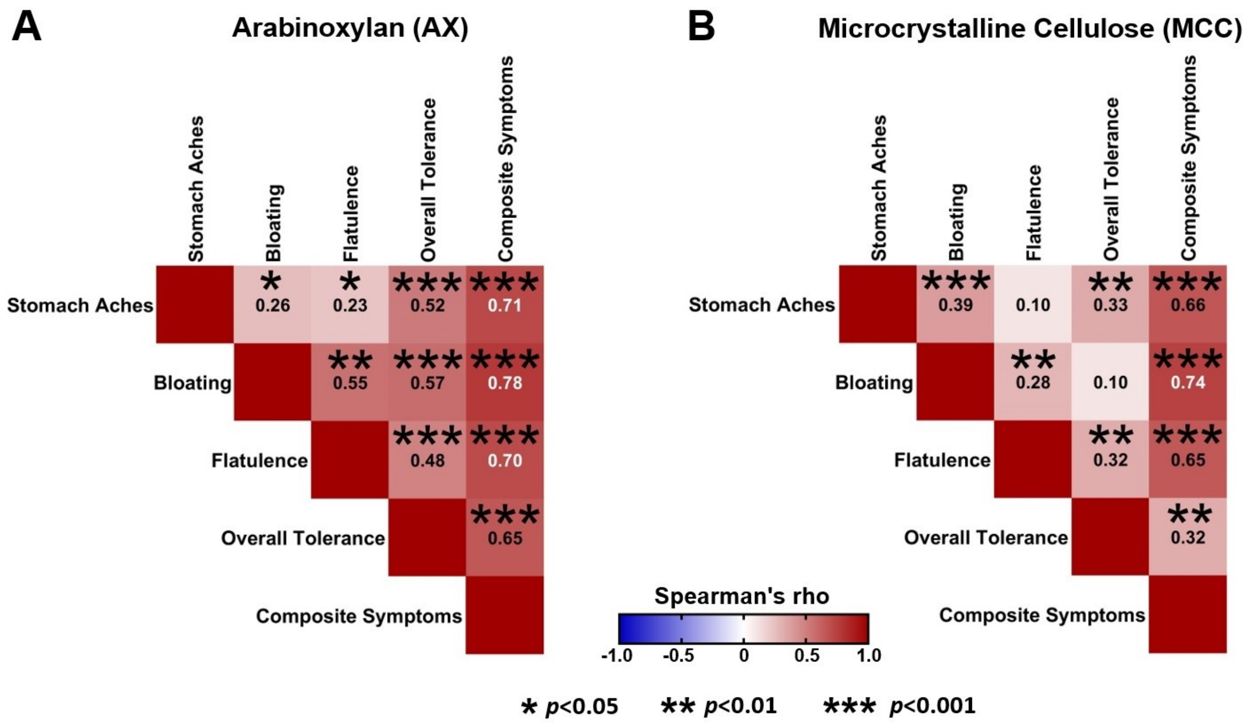

Figure S.5.2. Multicollinearity detected between treatment-induced changes in assessed gastrointestinal symptoms. Heatmaps show Spearman's correlations between changes in individual and composite symptoms (Δ weeks 1 to 6) during **(A)** AX and **(B)** MCC consumption.

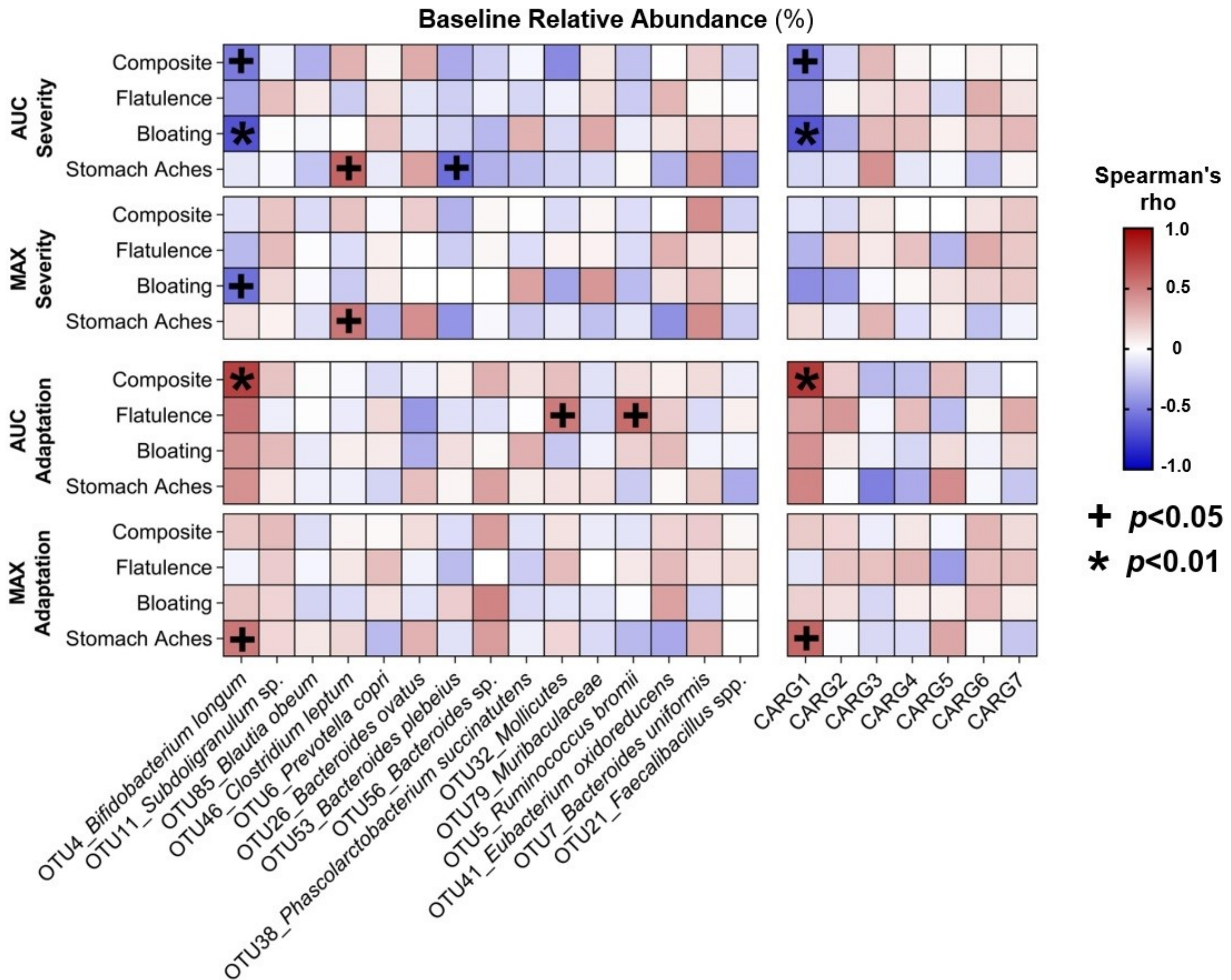

Figure S.5.3. Associations between severity and adaptation scores and pre-treatment abundances of AX-responsive bacterial taxa in fecal samples. Heatmaps show Spearman's correlations between MAX and AUC severity and adaption scores and the baseline relative abundances of all CARGs and those OTUs significantly affected by AX. Statistical significance was considered at $p<0.01$. AUC, area under the curve; AX, arabinoxylan; CARG, co-abundance response group; OTU, operational taxonomic unit.

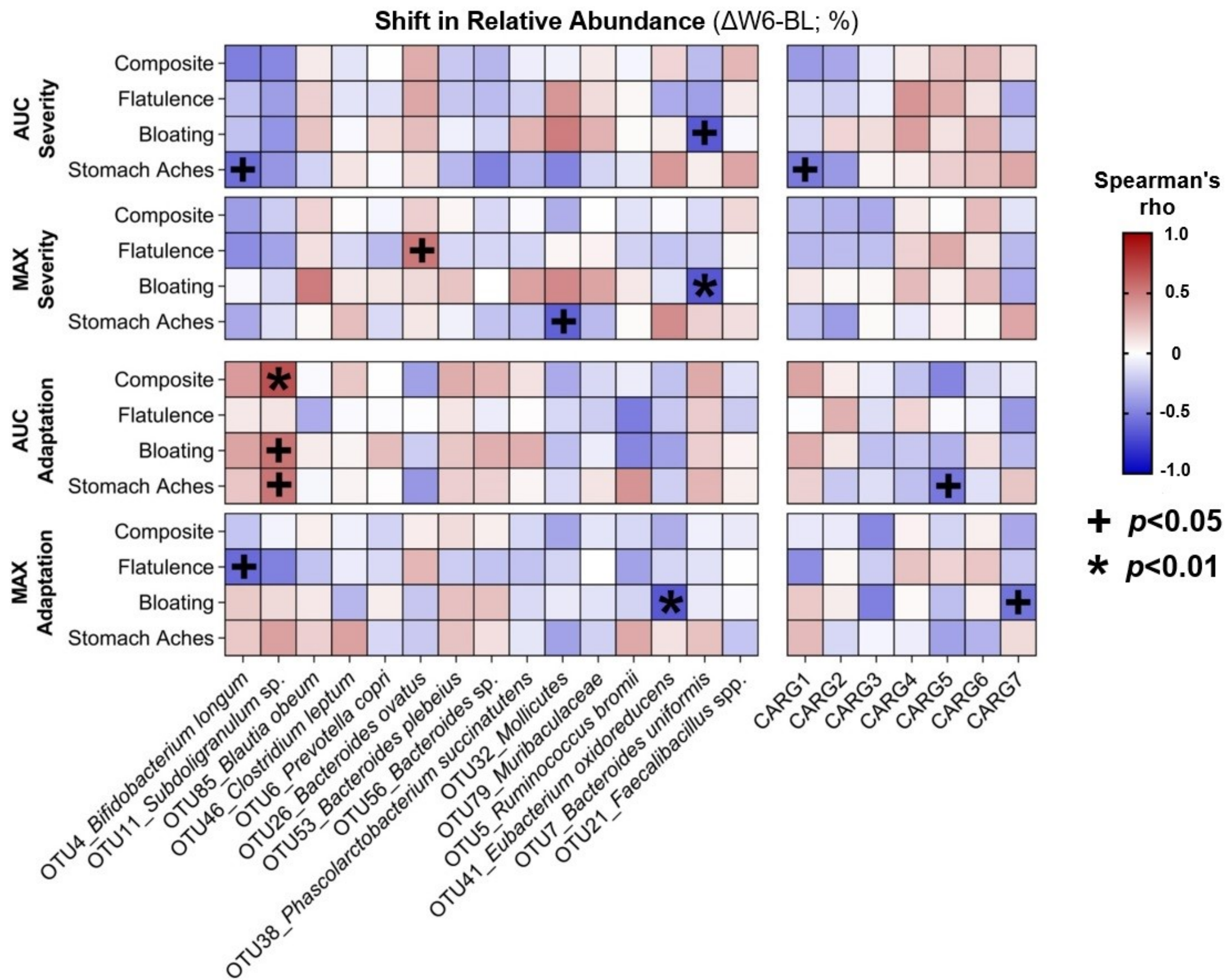

Figure S.5.4. Severity and adaptation scores correlated with the shifts in AX-responsive bacterial taxa in fecal samples. Heatmaps show Spearman's correlations between MAX and AUC severity and adaption scores and shifts (week 6 – baseline) in the relative abundance of all CARGs and those OTUs significantly affected by AX. Statistical significance was considered at p<0.01. AUC, area under the curve; AX, arabinoxylan; CARG, co-abundance response group; OTU, operational taxonomic unit.

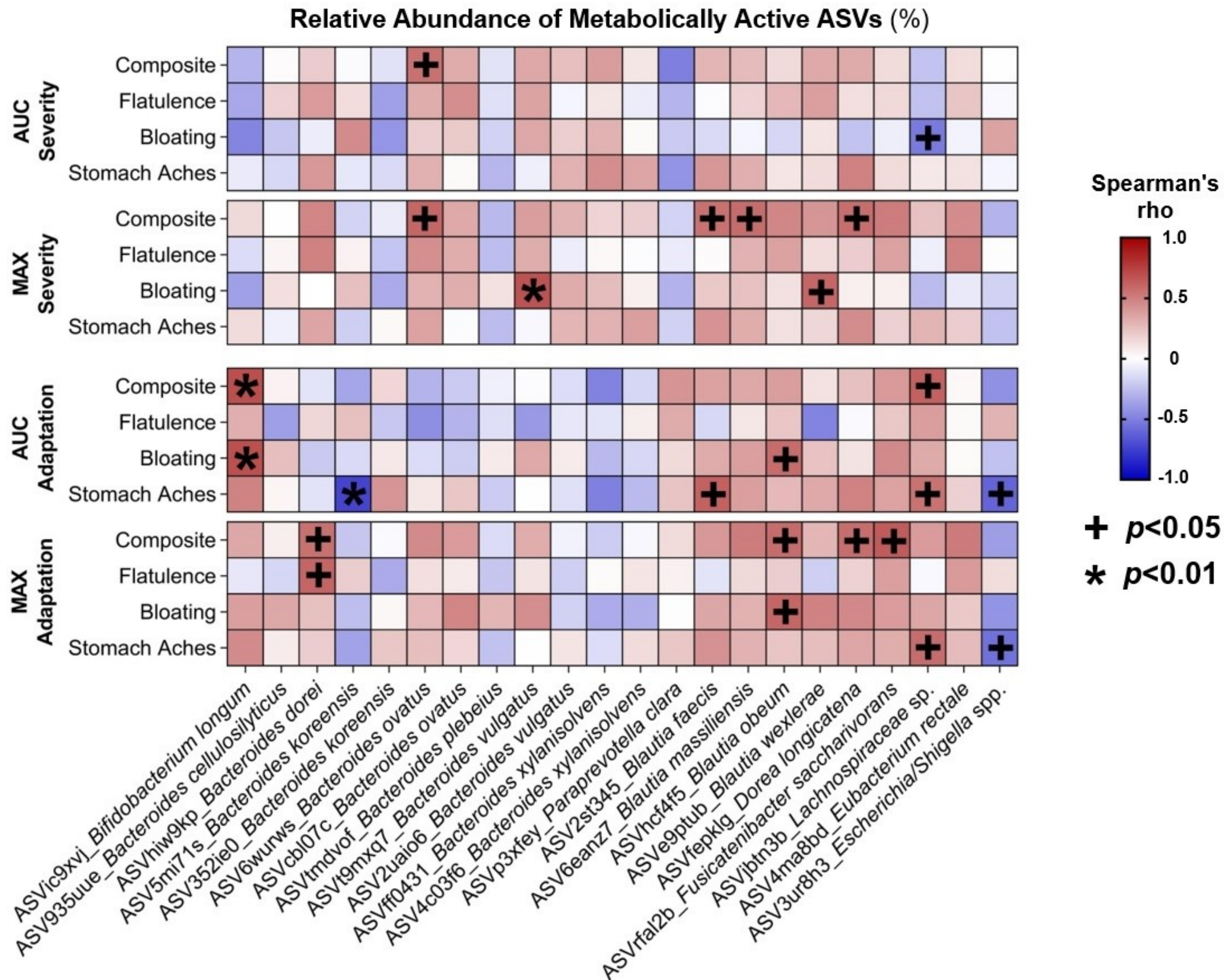

Figure S.5.5. Associations between severity and adaptation scores and the bacterial taxa that utilize AX *ex vivo*. Heatmaps show Spearman's correlations between MAX and AUC severity and adaption scores and the relative abundance of those ASVs most metabolically active during incubation with AX (average relative abundance >1.0%). Statistical significance was considered at $p<0.01$. ASV, amplicon sequence variant; AUC, area under the curve; AX, arabinoxylan.

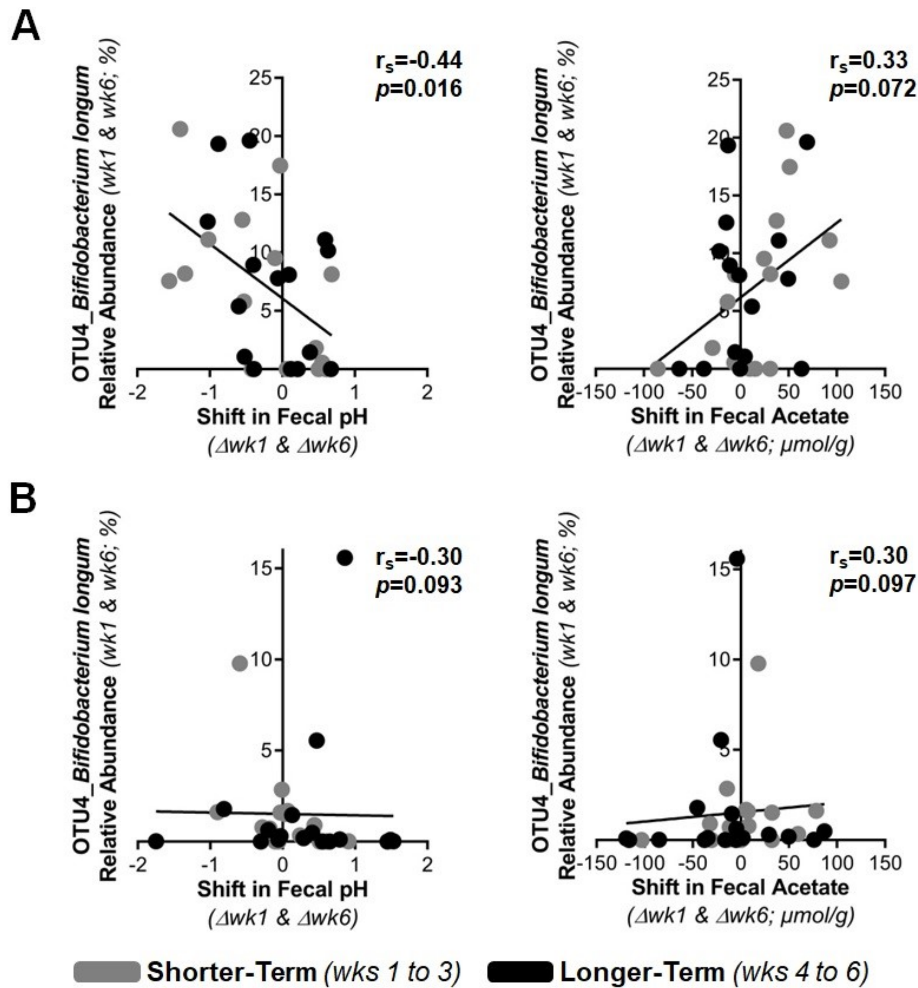

Figure S.5.6. Associations between *Bifidobacterium longum* abundance and fecal pH and acetate shifts during AX and MCC consumption. Spearman's correlations between the relative abundance of Bifidobacterium longum (OTU4) (weeks 1 and 6) and fecal pH and acetate shifts (Δ weeks 1 and 6) during **(A)** AX and **(B)** MCC consumption. Gray and black dots specifying shorter- (weeks 1 to 3) and longer- (weeks 4 to 6) term time points, respectively. The best-fitting line is the linear regression line. Statistical significance was considered at $p<0.05$. AX, arabinoxylan; MCC, microcrystalline cellulose; OTU, operational taxonomic unit.

CHAPTER 6: Precision microbiome modulation with discrete dietary fiber structures directs short-chain fatty acid production

A version of **Chapter 6** of this thesis was published as: Deehan EC, Yang C, Perez-Muñoz ME, Nguyen NK, Cheng CC, Triador L, Zhang Z, Bakal JA, Walter J. Precision microbiome modulation with discrete dietary fiber structures directs short-chain fatty acid production. *Cell Host & Microbe*. 2020;27(3):389-404.e6.

6.1 Introduction

The diverse microbial communities that humans harbor in their gastrointestinal (GI) tract have profound impacts on health. From an evolutionary perspective, the net effect of the gut microbiota is beneficial for the host. However, studies in animal models also suggest a causative role of the microbiome in the development of non-communicable diseases (NCDs)[553]. Although the exact factors that drive NCDs are unknown, most NCDs associate with both a Western-type diet and microbiome alterations (dysbioses) characterized by reduced diversity, blooms of opportunistic pathogens, and imbalanced ratio of beneficial to detrimental metabolites[18]. Western-type diets are characterized by high intakes of animal proteins, fats, and refined carbohydrates and low intakes of dietary fibers (DFs)[266]. Low DF consumption depletes gut microbiome diversity[4] and enhances the production of detrimental metabolites[121,122], with epidemiological and intervention studies identifying insufficient DF intake as a factor contributing to NCD development[267]. These observations implicate interactions between DF and the gut microbiota as a central mechanism in maintaining optimal health.

Plant-based foods deliver a diverse array of DFs to the gut microbiota that favorably shapes its metabolism[442]. These include nonstarch polysaccharides, oligosaccharides, and resistant starches (RSs), all of which display substantial structural heterogeneity and serve as microbiota-accessible carbohydrates[273]. *In vitro*[116,283] and *in vivo*[69,554] studies have shown that structural differences of DFs dictate the microbes involved in their degradation and the effects on the bacterial community, which are often specific though difficult to predict[63,73]. Fermentation of DF produces short-chain fatty acids (SCFAs; acetate, propionate, and butyrate), which are

largely considered beneficial but differ by their physiological effects[31]. Mechanistic studies in animal models showed beneficial effects of butyrate in maintaining GI barrier integrity, quenching oxygen at the epithelial interface, and exerting immune-modulating effects[198,555], while propionate has been shown to induce satiety through induction of anorectic hormones and intestinal gluconeogenesis (IGN), which also influences glucose metabolism[153,213]. Direct evidence in humans is limited, but butyrate is considered to be anti-carcinogenic and anti-inflammatory[556], while propionate has been shown to induce satiety[518] and improve glucose metabolism[519,520]. Therefore, one would predict that a targeted change in the molar ratio of SCFAs would alter the physiological, metabolic, and immunological relationship between the gut microbiota and human host[300].

The observations described above point to opportunities by which composition and functions of the gut microbiota could be selectively modulated by DFs. In 2014, Hamaker and Tuncil introduced a conceptual framework that proposes that 'discrete structures' within DF molecules (which they defined as unique chemical structures that align with gene clusters encoded in the genomes of specific microbial species) could be used to obtain predictable changes in microbiota composition to either maintain healthy or correct dysbiotic microbial populations[36]. Although a promising concept, the authors themselves urged caution that the framework might suffer from an oversimplification of complex ecological interactions within microbiomes[36]. Species within gut bacterial communities show both functional redundancy, where different species possess the same traits facilitated by horizontal gene transfer[557], and high strain-to-strain variability in important traits[443]. Species also do not function in isolation but form complex networks through mutualistic and competitive interactions[98]. In addition, both gut microbiomes and their response to DF are highly individualized[67,69], and they are homeostatic and resilient to change[98]. From a more practical perspective, the exact DF dose required for reliable changes, and if such doses are tolerable by modern-day humans, are unknown[300]. All these factors question whether discrete DF structures could be used to induce targeted and predictable alterations in humans.

In **Chapter 6**, we tested the hypothesis that small discrete differences in the chemical structure of DF can be used to direct changes in fecal microbiota composition and its functions. To achieve this, a randomized controlled trial was performed in humans to compare the effects and dose-response relationships of three type-IV resistant starches (RS4s) on fecal microbiota

composition, SCFA profiles, and perceived GI tolerance. RSs were chosen as the DF source as they have well-characterized, substrate-specific effects on the human gut microbiome[69,554,558], while the use of RS4s with well-characterized chemical modifications allowed the elucidation of specific structure-function relationships between DF and the microbiome. RS further has exciting potential for knowledge translation for the design of food products with high DF doses.

6.2 Methods

6.2.1 Human Subjects

This study was prospectively registered with ClinicalTrials.gov (identifier: NCT03255603) and was conducted at the University of Alberta Human Nutrition Research Unit in Edmonton, Canada between September 2017 and February 2018 in accordance with the principles of the Declaration of Helsinki. All procedures involving human subjects were approved by the Health Research Ethics Board of the University of Alberta (Approval Number: Pro00069884).

Written informed consent was obtained from all study subjects prior to enrollment into the study. Study subjects included healthy males and pre-menopausal, non-pregnant or lactating females aged 18 to 50 years that were recruited using campus-wide flyers, mailings to specific Listservs, local events, and word of mouth. Exclusion criteria included: (1) patient history of GI diseases or surgeries; (2) use of antibiotics 3-months prior to the start of the study; (3) chronic use of anti-hypertensive, lipid-lowering, anti-diabetic, analgesic, or laxative medications; (4) use of probiotic or prebiotic supplements; (5) intolerance to corn, potato, or tapioca; (6) vegetarian; (7) smoking; (8) alcohol intake ≥8 drinks/week; (9) more than 5-hours of moderate-vigorous exercise per week. After exclusions and replacements of subjects that withdrew because of time constraints (Tapioca RS4, n=1) and forgetting to take the supplement (Potato RS4, n=2), a total of 40 adult subjects (n=10 per arm), including 20 male and 20 female with a mean age of 28.4 ± 8.1 years, completed the dietary intervention and were included in final data analyses (**Table S.6.2**).

6.2.2 Experimental Design and Randomization

The trial used a randomized, double-blinded, placebo-controlled, parallel 4-arm, 4-week dose-escalation design (**Figure 6.1.A**). Random allocation was done using stratified random assignment based on sex, with 5 males and 5 females being assigned to each of the three

treatment arms (3 structurally distinct RS4s) or the placebo arm (digestible corn starch). Two separate random allocation sequences (male and female sequence) were generated (by a study investigator not involved in subject recruitment and allocation) using the website Randomization.com with four randomly permuted blocks (www.randomization.com), and then concealed using two lists of randomly generated codes. The sample size (*n*=10 per arm) was determined by referencing previous studies that successfully assessed the effect of DF on GI microbiome composition and GI symptoms[69,529,534,559-561].

Five weekly clinic visits were held for each subject (**Figure 6.1.A**). Potential subjects completed an initial telephone pre-screening followed by a baseline visit (week 0) to confirm eligibility. Upon enrollment, subjects were assigned to the next available randomization code by study investigators blinded to the predetermined allocation sequences, and then instructed to consume the corresponding RS4 or placebo supplement daily for four weeks, with the DF dose provided strictly as RS being raised weekly (Week 1: 10 g/day, Week 2: 20 g/day, Week 3: 35 g/day, Week 4: 50 g/day). The starches were administered as a supplement, divided into 2 to 3 servings, and then incorporated into water or other preferred drinks and foods without cooking.

6.2.3 Dietary Supplementation

The three RS4s and placebo (digestible starch) were all manufactured and provided by Ingredion Incorporated (Bridgewater, NJ, USA) as single batches. Supplement specifications, including their chemical structure, are provided in **Table S.6.1** and **Figure S.6.1**. Maize RS4 (VERSAFIBE™ 2470) is a high-amylose maize starch subjected to acid hydrolysis to remove nonenzyme-resistant material, and then to annealing treatments in order to reorganize and increase the stability of the native granule structure[562]. Potato RS4 (VERSAFIBE™ 1490) and Tapioca RS4 (VERSAFIBE™ 3490) are native potato and tapioca starches subjected to a phosphorus oxychloride treatment that reduces digestibility by cross-linking starch molecules at the surface of the starch granule, creating a slightly rough surface in comparison with the native starch[563]. The native corn starch used as the placebo is AMIOCA™ powder starch, a high amylopectin starch that should be rapidly digested and absorbed proximally in the small intestine, which prevents its availability for microbial fermentation in the colon, making it an ideal placebo when characterizing the microbial response to RS.

The supplements were identical in appearance (white powders), and weekly doses were provided in sealed opaque bags that contained individually packaged, ready-to-use daily sachets

that provided the desired doses of DF (*i.e.* 10 g/day to 50 g/day). The absolute amount was dependent on each supplement's DF content based on measurements with AOAC 2009.01 and adjusted for moisture content. The amount of placebo (digestible corn starch) packaged was equal to the mean amount of the RS4s used in the three treatment arms. Packaging, coding (*i.e.* 'Starch 1' to 'Starch 4'), and the unblinding upon completion of data collection were carried out by an individual not involved in the study. Subjects were instructed to return all provided sachets at their weekly visits, where the remaining portion of unconsumed supplement was weighed to assess treatment protocol adherence.

6.2.4 Lifestyle and Anthropometrics Assessments

Subjects were asked to maintain their habitual diet and physical activity level during the study, and instructed to avoid foods known to cause GI symptoms, such as cabbage, artichokes, onions, beans, lentils, wheat bran, prunes, and plum juice[529]. To assess dietary intake maintenance, subjects completed two 24-hour recalls, both at baseline and during week 4, using the Canadian version of the Automated Self-Administered 24-hour Dietary Assessment Tool (ASA24-Canada-2016), a method perceived to be less burdensome than other 24-hour recall methods[564,565]. Anthropometrics (height, weight, and body mass index [BMI]), physical activity (7-day total metabolic equivalent of task score), and perceived stress (1-month total perceived stress score) were assessed at baseline and during week 4 using the validated International Physical Activity Questionnaire and Perceived Stress Scale, respectively[566,567].

6.2.5 GI Tolerability and Bowel Habit Assessments

GI tolerability was assessed during all five clinical visits using a questionnaire to rate the severity of specific GI symptoms six days prior to the visit: nausea, GI rumblings, abdominal pain, bloating, flatulence, and diarrhea. The severity of each symptom was reported on a 3-points scale, with '0' denoting 'no symptoms/no more than usual', '1' denoting 'somewhat more than usual', and '2' denoting 'much more than usual'. A composite GI tolerability score was then calculated as the sum of each individual symptom score, with a range from 0 to 12 (representing complete tolerance and poor tolerance, respectively)[568,569]. Subjects also completed a bowel movement habit diary at baseline and over the two days preceding each clinic visit, recording bowel movement frequency, fecal consistency using the Bristol Stool Scale (scale of 1 [hard] to 7 [liquid]), and subjectively rating perceived fecal hardness (scale of 1 [soft] to 4 [very hard]), straining during a bowel movement, discomfort during a bowel movement, and a sensation of

incomplete evacuation (all with a scale of 1 [none] to 4 [severe])[568]. A mean daily score was calculated for each bowel movement habit prior to statistical analyses.

6.2.6 Fecal Microbiome Sequencing

Subjects collected fecal samples at baseline and the end of each week using a stool specimen container (Fisher, Canada), and delivered them to the investigators within 4-hours of defecation for immediate processing. Aliquots of fecal material, 1:10 fecal homogenates in phosphate-buffered saline (for DNA extraction), and 1:5 fecal homogenates in 5% phosphoric acid (for SCFA analysis) were immediately frozen (-80ºC) and stored until further processing. Bacterial DNA was extracted from fecal homogenates as previously described[69] with slight modifications: a reduction in the lysis step to 15 minutes and an elimination of the InhibitEX tablet provided in the QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany) were performed as recommended by Costea and colleagues[570].

Composition of the bacterial community in fecal samples was characterized using 16S rRNA gene amplicon sequencing. PCR targeting the V5-V6 region of the 16S rRNA gene with primers 784F [5'-RGGATTAGATACCC-3'] and 1064R [5'-CGACRRCCATGCANCACCT-3'], and subsequent amplicon sequencing (Illumina MiSeq platform v3 kit producing 300-bp paired-end sequences) was performed at the University of Minnesota Genomics Center, with DNA from all 200 fecal samples being included in a single sequencing run. Amplicon sequencing produced a total of 14,637,282 raw sequences (average = 73,554; minimum = 34,324 and maximum = 109,526). To make the dataset manageable, R1 and R2 fastq files were randomly subsampled, based on the sample with the lowest amount of reads, to obtain 30,000 matching reads using an in-house python script. Raw reads were trimmed to 210 bases long using FASTX-toolkit (hannonlab.cshl.edu/fastx_toolkit/index.html). R1 and R2 ends were quality filtered and paired using the merge-illumina-pairs application from Illumina utils[571]. Sequences that did not meet the quality criteria (p value of 0.03, enforced Q30 check, perfect matching to primers, and no ambiguous nucleotides allowed) were discarded. One sample collected after consuming the 50 g/day dose of Tapioca RS4 didn't amplify; therefore data from the subject's 35 g/day dose was carried forward. After trimming and quality filtering, a total of 4,523,790 paired sequences were obtained (average= 22,619 ± 443; minimum = 21,103 and maximum = 23,877). Sequences from all samples were compiled and dereplicated using Usearch v.10[572]. Subsequently, singletons were discarded, chimeras removed, OTUs clustered at 98%

identity, representative sequences for OTUs were selected and an OTU table was generated using Usearch v.10[572]. Non-chimeric sequences were binned by sample/subject and submitted to Ribosomal Database Project Classifier[457] for taxonomic assignment. OTUs were assigned taxonomy using Silva database (release 132)[573] and sequence identity confirmed using NCBI blastn[574], EzBioCloud[459], and Ribosomal Database Project Seqmatch[575]. Counts were transformed to relative abundance. Taxa with a mean relative abundance of ≤0.10% were removed from the dataset prior to statistical analyses. Diversity analyses were performed using Qiime[576] and Qiime2[577].

To determine groups of interacting OTUs in their response to RS4 supplementation, CARGs were determined from the top OTUs impacted by the dietary intervention (dose/interaction effect unadjusted *p* value less than 0.2; 2-way repeated-measures analysis of variance [rANOVA])[466]. Among the three treatment groups (*i.e.* not including placebo), Spearman's correlation analysis was performed between the shifts in these OTUs (*i.e.* 0 g/day to 50 g/day) to construct a correlation matrix. Hierarchical clustering was then performed on the matrix and a tree was built based on this matrix using the Ward algorithm. Differences between distinct branches of the Hierarchical tree, and thus individual CARGs, were determined by PERMANOVA (using a less-stringent cut-off of $p \leq 0.1$) and by visual inspection of the Hierarchical tree in order to separate OTU clusters that displayed clear differences in their response[466]. Relative abundance of each CARG was calculated as the sum of the OTUs within each CARG prior to statistical analyses.

6.2.7 Fecal SCFA Quantification

SCFAs were analyzed at the Agricultural, Food and Nutritional Science chromatography core facility of the University of Alberta as previously described[454], with modifications. Briefly, previously acidified fecal homogenates were thawed and centrifuged at 20,000 x g for 20 min; 1000 µl of supernatant was removed and added to 200 µl of internal standard (5% phosphoric acid containing 0.3% of 4-methyl-valeric acid [116.20 g/mol]). The mixture (0.2 µl) was injected onto a gas chromatograph (Bruker SCION 456-GC, Bruker Corporation, Billerica, MA, USA) and SCFAs were separated on a capillary column (Stabilwax-DA, 30 m X 0.53 mm inner diameter X 0.5 µm film thickness, Restek Corporation, Bellefonte, PA, USA) and detected with a flame ionization detector. Injector and detector temperatures were 170ºC and 190ºC, respectively. The column temperature was held at 90ºC for 0.1 min, increased at a rate of

10ºC/min to 170ºC, and then held for 2 minutes. SCFA quantification was done by calculating response factors for each SCFA relative to 4-methyl-valeric acid using the injections of pure standards. Total SCFAs were determined as the sum of acetate, propionate, and butyrate, while the relative proportion of each SCFA was determined by $\frac{\text{Individual SCFA}}{\text{Total SCFA}} * 100$. Total BCFAs were determined as the sum of isobutyrate and isovalerate.

6.2.8 *In Vitro* Assessment of Growth and Adherence

Four representative strains of human fecal origin from species known to respond to RSs, *B. adolescentis* IVS-1[357], *E. rectale* DSM 17629[102], *R. bromii* L2-63[430], and *P. distasonis* ATCC 8503[578], were grown in YCFA medium as previously described[430], supplemented with a filter-sterilized (0.22µm) carbohydrate mixture (0.1% glucose, 0.1% fructose, 0.1% galactose, and 0.1% maltose; w/v). Cultures were grown at 37°C under anaerobic conditions (5% CO2, 5% H2, and 90% N2). YCFA agar plates were made by adding equal volumes (1:1; v/v) of YCFA media and autoclaved 3% agar. When indicated, the carbohydrate mixture was replaced with 0.2% RS (w/v) that was 'predigested' with an *in vitro* process supposed to mimic human digestion as previously described[454].

Adherence to the RS granules was determined by methods previously described by Leitch *et al*., with slight modifications[94]. First, 20mg of each predigested RS were weighed into separate 1.5mL microcentrifuge tubes and sterilized using a 24-hour, 70% ethanol treatment followed by a 15 minute UV irradiation treatment. Overnight cultures of each strain were standardized to an OD_{600} of 0.5, and then 1mL was added into the microcentrifuge tube containing the RS. After being incubated at room temperature for 15 minutes with agitation (350 rpm), the non-attached and loosely attached bacteria were removed prior by washing with sterile PBS (4 times), then PBS containing 0.1% Tween 80 (2 times), and then a wash with sterile PBS to remove residual Tween 80. The centrifugation time between each washing was reduced to 30 seconds at 700 *g*[94]. Strain adherence to the RS was determined by quantifying CFUs on YCFA agar plates after either 2 days (*B. adolescentis* IVS-1, *E. rectale* 17629, and *R. bromii* L2-63) or 4 days (*P. distonsonis* 8503; slower growth rate on YCFA) of incubation at 37°C under anaerobic conditions. Assays were performed in triplicate.

Growth on RS substrates was determined (in triplicate) by inoculating overnight bacterial cultures (1%; v/v) into 10mL YCFA supplemented with the respective carbohydrate source, and growth was assessed through Optical density readings (OD_{600nm}) after tubes were vortexed for 10

seconds and then left standing for 5 minutes. RSs were sterilized by gamma irradiation (≥10kGy) prior to the assays. Samples were measured at intervals up to 48 hours for *B. adolescentis* IVS-1, *E. rectale* 17629, and *R. bromii* L2-63, and 168 hours for *P. distasonis* 8503 (slower growth rate of growth in YCFA).

6.2.9 Statistical Analyses

6.2.9.1 Statistical Analysis Software

Statistical analyses were performed using R Stats Software version 3.5.1 (R Core Team, Vienna, Austria), with ANOVAs and Spearman's rank-order correlations performed using GraphPad Prism version 8.3 (GraphPad Software, San Diego, CA, USA).

6.2.9.2 Missing Data and Outliers

When applicable, missing data were imputed by carrying the previous observation forward, assuming that no change occurred as the DF dose increased, as previously described[579]. No outliers were removed from statistical analyses.

6.2.9.3 Subject Characteristics at Baseline

To determine differences between intervention groups for the assessed characterizes (*e.g.* age, BMI, ethnicity) at baseline, either one-way ANOVA (continuous variables) or Fisher's exact tests (count variables) were applied. Data normality of continuous variables were assessed by Shapiro-Wilk test and inspection of QQ plots. If indicated, data were square-root transformed prior to statistical analysis using one-way ANOVA.

6.2.9.4 Anthropometric and Lifestyle Characteristics

Statistical significance of changes from baseline to week 4 in the assessed anthropometric (weight, BMI) and lifestyle (perceived stress, physical activity, and diet) characteristics were determined by 2-way rANOVA followed by Holm-Šídák multiple comparison test to correct for multiple pairwise comparisons within each group relative to baseline. Statistical significance was considered at $p<0.05$.

6.2.9.5 Analysis of GI Tolerability and Bowel Habits

GI tolerability and bowel movement habit data are either ordinal or derived from ordinal data (*i.e.* sum or mean) and consequentially are likely to be non-normally distributed. Therefore, GEE with repeated-measures models[465] were applied using R to assess the overall effect of treatment or dose on composite GI tolerability score and bowel movement habit data. When an overall significant effect was observed, within-group pairwise comparisons were applied using

estimated marginal means[580] followed by FDR corrections. Statistical significance was considered at FDR-adjusted q values <0.05. Individual GI symptom data were further analyzed using cumulative link models[581] to individually assess the effect of treatment and dose, where $p<0.05$ was considered statistically significant.

6.2.9.6 Analysis of Fecal Short-Chain Fatty Acids

To determine the statistical significance of within-group changes at each treatment dose (*i.e.* 10 g/day to 50 g/day) relative to baseline (0 g/day), individual and total SCFA and BCFA concentrations, SCFA proportions, and BCFA to SCFA ratio were analyzed using 2-way rANOVA followed by Holm-Šídák multiple comparison test. To determine whether the overall changes in fecal SCFAs induced by the four treatments were significantly different between each intervention group, acetate, propionate, and butyrate concentrations and relative proportions, as well as BCFA to SCFA ratio data were analyzed using ordinary 2-way ANOVA followed by Holm-Šídák multiple comparison test. For these analyses, all four delta values from each treatment dose (*i.e.* Δ10-0g to Δ50-0g) and for each subject were treated equally as replicates. Application of this statistical approach, therefore, assessed differences in the overall change in SCFAs during the intervention without consideration of supplementation dose, with an assumption that normal fluctuations over time would report a mean change near zero; while consistent, dose-dependent changes would report a clear mean positive or negative change. Further, even though ANOVA are considered to be a robust statistical approach for the analysis of data that may violate the general assumption of normal distribution[582,583], normality of ANOVA residuals were assessed using Shapiro-Wilk test and inspection of QQ plots. If the residuals were not normally distributed, square root transformations of SCFA or BCFA data were done prior to statistical analysis with ANOVA. Statistical significance was considered at $p<0.05$.

6.2.9.7 Analysis of Bacterial Community Composition

To determine the statistical significance of the changes observed in bacterial β- and α-diversity metrics, both temporal within-treatment group (effect of dose relative to baseline) and between-treatment groups (differences of shifts induced by RS4s and placebo at each dose), 2-way rANOVA were applied followed by Holm-Šídák multiple comparison tests. If the residuals were not normally distributed, square root transformations of diversity metrics data were done prior to statistical analysis with ANOVA. Non-metric multidimensional scaling plots,

PERMANOVA, and multivariate dispersion analyses (PERMDISP) were performed using the metaMDS, Adonis, and betadisper functions, respectively, from the vegan package in R[463] to determine the statistical significance of RS4-induced changes in inter-subject β-diversity when compared to baseline and to the analogous changes by placebo. Statistical significance was considered at $p<0.05$.

Furthermore, statistical significance of the changes observed in relative abundance of bacterial taxa and CARGs, both temporal within-treatment group (effect of dose relative to baseline) and between-treatment groups (differences of shifts induced by the RS4s relative to placebo), was determined by 2-way rANOVA and ordinary 2-way ANOVA (where the 4 delta values for each dose were treated equally as replicates, *i.e.* Δ10-0g to Δ50-0g), respectively. To control for multiple comparisons, FDR corrections were applied to p values using Prism, whereby statistical significance was considered at FDR-adjusted q values <0.05.

6.2.9.8 Fecal Microbiome and SCFA Correlations

Dose-response relationships were evaluated using Spearman's correlations to assess monotonic relationships between treatment doses (*i.e.* 0 g/day to 50 g/day) and fecal microbial abundance and SCFA concentrations. Statistical significance was considered at $p<0.05$. Spearman's correlations were further applied to assess correlations between changes in fecal microbial abundance and the relative proportion of fecal SCFAs. To account for the extensive comparisons made, FDR corrections were applied to p values using Prism, whereby statistical significance was considered at FDR-adjusted q values <0.05.

6.2.10 Data Availability

The 16S rRNA sequencing data have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive. The accession number for the sequencing data reported in this paper is BioProject: PRJNA560950.

6.3 Results

6.3.1 Intervention Trial Comparing the Effect of Different RS4s

A randomized double-blinded, placebo-controlled, parallel four-arm dose-response study was performed in 40 healthy individuals (n=10 per arm) to compare the effects of three RS4s (maize, potato, or tapioca derived) and one digestible corn starch (placebo) on the fecal microbiota in humans (**Figure 6.1.A**; see **Table S.6.1** for supplement specifications). The RS4s

differed in chemical structure and granule size (**Figure S.6.1**). While Maize RS4 (VERSAFIBE™ 2470) was produced through an annealing and acid treatment of high-amylose maize starch (leading to a restructured starch granule)[569], Potato RS4 (VERSAFIBE™ 1490) and Tapioca RS4 (VERSAFIBE™ 3490) were produced by phosphate cross-linking the native starches (generating inter-starch ester linkages)[584]. Subjects consumed the starches for four weeks to achieve a gradual weekly increase of DF to 10 g/day, 20 g/day, 35 g/day, and 50 g/day, and an equivalent amount of the placebo (**Figure 6.1.A**).

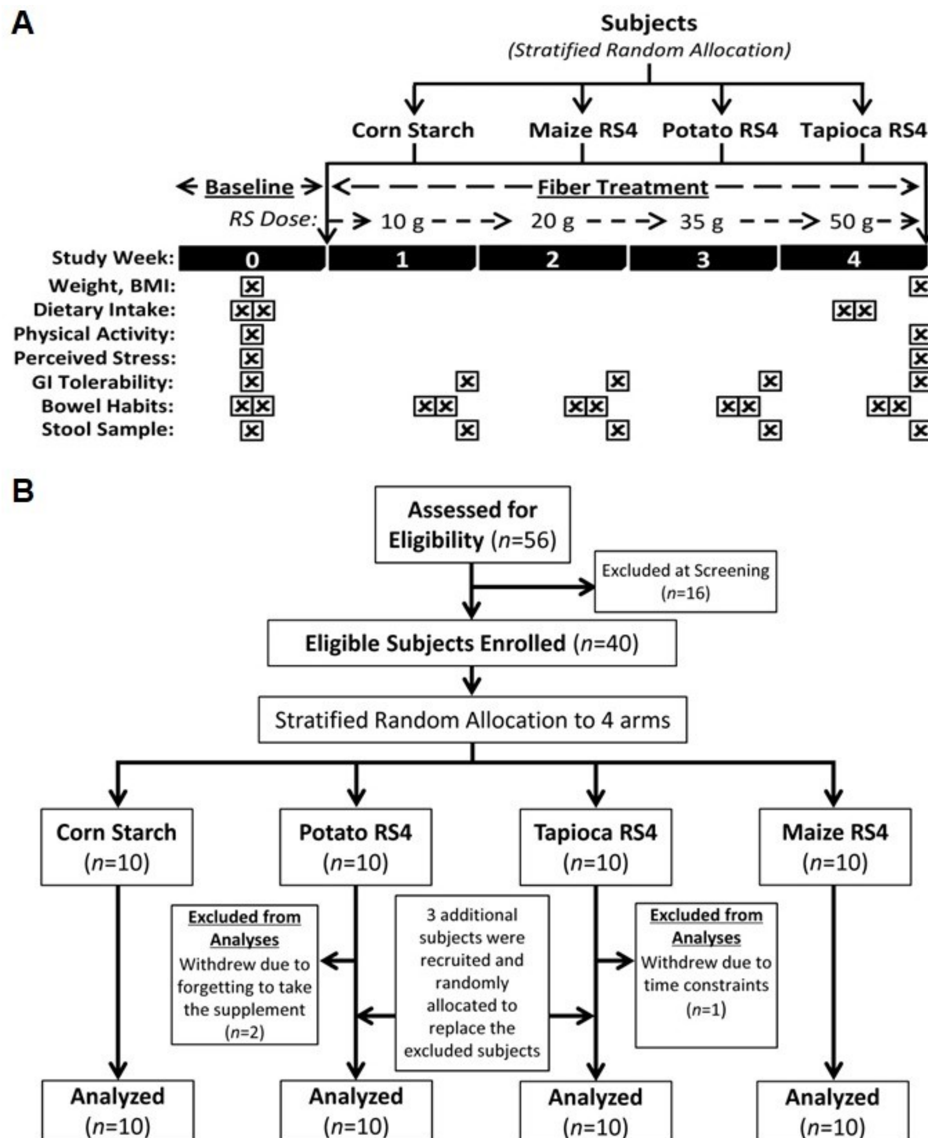

Figure 6.1. Study design and flow diagram. (A) Study design of the human trial. **(B)** Flow diagram of subject recruitment. See **Tables S.6.2 and S.6.3** for subject characteristics.

Protocol adherence rates were high at 98.9 ± 2.9% with no differences between groups. Three subjects withdrew from the study due to time constraints (Tapioca RS4, *n*=1) and forgetting the supplement (Potato RS4, *n*=2), thus additional subjects were enrolled and randomly aliquoted to these arms (**Figure 6.1.B**). Data analyses were limited to the 40 subjects that completed the protocol, which included 20 males and 20 females (5 each per arm) aged 28.4 ± 8.1 years and body mass index of 24.0 ± 3.2 kg/m^2 (**Table S.6.2**). Anthropometrics, physical activity, perceived stress, and dietary intake did not change during the intervention, except for additional DF provided as RS4 in the treatment groups on top of the average 18 g/day intake of DF reported by the study cohort (**Table S.6.3**).

6.3.2 High Doses of RS4 Show Acceptable GI Tolerance

Composite GI tolerability scores (sum of nausea, flatulence, bloating, GI rumbling, abdominal pain, and diarrhea, where higher scores equal poorer tolerance) were increased by all treatments, with clear dose-responses observed (dose-effect p<0.0001, generalized estimating equation [GEE] model; **Figure S.6.2.A**). Maize and Tapioca RS4s, and the placebo (digestible starch), caused moderate yet significant 1.6-2.8-point mean increases in composite tolerability scores at doses ≥35 g/day (p<0.05). In contrast, Potato RS4 did not affect composite tolerability scores, and no differences were detected between groups (treatment effect p=0.19). Of the 6 GI symptoms assessed, flatulence, bloating, GI rumbling, and abdominal pain were significantly affected by RS4 treatment (dose-effect p<0.0001, cumulative link model; **Figure S.6.2.B**).

In a separate measurement, the effects of RS4 on bowel habits were assessed (*i.e.* frequency, consistency, fecal hardness, straining, discomfort, and incomplete evacuation). Only Potato RS4 induced mild yet significant increases in bowel movement frequency at 50 g/day and decreases in fecal hardness at ≥35 g/day relative to baseline (p<0.05, GEE model; **Table S.6.4**). Enhanced laxation can be explained by Potato RS4 remaining largely unfermented by the gut microbiota[585], as laxation effects are primarily attributable to non-fermentable DFs[309]. Overall, these findings, together with findings from other RS interventions trials[586], suggest that modern-day humans without functional GI disorders are able to tolerate high daily doses of RS up to 50 grams, as only mild to moderate increases in GI symptoms and minimal changes in bowel habits were detected.

6.3.3 RS4s Differ Markedly in their Effects on Gut Microbiota Composition

6.3.3.1 Overall Fecal Microbiota Composition and Diversity

Characterization of fecal bacterial communities was performed by 16S rRNA gene sequencing. Maize and Tapioca RS4s increased inter-subject variation (individuality) in microbiome composition (β-diversity) when compared to baseline and to placebo ($p \leq 0.003$, 2-way rANOVA; **Figures 6.2.A and S.6.3.A**). For Maize RS4, inter-subject variation increased at doses ≥20 g/day. In contrast, for Tapioca RS4, inter-subject variation decreased at 10 g/day, but then increased at 50 g/day.

We then tested if RS4 consumption induced community-wide effects on microbiota composition. Non-metric multidimensional scaling analysis of Bray-Curtis distances showed differences ($p<0.05$, PERMANOVA) in the fecal bacterial community of individuals consuming Maize and Tapioca RS4s when compared to baseline and to placebo (**Figures S.6.3.B and S.6.3.C**). These dissimilarities were likely due to diverging centroids, as no differences were detected in community dispersion ($p>0.05$, PERMDISP). Tapioca RS4 also induced a significant shift in intra-subject β-diversity at 35 g/day and 50 g/day as compared to placebo (**Figure 6.2.B**).

Maize and Tapioca RS4 reduced the α-diversity (Shannon index) of the bacterial community at ≥20 g/day and ≥35 g/day, respectively, relative to baseline and placebo ($p<0.05$ 2-way rANOVA; **Figures 6.2.C and 6.2.D**). For both RS4s, the reduction in α-diversity was due to a decrease in community evenness (Pielou evenness index) (**Figures 6.2.E and 6.2.F**) and not due to a reduction of operational taxonomic units (OTUs; at 98% sequence similarity) within- or between-groups (**Figures 6.2.G and 6.2.H**).

Overall, the gut microbiome analysis showed that higher doses of Maize and Tapioca RS4 alter the fecal bacterial community by increasing interpersonal variation, shifting community composition, and reducing community evenness. The placebo and Potato RS4 did not affect β- or α-diversity, supporting the notion that the latter likely remained largely unfermented.

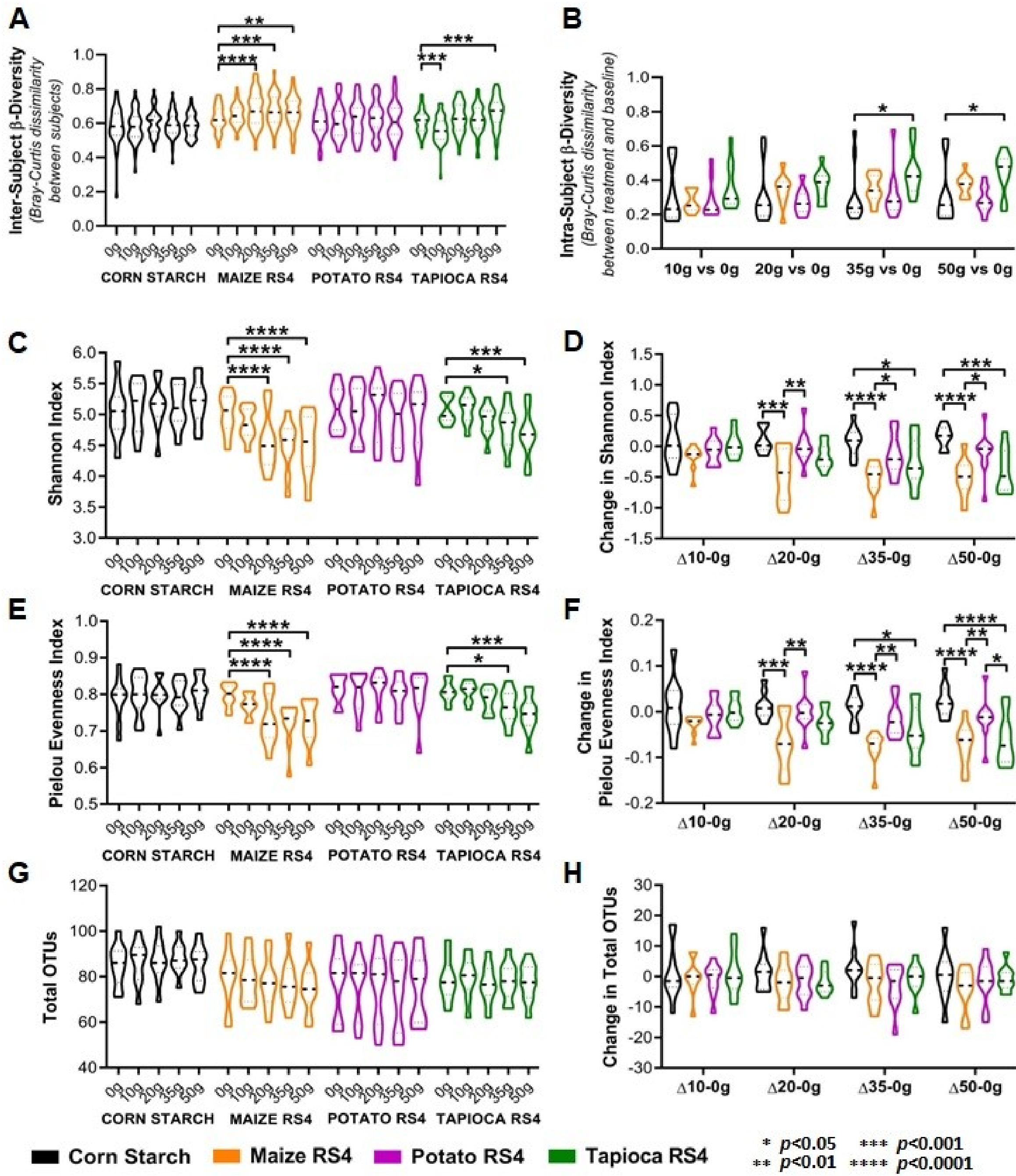

Figure 6.2. Effects of different RS4s and placebo on fecal bacterial diversity. Violin plots of Bray Curtis distances between **(A)** the fecal microbiomes of subjects at each dose/time-point (inter-individual β-diversity), and **(B)** each subject's fecal microbiome at baseline and during treatment (intra-individual; **(B)** was square root-transformed prior to analysis) (see **Figure S.6.3** for additional analyses of β-diversity using PERMANOVA). Violin plots showing the α-diversity of the fecal bacterial community at each dose, displayed as **(C)** Shannon index, **(E)** Pielou evenness index, and **(G)** total operational taxonomic units (OTUs). Violin plots showing the shift in diversity at each dose relative to baseline, displayed as **(D)** Shannon index, **(F)** Pielou evenness index, and **(H)** total OTUs. Data analyzed using 2-way rANOVA (with Holm-Šídák correction).

6.3.3.2 Taxonomic Composition of the Fecal Microbiota

In line with the changes observed in β- and α-diversity, Maize and Tapioca RS4 changed the relative abundance of bacterial taxa (overall dose/interaction effect $p<0.05$ and Benjamini-Hochberg's false discovery rate [FDR] corrected pairwise comparison $q<0.05$, 2-way rANOVA), while virtually no effects were detected for Potato RS4 and placebo (**Figure 6.3**). The effects were distinct and almost completely substrate-specific. Maize RS4 enriched OTUs related to *Eubacterium rectale* (OTU1), *Oscillibacter* spp. (OTU559), and an OTU within the *Ruminococcaceae* family with 100% similarity to database entries annotated as *Ruminococcus* spp. and *Anaeromassilibacillus* spp.[587] (OTU27; herein referred to as *Ruminococcus* spp.). In contrast, Tapioca RS4 enriched the family *Porphyromonadaceae*, the genus *Parabacteroides,* and OTUs related to *Parabacteroides distasonis* (OTU21), *Parabacteroides* spp. (OTU49), *Faecalibacterium prausnitzii* (OTU32), and *Eisenbergiella* spp. (OTU9). These enrichments were all substrate-specific, although non-significant increases in *E. rectale* and *Oscillibacter* spp. were also observed for Tapioca RS4. In addition, *Bifidobacterium adolescentis* (OTU3) showed an enrichment that approached statistical significance ($q<0.07$) for both Maize and Tapioca RS4.

Maize and Tapioca RS4s also led to negative shifts. Maize RS4 reduced OTUs related to *Ruminococcus callidus* (OTU57), *Agathobaculum butyriciproducens* (OTU84) and *Adlercreutzia equolifaciens* (OTU100). Tapioca RS4 reduced an unclassified genus of *Ruminococcaceae* and OTUs related to *Eubacterium hallii* (OTU67) and *Clostridium viride* (OTU132) (**Figure 6.3**). In contrast to the substrate-specific enrichments by Maize and Tapioca RS4, many of the reductions induced by Maize RS4 were also observed by Tapioca RS4, and vice versa, although none reached statistical significance in both groups. Overall, these findings suggest that structural differences between Maize and Tapioca RS4 selectively increase the fitness of specific OTUs, while reductions in taxa are also detected but appear less specific.

6.3.3.3 Identification of co-abundance response groups (CARGs)

Bacterial taxa often cooperate during DF degradation, establishing syntrophic interactions through cross-feeding, potentially establishing ecological guilds around primary degraders[540]. Potential interactions between bacterial taxa in their response to the 50 g/day dose were assessed using co-occurrence network analysis[466]. The analysis showed that the 55 OTUs most affected by RS4 treatment (dose/interaction effect unadjusted $p<0.2$, 2-way rANOVA) clustered into seven CARGs (**Figure S.6.4**). As observed with the shifts in OTU abundances, the responses in

CARGs were also substrate-specific. Maize RS4 increased the relative abundance of CARG6 ($q \geq 0.011$, 2-way rANOVA; **Figure 6.3**), which contained *E. rectale* as the only significantly enriched OTU. In contrast, Tapioca RS4 increased the abundance of CARG1 ($q \geq 0.001$), which contained *P. distasonis* and *Bifidobacterium* species known to utilize starch (*B. adolescentis* (OTU3) and *B. angulatum* (OTU68)[588]), and CARG4 ($q \geq 0.004$), which contained *Eisenbergiella* spp. as the only significantly enriched OTU. Both Maize and Tapioca RS4 reduced the abundance of CARG7 at ≥35 g/day and 50 g/day ($q \geq 0.02$), respectively.

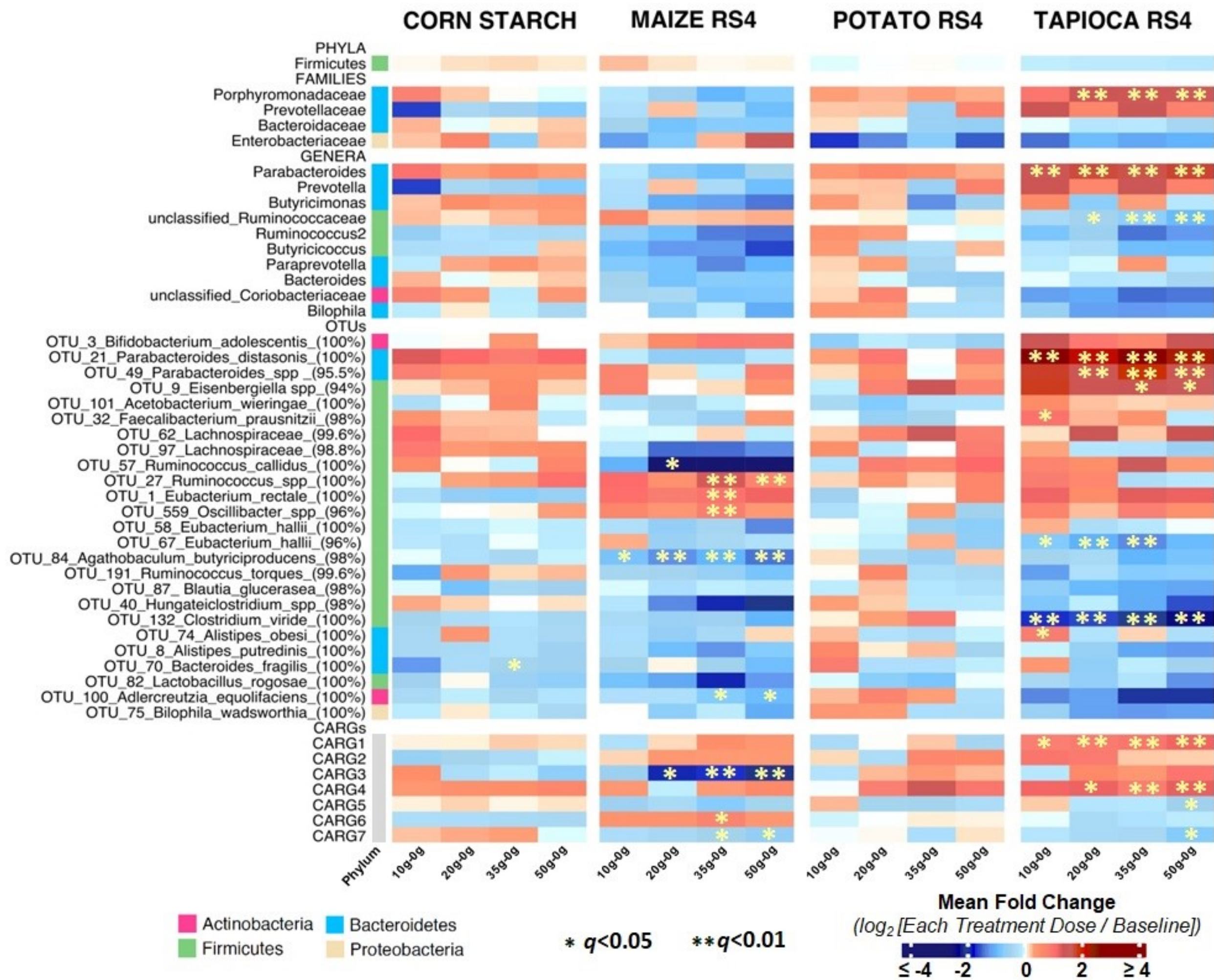

Figure 6.3. Shifts in the abundance of bacterial taxa and CARGs in response to RS4s and placebo. Heatmap of the mean $\log_2$-transformed fold change from baseline of phyla, families, genera, and operational taxonomic units (OTUs) that showed significant overall dose and/or interaction effects (unadjusted $p<0.05$; 2-way rANOVA), and the identified co-abundance response groups (CARGs; see **Figure S.6.4** for CARGs identification and **Figure S.6.5** for individual response magnitudes). Statistical significance of changes from baseline at each dose and within each treatment were determined using untransformed data by applying 2-way rANOVA (with FDR correction); $q<0.05$ considered significant.

Overall, the CARG analysis supports our conclusion from above that Maize and Tapioca RS4-induced enrichments are highly substrate-specific, while the reductions detected are less substrate-dependent. Furthermore, CARG1 contained several inter-correlated species of *Parabacteroides* and Actinobacteria (*Bifidobacterium* and *Collinsella*). This suggests that the degradation of Tapioca RS4 involves bacterial cross-feeding, which has been described for other RSs[430,554,589], although the bacterial species involved were different.

6.3.4 RS4 Chemistry Determines Output of Fecal SCFAs

Although RS4 consumption did not alter total SCFA concentrations (p>0.1, 2-way rANOVA), the different RS4s varied in their effect on individual SCFAs. Within-group comparisons revealed that Maize RS4 selectively increased butyrate concentrations (p=0.05) and relative proportions (percent of total SCFAs) (p=0.015) when compared to baseline, particularly at the 35 g/day dose (**Figure 6.4.A**). In contrast, Tapioca RS4 increased propionate concentrations relative to baseline at 35 g/day (p=0.04). These findings were confirmed by between-group comparisons, which showed that Maize RS4 elevated the relative proportion of butyrate (p=0.037, 2-way ANOVA treating delta values equally), while reducing the proportion of propionate (p=0.001). Tapioca RS4 increased propionate concentrations when compared to Maize RS4 (p=0.02) (**Figures 6.4.B and 6.4.C**). Neither placebo (digestible starch) nor Potato RS4 changed SCFA levels or relative proportions. The latter is in accordance with the absence of *in vivo* fermentation of Potato RS4 in rats[585].

Reductions in total and individual branched-SCFAs (BCFAs; isobutyrate and isovalerate) concentrations were detected at doses ≥35 g/day when all treatment arms were considered (dose-effect p≤0.014, 2-way rANOVA; **Figure 6.4.A**), but reductions for individual treatments, although detectable, did not reach significance. Significant reductions in the ratio between BCFAs and SCFAs were observed, particularly at the 35 g/day dose, for Tapioca RS4 (p=0.005), while reductions approached statistical significance for Maize RS4 (p=0.07) relative to baseline (**Figure 6.4.D**). When comparing between groups, Tapioca RS4 reduced the BCFA to SCFA ratio relative to Potato RS4 and placebo (p≤0.02, 2-way ANOVA treating delta values equally) (**Figure 6.4.E**). In summary, it appears that both Maize and Tapioca RS4s upregulate saccharolytic fermentation with specificity to which SCFA (*i.e.* butyrate or propionate) was elevated, at the expense of BCFAs that are indicative of proteolytic fermentation[122,590].

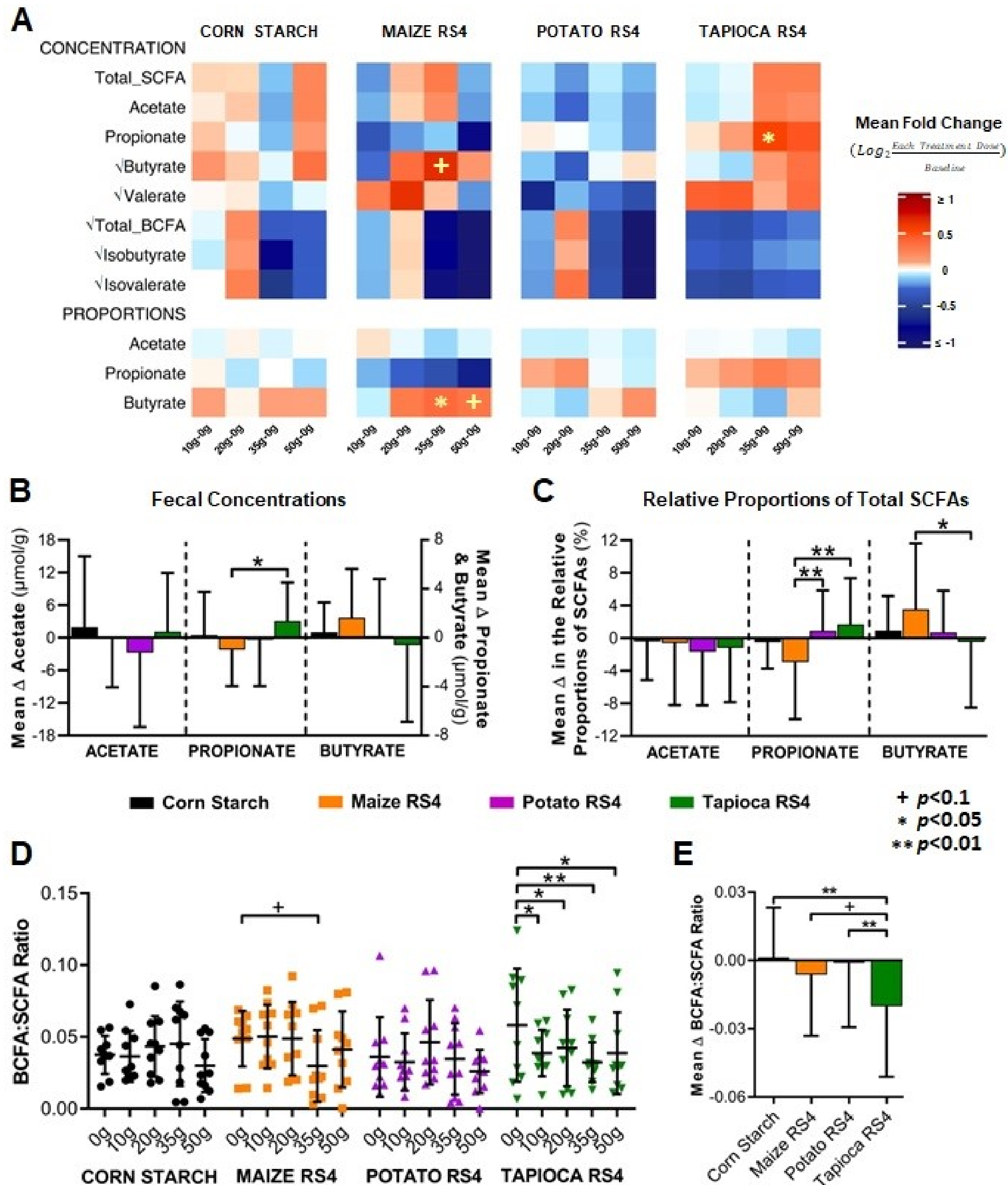

Figure 6.4. Modulation of fecal SCFAs through RS4s and placebo. (A) Heatmap of the mean $\log_2$-transformed fold change from baseline of SCFA concentrations (µmol/g feces) and the relative proportions of acetate, propionate, and butyrate relative to total SCFAs. Bar plots of the mean change from baseline considering all doses for **(B)** concentrations and **(C)** relative proportions of SCFAs. Ratio of total branched short-chain to short-chain fatty acids (BCFA:SCFA) at **(D)** each supplementation dose and the **(E)** mean shift from baseline in BCFA:SCFA considering all doses. Symbols represent individual samples; lines represent mean ± SD. Data analyzed for **(A and D)** using 2-way rANOVA (with Holm-Šídák correction), and for **(B, C, and E)** using ordinary 2-way ANOVA (with Holm-Šídák correction) where the 4 delta values (*i.e.* Δ10-0g to Δ50-0g) for each subject were treated equally as replicates. √, square root transformed prior to statistical analysis.

6.3.5 Effects of RS4s were Dose-Dependent

To examine RS4 dose-response relationships, we conducted Spearman's correlations between doses (*i.e.* 0 g/day to 50 g/day) and the abundance of the OTUs that showed the largest increase (>0.75% mean increase in relative abundance), all CARGs, and concentrations of SCFAs (**Figure 6.5.A**). Consistent with the substrate-specific effects detected above, the dose of Maize RS4 positively correlated with *E. rectale* (OTU1; r_s=0.34, p=0.015), CARG6 (r_s=0.26, p=0.066), and butyrate (r_s=0.31, p=0.03), while the dose of Tapioca RS4 positively correlated with *P. distasonis* (OTU21; r_s=0.39, p=0.005), CARG1 (r_s=0.33, p=0.019), and propionate (r_s=0.28, p=0.049). Dose-response relationships were not detected for Potato RS4 or placebo.

For additional insight into the magnitude of RS4 dose-dependent effects, we plotted the mean abundance or concentration of OTUs (>0.75% increase), CARGs, and SCFAs at all time-points, as well as absolute changes of these variables relative to previous time-points. As shown in **Figures 6.5.B and 6.5.C**, all OTUs enriched by Maize RS4, as well as the increase of OTU3 (*B. adolescentis*) and CARG6, exhibited a mean that plateaued at 35 g/day. A plateau at 35 g/day was also observed by three OTUs (OTU21, OTU49, and OTU9) enriched by Tapioca RS4, as well as OTU3. While the increase of OTU32 (*F. prausnitzii*) with Tapioca RS4 peaked at 10 g/day, the increase in CARG1 did not reach a plateau at any of the doses tested. Interestingly, the increased concentrations of butyrate and propionate induced by Maize and Tapioca RS4, respectively, also showed means that plateaued at 35 g/day (**Figure 6.5.D**). Overall, these findings suggest that RS4-induced effects on the gut microbiota are dose-dependent, with the average response of most variables detected plateauing at a dose of 35 g/day for Maize and Tapioca RS4s.

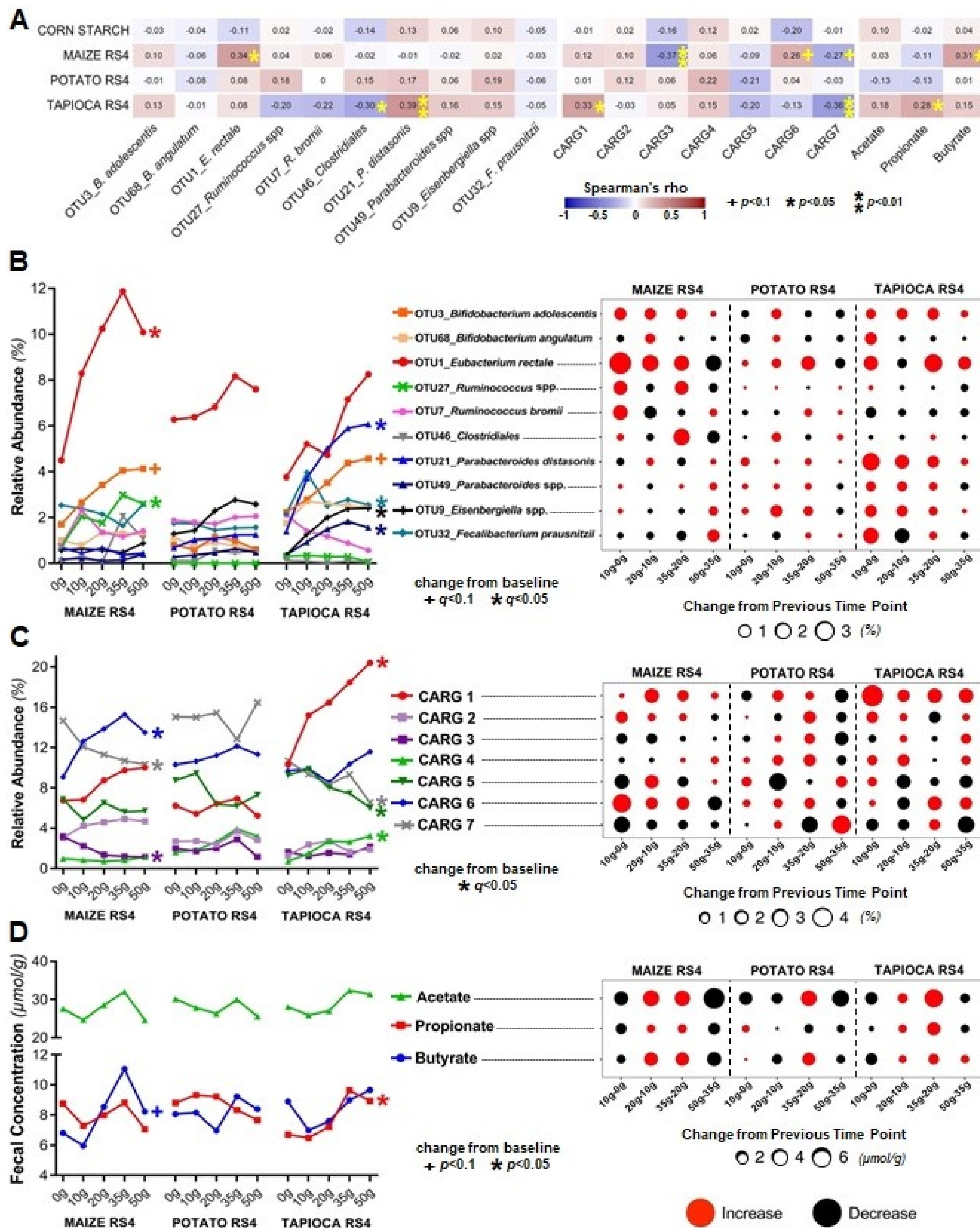

Figure 6.5. Dose-dependent effects of RS4 treatment on fecal bacterial composition and function. (A) Dose-response relationships were evaluated using Spearman's correlations between doses (*i.e.* 0 g/day to 50 g/day) and the abundances of operational taxonomic units (OTUs) with mean enrichments >0.75% relative abundance, all co-abundance response group (CARGs), and concentrations of principal short-chain fatty acids (SCFAs; μmol/g feces). *Legend continued on the next page.*

Line graphs show dose-responses of the **(B)** OTUs, **(C)** CARGs, and **(D)** SCFAs (see **Figure S.6.6** for dose-response plots of each subject treated with Maize and Tapioca RS4). Bubble plots show changes between doses (*e.g.* Δ10-0g and Δ50-35g), where red and black circles represent positive and negative changes, respectively, and circle size represents the magnitude of change. Statistical significance of changes relative to baseline was determined within each treatment group using 2-way rANOVA, where pairwise comparisons were corrected with either FDR (OTU and CARG) or Holm-Šídák (SCFA).

6.3.6 Individualized Effects of RS4 Consumption

As described in previous RS intervention studies[67,69], the effects observed were shown to be individualized, such as increases in *B. adolescentis* (OTU3), *Parabacteroides* spp. (OTU49), and *Eisenbergiella* spp. (OTU9) (**Figure S.6.5.A**). However, some of the detected responses were remarkably consistent. For example, consumption of Maize RS4 enriched *E. rectale* (OTU1) in all ten subjects, while CARG6 increased in nine subjects. The effects of Tapioca RS4 were also consistent, leading to an enrichment of *P. distasonis* (OTU21) in all subjects, while CARG1 increased in all but one subject. The magnitudes of these responses were, however, individualized, ranging from an increase of 53% to 535% from baseline for *E. rectale* and an increase of 116% to 21,183% from baseline for *P. distasonis*. Interestingly, a clear co-exclusion pattern was detected between the OTUs classified as *Ruminococcus bromii* (OTU7) and *Ruminococcus* spp. (OTU27), which differed in their response to Maize RS4 (**Figure S.6.5.B**), pointing to competitive differences between closely related OTUs as a potential driver for individualized effects.

The increase in Butyrate with Maize RS4 was also quite consistent (**Figure S.6.5.C**), showing increases in the relative proportion of butyrate in all but two subjects. Tapioca RS4 only increased the relative proportion of propionate in six subjects. However, one must consider that some of these inconsistencies might arise from fecal SCFA measurements being less sensitive to detect changes in colonic SCFA production due to host absorption[273].

The optimal dose of Maize and Tapioca RS4 to show maximum effects on OTUs or SCFAs were further individualized. An assessment of individual dose-response curves revealed that although the averages of most effects plateaued at 35 g/day, doses for maximum effect differed among individuals. For instance, the effects of Maize RS4 on *E. rectale*, CARG6, and butyrate (**Figure S.6.A**), and those of Tapioca RS4 on *P. distasonis*, CARG1, and propionate (**Figure S.6.6.B**), continued to be enhanced by the 50 g/day dose for nearly a third of subjects.

Overall, these findings showed that the dominant substrate-specific and dose-dependent effects of the RS4s are remarkably consistent (*e.g.* CARG1, CARG6, OTU1, and OTU21). However, the findings also emphasize that both the magnitude of compositional and functional responses, and the DF dose required to achieve these effects, are individualized.

6.3.7 Selective Effects of RS4s on Microbiota Composition Explain Responses in SCFAs

To determine whether RS4-induced shifts in the output of SCFAs were linked to the specific effects on bacterial taxa, we conducted Spearman's correlation analyses (**Figure 6.6**). Maize-RS4-induced shifts in butyrate proportions were positively correlated with increases in *E. rectale* (OTU1; r_s=0.41, q=0.07), a major butyrate-producer[477], and negatively correlated with increases in *R. bromii* (OTU7; r_s=-0.49, q=0.02). In contrast, Tapioca-RS4-induced shifts in propionate proportions were negatively correlated with increases in *Eisenbergiella* spp. (OTU9; r_s=-0.55, q=0.007), while being positively correlated with increases in *P. distasonis* (OTU21; r_s=0.49, q=0.03), an important succinate-producing bacterium. Succinate is promptly converted to propionate by other commensal bacteria[591], providing an explanation for this association. Several of these correlations within groups were also detectable in the whole dataset; changes in butyrate correlated with *E. rectale* shifts (r_s=0.29, q=0.004) and changes in propionate correlated with *P. distasonis* shifts (r_s=0.30, q=0.004). These findings suggest that the shifts in SCFA output are the result of a targeted and structure-dependent effect of the RS4s on microbiota members that possess the pathways to both utilize the RS4 and generate the respective SCFA.

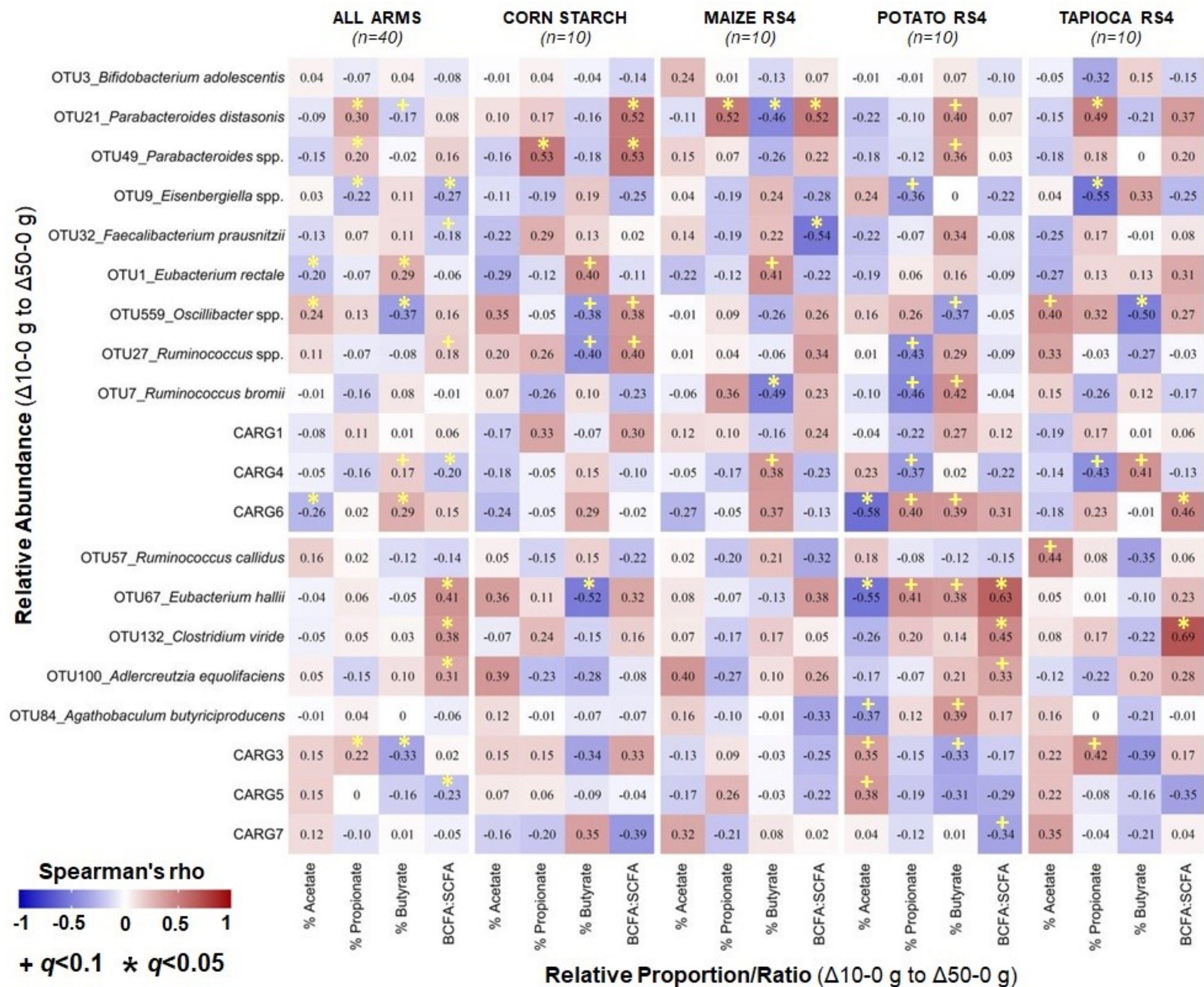

Figure 6.6. Associations between shifts of bacterial abundances and changes in the relative proportions of SCFAs. Spearman's correlations (with FDR correction) assess associations between shifts in bacterial composition and relative proportions of SCFAs, where all subjects' delta values (*i.e.* Δ10-0g to Δ50-0g) from the four intervention arms were analyzed together (n=40) and as separate arms (n=10). BCFA:SCFA, total branched short-chain to short-chain fatty acid ratio; CARG, co-abundance response group; OTU, operational taxonomic unit.

6.3.8 Specific Effects of RS4s can be Explained by Selective Bacterial Adherence and Substrate Utilization

To determine the mechanisms that led to the substrate-specificity of RS4s, we compared the adherence and utilization ability of 4 strains from amylolytic species that were representative of OTUs enriched by Maize and Tapioca RS4; *B. adolescentis* IVS-1, *E. rectale* 17629, *R. bromii* L2-63, and *P. distasonis* 8503. The analysis showed that these strains varied in their ability to bind and utilize different RSs (**Figure S.6.7**). *B. adolescentis* IVS-1, *R. bromii* L2-63,

and *P. distasonis* 8503 were all able to adhere to the granules of all RS4s *in vitro*, while *E. rectale* 17629 adhered only to Maize RS4 but not Potato or Tapioca RS4 (**Figure 6.7.A**). In addition, although all strains were able to utilize Maize RS4 for growth, only *B. adolescentis* IVS-1 and *P. distasonis* 8503, but not *E. rectale* 17629 and *R. bromii* L2-63, were able to grow on Tapioca RS4 (**Figure 6.7.B**). In summary, the *in vitro* experiments revealed substrate-specific differences in the adherence and utilization of Tapioca RS4 that were in line with our *in vivo* findings, providing a potential mechanism for the specific effects of different RS4s in the human trial.

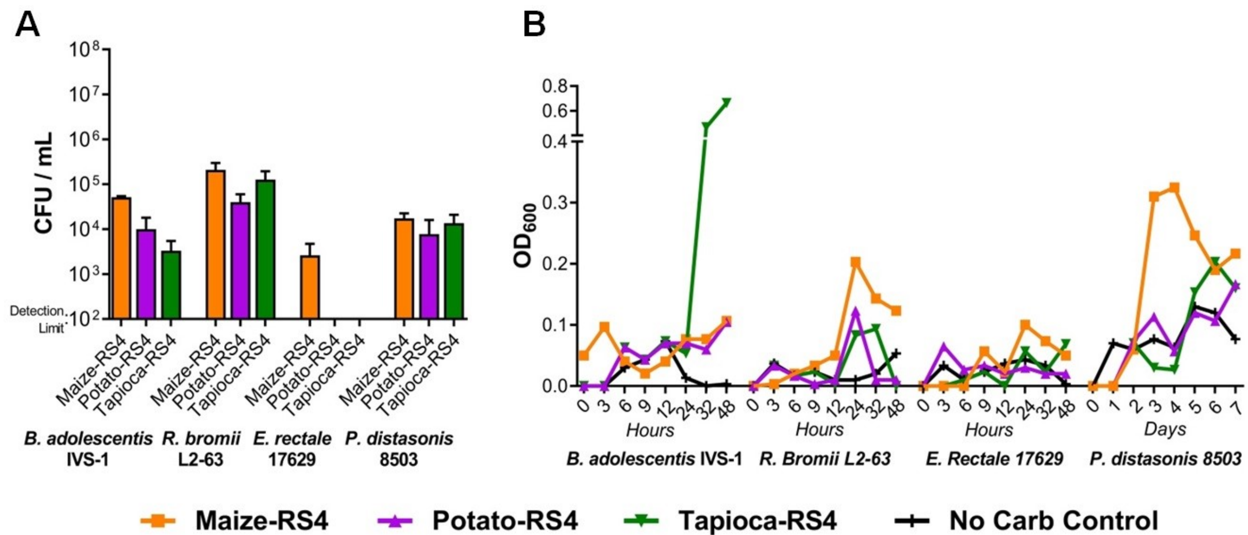

Figure 6.7. *In vitro* assessment of RS4 adherence and utilization by representative human-gut-derived amylolytic bacteria. (A) Total CFUs (CFU/ml) of *Bifidobacterium adolescentis* IVS-1, *Ruminococcus bromii* L2-63, *Eubacterium rectale* 17629, and *Parabacteroides distasonis* 8503 recovered from RS4s after *in vitro* binding assay with the respective RS4. **(B)** Growth curves (OD_{600}) of *B. adolescentis* IVS-1, *R. bromii* L2-63, *E. rectale* 17629, and *P. distasonis* 8503 in YCFA medium containing either 0.2% of the indicated RS4 or no carbohydrate (control).

6.4 Discussion

This study revealed that discrete structural differences between two DFs can induce substantial yet distinct effects on overall gut microbiota diversity, composition, and functions, leading to selective enrichments of a few bacterial taxa that possess adaptations towards the respective substrates. These compositional responses were linked to directed changes in SCFA output towards either butyrate or propionate. Dominant compositional and functional effects of RS4s were dose-dependent and plateaued at a 35 g/day dose in the overall population. Even though all effects showed inter-individual variation, the dominant RS4-induced changes were

remarkably consistent. The doses necessary for RS4s to maximize effects on the fecal microbiome were further on average well tolerated.

In ecological terms, DFs constitute resources in the GI tract that support the growth of primary degraders able to utilize them directly and microbes that benefit through cross-feeding of pubic goods released during degradation. The selective enrichment of only a limited number of bacterial taxa in our study suggests that within the diverse microbiota, only few microbes possess the specialized adaptations needed to competitively access and utilize the molecular structures of Maize and Tapioca RS4. Accordingly, the microbes enriched are known to efficiently utilize starch[430,588,592,593], but they differ in their ability to bind to the RS4s, and also potentially in their ability to access their distinct crystalline and cross-linked structures. The bacteria enriched by Maize RS4, such as *B. adolescentis*, *E. rectale*, *Oscillibacter*, and *Ruminococcus* related taxa, were also detected in previous studies supplementing with granular and retrograded crystalline starches[67-69,554,594,595]. This suggests that re-structuring of the high-amylose maize granule (by annealing and acid treatment) to produce Maize RS4 does not alter the microbial affinity to this substrate. Interestingly, the same bacterial taxa (*B. adolescentis*, *R. bromii*, and *E. rectale*) are also able to selectively colonize RS2 granules, which might constitute an important factor for competitive substrate utilization[94].

In contrast, our findings showed that *E. rectale* was completely unable to bind Tapioca RS4, and *E. rectale* and *R. bromii* were both limited in their growth on the substrate, while *B. adolescentis* and *P. distasonis* both showed good adherence and utilization of Tapioca RS4. Phosphate cross-linking acts on the surface of the tapioca granule generating additional inter-starch ester linkages that produce a slightly rough textured surface[563]. Based on our *in vitro* findings, we speculate that the ester linkages specifically impede surface-attachment by *E. rectale* and resource utilization by *Ruminococcus* species, conferring a competitive advantage to *P. distasonis*. Selective enrichment of *P. distasonis* has also been shown for cross-linked wheat starch[69,110] and butyrate esterified maize starch[596,597], suggesting that this species possesses specialized traits to access esterified starches. The only species that seemed able to bind and utilize both crystalline and cross-linked starches was *B. adolescentis*, which is in agreement with the consistent enrichment of this species in human intervention trials[67,69,110,554]. Although differences in chemical modifications of RS4s likely explain the specificity in microbiome response, other factors, such as particle size, might also contribute. This would explain the lack

of Potato RS4 fermentation in our and previous studies[585,598], as this starch has the largest particle size, which reduces potential attachment sites per particle volume[599].

The effects of Maize and Tapioca RS4 were remarkably specific, with most taxa impacted by one RS4 showing no response with the other, and several taxa (*P. distasonis*, *F. prausnitzii, Ruminococcus* spp.) even showing opposite responses (**Figure 6.3**). This was in contrast to the taxa that decreased in abundance, which virtually all responded in both RS4s. This finding suggests inhibition as a likely mechanism for these reductions, potentially attributable to colonic environment changes, which are likely less specific than direct competition. A potential mechanism for this inhibition is the increased production of SCFAs, which possess antimicrobial activity themselves (*e.g.* acetate and propionate[600]) and also acidify the environment inhibiting the growth of pH-sensitive taxa like *Bacteroides fragilis*[76]. On a more speculative note, SCFAs up-regulate phage production, which might be reducing the abundance of taxa in the community[601]. It appears that while discrete DF structures enrich specific features of the gut microbiota (including SCFAs); reductions (taxa and BCFAs) are much less specific.

It is of substantial interest that RS4 treatments not only modulated gut microbiota composition, but also its metabolism, with strong correlations between compositional shifts and SCFAs that reflect the organisms' metabolic capacities. The RS4-induced taxa that showed strong links to butyrate and propionate shifts, *E. rectale* and *P. distasonis*, encode the metabolic pathways for butyrate and succinate production, with the latter being readily converted to propionate[477]. Correlations of SCFAs with CARGs (*i.e.* CARG6 and CARG1) were not significant, which suggests that RS4-induced changes of SCFAs are more dependent on individual taxa and not complex ecological guilds. Although physiological effects of BCFAs have not been elucidated, their reductions do indicate that fermentation of RS4 inhibits colonic protein fermentation, an effect considered beneficial[122,590]. In summary, our results suggest that discrete DF structures can be developed to guide the output of specific SCFAs with non-specific reductions of BCFAs.

The dose-response study design allowed us to identify microbiome features that exhibited dose-dependent responses to RS4s, and the approximate doses needed to maximize these effects. We can only speculate about the reason for the detected thresholds, but it is possible that the ecosystem is saturated with 35 g/day of RS4, with taxa reaching maximum growth rates or experiencing limitations in other essential nutrients that limit their expansion. Although our

study does not allow direct inferences on health, our findings do provide a basis for tailoring the use of DF to enhance desired effects that are relevant to health. This is pertinent as DF doses used by a vast majority of human trials in the literature are considerably lower than 35 g/day[437].

Given the substantial degree of individuality in both gut microbiota composition[59] and response to diet[438], we were surprised by how consistent and reproducible several of our findings were. This was particularly evident in the enrichments of *E. rectale* and *P. distasonis*, which were observed in every single subject while on Maize and Tapioca RS4, respectively. This consistency might have resulted in the directed outputs of butyrate and propionate, which were correlated with these taxa. In addition, comparisons of our findings with those in the literature revealed that the effects of RSs are remarkably reproducible in studies performed in different cohorts, countries, and even continents[67-69,554,594,595]. For example, *B. adolescentis, E. rectale*, *R. bromii*, and *Ruminococcus* spp. (OTU27), the latter being 100% identical to seq100 detected by Baxter *et al.*[554], have been consistently enriched by crystalline RSs (RS2 and RS3), while phosphate cross-linked RS4s consistently enriched for *P. distasonis* and *B. adolescentis*[69,110]. This overlap is remarkable in the light of inter-individual variation and the lack of conserved core-species in human microbiomes[602], as well as the low reproducibility in gut microbiome studies due to methodological differences[570]. It further suggests phylogenetic niche conservatism in bacterial species in relation to the ability to adhere and utilize RS, which implies that within-species strain-level genomic and functional differences and functional redundancies among unrelated species are low as they relate to the genes required to utilize RS.

Despite these consistent findings in dominant responses, there was still clear inter-subject variation. Even in taxa consistently enriched, magnitudes and the doses to achieve maximum changes differed, while several taxa showed an even higher degree of interpersonal variation. The most interesting taxon in this respect was arguably *E. rectale*, which was consistently enhanced with Maize RS4, while also showing a very strong response in three individuals with Tapioca RS4. In addition, *B. adolescentis* clearly increased in only around a third of individuals, consistent with previous findings[68,69]. This variation might be due to strain-to-strain differences in the ability of *B. adolescentis* to adhere and utilize RS[588,603]. Although overall well tolerated, RS4-induced symptoms were also individualized (**Figure S.6.2**). Therefore, even though our findings clearly suggest that targeted effects of RS4s can be achieved in a human population, a "one size fits all" approach is unlikely to be universally successful at remodeling dysbiotic

patterns, indicating there is still scope for designing personalized treatments to maximize both health effects and GI tolerance[604].

This study revealed important insight for the use of RS4s for microbiota-directed interventions to improve health. In general, Maize and Tapioca RS4s induce gut microbiota responses that are likely beneficial, enhancing saccharolytic fermentation at the expense of detrimental proteolysis (*i.e.* SCFA vs. BCFA production) without reducing the overall number of taxa. In addition, our findings provide a basis for a more 'intelligent' use of Maize and Tapioca RS4 to achieve targeted manipulation of dysbiotic gut communities to yield specific health endpoints. This strategy could be further extended to fecal microbiota transplantations with an aim to provide substrates for selective niche opportunities of specific bacterial species predicted to provide benefits. For instance, Maize RS4 might be applicable for correcting the dysbiosis seen in type II diabetes, where *E. rectale* has been shown to be low in abundance[605] and positively associated with improved glycemic control[8,53]. Furthermore, the immunoregulatory properties of butyrate[31] makes Maize RS4 a candidate for the treatment and/or prevention of colorectal cancer, inflammatory bowel disease, and obesity-associated inflammation. Tapioca RS4, on the other hand, could be used to correct dysbiotic communities where *P. distasonis* abundance is reduced, such as obesity and non-alcoholic fatty liver disease[606,607]. To this end, treatment with live *P. distasonis* has recently been shown in mice to decrease high-fat diet induced weight gain, hyperglycemia, and hepatic steatosis[591]. Propionate could be further targeted by Tapioca RS4 for the treatment of insulin resistance and obesity, owing to its upregulation of IGN[153] and anorectic hormones[213]. Mixtures of Maize and Tapioca RS4 could be used to increase both butyrate and propionate production simultaneously, which would be particularly relevant for the treatment of obesity and its dysregulated immunometabolism. Given the technological attributes of RSs in the production of flour-based foods[86], they could be readily incorporated into medical foods for specific patient populations and the general food supply.

Overall, findings in **Chapter 6** of this dissertation provide critical evidence in support of a mechanistic framework for intelligent manipulation of the colonic microbiota with discrete DF structures[36]. Our correlation analyses indicate that key aspects of the framework apply to the competitive constraints of a human microbiome, as DF structures do in fact align with phenotypes of specific microbes that differ in their metabolic pathways, directing the output of physiologically relevant metabolites. The ability to employ small differences in DF chemical

structure to achieve substantial, highly selective, and tractable effects on the gut microbiome paves the way for the development of precision approaches that could involve designer carbohydrates that target functional outputs relevant to health. The notable dose-dependency of DF-induced effects established herein permits a more systematic and precise modulation of the microbiome, information that is for the most part lacking in the field. Although approaches could be optimized through personalization, the consistency of the main findings and their high reproducibility among other published studies suggests that microbiome-modulating strategies based on discrete DF structures could be successfully deployed on a population-wide basis.

6.5 References

4. Sonnenburg ED, Smits SA, Tikhonov M, Higginbottom SK, Wingreen NS, Sonnenburg JL. Diet-induced extinctions in the gut microbiota compound over generations. *Nature.* 2016;529(7585):212-15.
8. Martínez I, Lattimer JM, Hubach KL*, et al.* Gut microbiome composition is linked to whole grain-induced immunological improvements. *ISME J.* 2013;7(2):269-80.
18. Walker AW, Lawley TD. Therapeutic modulation of intestinal dysbiosis. *Pharmacol Res.* 2013;69(1):75-86.
31. Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. *Cell.* 2016;165(6):1332-45.
36. Hamaker BR, Tuncil YE. A perspective on the complexity of dietary fiber structures and their potential effect on the gut microbiota. *J Mol Biol.* 2014;426(23):3838-50.
53. Zeevi D, Korem T, Zmora N*, et al.* Personalized Nutrition by Prediction of Glycemic Responses. *Cell.* 2015;163(5):1079-94.
59. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. *Nature.* 2012;489(7415):220-30.
63. Flint HJ, Duncan SH, Scott KP, Louis P. Links between diet, gut microbiota composition and gut metabolism. *Proc Nutr Soc.* 2015;74(1):13-22.
67. Venkataraman A, Sieber JR, Schmidt AW, Waldron C, Theis KR, Schmidt TM. Variable responses of human microbiomes to dietary supplementation with resistant starch. *Microbiome.* 2016;4(1):33.
68. Walker AW, Ince J, Duncan SH*, et al.* Dominant and diet-responsive groups of bacteria within the human colonic microbiota. *ISME J.* 2011;5(2):220-30.
69. Martínez I, Kim J, Duffy PR, Schlegel VL, Walter J. Resistant starches types 2 and 4 have differential effects on the composition of the fecal microbiota in human subjects. *PLoS One.* 2010;5(11):e15046.
73. Davis LMG, Martínez I, Walter J, Goin C, Hutkins RW. Barcoded pyrosequencing reveals that consumption of galactooligosaccharides results in a highly specific bifidogenic response in humans. *PLoS One.* 2011;6(9):e25200.
76. Duncan SH, Louis P, Thomson JM, Flint HJ. The role of pH in determining the species composition of the human colonic microbiota. *Environ Microbiol.* 2009;11(8):2112-22.

86. Raigond P, Ezekiel R, Raigond B. Resistant starch in food: a review. *J Sci Food Agr.* 2015;95(10):1968-78.
94. Leitch ECM, Walker AW, Duncan SH, Holtrop G, Flint HJ. Selective colonization of insoluble substrates by human faecal bacteria. *Environ Microbiol.* 2007;9(3):667-79.
98. Coyte KZ, Schluter J, Foster KR. The ecology of the microbiome: Networks, competition, and stability. *Science.* 2015;350(6261):663-66.
102. Barcenilla A, Pryde SE, Martin JC*, et al.* Phylogenetic relationships of butyrate-producing bacteria from the human gut. *Appl Environ Microbiol.* 2000;66(4):1654-61.
110. Upadhyaya B, McCormack L, Fardin-Kia AR*, et al.* Impact of dietary resistant starch type 4 on human gut microbiota and immunometabolic functions. *Sci Rep.* 2016;6:28797.
116. Yang J, Martínez I, Walter J, Keshavarzian A, Rose DJ. *In vitro* characterization of the impact of selected dietary fibers on fecal microbiota composition and short chain fatty acid production. *Anaerobe.* 2013;23:74-81.
121. Russell WR, Gratz SW, Duncan SH*, et al.* High-protein, reduced-carbohydrate weight-loss diets promote metabolite profiles likely to be detrimental to colonic health. *Am J Clin Nutr.* 2011;93(5):1062-72.
122. Windey K, De Preter V, Verbeke K. Relevance of protein fermentation to gut health. *Mol Nutr Food Res.* 2012;56(1):184-96.
153. De Vadder F, Kovatcheva-Datchary P, Goncalves D*, et al.* Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. *Cell.* 2014;156(1-2):84-96.
198. Arpaia N, Campbell C, Fan X*, et al.* Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature.* 2013;504(7480):451-55.
213. Psichas A, Sleeth ML, Murphy KG*, et al.* The short chain fatty acid propionate stimulates GLP-1 and PYY secretion via free fatty acid receptor 2 in rodents. *Int J Obes.* 2015;39(3):424-29.
266. Makki K, Deehan EC, Walter J, Bäckhed F. The Impact of Dietary Fiber on Gut Microbiota in Host Health and Disease. *Cell Host Microbe.* 2018;23(6):705-15.
267. Reynolds A, Mann J, Cummings J, Winter N, Mete E, Te Morenga L. Carbohydrate quality and human health: a series of systematic reviews and meta-analyses. *Lancet.* 2019;393(10170):434-45.
273. Deehan EC, Duar RM, Armet AM, Perez-Muñoz ME, Jin M, Walter J. Modulation of the Gastrointestinal Microbiome with Nondigestible Fermentable Carbohydrates To Improve Human Health. *Microbiol Spectr.* 2017;5(5).
283. Reichardt N, Vollmer M, Holtrop G*, et al.* Specific substrate-driven changes in human faecal microbiota composition contrast with functional redundancy in short-chain fatty acid production. *ISME J.* 2018;12(2):610-22.
300. Tannock GW, Liu Y. Guided dietary fibre intake as a means of directing short-chain fatty acid production by the gut microbiota. *J Roy Soc New Zeal.* 2019.
309. McRorie JWJ, McKeown NM. Understanding the Physics of Functional Fibers in the Gastrointestinal Tract: An Evidence-Based Approach to Resolving Enduring Misconceptions about Insoluble and Soluble Fiber. *J Acad Nutr Diet.* 2017;117(2):251-64.
357. Krumbeck JA, Rasmussen HE, Hutkins RW*, et al.* Probiotic *Bifidobacterium* strains and galactooligosaccharides improve intestinal barrier function in obese adults but show no synergism when used together as synbiotics. *Microbiome.* 2018;6(1):121.

430. Ze X, Duncan SH, Louis P, Flint HJ. *Ruminococcus bromii* is a keystone species for the degradation of resistant starch in the human colon. *ISME J.* 2012;6(8):1535-43.
437. Armet AM, Deehan EC, Thöne JV, Hewko SJ, Walter J. The Effect of Isolated and Synthetic Dietary Fibers on Markers of Metabolic Diseases in Human Intervention Studies: A Systematic Review. *Adv Nutr.* 2020;11(2):420–38.
438. Healey GR, Murphy R, Brough L, Butts CA, Coad J. Interindividual variability in gut microbiota and host response to dietary interventions. *Nutr Rev.* 2017;75(12):1059-80.
442. Cockburn DW, Koropatkin NM. Polysaccharide Degradation by the Intestinal Microbiota and Its Influence on Human Health and Disease. *J Mol Biol.* 2016;428(16):3230-52.
443. De Filippis F, Pasolli E, Tett A*, et al.* Distinct Genetic and Functional Traits of Human Intestinal *Prevotella copri* Strains Are Associated with Different Habitual Diets. *Cell Host Microbe.* 2019;25(3):444-53.e3.
454. Jin M, Kalainy S, Baskota N*, et al.* Faecal microbiota from patients with cirrhosis has a low capacity to ferment non-digestible carbohydrates into short-chain fatty acids. *Liver Int.* 2019;39(8):1437-47.
457. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol.* 2007;73(16):5261-67.
459. Yoon S-H, Ha S-M, Kwon S*, et al.* Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol.* 2017;67(5):1613-17.
463. vegan: Community Ecology Package [computer program]. Version R-package version 2.5-52019.
465. Højsgaard S, Halekoh U, Yan J. The R Package geepack for Generalized Estimating Equations. *J Stat Softw.* 2005;15(2).
466. Tong X, Xu J, Lian F*, et al.* Structural Alteration of Gut Microbiota during the Amelioration of Human Type 2 Diabetes with Hyperlipidemia by Metformin and a Traditional Chinese Herbal Formula: a Multicenter, Randomized, Open Label Clinical Trial. *mBio.* 2018;9(3):e02392-17.
477. Louis P, Flint HJ. Formation of propionate and butyrate by the human colonic microbiota. *Environ Microbiol.* 2017;19(1):29-41.
518. Chambers ES, Viardot A, Psichas A*, et al.* Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults. *Gut.* 2015;64(11):1744-54.
519. Chambers ES, Byrne CS, Morrison DJ*, et al.* Dietary supplementation with inulin-propionate ester or inulin improves insulin sensitivity in adults with overweight and obesity with distinct effects on the gut microbiota, plasma metabolome and systemic inflammatory responses: a randomised cross-over trial. *Gut.* 2019;68(8):1430-38.
520. Venter C, Vorster H, Cummings JH. Effects of dietary propionate on carbohydrate and lipid metabolism in healthy volunteers. *Am J Gastroenterol.* 1990;85(5):549-53.
529. Cherbut C, Michel C, Raison V, Kravtchenko T, Severine M. Acacia Gum is a Bifidogenic Dietary Fibre with High Digestive Tolerance in Healthy Humans. *Microb Ecol Health Dis.* 2003;15(1):43-50.
534. Mego M, Accarino A, Tzortzis G*, et al.* Colonic gas homeostasis: Mechanisms of adaptation following HOST-G904 galactooligosaccharide use in humans. *Neurogastroenterol Motil.* 2017;29(9):e13080.

540. Lam YY, Zhang C, Zhao L. Causality in dietary interventions-building a case for gut microbiota. *Genome Med.* 2018;10(1):62.
553. Valdes AM, Walter J, Segal E, Spector TD. Role of the gut microbiota in nutrition and health. *BMJ.* 2018;361:k2179.
554. Baxter NT, Schmidt AW, Venkataraman A, Kim KS, Waldron C, Schmidt TM. Dynamics of Human Gut Microbiota and Short-Chain Fatty Acids in Response to Dietary Interventions with Three Fermentable Fibers. *mBio.* 2019;10(1):e02566-18.
555. Rivera-Chavez F, Zhang LF, Faber F*, et al.* Depletion of Butyrate-Producing *Clostridia* from the Gut Microbiota Drives an Aerobic Luminal Expansion of *Salmonella*. *Cell Host Microbe.* 2016;19(4):443-54.
556. van der Beek CM, Dejong CHC, Troost FJ, Masclee AAM, Lenaerts K. Role of short-chain fatty acids in colonic inflammation, carcinogenesis, and mucosal protection and healing. *Nutr Rev.* 2017;75(4):286-305.
557. Moya A, Ferrer M. Functional Redundancy-Induced Stability of Gut Microbiota Subjected to Disturbance. *Trends Microbiol.* 2016;24(5):402-13.
558. DeMartino P, Cockburn DW. Resistant starch: impact on the gut microbiome and health. *Curr Opin Biotechnol.* 2020;61:66-71.
559. Calame W, Weseler AR, Viebke C, Flynn C, Siemensma AD. Gum arabic establishes prebiotic functionality in healthy human volunteers in a dose-dependent manner. *Br J Nutr.* 2008;100(6):1269-75.
560. So D, Whelan K, Rossi M*, et al.* Dietary fiber intervention on gut microbiota composition in healthy adults: a systematic review and meta-analysis. *Am J Clin Nutr.* 2018;107(6):965-83.
561. van den Heuvel EG, Wils D, Pasman WJ, Bakker M, Saniez M-HH, Kardinaal AF. Short-term digestive tolerance of different doses of NUTRIOSE® FB, a food dextrin, in adult men. *Eur J Clin Nutr.* 2004;58(7):1046-55.
562. Brumovsky JO, Thompson DB. Production of Boiling-Stable Granular Resistant Starch by Partial Acid Hydrolysis and Hydrothermal Treatments of High-Amylose Maize Starch. *Cereal Chem.* 2001;78(6):680-89.
563. Chen Q, Yu H, Wang L*, et al.* Recent progress in chemical modification of starch and its applications. *RSC Adv.* 2015;5(83):67459-74.
564. Thompson FE, Dixit-Joshi S, Potischman N*, et al.* Comparison of Interviewer-Administered and Automated Self-Administered 24-Hour Dietary Recalls in 3 Diverse Integrated Health Systems. *Am J Epidemiol.* 2015;181(12):970-78.
565. Csizmadi I, Kahle L, Ullman R*, et al.* Adaptation and evaluation of the National Cancer Institute's Diet History Questionnaire and nutrient database for Canadian populations. *Public Health Nutr.* 2007;10(1):88-96.
566. Mäder U, Martin BW, Schutz Y, Marti B. Validity of four short physical activity questionnaires in middle-aged persons. *Med Sci Sports Exerc.* 2006;38(7):1255-66.
567. Cohen S, Williamson GM. Perceived stress in a probability sample of the United States. In: Spacapan S, Oskamp S, eds. *The Social Psychology of Health. The Claremont Symposium on Applied Social Psychology.* Thousand Oaks, CA: Sage Publications, Inc.; 1988.
568. Maki KC, Rains TM, Kelley KM, Cook CM, Schild AL, Gietl E. Fibermalt is well tolerated in healthy men and women at intakes up to 60 g/d: a randomized, double-blind, crossover trial. *Int J Food Sci Nutr.* 2013;64(3):274-81.

569. Stewart ML, Wilcox ML, Bell M, Buggia MA, Maki KC. Type-4 Resistant Starch in Substitution for Available Carbohydrate Reduces Postprandial Glycemic Response and Hunger in Acute, Randomized, Double-Blind, Controlled Study. *Nutrients.* 2018;10(2):129.
570. Costea PI, Zeller G, Sunagawa S*, et al.* Towards standards for human fecal sample processing in metagenomic studies. *Nat Biotechnol.* 2017;35(11):1069-76.
571. Eren AM, Vineis JH, Morrison HG, Sogin ML. A filtering method to generate high quality short reads using illumina paired-end technology. *PLoS One.* 2013;8(6):e66643.
572. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods.* 2013;10(10):996-98.
573. Westram R, Bader K, Prüsse E*, et al.* ARB: a software environment for sequence data. In: de Bruijn FJ, ed. *Handbook of Molecular Microbial Ecology, Volume I: Metagenomics and Complementary Approaches*. 1 ed: John Wiley & Sons, Inc; 2011:399-406.
574. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *J Mol Biol.* 1990;215(3):403-10.
575. Michigan State University. RDP (Ribosomal Database Project). *RDP Release 11, Update 5.* 2016. September 30:Available at: www.rdp.cme.msu.edu/seqmatch/seqmatch_intro.jsp;jsessionid=900158E5787CA0B0CFB591C737B90E9B.radian.
576. Caporaso JG, Kuczynski J, Stombaugh JI*, et al.* QIIME allows analysis of high-throughput community sequencing data. *Nat Methods.* 2010;7(5):335-36.
577. Hall M, Beiko RG. 16S rRNA Gene Analysis with QIIME2. *Methods Mol Biol.* 2018;1849:113-29.
578. Sakamoto M, Benno Y. Reclassification of *Bacteroides distasonis*, *Bacteroides goldsteinii* and *Bacteroides merdae* as *Parabacteroides distasonis* gen. nov., comb. nov., *Parabacteroides goldsteinii* comb. nov. and *Parabacteroides merdae* comb. nov. *Int J Syst Evol Microbiol.* 2006;56(Pt 7):1599-605.
579. Streiner D, Geddes J. Intention to treat analysis in clinical trials when there are missing data. *Evid Based Ment Health.* 2001;4(3):70-71.
580. Lenth R, Singmann H, Love J, Buerkner P, Herve M. Estimated Marginal Means, aka Least-Squares Means. 2019. Available at: www.cran.r-project.org/web/packages/emmeans/emmeans.pdf.
581. Christensen RHB. Cumulative Link Models for Ordinal Regression with the R Package ordinal. 2018. Available at: www.cran.r-project.org/web/packages/ordinal/vignettes/clm_article.pdf.
582. Schmider E, Ziegler M, Danay E, Beyer L, Bühner M. Is It Really Robust? Reinvestigating the Robustness of ANOVA Against Violations of the Normal Distribution Assumption. *Methodology.* 2010;6(4):147-51.
583. Harwell MR, Rubinstein EN, Hayes WS, Olds CC. Summarizing Monte Carlo Results in Methodological Research: The One- and Two-Factor Fixed Effects ANOVA Cases. *J Educ Stat.* 1992;17(4):315-39.
584. Stewart ML, Zimmer JP. A High Fiber Cookie Made with Resistant Starch Type 4 Reduces Post-Prandial Glucose and Insulin Responses in Healthy Adults. *Nutrients.* 2017;9(3):237.

585. Coulon DB, Page R, Raggio AM*, et al.* Novel Resistant Starch Type 4 Products of Different Starch Origins, Production Methods, and Amounts are not Equally Fermented when Fed to Sprague-Dawley Rats. *Mol Nutr Food Res.* 2020;64(2):e1900901.
586. Wang Y, Chen J, Song Y-H*, et al.* Effects of the resistant starch on glucose, insulin, insulin resistance, and lipid parameters in overweight or obese adults: a systematic review and meta-analysis. *Nutr Diabetes.* 2019;9(1):19.
587. Guilhot E, Alou MT, Lagier J-C*, et al.* Genome sequence and description of *Anaeromassilibacillus senegalensis* gen. nov., sp. nov., isolated from the gut of patient with kwashiorkor. *New Microbes New Infect.* 2017;17:54-64.
588. Duranti S, Turroni F, Lugli GA*, et al.* Genomic characterization and transcriptional studies of the starch-utilizing strain *Bifidobacterium adolescentis* 22L. *Appl Environ Microbiol.* 2014;80(19):6080-90.
589. Cerqueira FM, Photenhauer AL, Pollet RM, Brown HA, Koropatkin NM. Starch Digestion by Gut Bacteria: Crowdsourcing for Carbs. *Trends Microbiol.* 2019;28(2):95-108.
590. Korpela K. Diet, Microbiota, and Metabolic Health: Trade-Off Between Saccharolytic and Proteolytic Fermentation. *Annu Rev Food Sci Technol.* 2018;9:65-84.
591. Wang K, Liao M, Zhou N*, et al. Parabacteroides distasonis* Alleviates Obesity and Metabolic Dysfunctions via Production of Succinate and Secondary Bile Acids. *Cell Rep.* 2019;26(1):222-35 e5.
592. Xu J, Mahowald MA, Ley RE*, et al.* Evolution of symbiotic bacteria in the distal human intestine. *PLoS Biol.* 2007;5(7):e156.
593. Cockburn DW, Orlovsky NI, Foley MH*, et al.* Molecular details of a starch utilization pathway in the human gut symbiont *Eubacterium rectale*. *Mol Microbiol.* 2015;95(2):209-30.
594. Abell GCJ, Cooke CM, Bennett CN, Conlon MA, McOrist AL. Phylotypes related to *Ruminococcus bromii* are abundant in the large bowel of humans and increase in response to a diet high in resistant starch. *FEMS Microbiol Ecol.* 2008;66(3):505-15.
595. Vital M, Howe A, Bergeron N, Krauss RM, Jansson JK, Tiedje JM. Metagenomic Insights into the Degradation of Resistant Starch by Human Gut Microbiota. *Appl Environ Microbiol.* 2018;84(23):e01562-18.
596. Clarke JM, Topping DL, Christophersen CT*, et al.* Butyrate esterified to starch is released in the human gastrointestinal tract. *Am J Clin Nutr.* 2011;94(5):1276-83.
597. Le Leu RK, Winter JM, Christophersen CT*, et al.* Butyrylated starch intake can prevent red meat-induced O^6-methyl-2-deoxyguanosine adducts in human rectal tissue: a randomised clinical trial. *Br J Nutr.* 2015;114(2):220-30.
598. Dahl WJ, Ford AL, Ukhanova M*, et al.* Resistant potato starches (type 4 RS) exhibit varying effects on laxation with and without phylum level changes in microbiota: A randomised trial in young adults. *J Funct Foods.* 2016;23:1-11.
599. Tester R, Qi X, Karkalas J. Hydrolysis of native starches with amylases. *Anim Feed Sci Technol.* 2006;130(1-2):39-54.
600. Mani-López E, García HS, López-Malo A. Organic acids as antimicrobials to control *Salmonella* in meat and poultry products. *Food Res Int.* 2012;45(2):713-21.
601. Oh J-H, Alexander LM, Pan M*, et al.* Dietary Fructose and Microbiota-Derived Short-Chain Fatty Acids Promote Bacteriophage Production in the Gut Symbiont *Lactobacillus reuteri*. *Cell Host Microbe.* 2019;25(2):273-84 e6.

602. Martínez I, Muller CE, Walter J. Long-term temporal analysis of the human fecal microbiota revealed a stable core of dominant bacterial species. *PLoS One.* 2013;8(7):e69621.
603. Crittenden R, Laitila A, Forssell P*, et al.* Adhesion of bifidobacteria to granular starch and its implications in probiotic technologies. *Appl Environ Microbiol.* 2001;67(8):3469-75.
604. Kolodziejczyk AA, Zheng D, Elinav E. Diet-microbiota interactions and personalized nutrition. *Nat Rev Microbiol.* 2019;17:742–53.
605. Qin J, Li Y, Cai Z*, et al.* A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature.* 2012;490(7418):55-60.
606. Del Chierico F, Nobili V, Vernocchi P*, et al.* Gut microbiota profiling of pediatric nonalcoholic fatty liver disease and obese patients unveiled by an integrated meta-omics-based approach. *Hepatology.* 2017;65(2):451-64.
607. Verdam FJ, Fuentes S, de Jonge C*, et al.* Human intestinal microbiota composition is associated with local and systemic inflammation in obesity. *Obesity.* 2013;21(12):E607-E15.

6.6 Supplementary Material

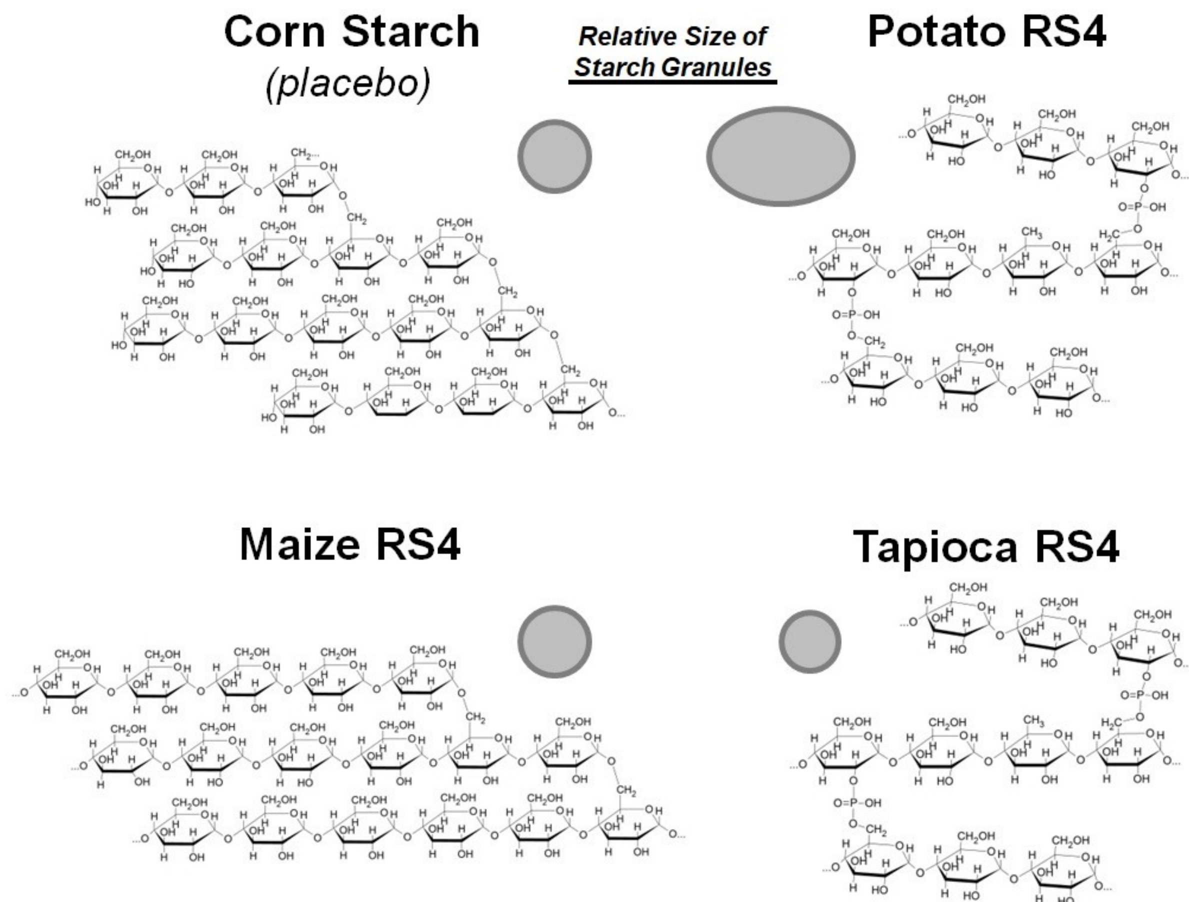

Figure S.6.1. Schematic diagrams of the chemical structure and relative granule size of each supplement.

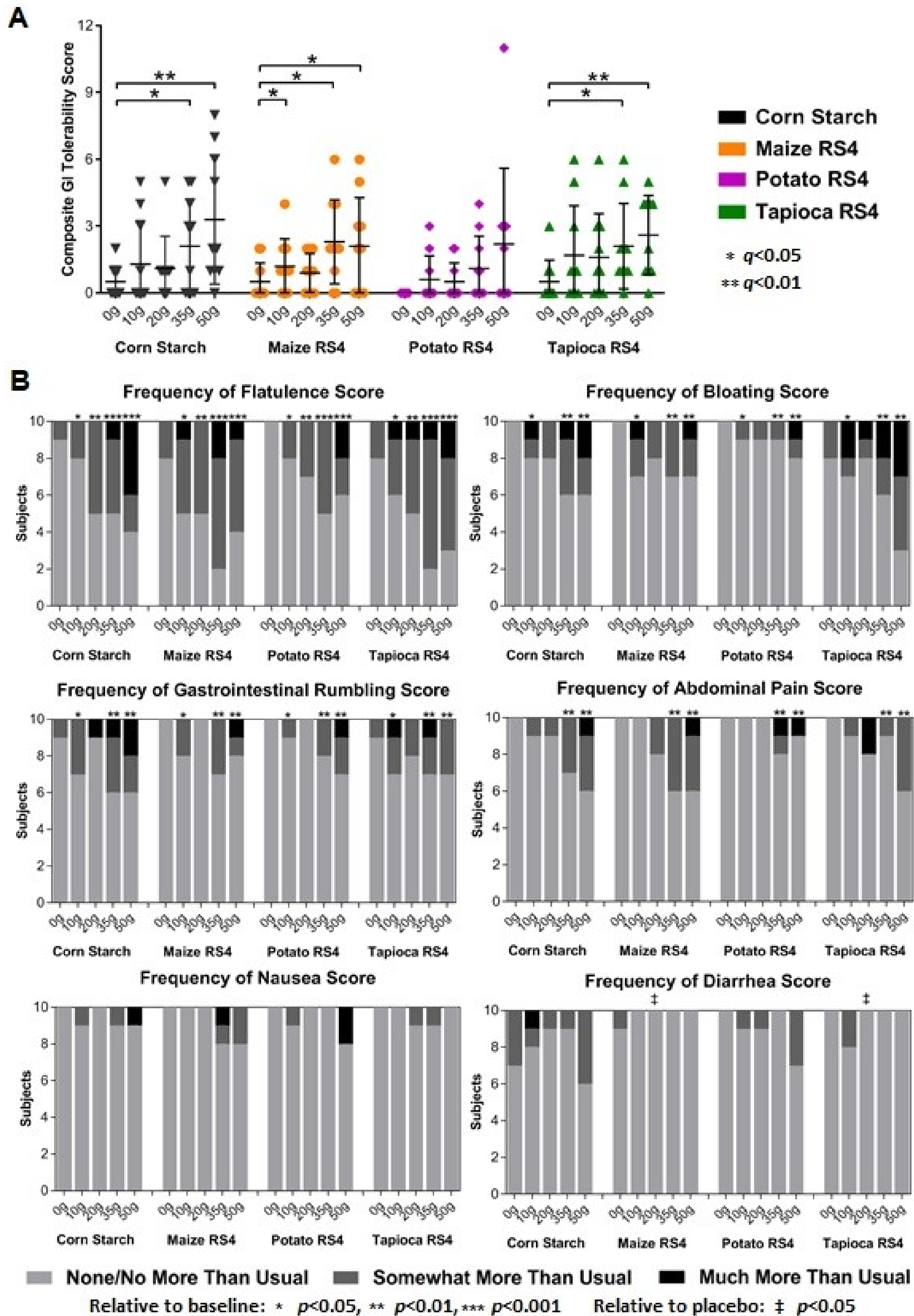

Figure S.6.2. Composite GI tolerability scores and the frequency of GI symptoms were affected by RS4 and placebo consumption, with high individual variability. *Legend continued on the next page.*

(A) Composite GI tolerability score data were analyzed by generalized estimating equation models (with FDR correction) to assess differences within- and between-groups. All treatments, apart from Potato RS4, significantly increased composite scores relative to baseline, with no differences detected relative to placebo (Corn Starch). Symbols represent individual tolerance reports; lines represent mean ± SD. **(B)** Frequency of individual GI symptoms were analyzed by cumulative link models relative to baseline (considering all four treatment groups) and to placebo (considering all 5 time-points).

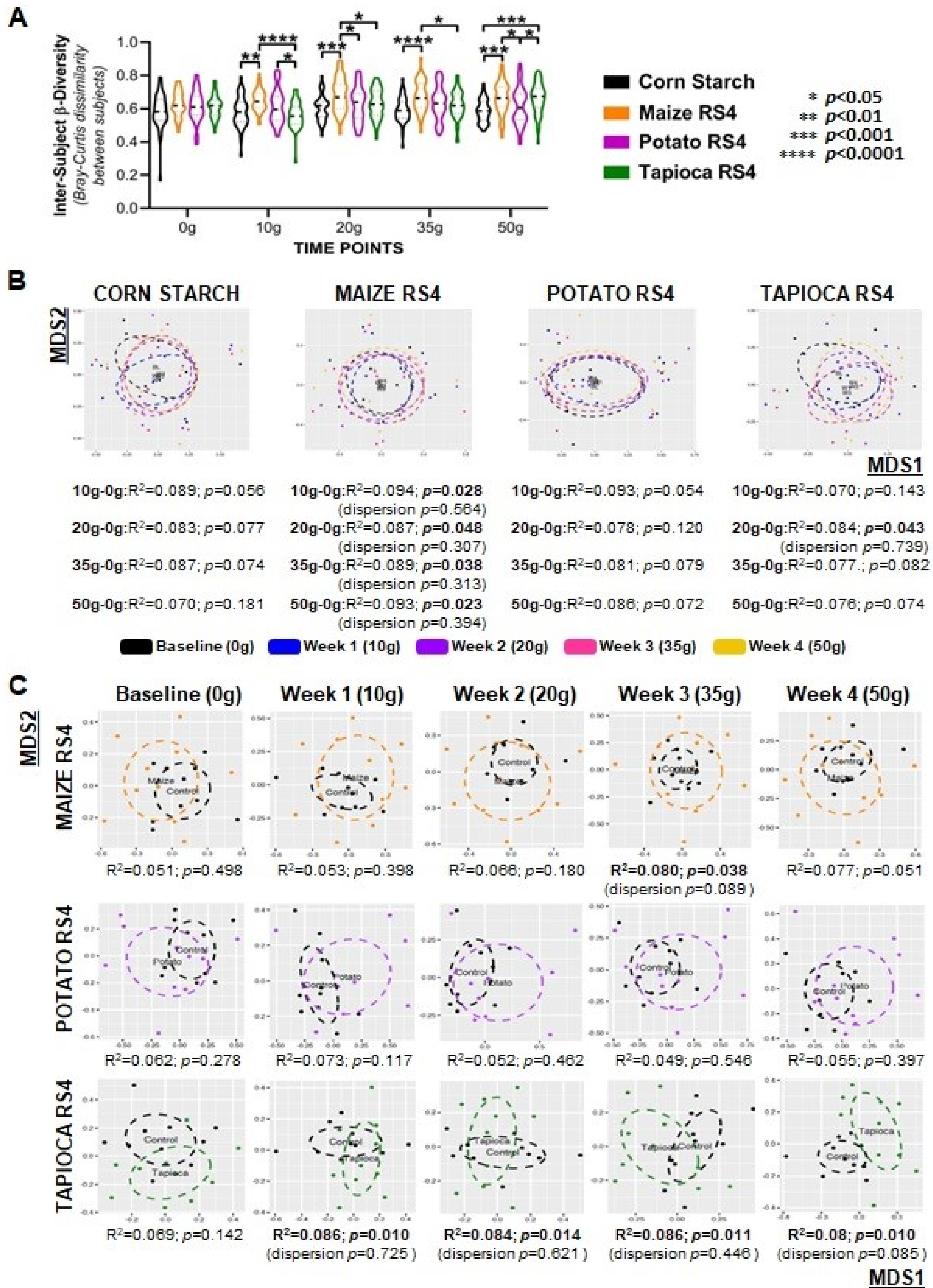

Figure S.6.3. Effects of different RS4s and placebo fecal bacterial β-diversity. *Legend continued on the next page.*

(A) Violin plots of Bray Curtis distances comparing between-treatment groups the fecal microbiomes of subjects at each dose/time-point (inter-subject β-diversity). Non-metric multidimensional scaling (NMDS) plots based on Bray Curtis distance metrics of RS4s and placebo at each dose (*i.e.* 0 g/day to 50 g/day) showing changes in the distance between subjects as DF dose increased for **(B)** each treatment relative to their baseline and **(C)** between RS4 treatments relative to placebo. Data analyzed for **(A)** using 2-way rANOVA (with Holm-Šídák correction) and for **(B and C)** using PERMANOVA and PERMDISP.

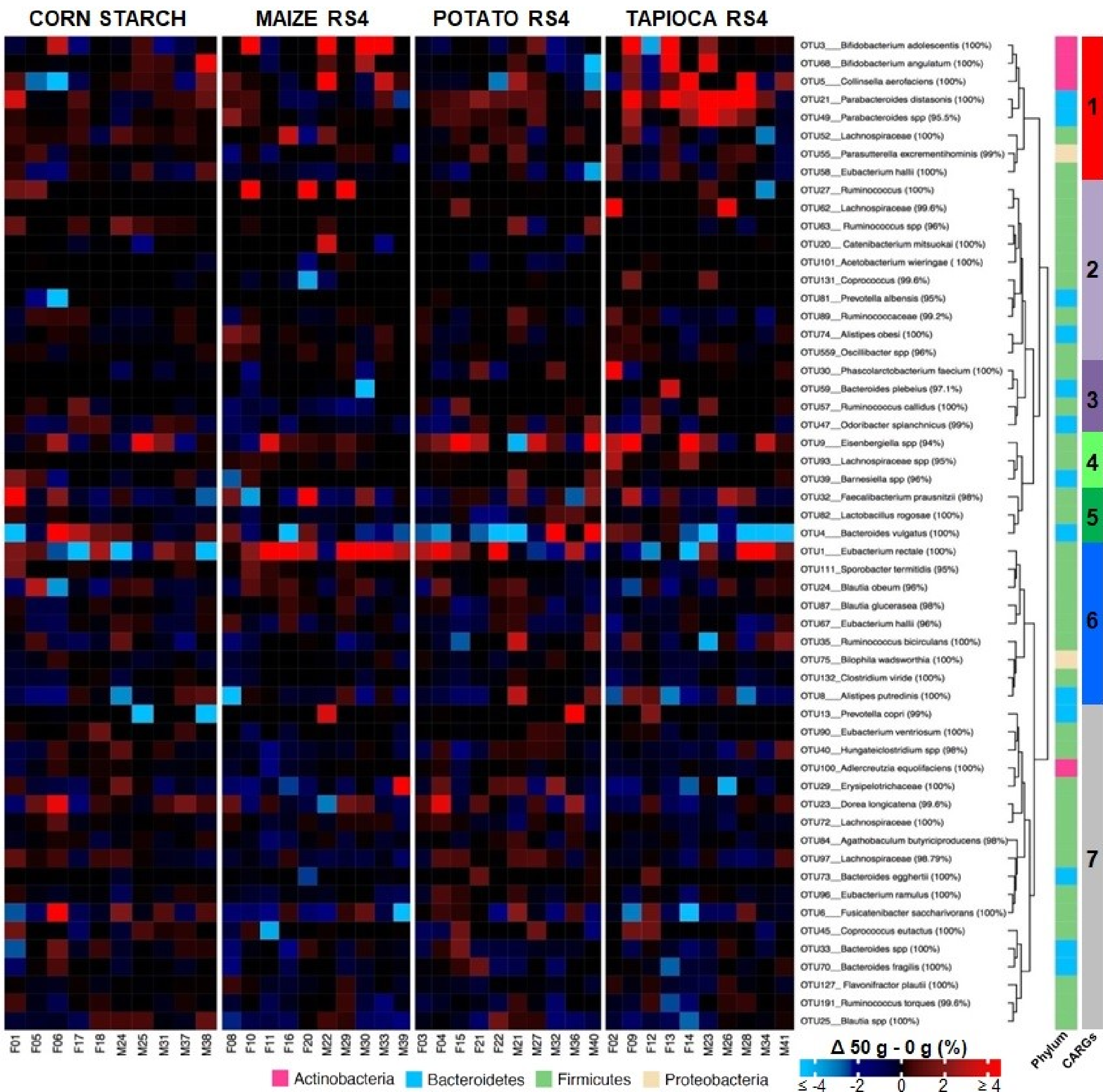

Figure S.6.4. Identification of co-abundance response groups (CARGs). Heatmap of shifts from baseline to week 4 (0 g/day to 50 g/day) in relative abundance of 55 operational taxonomic units (OTUs) affected by RS4 treatment (dose/interaction effect unadjusted $p<0.20$, 2-way rANOVA). OTUs were clustered by the Ward cluster algorithm based on Spearman's correlation distances that considered shifts across the 3 treatment arms (without placebo). Then OTUs were grouped on the Spearman's Hierarchical tree into 7 CARGs by PERMANOVA ($p\leq0.1$; CARGs 1-5) and visual inspection of the tree (CARGs 6-7).

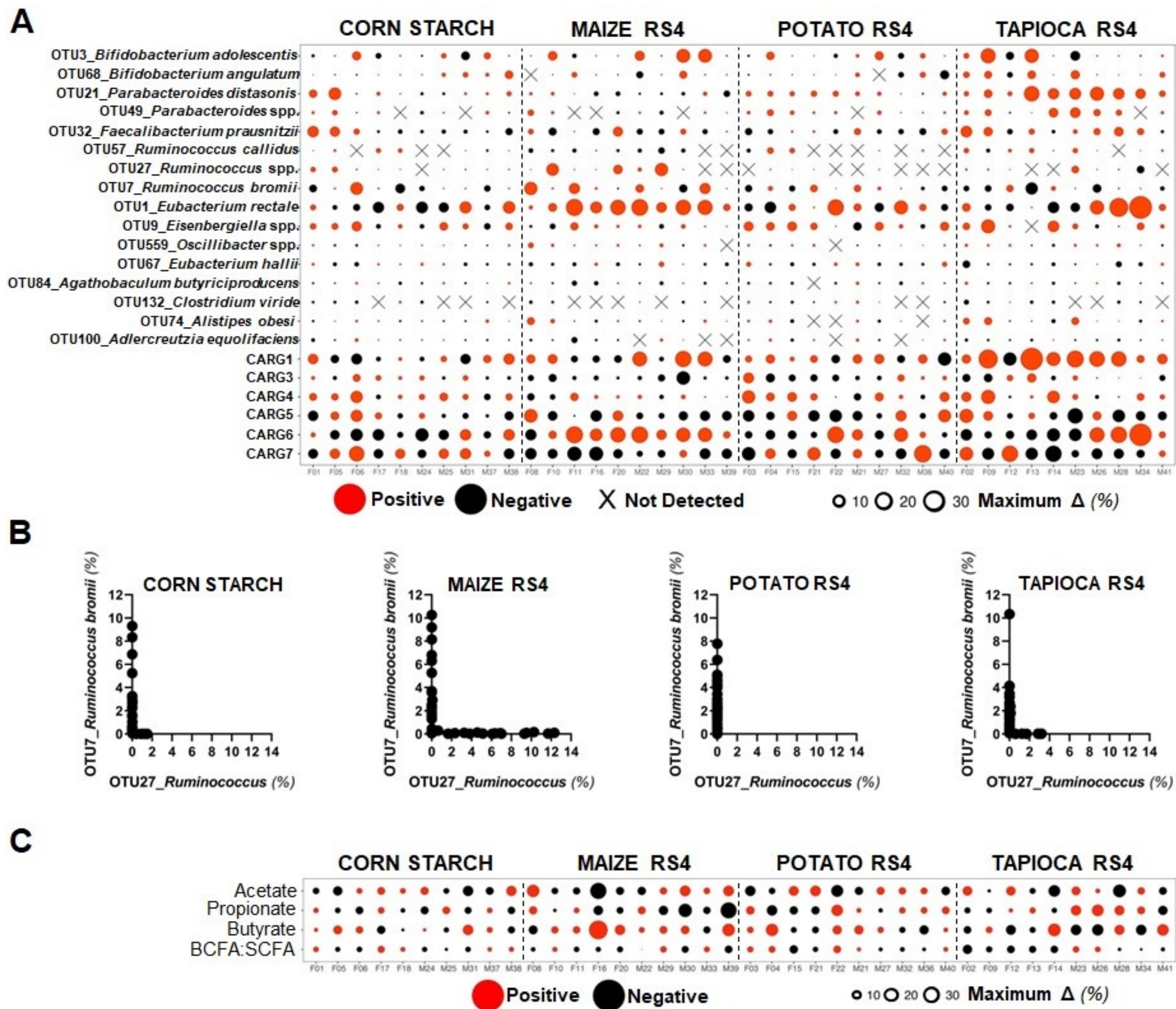

Figure S.6.5. Individual differences in the magnitude of OTU, CARG, and SCFA response to RS4s and placebo. (A) Bubble plots showing maximum differences in relative abundance of OTUs and CARGs (percentage of total microbiota composition) detected during Maize and Tapioca RS4 treatment relative to baseline. **(B)** Scatter plots showing the association between the relative abundance of OTU7 (*Ruminococcus bromii*) and OTU27 (*Ruminococcus* spp.; also classified as *Anaeromassilibacillus* spp.) during the study (*i.e.* 0 g to 50 g), which suggests a potential co-exclusion relationship between these OTUs in response to Maize RS4. **(C)** Bubble plots showing maximum differences in relative proportion of acetate, propionate, butyrate (percentage of total SCFA) and in ratio of BCFA:SCFA during RS4 treatment relative to baseline. For **(A and C)**, the red circles represent a mean positive change in relative abundance during the intervention; black circles represent a mean negative change. The circle size is proportional to the magnitude of the largest difference relative to baseline (dependent on direction of mean change). BCFA:SCFA, total branched short-chain to short-chain fatty acid ratio; CARG, co-abundance response group; OTU, operational taxonomic unit.

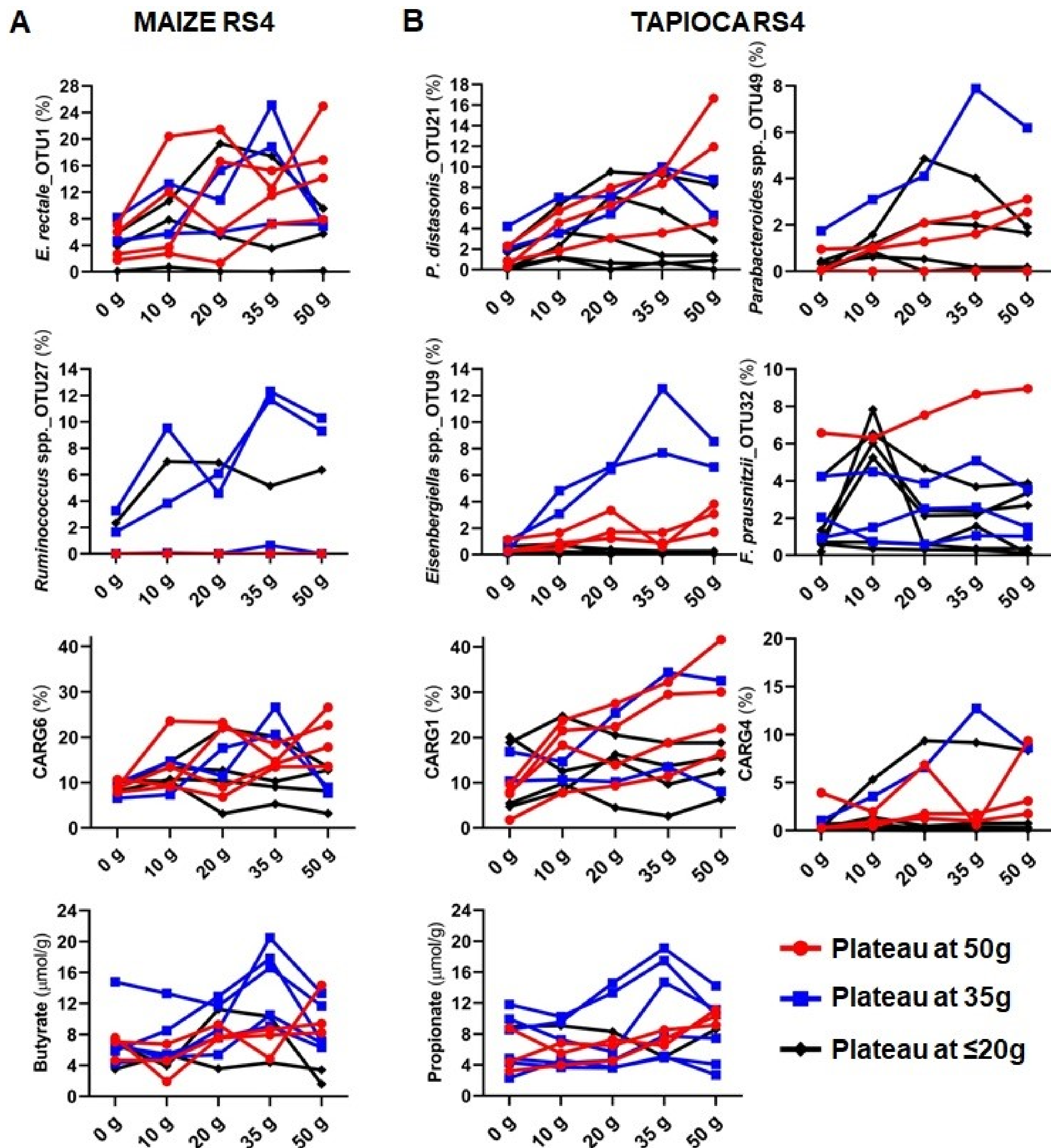

Figure S.6.6. Individual dose-dependent effects of Maize and Tapioca RS4 on fecal bacterial composition and function. Individual dose-response curves of each operational taxonomic unit (OTU; mean change >0.75% relative abundance), co-abundance response group (CARG), and short-chain fatty acid that showed an increase (in either relative abundance or concentration) during **(A)** Maize RS4 or **(B)** Tapioca RS4 treatment. Red lines represent a maximum increase in relative abundance or concentration detected at 50 g/day, blue lines represent a maximum increase detected at 35 g/day, and black lines represent a maximum increase detected at a dose ≤20 g/day.

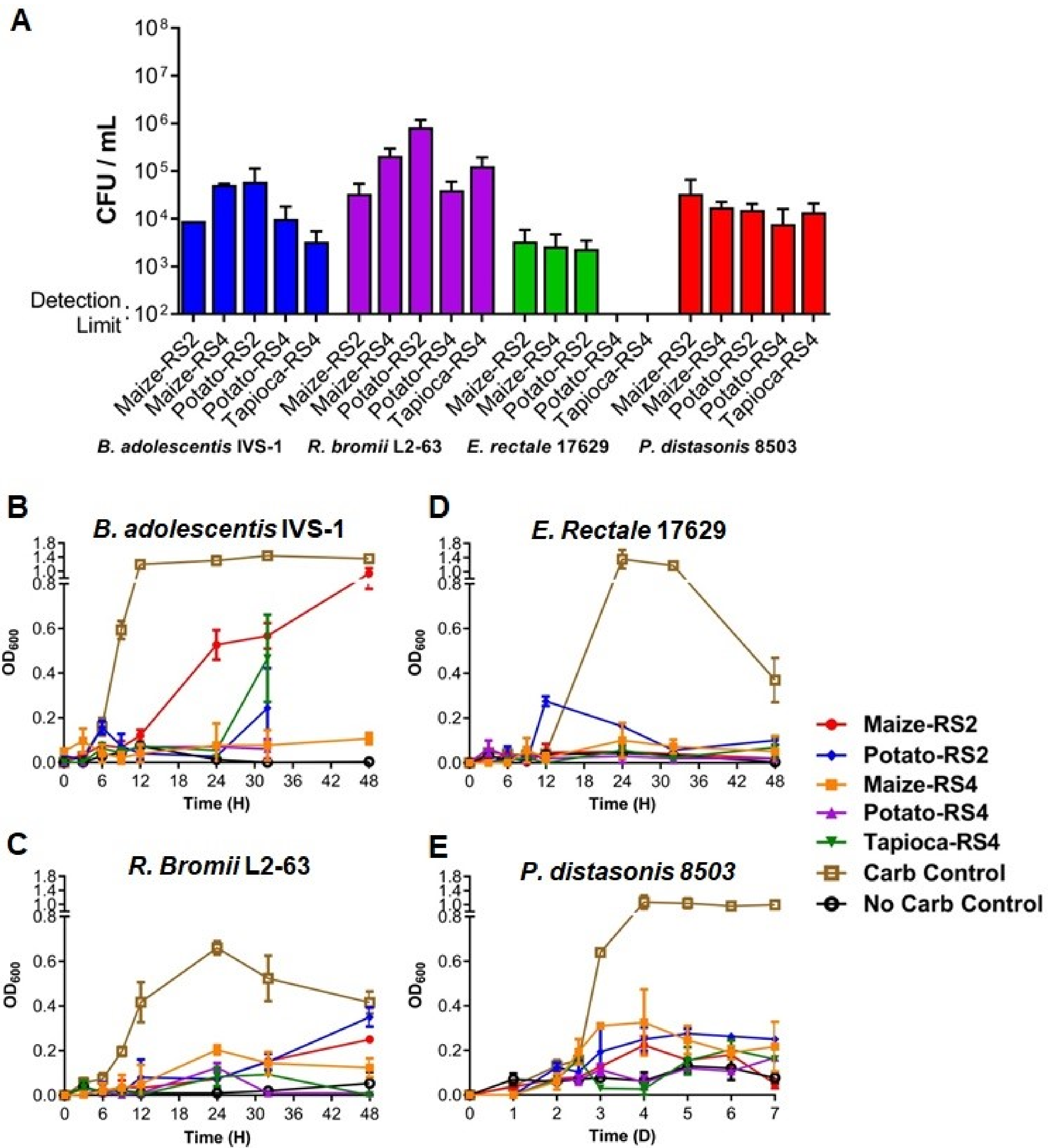

Figure S.6.7. Assessing the adherence and utilization of human-gut-derived amylolytic bacteria on type-II and type-IV RSs. (A) Total CFUs (CFU/ml) of *Bifidobacterium adolescentis* IVS-1, *Ruminococcus bromii* L2-63, *Eubacterium rectale* DSM 17629, and *Parabacteroides distasonis* ATCC 8503 recovered from RS4s and RS2s after in vitro binding assay with the respective RS. Growth curves (OD_{600}) of **(B)** *B. adolescentis* IVS-1, **(C)** *R. bromii* L2-63, **(D)** *E. rectale* DSM 17629, and **(E)** *P. distasonis* ATCC 8503 in YCFA medium containing either 0.2% of the indicated RS, a carbohydrate mixture (positive control), or no carbohydrate (negative control).

Table S.6.1. Supplement specifications

Treatment Name	Product Name	Chemical Processing	Chemical Modifications	Total Fiber [a] *(dwb/as-is - %)*
Corn Starch *(placebo)*	AMIOCA™ TF	None; native high amylopectin corn starch	None	0.0/0.0
Maize RS4	VERSAFIBE™ 2470	Acid hydrolysis & annealing of native high-amylose maize starch	Removed digestible material & reorganized starch granule structure	65.0/58.2
Potato RS4	VERSAFIBE™ 1490	Phosphorylation of native potato starch with phosphorus oxychloride	Cross-linked the surface of the starch granule	90.0/78.7
Tapioca RS4	VERSAFIBE™ 3490	Phosphorylation of native tapioca starch with phosphorus oxychloride	Cross-linked the surface of the starch granule	96.0/85.3

[a] Total dietary fiber content was determined by AOAC 2009.01. as-is, adjusted for moisture content; dwb, dry weight basis.

Table S.6.2. Subject characteristics at baseline

Characteristic	Treatments				Between Group
	Corn Starch	Maize RS4	Potato RS4	Tapioca RS4	*p* value [a]
Population (*n*)	10	10	10	10	
Gender (M/F)	5/5	5/5	5/5	5/5	
Age (y) [b]	27 ± 7.8	25 ± 8.3	31 ± 8.4	29 ± 7.7	0.44
Height (cm)	173.5 ± 10.1	170.5 ± 9.3	169.5 ± 9.0	173.2 ± 10.0	0.74
Weight (kg) [b]	72.3 ± 10.4	66.7 ± 12.7	71.6 ± 14.0	73.4 ± 18.2	0.71
BMI (kg/m^2) [b]	24.1 ± 3.7	22.8 ± 2.1	24.7 ± 2.8	24.3 ± 3.9	0.56
Perceived stress score	4.2 ± 3.1	4.1 ± 1.8	4.7 ± 3.2	5.4 ± 2.2	0.68
MET score [b]	3741 ± 2395	2794 ± 1568	2326 ± 2402	2507 ± 1837	0.29
Ethnicity					0.13
Caucasian	8 (80%)	3 (30%)	4 (40%)	5 (50%)	
Asian	1 (10%)	7 (70%)	5 (50%)	4 (40%)	
Other	1 (10%)	0 (0%)	1 (10%)	1 (10%)	
Education level					0.96
High school diploma	2 (20%)	4 (40%)	2 (20%)	3 (30%)	
Bachelor's degree	5 (50%)	3 (30%)	4 (40%)	3 (30%)	
Graduate degree	2 (20%)	1 (10%)	3 (30%)	3 (30%)	
Other	1 (10%)	2 (20%)	1 (10%)	1 (10%)	
Employment status					0.31
Student	7 (70%)	6 (60%)	5 (50%)	3 (30%)	
Employed	3 (30%)	3 (30%)	5 (50%)	7 (70%)	
Unemployed	0 (0%)	1 (10%)	0 (0%)	0 (0%)	
Household income					0.23
Less than $40,000	5 (50%)	3 (30%)	2 (20%)	3 (30%)	
$40,000 -- $69,000	0 (0%)	5 (50%)	3 (30%)	2 (20%)	
$70,000 -- $99,000	2 (2%)	2 (20%)	1 (10%)	2 (20%)	
$100,000 or more	3 (30%)	0 (0%)	4 (40%)	3 (30%)	

[a] Data were analyzed using either one-way ANOVA (continuous variable) or Fisher's exact test (count variable). No significant differences between the intervention arms at baseline ($p>0.05$). Data reported as mean ± SD or count (%). BMI, body mass index; MET, metabolic equivalent. [b] Square root transformed prior to statistical analysis with one-way ANOVA.

Table S.6.3. Changes in anthropometric and lifestyle characteristics after the 4-week intervention

Δ Characteristic (W4 - BL)	Treatments: Corn Starch	Maize RS4	Potato RS4	Tapioca RS4	Treatment *p* value [a]	Time *p* value	Interaction *p* value
Weight (kg)	0.0 ± 1.1	0.9 ± 1.1	-0.3 ± 1.5	0.4 ± 1.1	0.753	0.195	0.174
BMI (kg/m²)	0.0 ± 0.4	0.3 ± 0.4	-0.1 ± 0.5	0.1 ± 0.4	0.620	0.163	0.224
Perceived stress	1.4 ± 2.5	-0.2 ± 2.1	-0.3 ± 2.2	0.4 ± 1.6	0.624	0.337	0.269
MET score	105 ± 1162	-230 ± 1326	-852 ± 1268	-423 ± 594	0.168	0.057	0.303
Dietary Intake							
Energy (kcal)	-107 ± 569	348 ± 897	-103 ± 518	234 ± 391	0.652	0.350	0.258
Carbohydrate (g)	4.1 ± 57.5	18.7 ± 102.4	-14.0 ± 49.1	16.7 ± 68.2	0.914	0.582	0.730
Total sugar (g)	4.1 ± 41.3	-6.6 ± 57.2	-12.4 ± 47.8	16.7 ± 35.9	0.535	0.950	0.521
Total fiber [b] (g)	1.2 ± 6.8	-0.2 ± 6.9	-0.2 ± 9.0	-2.2 ± 9.1	0.521	0.856	0.939
Protein (g)	-13.5 ± 41.0	27.6 ± 49.9	-17.1 ± 26.2	20.1 ± 33.3	0.212	0.488	**0.025**
Total fat (g)	-2.0 ± 45.4	17.4 ± 46.9	3.0 ± 36.0	4.5 ± 28.0	0.382	0.369	0.733
SFA (g)	-1.5 ± 15.6	3.9 ± 16.9	2.1 ± 18.1	2.7 ± 12.0	0.196	0.476	0.886
USFA (g)	0.0 ± 27.6	12.8 ± 28.5	0.2 ± 16.5	0.9 ± 15.5	0.618	0.341	0.537
Cholesterol (g)	-53.0 ± 230.3	155.2 ± 319.4	-19.4 ± 202.3	55.2 ± 238.8	0.115	0.391	0.275

[a] Data were analyzed using two-way rANOVA (with Holm-Šídák correction). No significant changes within- or between-treatment groups after correction for multiple comparisons. Data reported as mean change from baseline ± SD. BMI, body mass index; MET, metabolic equivalent, SFA, saturated fatty acids; USFA, unsaturated fatty acids. [b] Total dietary fiber provided by the diet without the added fiber supplement.

Table S.6.4. Effect of RS4 treatments and placebo on bowel movement habits

Treatments	Study Weeks Baseline (0g)	Week 1 (10g)	Week 2 (20g)	Week 3 (35g)	Week 4 (50g)	Treatment Effect *p* value [a]
Frequency (stools/day)						
Corn Starch	1.5 ± 0.7	1.7 ± 0.7	1.9 ± 1.4	1.7 ± 0.6	1.6 ± 0.7	–
Maize RS4	1.1 ± 0.6	1.2 ± 0.6	1.4 ± 0.8	1.1 ± 0.7	1.3 ± 0.7	0.077
Potato RS4	1.6 ± 0.6	1.8 ± 0.6	1.3 ± 0.3	1.8 ± 1.1	**1.9 ± 0.7***	1.000
Tapioca RS4	1.2 ± 1.0	1.3 ± 0.9	1.6 ± 1.0	1.3 ± 0.9	1.6 ± 0.9	0.416
Bristol stool scale [b]						
Corn Starch	4.1 ± 1.0	4.1 ± 0.7	4.2 ± 1.1	3.4 ± 1.1	4.1 ± 1.0	–
Maize RS4	4.0 ± 0.8	4.0 ± 1.0	3.8 ± 0.8	3.7 ± 0.9	3.5 ± 0.8	0.520
Potato RS4	3.8 ± 1.1	3.8 ± 1.0	3.7 ± 0.9	3.9 ± 0.8	4.1 ± 1.1	0.690
Tapioca RS4	3.8 ± 1.1	4.1 ± 1.0	3.9 ± 0.6	4.2 ± 0.7	3.8 ± 0.9	0.930
Fecal hardness [c]						
Corn Starch	1.8 ± 0.6	1.6 ± 0.5	1.6 ± 0.6	2.0 ± 0.8	1.7 ± 0.6	–
Maize RS4	1.6 ± 0.5	1.8 ± 0.6	1.6 ± 0.4	1.9 ± 0.6	1.6 ± 0.6	0.679
Potato RS4	2.1 ± 0.5	1.8 ± 0.4	1.9 ± 0.6	**1.8 ± 0.4***	**1.6 ± 0.5***	0.574
Tapioca RS4	2.0 ± 0.7	1.7 ± 0.5	1.7 ± 0.4	1.6 ± 0.5	1.7 ± 0.5	0.965
Straining [d]						
Corn Starch	1.3 ± 0.4	1.6 ± 0.5	1.4 ± 0.4	1.8 ± 0.8	1.5 ± 0.3	–
Maize RS4	1.6 ± 0.5	1.5 ± 0.6	1.4 ± 0.5	1.6 ± 0.4	1.8 ± 0.7	0.682
Potato RS4	1.7 ± 0.6	1.6 ± 0.4	1.6 ± 0.7	1.6 ± 0.5	1.4 ± 0.5	0.713
Tapioca RS4	1.8 ± 0.8	1.4 ± 0.5	1.5 ± 0.6	1.4 ± 0.5	1.5 ± 0.5	0.920
Discomfort [d]						
Corn Starch	1.4 ± 0.4	1.7 ± 0.8	1.2 ± 0.4	1.7 ± 0.7	1.7 ± 0.6	–
Maize RS4	1.3 ± 0.5	1.4 ± 0.7	1.3 ± 0.4	1.3 ± 0.4	1.6 ± 0.6	0.419
Potato RS4	1.3 ± 0.5	1.3 ± 0.4	1.4 ± 0.5	1.2 ± 0.4	1.2 ± 0.5	0.183
Tapioca RS4	1.2 ± 0.5	1.2 ± 0.3	1.2 ± 0.5	1.1 ± 0.2	1.2 ± 0.5	**0.023**
Incomplete evacuation [d]						
Corn Starch	1.5 ± 0.5	1.7 ± 0.8	1.5 ± 0.6	1.6 ± 0.6	1.3 ± 0.4	–
Maize RS4	1.4 ± 0.7	1.3 ± 0.6	1.1 ± 0.1	1.2 ± 0.2	1.6 ± 0.9	0.330
Potato RS4	1.5 ± 0.6	1.3 ± 0.4	1.6 ± 0.6	1.5 ± 0.5	1.6 ± 0.5	0.880
Tapioca RS4	1.6 ± 0.7	1.2 ± 0.4	1.6 ± 0.8	1.3 ± 0.4	1.3 ± 0.5	0.400

[a] Data were analyzed by generalized estimating equation models to assess the effect of treatment and dose, with FDR corrected pair-wise comparisons within each treatment to assess change from baseline, *p<0.05. Data reported as mean ± SD. [b] Rated on a scale of 1 (hard) to 7 (liquid); [c] Rated on a scale of 1 (soft) to 4 (very hard); [d] Rated on a scale of 1 (none) to 4 (severe).

CHAPTER 7: Conclusions, future directions, and implications

Parts of this chapter are published or submitted for publication.

7.1 Executive Summary

Increasing evidence suggests that obesity and its associated comorbidities are associated with diets low in dietary fibers (DFs) that are accessible to the gut microbiota and a disruption of host-microbiota symbiosis[27,273]. By applying purified fermentable DFs, preclinical studies in animal models have established physiological mechanisms underlying the link between DF, gut microbiota, and the development of numerous chronic diseases[31,272]. However, despite convincing evidence reported by epidemiological and animal studies[267,269,272], the use of purified DFs in human randomized controlled trials (RCTs) has produced conflicting results with extensive inter-study heterogeneity[84,222,277]. Therefore, important questions remain on whether DFs can confer reliable physiological benefits in humans when a reductionist approach is used with purified DFs, what the efficacious doses are, and whether such doses are tolerable by modern humans. The research in my thesis addressed some of the knowledge gaps in the field.

By applying systematic review methodology, findings in **Chapter 3** suggest that purified DFs, as currently applied, may be effective therapies for improving cholesterol concentrations and insulin resistance. However, achieving an attenuation of dysglycemia and systemic inflammation might require more targeted applications, where specific purified DFs are supplemented at higher doses, for longer durations, and used to replace digestible carbohydrates. Although a strength of this systematic review was, at 77 studies, the breadth of DF interventions reviewed, these findings were still based on an insufficient number of interventions as few studies efficiently applying purified DFs. Therefore, future studies are needed that assess the immunometabolic effects of well-characterized DFs at relevant doses (*i.e.* above 20 g/day) on specific clinical endpoints to substantiate whether a targeted approach would improve the efficacy of purified DFs.

While purified fermentable DFs have been shown to beneficially modulate the composition of the gut microbiota and its metabolite outputs, responses are highly variable. As person-specific microbial responses to DF might impart clinical ramifications, it would be advantageous to determine whether compositional features of the gut microbial community can predict the output of health-relevant metabolites such as short-chain fatty acids (SCFAs). Using a

human RCT, **Chapter 4** showed that high-dose supplementation with arabinoxylan (AX), but not microcrystalline cellulose (MCC), induced global shifts to bacterial community composition and promoted *Bifidobacterium longum*, *Prevotella copri*, and propionate, which are considered beneficial microbes and a health-relevant SCFA, respectively. While responses were highly individualized, shifts in propionate were predicted by compositional shifts and to lesser degree baseline microbiota composition, with consideration of the entire microbiota performing better than few individual taxa. These findings were consistent with the hypothesis that AX-induced shifts in fecal microbiota composition were linked to the output of fecal SCFAs. This is exciting as it suggests that the microbial output of SCFAs from the fermentation of DF can be predicted by an individual's pre-treatment microbiota. However, larger intervention studies are needed to develop robust machine learning algorithms that predict SCFA responses and more importantly clinical responses to purified DFs.

Work in **Chapter 5** showed that the amounts of AX consumed in **Chapter 4**, which induced physiologically relevant changes in fecal microbial community composition and SCFAs, were tolerable by modern humans. While AX caused gastrointestinal symptoms when compared to non-fermentable MCC, effects were transient with individuals adapting to tolerate AX by the end of the intervention. It was hypothesized in **Chapter 2** that AX-induced symptoms would be linked to both baseline diet history and fecal microbiota compositional and SCFA shifts. Consistent with this hypothesis, the severity and adaption of gastrointestinal symptoms were individualized and linked to the consumption of whole grains and animal-based foods. However, contrary to this hypothesis, associations were not observed between symptom scores and AX-induced changes in fecal microbiota composition. Instead, adaption, but not severity, responses showed detectable links to the relative abundance of *B. longum* at baseline, within the bacterial community that actively utilized AX *ex vivo*, and AX-induced shifts in fecal acetate (the primary SCFA produced by bifidobacteria[541]). These findings are relevant because they provide a basis for improved tolerance of DF at efficacious doses. Considering the associations between *B. longum* and gastrointestinal symptoms, future studies should assess whether synbiotic (*B. longum* plus AX) supplementation enhances the tolerance of AX, especially for individuals that are intolerant.

Finally, by employing a dose-response trial with three chemically modified resistant starches (RS4s), work in **Chapter 6** confirmed the hypothesis that small discrete differences in

the chemical structure of RS4 could be used in humans to direct changes in fecal microbiota composition and its SCFA output. Specifically, the human intervention study revealed that crystalline and phosphate-cross-linked starches induce divergent effects on the gut microbiota that associated with physiologically relevant shifts in the output of either propionate or butyrate. The dominant RS4-induced effects were also shown to be remarkably consistent within treatment groups and dose-dependent plateauing at 35 g/day. Overall, these findings support the potential of using specific doses of purified DFs with discrete structures to achieve targeted manipulations of gut microbiota confirmations relevant to health. However, larger human interventions are needed to determine whether such targeted manipulations of the gut microbiota would yield RS4-specific and predictable health outcomes.

Together, the findings discussed in this dissertation provide evidence that DFs can confer reliable physiological benefits in humans when a reductionist approach is used with purified DFs if more targeted approaches are applied. Findings also provide a basis for the development of more precise nutritional strategies based on purified DFs that selectively modulate the gut microbiota, in terms of both composition and functions relevant to health, and improve immunometabolic effects in humans.

7.2 Targeted Applications of Purified DFs to Improved Human Health

Although the last five decades of research into DFs have yielded detailed knowledge of how DFs exert their potential physiological benefits, disagreements remain on the health benefits of purified DFs due to extensive heterogeneity between human intervention studies[84,222,277]. We, as a field, are just starting to understand how purified DFs can be applied more efficiently to achieve targeted health benefits that are both dependent and independent of the gut microbiota. One consistent finding of the research discussed in this dissertation that is of significance to the field is that higher doses of specific DF structures are likely necessary for more reliable physiological benefits in humans. In addition, individualized responses to purified DFs need to be considered. These key findings are discussed in more detail in the sections below.

7.2.1 Efficacious Doses of Purified DFs

While findings from observational studies provide evidence for dose-response relationships between the consumption of DF from plant foods and risk of developing numerous chronic diseases[267,418], questions remain on what the efficacious doses of purified DFs are for

reliable physiological effects. From the systematic review of purified DF interventions, findings in **Chapter 3** showed that studies with supplementation amounts >20 g/day had higher proportions of significant immunometabolic benefits. Furthermore, as described in **Appendix A**, high-dose supplementation with either AX or MCC at 25 g/day (females) and 35 g/day (males) markedly improved measures of satiety and insulin resistance or intestinal and systemic inflammation, respectively. Observations from dose-escalation trials, while limited, also provide evidence that satiety[390,608] and anti-inflammatory[361,403,417] responses are dose-dependent with significant effects detected when supplementing at around 25 g/day. When added to basal DF levels of ~15 g/day provided by background diets, these supplemental amounts exceed current dietary recommendations of around 30 g/day[12]. Instead, these amounts align more closely with recent suggestions that at least 50 g/day are required for consistent health benefits linked to DF[266,302,303]. Overall, the findings of this thesis, together with reports in the literature, suggest that in general the efficacious dose, when supplementing purified DFs, is at least 20 g/day. This is concerning as nearly 50% of the interventions reviewed in **Chapter 3** employed doses ≤10 g/day, which might be a key contributing factor to the uncertainty that surrounds the efficacy of purified DFs. It would therefore be advantageous for future DF studies to restrain from employing doses below 20 g/day unless higher doses are used in parallel to ascertain minimum efficacious doses.

Achieving amounts of DF closer to 50 g/day through DF-rich plant foods is certainly the most advantageous in part because there is little evidence supporting a link between increased DF consumption from a variety of plant foods and severe gastrointestinal symptoms in healthy individuals[42]. Contrary to a whole, plant-based diet, excessive symptoms have been reported at even moderate intakes of certain purified DFs[528] and, as a result, concerns remain as to whether modern humans could tolerate efficacious doses of purified DF[300]. Findings of **Chapters 5 and 6** are therefore highly relevant as they demonstrate that modern humans are capable of tolerating the amounts of purified DF needed to selectively modulate the human gut microbiota, in terms of both composition and functions relevant to health (*i.e.* AX and RS4s), and attenuate measures of insulin resistance and intestinal inflammation (*i.e.* AX and MCC). Similar findings are also described for other purified DFs, where supplementing psyllium, resistant dextrin, and RS type-II (RS2) at doses ≥25 g/day have been shown to be tolerable and improve measures of inflammation (psyllium[403]), insulin resistance (resistant dextrin[340,343], RS2[351,356]), and cholesterol

concentrations (psyllium[342], resistant dextrin[340,343]). This literature, together with findings in this dissertation, suggests that modern humans can indeed tolerate the supplementation of several purified DFs at more physiologically relevant levels, making closing the fiber gap feasible.

One promising finding of the research discussed in **Chapter 5** was that AX-induced symptoms were transient with subjects adapting to tolerate the high AX doses by the end of treatment. In accordance with these findings, previous DF intervention studies supplementing inulin, acacia gum, and NUTRIOSE® FB at comparable doses have also described a similar reversion of gastrointestinal symptoms[325,531,532,609]. These findings suggest that, within around four weeks of treatment, modern humans adapt to tolerate the sustained consumption of purified DF, a likely necessary trait for maintaining their physiological effects and closing the fiber gap. However, one strength of the research presented in **Chapter 5**, when compared to studies in the literature, was the systematic integration of microbiota data with reported gastrointestinal symptoms. This analysis revealed strong connections between adaption responses and both the relative abundance of *B. longum* and shifts in fecal acetate. Links between adaptation and the gut microbiota are exciting because they provide a basis for understanding the tolerance of purified DFs and for the development of strategies aimed to improve the tolerance of purified DFs at more efficacious doses. The findings also have implications for the treatment of functional gastrointestinal disorders as, beyond depriving the gut microbiota of favorable fermentable oligo-, di-, mono-saccharides and polyols[610], nutritional strategies such as synbiotics (*B. longum* plus AX) could be developed to maximize the gut microbiota's ability to potentially improve debilitating gastrointestinal symptoms.

7.2.2 Efficacious Physicochemical Properties of Purified DFs

As DF is a general group of heterogeneous compounds with increasingly diverse structures and physicochemical properties, questions remain on which DF-types or properties should be considered for specific health outcomes. In terms of physicochemical properties, evidence of clinical efficacy has mainly been shown for viscous DFs and improved lipid and glucose metabolism[413,611,612], with viscous β-glucans and psyllium (at 3 g/day and 7 g/day, respectively) having approved Food and Drug Administration health claims for reduced cardiovascular disease risk[223]. As these conclusions are in accordance with findings in **Chapter 3**, future research should be focused on determining what the efficacious doses of viscous DF ingredients are in order to attain health claims for their physiological effects. Findings in

Chapter 3 also provide new and exciting evidence that suggests mixed plant cell wall (MPCW) DFs can be as effective as viscous DFs at improving insulin resistance and cholesterol concentrations. However, as interventions are limited, additional research is needed to substantiate both the effects of MPCW DFs and underling mechanisms behind these effects. Since MPCW DFs are often derived from the manufacturing or processing of plant foods[278,613], termed upcycling, increased applications of MPCW DFs in research and ultimately foods would have further implications for environmental sustainability.

In contrast to viscous and MPCW DFs, findings in **Chapter 3** suggest that the physiological effects of consuming soluble, nonviscous DFs are largely unreliable. These inconsistencies are important to acknowledge for a few reasons. First, a number of nonviscous DFs, including inulin, oligofructose, galactooligosaccharides, xylooligosaccharides, and polydextrose, have been classified as either prebiotics or 'candidate prebiotics'[46] - defined accordingly as 'a substrate that is selectively utilized by host microorganisms conferring a health benefit' by the most recent ISAPP consensus statement[45]. Thus questioning whether prebiotics, as hitherto used in the reviewed studies, actually confer immunometabolic benefits upon host health[45,46]. Second, from a practical perspective, purified DFs that exhibit soluble, nonviscous properties can be incorporated into several drink and food systems at relatively very high dosages while still preserving organoleptic characteristics[614,615]. Finally, while supplementation with inulin and resistant oligosaccharides at doses >20 g/day would not be feasible due to adverse gastrointestinal symptoms, several other nonviscous DFs have shown acceptable tolerance at more physiologically relevant doses well above 20 g/day, such as acacia gum, soluble corn fibre, polydextrose, and NUTRIOSE® FB[325,528,529,616]. Considering the practicality of soluble, nonviscous DFs, further investigation is needed to ascertain whether supplementation at higher doses and for longer durations would produce reliable health effects, or whether these DF-types are simply not viable options for the treatment and prevention of chronic disease.

7.2.3 Individualised Responses to Purified DFs

Given the importance of DF fermentation by an individual's gut microbiota, as discussed in **Chapter 1.2**, personalized gut microbiota configurations would diversify the microbial consortium involved in DF fermentation, which could alter the generation of bioactive compounds such as SCFAs, thus resulting in heterogenous clinical responses to DF. As several soluble, nonviscous DFs are prebiotic[46], the inconsistencies reported in **Chapter 3** may be a

consequence of how or whether a specific DF structure is metabolized by the microbiota. Findings in **Chapter 4** are therefore pertinent as they suggest that the outputs of bioactive compounds during DF fermentation are predictable when compositional features of the global gut microbial community are considered, as opposed to few individual taxa. Even though, upon further analysis in **Appendix A**, health outcomes were not explained by predictable SCFA responses, effects on satiety were shown to be predicted by the bacterial consortia that utilized AX. While predictive modeling has yet to be extensively applied to the DF field, previous studies have demonstrated that, through mathematical modeling based on microbiome features, individualized physiological responses to foods are predictable[10,53,617]. Overall, the findings discussed in this dissertation, together with the literature, provide a rationale for the development of robust machine learning algorithms based on an ecological framework that allows for the personalization of purified DF applications in a move towards precision nutrition and precision medicine.

7.2.4 Mixtures of Purified DFs

As both microbial and physiological responses to specific DF structures are shown to be individualized, would mixtures of purified DFs be an avenue for diversified microbial responses and more reliable health effects in humans? For instance, mixtures of maize and tapioca RS4, as discussed in **Chapter 6**, could be used to enhance the output of butyrate and propionate, which might have greater therapeutic potential for improving obesity-related immunometabolic dysregulations than a singular RS4. Similarly, mixtures of AX and MCC, as discussed in **Appendix A**, might also have relevance for the prevention or treatment of dysregulated immunometabolism that underlies the pathophysiology of obesity-associated comorbidities. Considering *in vitro* human fecal fermentation studies have reported that mixtures of rapidly and slowly fermented DFs generally reduce the overall rate of gas production[445,525], DF mixtures might be an additional avenue for improving the gastrointestinal tolerance of inulin, resistant oligosaccharides, and other purified DFs. While mixtures of purified DFs are previously shown to improve human health[280,378,405,417], carefully designed human intervention trials are needed to ascertain whether the physiological effects of specific DF mixtures are additive, synergistic, or even antagonistic. If the effects of purified DFs are indeed additive or synergistic, lessening individualized responses, then the development of a 'one size fits all' approach based on DF mixtures might be feasible for the prevention and/or treatment of chronic diseases.

7.3 Predictable Modulations of the Gut Microbiota with Specific Purified DFs

Whole, plant foods are comprised of several structurally distinct DFs and phytochemicals that are to varying agrees accessible to the gut microbiota[442,618], thereby supporting a potentially more diverse microbial response when consumed[78]. In contrast, the constituents of purified DFs can be limited, with synthesized designer carbohydrates having up to one specific DF structure[36,286]. Therefore, what differentiates purified DFs from plant foods is the precision to which purified DFs can potentially manipulate the gut microbiota[36,286]. Findings in **Chapters 4 and 6** are relevant as they provide evidence that such targeted and predictable manipulations of gut microbiota composition and its output of health-relevant metabolites can be achieved despite ecological constraints of the gut microbiota, through the use of specific doses of discrete DF structures. Reproducible manipulations of the gut microbiota have been further described of other purified DFs[36,286]. For instance, the application of simple structured inulin, oligofructose, or galactooligosaccharides have been shown to target and promote multiple *Bifidobacterium* species[66,73,106,535,619,620], while more structurally-complex AXs and crystalline RSs have shown higher degrees of specificity for *B. longum*[478-480] and *B. adolescentis*[67,69,554], respectively. Overall, these findings suggest that, while both plant foods and purified DFs modulate the gut microbial community in ways consider beneficial, intelligent manipulation of the gut microbiota would be feasible with purified DFs.

Although such manipulations of the gut microbiota for the promotion of health or treatment of chronic disease are conceivable[36,286], attainment of this goal and translation of these findings into therapies for the clinic would require a great deal of research in the following areas. First, research is needed that characterizes discrete DFs structures[36], determines which bacterial species encode the genetic repertoire and traits required for accessing and degrading the DF[36,430] and the degree to which these genes and traits are conserved among bacterial strains[443,481,482,486,489,603], elucidates cross-feeding interaction[98,540], and establishes potential secondary fermenters and metabolite utilizers[273,430,441,442]. Second, as metagenomic and *in vitro* findings do not necessarily translate into observed responses under the competitive constraints of the human colon environment[69,439], well-controlled human interventions across different populations are further needed to establish which bacterial taxa and metabolites are promoted consistently by purified DFs. Finally, more sensitive *ex vivo* approaches such as stable isotope probing[77] or bio-orthogonal non-canonical amino acid tagging[506] may be required to detect

relevant ecological interactions, since not every species that contributes to DF fermentation becomes enriched in feces, and vice versa[273,537]. Through systematic integration of the compositional and functional datasets, one could discern which bacteria or metabolites are reliable promoted by a purified DF and ultimately the development of a 'DF-microbiota catalog' that categorizes the effects of specific purified DFs on the human gut microbiota.

As preclinical and human studies better-define which microbiota-specific endpoints affect surrogate outcomes in humans, an established 'DF-microbiota catalog' would lead to the development of purified DF-based therapies that promote target organisms and/or metabolites in an attempt to treat human disease. One illustration of this potential is the treatment of multiple sclerosis (MS). Previous studies have shown that abundances of *Parabacteroides distasonis* are reduced in MS patients and that the bacterium promotes anti-inflammatory responses, simulating regulatory T cells in monocolonized mice[621]. In addition, propionate levels have also been shown to be reduced in MS patients, where propionate supplementation further promoted anti-inflammatory responses in MS patients[622]. Therefore, one feasible purified DF-based therapy for the treatment of MS might be supplementing Tapioca RS4 at 35 g/day, as it selectively and dose-dependently promoted *P. distasonis* and propionate. The development of purified DF-based therapies, like that proposed for the treatment of MS, is already underway. Both researchers[36,300,623] and companies, such as BCD Bioscience Incorporated[624] and Kaleido Biosciences Incorporated[625], are working towards identifying discrete DF structures that can be leveraged to modulate the human microbiome for improved health, thus paving the way for microbiome-based precision medicine using purified DFs.

7.4 Applications of Purified DFs

As future human intervention trials begin to decipher which purified DF ingredients can improve the health of humans, and at what amounts these effects are reliable, it is important to discern how purified DFs should be applied to achieve the desired health outcomes. Similar to the research methodology described in this dissertation, purified DFs, or mixture thereof, could be provided daily as powdered or liquid supplements to reach efficacious amounts. Furthermore, microbiota- and immunometabolic-targeted medical foods could be formulated, where precise DFs are supplied at high doses to selectively promote putatively beneficial bacteria, direct SCFA production, and induce targeted health effects in human with sustained consumption. While it

remains insufficiently understood whether longstanding adherence to DF supplements or medical foods would transpire for sustained physiological benefits, research of the long-term adherence to prescribed dietary supplements, where adherence rates were often reported at ≤ 50%[626-628], suggests that such an approach would not be widely successful.

In **Chapter 1.1**, an alternative approach to dietary supplements was proposed, whereas purified DFs could be incorporated into regularly consumed foods such as white flour-rich products[13] in order to reach recommended DFs amounts[27,276,277]. Indeed, a variety of purified DFs are already commercially available with physicochemical attributes that allow for their application in refined foods, at even high amounts, without affecting product quality[27,278]. For instance, preliminary findings from my internship with Ingredion Incorporated suggest that RS4s can enrich the DF content of muffin and pretzel bun formulations over 10-fold while still maintaining an acceptable product[629], with other research groups showing comparable findings[351,569,584,630,631]. In addition to achieving targeted immunometabolic effects with physicochemically-distinct purified DFs, a general replacement of refined, readily digestible ingredients with non-digestible DFs would reduce the total caloric value of foods, as DFs provide ≤50% of the calories provided by digestible carbohydrates[42]. Furthermore, replacement of readily digestible carbohydrates with DFs could attenuate the negative effects observed for digestible carbohydrates on measures of systemic inflammation and glucose homeostasis[314,315,437], which is in agreement with findings in **Chapter 3**. Overall, the incorporation of purified DFs in regularly consumed foods, as opposed to dietary supplements, is a promising approach for achieving both physiologically relevant doses of DF and health benefits linked to DF.

While findings discussed in this dissertation are in overall favor of pursuing purified DFs as a potential strategy for closing the fiber gap, we, as a field, are just starting to understand how to efficiently apply purified DFs for reliably health benefits. Research into the potential adverse effects of long-term purified DF consumption, beyond commonly described gastrointestinal symptoms, remains limited. Recent work by Singh and colleagues suggests that such applications should be approached with great caution, as, in mice, an inulin-rich high-fat diet was shown to prevent dysregulated immunometabolism but induce cholestatic liver cancer[632]. High levels of DF may also be contraindicated for select patient populations, such as individuals with an ileostomy or colostomy[633] or patients with irritable bowel syndrome and visceral

hypersensitivity[634]. Finally, it is important to acknowledge that purified DFs are seldomly consumed in isolation. Therefore, government regulations should consider the background food or beverage matrix when determining if nutrient content or structure/function claims are suitable for DF-enriched products. For instance, it is misleading to a health-focused consumer when a product is marketed as a 'good source of fiber' when it also has a high glycemic index due to its elevated added sugar content. Overall, prior to a more systematic application of purified DFs in the food supply, additional research in necessary to elucidate the potential benefits and risks of enriching regularly consumed foods with purified DFs. Such research should include rigorously designed RCTs with clear health- and microbiome-related outcomes that compare the effects of DF-enriched foods to their unenriched counterparts and comparable whole, plant-based foods, and that further assess potential adverse effects of long-term purified DF consumption.

7.5 Limitations

Although the findings discussed in this dissertation provide evidence that purified DFs could exert more reliable effects in humans with targeted applications, there are some limitations to the discussed research that should be acknowledged in addition to the methodological constraints already discussed. First, study cohorts were relatively modest in size and comprised of primarily University of Alberta students and employees, which may limit the generalizability of research findings to more diverse populations. A further limitation is that the diets of the study participant were self-selected and non-standardized. This allowed us to study a 'real-life' scenario, where high doses of powdered supplements were consumed ad libitum as it would be in a real-world setting. However, to accomplish the high-dose supplementation, study participants changed their habitual diet, at least to some extent, by consuming certain foods and drinks, like eggs, yogurt, water, and smoothies, more often. Other lifestyle habits, such as physical activity, may have also changed during the intervention. To control for this, repeated 24-hr recalls[564] and physical activity questionnaires[566] were completed throughout the intervention to detect deviations from baseline lifestyle habits, and considered when analyzing the data. As powdered supplements were not uniformly added to the diet, personal differences in DF supplementation, such as the amount consumed, when it was consumed, and how it was incorporated into the diet, are may also have influenced study findings. To overcome these limitations, rigorously designed studies are needed that either apply microbiota- and immunometabolic-targeted medical foods

and control the background diet or provide participants with a complete diet that includes DF-enriched foods.

Microbiota findings discussed in this dissertation were extrapolated from the self-sampled feces of research participants, which also has inherent limitations. Fecal samples essentially contain the end-products of a highly complex degradation of non-digestible dietary and host-derived compounds by the gut microbiota after host absorption[635]. Therefore, the rate and location of microbial fermentation (*e.g.* proximal or distal colon) can not be determined by the data, which could influence the gastrointestinal tolerance and physiological effects of DF[447,526]. Although low-cost and non-invasive, the fecal microbiota and SCFA concentrations may also not be representative of the microbial community that resides, or the amount of SCFA produced, in the proximal colon where DF fermentation is usually more profound[273,635-637]. In addition, even though the lapse of time between defecation and processing was less than four hours, variation in this time could influence fecal microbiota composition and SCFA concentrations due to undesirable microbial growth and a loss of volatile SCFAs[638-640]. One innovative approach that could be applied in future human interventions to overcome these challenges would be the use of ingestible gas-sensing or microbiota-sampling capsule[521,641,642]. Such technologies would allow for the sampling of gases, pH, SCFAs, and potentially other health-relevant metabolites along the gastrointestinal tract, as well as the precise sampling gut microbiota at pre-determined sites[521,642].

Although not a methodological limitation per se, the exploratory RCT discussed in **Chapters 4 and 5** and **Appendix A** was originally part of a large parallel four-armed RCT that aimed to compare the effects of four structurally-distinct DFs (*i.e.* AX, acacia gum, RS4, and MCC) on the gut microbiota and human health, referred to as The Alberta FYBER (Feed Your gut Bacteria morE fibeR) Study. In response to requests by reviewers of a grant application, the AX arm was separated from the original RCT, and data from the 15 protocol completers were analyzed independently. While AX-induced effects on the gut microbiota, symptoms, and health endpoints were still detected relative to MCC with the modest sample size, potentially because of the high DF doses provided, changes to the design could have improved the research. For instance, a cross-over design would have been feasible with only AX and MCC, thus allowing each subject to serve as their own control. Alternatively, by applying a parallel four-armed design, the long-chain, complex AX used herein could have been compared to a long-chain but

simple structured AX from sorghum or rice, an AX-oligosaccharide, and MCC. Such a design would have allowed for the elucidation of whether physicochemically-distinct DFs with similar, yet discrete chemical structures differ in their effects on the gut microbiota, gastrointestinal symptoms, and human health.

7.6 Overall Conclusions

In conclusion, findings presented in this dissertation suggest that, in general, purified DFs do hold merit for the treatment and prevention of obesity and its associated comorbidities and should therefore be pursued as an adjuvant to current whole, plant-based approaches. However, additional research in humans is imperative to determine whether targeted applications of well-characterized purified DFs, at explicitly higher doses, would indeed induce predictable and reliable physiological benefits. Precise and selective modulation of the gut microbiota should also be considered a valued attribute of structurally distinct purified DFs, and with an enhanced understanding of microbiome-disease relationships, this feature will be increasingly relevant for the treatment of chronic diseases. Overall, this dissertation provides a basis for the targeted application of purified DFs to selectively modulate gut microbiota composition and functions relevant to health, and to improve immunometabolic effects in human.

7.7 References

10. Kovatcheva-Datchary P, Nilsson A, Akrami R*, et al.* Dietary Fiber-Induced Improvement in Glucose Metabolism Is Associated with Increased Abundance of *Prevotella*. *Cell Metab.* 2015;22(6):971-82.
12. Jones JM. CODEX-aligned dietary fiber definitions help to bridge the ‘fiber gap’. *Nutr J.* 2014;13:34.
13. Fungwe TV, Bente L, Hiza H. The Food Supply and Dietary Fiber: Its Availability and Effect on Health. Center for Nutrition Policy and Promotion. November, 2007.
27. Deehan EC, Walter J. The Fiber Gap and the Disappearing Gut Microbiome: Implications for Human Nutrition. *Trends Endocrinol Metab.* 2016;27(5):239-42.
31. Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. *Cell.* 2016;165(6):1332-45.
36. Hamaker BR, Tuncil YE. A perspective on the complexity of dietary fiber structures and their potential effect on the gut microbiota. *J Mol Biol.* 2014;426(23):3838-50.
42. Dahl WJ, Stewart ML. Position of the Academy of Nutrition and Dietetics: Health Implications of Dietary Fiber. *J Acad Nutr Diet.* 2015;115(11):1861-70.

45. Gibson GR, Hutkins R, Sanders ME, *et al.* Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol.* 2017;14(8):491-502.
46. Gibson GR, Scott KP, Rastall RA, *et al.* Dietary prebiotics: current status and new definition. *Food Sci Tech Bull Funct Foods.* 2010;7(1):1-19.
53. Zeevi D, Korem T, Zmora N, *et al.* Personalized Nutrition by Prediction of Glycemic Responses. *Cell.* 2015;163(5):1079-94.
66. Dewulf EM, Cani PD, Claus SP, *et al.* Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. *Gut.* 2013;62(8):1112-21.
67. Venkataraman A, Sieber JR, Schmidt AW, Waldron C, Theis KR, Schmidt TM. Variable responses of human microbiomes to dietary supplementation with resistant starch. *Microbiome.* 2016;4(1):33.
69. Martínez I, Kim J, Duffy PR, Schlegel VL, Walter J. Resistant starches types 2 and 4 have differential effects on the composition of the fecal microbiota in human subjects. *PLoS One.* 2010;5(11):e15046.
73. Davis LMG, Martínez I, Walter J, Goin C, Hutkins RW. Barcoded pyrosequencing reveals that consumption of galactooligosaccharides results in a highly specific bifidogenic response in humans. *PLoS One.* 2011;6(9):e25200.
77. Tannock GW, Lawley B, Munro K, *et al.* RNA-stable-isotope probing shows utilization of carbon from inulin by specific bacterial populations in the rat large bowel. *Appl Environ Microbiol.* 2014;80(7):2240-47.
78. Holscher HD. Dietary fiber and prebiotics and the gastrointestinal microbiota. *Gut Microbes.* 2017;8(2):172-84.
84. Fuller S, Beck E, Salman H, Tapsell L. New Horizons for the Study of Dietary Fiber and Health: A Review. *Plant Foods Hum Nutr.* 2016;71(1):1-12.
98. Coyte KZ, Schluter J, Foster KR. The ecology of the microbiome: Networks, competition, and stability. *Science.* 2015;350(6261):663-66.
106. Vandeputte D, Falony G, Vieira-Silva S, *et al.* Prebiotic inulin-type fructans induce specific changes in the human gut microbiota. *Gut.* 2017;66(11):1968-74.
222. Buyken AE, Goletzke J, Joslowski G, *et al.* Association between carbohydrate quality and inflammatory markers: systematic review of observational and interventional studies. *Am J Clin Nutr.* 2014;99(4):813-33.
223. Food and Drug Administration H. Subpart E-Specific Requirements for Health Claims. Maryland, United States. April 1, 2016.
266. Makki K, Deehan EC, Walter J, Bäckhed F. The Impact of Dietary Fiber on Gut Microbiota in Host Health and Disease. *Cell Host Microbe.* 2018;23(6):705-15.
267. Reynolds A, Mann J, Cummings J, Winter N, Mete E, Te Morenga L. Carbohydrate quality and human health: a series of systematic reviews and meta-analyses. *Lancet.* 2019;393(10170):434-45.
269. Veronese N, Solmi M, Caruso MG, *et al.* Dietary fiber and health outcomes: an umbrella review of systematic reviews and meta-analyses. *Am J Clin Nutr.* 2018;107(3):436–44.
272. Kaczmarczyk MM, Miller MJ, Freund GG. The health benefits of dietary fiber: beyond the usual suspects of type 2 diabetes mellitus, cardiovascular disease and colon cancer. *Metabolism.* 2012;61(8):1058-66.

273. Deehan EC, Duar RM, Armet AM, Perez-Muñoz ME, Jin M, Walter J. Modulation of the Gastrointestinal Microbiome with Nondigestible Fermentable Carbohydrates To Improve Human Health. *Microbiol Spectr.* 2017;5(5).
276. Sonnenburg ED, Sonnenburg JL. The ancestral and industrialized gut microbiota and implications for human health. *Nat Rev Microbiol.* 2019;17(6):383-90.
277. Thompson SV, Hannon BA, An R, Holscher HD. Effects of isolated soluble fiber supplementation on body weight, glycemia, and insulinemia in adults with overweight and obesity: a systematic review and meta-analysis of randomized controlled trials. *Am J Clin Nutr.* 2017;106(6):1514-28.
278. Sharma SK, Bansal S, Mangal M, Dixit AK, Gupta RK, Mangal AK. Utilization of Food Processing By-products as Dietary, Functional and Novel Fibre: A Review. *Crit Rev Food Sci Nutr.* 2016;56(10):1647-61.
280. Zhao L, Zhang F, Ding X*, et al.* Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes. *Science.* 2018;359(6380):1151-56.
286. Cantu-Jungles TM, Hamaker BR. New View on Dietary Fiber Selection for Predictable Shifts in Gut Microbiota. *mBio.* 2020;11(1):e02179-19.
300. Tannock GW, Liu Y. Guided dietary fibre intake as a means of directing short-chain fatty acid production by the gut microbiota. *J Roy Soc New Zeal.* 2019.
302. O'Keefe SJ. The association between dietary fibre deficiency and high-income lifestyle-associated diseases: Burkitt's hypothesis revisited. *Lancet Gastroenterol Hepatol.* 2019;4(12):984-96.
303. O'Keefe SJD. The Need to Reassess Dietary Fiber Requirements in Healthy and Critically Ill Patients. *Gastroenterol Clin North Am.* 2018;47(1):219-29.
314. Jenkins DJ, Kendall CW, Augustin LS*, et al.* Glycemic index: overview of implications in health and disease. *Am J Clin Nutr.* 2002;76(1):266S-73S.
315. Ludwig DS. The glycemic index: physiological mechanisms relating to obesity, diabetes, and cardiovascular disease. *JAMA.* 2002;287(18):2414-23.
325. Pasman WJ, Wils D, Saniez M-HH, Kardinaal AF. Long-term gastrointestinal tolerance of NUTRIOSE® FB in healthy men. *Eur J Clin Nutr.* 2006;60(8):1024-34.
340. Li S, Guerin-Deremaux L, Pochat M, Wils D, Reifer C, Miller LE. NUTRIOSE dietary fiber supplementation improves insulin resistance and determinants of metabolic syndrome in overweight men: a double-blind, randomized, placebo-controlled study. *Appl Physiol Nutr Metab.* 2010;35(6):773-82.
342. Pal S, Khossousi A, Binns C, Dhaliwal S, Ellis V. The effect of a fibre supplement compared to a healthy diet on body composition, lipids, glucose, insulin and other metabolic syndrome risk factors in overweight and obese individuals. *Br J Nutr.* 2011;105(1):90-100.
343. Hashizume C, Kishimoto Y, Kanahori S, Yamamoto T, Okuma K, Yamamoto K. Improvement effect of resistant maltodextrin in humans with metabolic syndrome by continuous administration. *J Nutr Sci Vitaminol.* 2012;58(6):423-30.
351. Dainty SA, Klingel SL, Pilkey SE*, et al.* Resistant Starch Bagels Reduce Fasting and Postprandial Insulin in Adults at Risk of Type 2 Diabetes. *J Nutr.* 2016;146(11):2252-59.
356. Alfa MJ, Strang D, Tappia PS*, et al.* A Randomized Placebo Controlled Clinical Trial to Determine the Impact of Digestion Resistant Starch *MSPrebiotic*® on Glucose, Insulin, and Insulin Resistance in Elderly and Mid-Age Adults. *Front Med.* 2018;4:260.

361. King DE, Mainous AGr, Egan BM, Woolson RF, Geesey ME. Effect of psyllium fiber supplementation on C-reactive protein: the trial to reduce inflammatory markers (TRIM). *Ann Fam Med.* 2008;6(2):100-06.
378. Jensen C, Spiller G, Gates J, Miller A, Whittam J. The effect of acacia gum and a water-soluble dietary fiber mixture on blood lipids in humans. *J Am Coll Nutr.* 1993;12(2):147-54.
390. Pedersen C, Lefevre S, Peters V*, et al.* Gut hormone release and appetite regulation in healthy non-obese participants following oligofructose intake. A dose-escalation study. *Appetite.* 2013;66:44-53.
403. King DE, Egan BM, Woolson RF, Mainous AG, Al-Solaiman Y, Jesri A. Effect of a high-fiber diet vs a fiber-supplemented diet on C-reactive protein level. *Arch Intern Med.* 2007;167(5):502-06.
405. Haskell WL, Spiller GA, Jensen CD, Ellis BK, Gates JE. Role of water-soluble dietary fiber in the management of elevated plasma cholesterol in healthy subjects. *Am J Cardiol.* 1992;69(5):433-39.
413. Jovanovski E, Khayyat R, Zurbau A*, et al.* Should Viscous Fiber Supplements Be Considered in Diabetes Control? Results From a Systematic Review and Meta-analysis of Randomized Controlled Trials. *Diabetes Care.* 2019;42(5):755-66.
417. Johansson-Persson A, Ulmius M, Cloetens L, Karhu T, Herzig K-H, Önning G. A high intake of dietary fiber influences C-reactive protein and fibrinogen, but not glucose and lipid metabolism, in mildly hypercholesterolemic subjects. *Eur J Nutr.* 2014;53(1):39-48.
418. National Research Council. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients). Washington, D.C.: The National Academies Press; 2005.
430. Ze X, Duncan SH, Louis P, Flint HJ. *Ruminococcus bromii* is a keystone species for the degradation of resistant starch in the human colon. *ISME J.* 2012;6(8):1535-43.
437. Armet AM, Deehan EC, Thöne JV, Hewko SJ, Walter J. The Effect of Isolated and Synthetic Dietary Fibers on Markers of Metabolic Diseases in Human Intervention Studies: A Systematic Review. *Adv Nutr.* 2020;11(2):420–38.
439. Deehan EC, Yang C, Perez-Muñoz ME*, et al.* Precision Microbiome Modulation with Discrete Dietary Fiber Structures Directs Short-Chain Fatty Acid Production. *Cell Host Microbe.* 2020;27(3):389-404.e6.
441. Flint HJ, Duncan SH, Louis P. The impact of nutrition on intestinal bacterial communities. *Curr Opin Microbiol.* 2017;38:59-65.
442. Cockburn DW, Koropatkin NM. Polysaccharide Degradation by the Intestinal Microbiota and Its Influence on Human Health and Disease. *J Mol Biol.* 2016;428(16):3230-52.
443. De Filippis F, Pasolli E, Tett A*, et al.* Distinct Genetic and Functional Traits of Human Intestinal *Prevotella copri* Strains Are Associated with Different Habitual Diets. *Cell Host Microbe.* 2019;25(3):444-53.e3.
445. Tuncil YE, Nakatsu CH, Kazem AE*, et al.* Delayed utilization of some fast-fermenting soluble dietary fibers by human gut microbiota when presented in a mixture. *J Funct Foods.* 2017;32:347-57.
447. Rumpagaporn P, Reuhs BL, Kaur A, Patterson JA, Keshavarzian A, Hamaker BR. Structural features of soluble cereal arabinoxylan fibers associated with a slow rate of *in vitro* fermentation by human fecal microbiota. *Carbohydr Polym.* 2015;130:191-97.

478. Van den Abbeele P, Gérard P, Rabot S*, et al.* Arabinoxylans and inulin differentially modulate the mucosal and luminal gut microbiota and mucin-degradation in humanized rats. *Environ Microbiol.* 2011;13(10):2667-80.
479. Van den Abbeele P, Venema K, Van de Wiele T, Verstraete W, Possemiers S. Different human gut models reveal the distinct fermentation patterns of Arabinoxylan versus inulin. *J Agric Food Chem.* 2013;61(41):9819-27.
480. Crittenden R, Karppinen S, Ojanen S*, et al. In vitro* fermentation of cereal dietary fibre carbohydrates by probiotic and intestinal bacteria. *J Sci Food Agr.* 2002;82(8):781-89.
481. Rivière A, Moens F, Selak M, Maes D, Weckx S, De Vuyst L. The ability of bifidobacteria to degrade arabinoxylan oligosaccharide constituents and derived oligosaccharides is strain dependent. *Appl Environ Microbiol.* 2014;80(1):204-17.
482. Komeno M, Hayamizu H, Fujita K, Ashida H. Two Novel α-l-Arabinofuranosidases from *Bifidobacterium longum* subsp. *longum* Belonging to Glycoside Hydrolase Family 43 Cooperatively Degrade Arabinan. *Appl Environ Microbiol.* 2019;85(6):e02582-18.
486. Zhang M, Chekan JR, Dodd D*, et al.* Xylan utilization in human gut commensal bacteria is orchestrated by unique modular organization of polysaccharide-degrading enzymes. *Proc Natl Acad Sci USA.* 2014;111(35):E3708-17.
489. Dodd D, Mackie RI, Cann IK. Xylan degradation, a metabolic property shared by rumen and human colonic Bacteroidetes. *Mol Microbiol.* 2011;79(2):292-304.
506. Hatzenpichler R, Scheller S, Tavormina PL, Babin BM, Tirrell DA, Orphan VJ. *In situ* visualization of newly synthesized proteins in environmental microbes using amino acid tagging and click chemistry. *Environ Microbiol.* 2014;16(8):2568-90.
521. Kalantar-Zadeh K, Berean KJ, Burgell RE, Muir JG, Gibson PR. Intestinal gases: influence on gut disorders and the role of dietary manipulations. *Nat Rev Gastroenterol Hepatol.* 2019;16(12):733-47.
525. Koecher KJ, Noack JA, Timm DA, Klosterbuer AS, Thomas W, Slavin JL. Estimation and interpretation of fermentation in the gut: coupling results from a 24 h batch *in vitro* system with fecal measurements from a human intervention feeding study using fructo-oligosaccharides, inulin, gum acacia, and pea fiber. *J Agric Food Chem.* 2014;62(6):1332-37.
526. Kaur A, Rose DJ, Rumpagaporn P, Patterson JA, Hamaker BR. *In vitro* batch fecal fermentation comparison of gas and short-chain fatty acid production using "slowly fermentable" dietary fibers. *J Food Sci.* 2011;76(5):H137-H42.
528. Grabitske HA, Slavin JL. Gastrointestinal effects of low-digestible carbohydrates. *Crit Rev Food Sci Nutr.* 2009;49(4):327-60.
529. Cherbut C, Michel C, Raison V, Kravtchenko T, Severine M. Acacia Gum is a Bifidogenic Dietary Fibre with High Digestive Tolerance in Healthy Humans. *Microb Ecol Health Dis.* 2003;15(1):43-50.
531. Kruse HP, Kleessen B, Blaut M. Effects of inulin on faecal bifidobacteria in human subjects. *Br J Nutr.* 1999;82(5):375-82.
532. Babiker R, Merghani TH, Elmusharaf K, Badi RM, Lang F, Saeed AM. Effects of Gum Arabic ingestion on body mass index and body fat percentage in healthy adult females: two-arm randomized, placebo controlled, double-blind trial. *Nutr J.* 2012;11:111.
535. Davis LMG, Martínez I, Walter J, Hutkins RW. A dose dependent impact of prebiotic galactooligosaccharides on the intestinal microbiota of healthy adults. *Int J Food Microbiol.* 2010;144(2):285-92.

537. Nguyen NK, Deehan EC, Zhang Z, *et al.* Gut microbiota modulation with long-chain corn bran arabinoxylan in adults with overweight and obesity is linked to an individualized temporal increase in fecal propionate. *Microbiome.* 2020;8:118.
540. Lam YY, Zhang C, Zhao L. Causality in dietary interventions-building a case for gut microbiota. *Genome Med.* 2018;10(1):62.
541. Lee J-H, O'Sullivan DJ. Genomic Insights into Bifidobacteria. *Microbiol Mol Biol Rev.* 2010;74(3):378-416.
554. Baxter NT, Schmidt AW, Venkataraman A, Kim KS, Waldron C, Schmidt TM. Dynamics of Human Gut Microbiota and Short-Chain Fatty Acids in Response to Dietary Interventions with Three Fermentable Fibers. *mBio.* 2019;10(1):e02566-18.
564. Thompson FE, Dixit-Joshi S, Potischman N, *et al.* Comparison of Interviewer-Administered and Automated Self-Administered 24-Hour Dietary Recalls in 3 Diverse Integrated Health Systems. *Am J Epidemiol.* 2015;181(12):970-78.
566. Mäder U, Martin BW, Schutz Y, Marti B. Validity of four short physical activity questionnaires in middle-aged persons. *Med Sci Sports Exerc.* 2006;38(7):1255-66.
569. Stewart ML, Wilcox ML, Bell M, Buggia MA, Maki KC. Type-4 Resistant Starch in Substitution for Available Carbohydrate Reduces Postprandial Glycemic Response and Hunger in Acute, Randomized, Double-Blind, Controlled Study. *Nutrients.* 2018;10(2):129.
584. Stewart ML, Zimmer JP. A High Fiber Cookie Made with Resistant Starch Type 4 Reduces Post-Prandial Glucose and Insulin Responses in Healthy Adults. *Nutrients.* 2017;9(3):237.
603. Crittenden R, Laitila A, Forssell P, *et al.* Adhesion of bifidobacteria to granular starch and its implications in probiotic technologies. *Appl Environ Microbiol.* 2001;67(8):3469-75.
608. Guerin-Deremaux L, Pochat M, Reifer C, Wils D, Cho S, Miller LE. The soluble fiber NUTRIOSE induces a dose-dependent beneficial impact on satiety over time in humans. *Nutr Res.* 2011;31(9):665-72.
609. Vermorel M, Coudray C, Wils D, *et al.* Energy value of a low-digestible carbohydrate, NUTRIOSE® FB, and its impact on magnesium, calcium and zinc apparent absorption and retention in healthy young men. *Eur J Nutr.* 2004;43(6):344-52.
610. Huaman J-W, Mego M, Manichanh C, *et al.* Effects of Prebiotics vs a Diet Low in FODMAPs in Patients With Functional Gut Disorders. *Gastroenterology.* 2018;155(4):1004-07.
611. Dikeman CL, Fahey GC. Viscosity as related to dietary fiber: a review. *Crit Rev Food Sci Nutr.* 2006;46(8):649-63.
612. Lattimer JM, Haub MD. Effects of dietary fiber and its components on metabolic health. *Nutrients.* 2010;2(12):1266-89.
613. Elleuch M, Bedigian D, Roiseux O, Besbes S, Blecker C, Attia H. Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. *Food Chem.* 2011;124(2):411-21.
614. Lefranc-Millot C, Wils D, Roturier J-M, Le Bihan C, Saniez-Degrave M-H. NUTRIOSE® Soluble Fiber. In: Cho SS, Samuel P, eds. *Fiber Ingredients: Food Applications and Health Benefits*. 1st ed. Boca Raton (USA): CRC Press; 2009:19-40.
615. Ahmed W, Rashid S. Functional and therapeutic potential of inulin: A comprehensive review. *Crit Rev Food Sci Nutr.* 2019;59(1):1-13.

616. Housez B, Cazaubiel M, Vergara C, *et al.* Evaluation of digestive tolerance of a soluble corn fibre. *J Hum Nutr Diet.* 2012;25(5):488-96.
617. Berry SE, Valdes AM, Drew DA, *et al.* Human postprandial responses to food and potential for precision nutrition. *Nat Med.* 2020;26(6):964-73.
618. Rowland I, Gibson G, Heinken A, *et al.* Gut microbiota functions: metabolism of nutrients and other food components. *Eur J Nutr.* 2018;57(1):1-24.
619. Holscher HD, Bauer LL, Gourineni V, Pelkman CL, Fahey GCJ, Swanson KS. Agave Inulin Supplementation Affects the Fecal Microbiota of Healthy Adults Participating in a Randomized, Double-Blind, Placebo-Controlled, Crossover Trial. *J Nutr.* 2015;145(9):2025-32.
620. Vulevic J, Juric A, Tzortzis G, Gibson GR. A mixture of trans-galactooligosaccharides reduces markers of metabolic syndrome and modulates the fecal microbiota and immune function of overweight adults. *J Nutr.* 2013;143(3):324-31.
621. Cekanaviciute E, Yoo BB, Runia TF, *et al.* Gut bacteria from multiple sclerosis patients modulate human T cells and exacerbate symptoms in mouse models. *Proc Natl Acad Sci USA.* 2017;114(42):E8943.
622. Duscha A, Gisevius B, Hirschberg S, *et al.* Propionic Acid Shapes the Multiple Sclerosis Disease Course by an Immunomodulatory Mechanism. *Cell.* 2020;180(6):1067-80 e16.
623. ClinicalTrials.gov. National Library of Medicine (US). Identifier NCT02322112, The Alberta FYBER (Feed Your gut Bacteria morE fibeR) Study. 2019, Sep 25. Available at: https://clinicaltrials.gov/ct2/show/NCT02322112.
624. BCD Bioscience Incorporated. 2020. Available at: https://www.bcdbio.com/.
625. Kaleido Biosciences Incorporated. 2020.
626. Tham Y-L, Sexton K, Weiss HL, Elledge RM, Friedman LC, Kramer RM. The adherence to practice guidelines in the assessment of bone health in women with chemotherapy-induced menopause *J Support Oncol.* 2006;4(6):295-304.
627. Schiavo L, Scalera G, Pilone V, De Sena G, Ciorra FR, Barbarisi A. Patient adherence in following a prescribed diet and micronutrient supplements after laparoscopic sleeve gastrectomy: our experience during 1 year of follow-up. *J Hum Nutr Diet.* 2017;30(1):98-104.
628. Pfister AK, Welch CA, WuLu JTJ, Hager KA, Saville PD. An assessment of postmenopausal women's adherence to calcium with vitamin D supplements. *J Appl Res.* 2008;8(2):143-50.
629. Deehan EC, Sizemore M, Walter J, Stewart ML. Characterization of two structurally distinct resistant starch type IV in a blueberry muffin batter and a pretzel bun dough application. Poster presented at 7th International Dietary Fiber Conference 2018; June 4th-6th, 2018; Rotterdam, Netherlands.
630. Stewart ML, Zimmer JP. Postprandial glucose and insulin response to a high-fiber muffin top containing resistant starch type 4 in healthy adults: a double-blind, randomized, controlled trial. *Nutrition.* 2018;53:59-63.
631. Du Y, Wu Y, Xiao D, *et al.* Food prototype containing resistant starch type 4 on postprandial glycemic response in healthy adults. *Food Funct.* 2020;11(3):2231-37.
632. Singh V, Yeoh BS, Chassaing B, *et al.* Dysregulated Microbial Fermentation of Soluble Fiber Induces Cholestatic Liver Cancer. *Cell.* 2018;175(3):679-94 e22.
633. Akbulut G. Nutrition in Stoma Patients: A Practical View of Dietary Therapy. *Int J Hematol Oncol.* 2011;21(1):61-66.

634. Cozma-Petrut A, Loghin F, Miere D, Dumitrascu DL. Diet in irritable bowel syndrome: What to recommend, not what to forbid to patients! *World J Gastroenterol.* 2017;23(21):3771-83.
635. Louis P, Scott KP, Duncan SH, Flint HJ. Understanding the effects of diet on bacterial metabolism in the large intestine. *J Appl Microbiol.* 2007;102(5):1197-208.
636. Sartor RB. Gut microbiota: Optimal sampling of the intestinal microbiota for research. *Nat Rev Gastroenterol Hepatol.* 2015;12(5):253-54.
637. Gurry T, Nguyen LTT, Yu X, Alm EJ. Functional heterogeneity in the fermentation capabilities of the healthy human gut microbiota. *bioRxiv.* 2020.
638. Gorzelak MA, Gill SK, Tasnim N, Ahmadi-Vand Z, Jay M, Gibson DL. Methods for Improving Human Gut Microbiome Data by Reducing Variability through Sample Processing and Storage of Stool. *PLoS One.* 2015;10(8):e0134802.
639. Tedjo DI, Jonkers DM, Savelkoul PH*, et al.* The effect of sampling and storage on the fecal microbiota composition in healthy and diseased subjects. *PLoS One.* 2015;10(5):e0126685.
640. Cunningham JL, Bramstang L, Singh A*, et al.* Impact of time and temperature on gut microbiota and SCFA composition in stool samples. *PLoS One.* 2020;15(8):e0236944.
641. Salem MB, Aiche G, Rubbert L, Renaud P, Haddab Y. Design of a Microbiota Sampling Capsule using 3D-Printed Bistable Mechanism. *Conf Proc IEEE Eng Med Biol Soc.* 2018;2018:4868-71.
642. Tang Q, Jin G, Wang G*, et al.* Current Sampling Methods for Gut Microbiota: A Call for More Precise Devices. *Front Cell Infect Microbiol.* 2020;10:151.

BIBLIOGRAPHY

1. Logan AC, Jacka FN, Prescott SL. Immune-Microbiota Interactions: Dysbiosis as a Global Health Issue. *Curr Allergy Asthma Rep.* 2016;16(2):13.
2. Blaser MJ, Falkow S. What are the consequences of the disappearing human microbiota? *Nat Rev Microbiol.* 2009;7(12):887-94.
3. Segata N. Gut Microbiome: Westernization and the Disappearance of Intestinal Diversity. *Curr Biol.* 2015;25(14):R611-R13.
4. Sonnenburg ED, Smits SA, Tikhonov M, Higginbottom SK, Wingreen NS, Sonnenburg JL. Diet-induced extinctions in the gut microbiota compound over generations. *Nature.* 2016;529(7585):212-15.
5. Jew S, AbuMweis SS, Jones PJ. Evolution of the Human Diet: Linking Our Ancestral Diet to Modern Functional Foods as a Means of Chronic Disease Prevention. *J Med Food.* 2009;12(5):925-34.
6. Sonnenburg ED, Sonnenburg JL. Starving our microbial self: the deleterious consequences of a diet deficient in microbiota-accessible carbohydrates. *Cell Metab.* 2014;20(5):779-86.
7. Tap J, Furet J-P, Bensaada M*, et al.* Gut microbiota richness promotes its stability upon increased dietary fibre intake in healthy adults. *Environ Microbiol.* 2015;17(12):4954-64.
8. Martínez I, Lattimer JM, Hubach KL*, et al.* Gut microbiome composition is linked to whole grain-induced immunological improvements. *ISME J.* 2013;7(2):269-80.
9. Martínez I, Stegen JC, Maldonado-Gómez MX*, et al.* The gut microbiota of rural papua new guineans: composition, diversity patterns, and ecological processes. *Cell Rep.* 2015;11(4):527-38.
10. Kovatcheva-Datchary P, Nilsson A, Akrami R*, et al.* Dietary Fiber-Induced Improvement in Glucose Metabolism Is Associated with Increased Abundance of *Prevotella*. *Cell Metab.* 2015;22(6):971-82.
11. O'Keefe SJD, Li JV, Lahti L*, et al.* Fat, fibre and cancer risk in African Americans and rural Africans. *Nat Commun.* 2015;6:6342.
12. Jones JM. CODEX-aligned dietary fiber definitions help to bridge the 'fiber gap'. *Nutr J.* 2014;13:34.
13. Fungwe TV, Bente L, Hiza H. The Food Supply and Dietary Fiber: Its Availability and Effect on Health. Center for Nutrition Policy and Promotion. November, 2007.
14. Storey M, Anderson P. Income and race/ethnicity influence dietary fiber intake and vegetable consumption. *Nutr Res.* 2014;34(10):844-50.
15. Abdullah MMH, Gyles CL, Marinangeli CPF, Carlberg JG, Jones PJH. Cost-of-illness analysis reveals potential healthcare savings with reductions in type 2 diabetes and cardiovascular disease following recommended intakes of dietary fiber in Canada. *Front Pharmacol.* 2015;6:167.
16. Walter J, Ley RE. The human gut microbiome: ecology and recent evolutionary changes. *Annu Rev Microbiol.* 2011;65:411-29.
17. Schroeder BO, Bäckhed F. Signals from the gut microbiota to distant organs in physiology and disease. *Nat Med.* 2016;22(10):1079-89.
18. Walker AW, Lawley TD. Therapeutic modulation of intestinal dysbiosis. *Pharmacol Res.* 2013;69(1):75-86.

19. Brahe LK, Astrup A, Larsen LH. Can We Prevent Obesity-Related Metabolic Diseases by Dietary Modulation of the Gut Microbiota? *Adv Nutr.* 2016;7(1):90-101.
20. Olle B. Medicines from microbiota. *Nat Biotechnol.* 2013;31(4):309–15.
21. David LA, Maurice CF, Carmody RN*, et al.* Diet rapidly and reproducibly alters the human gut microbiome. *Nature.* 2014;505(7484):559-63.
22. Wu GD, Chen J, Hoffmann C*, et al.* Linking long-term dietary patterns with gut microbial enterotypes. *Science.* 2011;334(6052):105-08.
23. Wu GD, Compher C, Chen EZ*, et al.* Comparative metabolomics in vegans and omnivores reveal constraints on diet-dependent gut microbiota metabolite production. *Gut.* 2016;65(1):63-72.
24. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr.* 1995;125(6):1401-12.
25. Bach J-F. The effect of infections on susceptibility to autoimmune and allergic diseases. *N Engl J Med.* 2002;347(12):911-20.
26. Bickler SW, DeMaio A. Western diseases: current concepts and implications for pediatric surgery research and practice. *Pediatr Surg Int.* 2008;24(3):251-5.
27. Deehan EC, Walter J. The Fiber Gap and the Disappearing Gut Microbiome: Implications for Human Nutrition. *Trends Endocrinol Metab.* 2016;27(5):239-42.
28. Burkitt DP, Walker ARP, Painter NS. Dietary Fiber and Disease. *JAMA.* 1974;229(8):1068-74.
29. Eaton S, Eaton SI, Konner M. Paleolithic nutrition revisited: a twelve-year retrospective on its nature and implications. *Eur J Clin Nutr.* 1997;51(4):207-16.
30. Burkitt DP. Some diseases characteristic of modern Western civilization. *BMJ.* 1973;1(5848):274-78.
31. Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. *Cell.* 2016;165(6):1332-45.
32. Yatsunenko T, Rey FE, Manary MJ*, et al.* Human gut microbiome viewed across age and geography. *Nature.* 2012;486(7402):222-27.
33. Clemente JC, Pehrsson EC, Blaser MJ*, et al.* The microbiome of uncontacted Amerindians. *Sci Adv.* 2015;1(3):e1500183.
34. Schnorr SL, Candela M, Rampelli S*, et al.* Gut microbiome of the Hadza hunter-gatherers. *Nat Commun.* 2014;5:3654.
35. De Filippo C, Cavalieri D, Di Paola M*, et al.* Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci USA.* 2010;107(33):14691-96.
36. Hamaker BR, Tuncil YE. A perspective on the complexity of dietary fiber structures and their potential effect on the gut microbiota. *J Mol Biol.* 2014;426(23):3838-50.
37. King DE, Mainous AGr, Lambourne CA. Trends in dietary fiber intake in the United States, 1999-2008. *J Acad Nutr Diet.* 2012;112(5):642-68.
38. Hill DR, Newburg DS. Clinical applications of bioactive milk components. *Nutr Rev.* 2015;73(7):463-76.
39. Butto LF, Haller D. Functional relevance of microbiome signatures: The correlation era requires tools for consolidation. *J Allergy Clin Immunol.* 2017;139(4):1092-98.

40. Yang L, Lu X, Nossa CW, Francois F, Peek RM, Pei Z. Inflammation and intestinal metaplasia of the distal esophagus are associated with alterations in the microbiome. *Gastroenterology.* 2009;137(2):588-97.
41. Wu T, Zhang Z, Liu B*, et al.* Gut microbiota dysbiosis and bacterial community assembly associated with cholesterol gallstones in large-scale study. *BMC Genomics.* 2013;14:669.
42. Dahl WJ, Stewart ML. Position of the Academy of Nutrition and Dietetics: Health Implications of Dietary Fiber. *J Acad Nutr Diet.* 2015;115(11):1861-70.
43. Anderson JW, Baird P, Davis RHJ*, et al.* Health benefits of dietary fiber. *Nutr Rev.* 2009;67(4):188-205.
44. Bindels LB, Delzenne NM, Cani PD, Walter J. Towards a more comprehensive concept for prebiotics. *Nat Rev Gastroenterol Hepatol.* 2015;12(5):303-10.
45. Gibson GR, Hutkins R, Sanders ME*, et al.* Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol.* 2017;14(8):491-502.
46. Gibson GR, Scott KP, Rastall RA*, et al.* Dietary prebiotics: current status and new definition. *Food Sci Tech Bull Funct Foods.* 2010;7(1):1-19.
47. Roberfroid M. Prebiotics: the concept revisited. *J Nutr.* 2007;137(3 Suppl 2):830S-37S.
48. Katsnelson A. Core Concept: Prebiotics gain prominence but remain poorly defined. *Proc Natl Acad Sci USA.* 2016;113(50):14168-69.
49. Verspreet J, Damen B, Broekaert WF, Verbeke K, Delcour JA, Courtin CM. A Critical Look at Prebiotics Within the Dietary Fiber Concept. *Annu Rev Food Sci Technol.* 2016;7:167-90.
50. Shanahan F. Fiber man meets microbial man. *Am J Clin Nutr.* 2015;101(1):1-2.
51. Louis P, Flint HJ, Michel C. How to Manipulate the Microbiota: Prebiotics. *Adv Exp Med Biol.* 2016;902:119-42.
52. Quevrain E, Maubert M-A, Michon C*, et al.* Identification of an anti-inflammatory protein from *Faecalibacterium prausnitzii*, a commensal bacterium deficient in Crohn's disease. *Gut.* 2015;65(3):415-25.
53. Zeevi D, Korem T, Zmora N*, et al.* Personalized Nutrition by Prediction of Glycemic Responses. *Cell.* 2015;163(5):1079-94.
54. Everard A, Belzer C, Geurts L*, et al.* Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. *Proc Natl Acad Sci USA.* 2013;110(22):9066-71.
55. Atarashi K, Tanoue T, Oshima K*, et al.* Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature.* 2013;500(7461):232-36.
56. Round JL, Lee SM, Li J*, et al.* The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science.* 2011;332(6032):974-77.
57. Le Chatelier E, Nielsen T, Qin J*, et al.* Richness of human gut microbiome correlates with metabolic markers. *Nature.* 2013;500(7464):541-46.
58. Cotillard A, Kennedy SP, Kong LC*, et al.* Dietary intervention impact on gut microbial gene richness. *Nature.* 2013;500(7464):585-88.
59. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. *Nature.* 2012;489(7415):220-30.
60. LeBlanc JG, Chain F, Martin R, Bermudez-Humaran LG, Courau S, Langella P. Beneficial effects on host energy metabolism of short-chain fatty acids and vitamins produced by commensal and probiotic bacteria. *Microb Cell Fact.* 2017;16(1):79.

61. Canfora EE, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity. *Nat Rev Endocrinol.* 2015;11(10):577-91.
62. Zheng J, Ruan L, Sun M, Gänzle M. A Genomic View of Lactobacilli and Pediococci Demonstrates that Phylogeny Matches Ecology and Physiology. *Appl Environ Microbiol.* 2015;81(20):7233-43.
63. Flint HJ, Duncan SH, Scott KP, Louis P. Links between diet, gut microbiota composition and gut metabolism. *Proc Nutr Soc.* 2015;74(1):13-22.
64. Klijn A, Mercenier A, Arigoni F. Lessons from the genomes of bifidobacteria. *FEMS Microbiol Rev.* 2005;29(3):491-509.
65. Makras L, Falony G, Van der Meulen R, De Vuyst L. Production of organic acids from fermentation of mannitol, fructooligosaccharides and inulin by a cholesterol removing Lactobacillus acidophilus strain. *J Appl Microbiol.* 2006;100(6):1388-89.
66. Dewulf EM, Cani PD, Claus SP*, et al.* Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. *Gut.* 2013;62(8):1112-21.
67. Venkataraman A, Sieber JR, Schmidt AW, Waldron C, Theis KR, Schmidt TM. Variable responses of human microbiomes to dietary supplementation with resistant starch. *Microbiome.* 2016;4(1):33.
68. Walker AW, Ince J, Duncan SH*, et al.* Dominant and diet-responsive groups of bacteria within the human colonic microbiota. *ISME J.* 2011;5(2):220-30.
69. Martínez I, Kim J, Duffy PR, Schlegel VL, Walter J. Resistant starches types 2 and 4 have differential effects on the composition of the fecal microbiota in human subjects. *PLoS One.* 2010;5(11):e15046.
70. El Kaoutari A, Armougom F, Gordon JI, Raoult D, Henrissat B. The abundance and variety of carbohydrate-active enzymes in the human gut microbiota. *Nat Rev Microbiol.* 2013;11(7):497-504.
71. Flint HJ, Bayer EA, Rincon MT, Lamed R, White BA. Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. *Nat Rev Microbiol.* 2008;6(2):121-31.
72. Chung WSF, Walker AW, Louis P*, et al.* Modulation of the human gut microbiota by dietary fibres occurs at the species level. *BMC Biology.* 2016;14(1):3.
73. Davis LMG, Martínez I, Walter J, Goin C, Hutkins RW. Barcoded pyrosequencing reveals that consumption of galactooligosaccharides results in a highly specific bifidogenic response in humans. *PLoS One.* 2011;6(9):e25200.
74. Sawicki CM, Livingston KA, Obin M, Roberts SB, Chung M, McKeown NM. Dietary Fiber and the Human Gut Microbiota: Application of Evidence Mapping Methodology. *Nutrients.* 2017;9(2):125.
75. Walker AW, Duncan SH, McWilliam Leitch EC, Child MW, Flint HJ. pH and peptide supply can radically alter bacterial populations and short-chain fatty acid ratios within microbial communities from the human colon. *Appl Environ Microbiol.* 2005;71(7):3692-700.
76. Duncan SH, Louis P, Thomson JM, Flint HJ. The role of pH in determining the species composition of the human colonic microbiota. *Environ Microbiol.* 2009;11(8):2112-22.
77. Tannock GW, Lawley B, Munro K*, et al.* RNA-stable-isotope probing shows utilization of carbon from inulin by specific bacterial populations in the rat large bowel. *Appl Environ Microbiol.* 2014;80(7):2240-47.

78. Holscher HD. Dietary fiber and prebiotics and the gastrointestinal microbiota. *Gut Microbes.* 2017;8(2):172-84.
79. Slavin JL. Fiber and prebiotics: mechanisms and health benefits. *Nutrients.* 2013;5(4):1417-35.
80. Hipsley EH. Dietary "Fibre" and Pregnancy Toxaemia. *BMJ.* 1953;2(4833):420–22.
81. Trowell HC. Definition of dietary fiber. *Lancet.* 1974;1(7856):503.
82. Joint FAO/WHO Food Standards Programme. Secretariat of the CODEX Alimentarius Commission: CODEX Alimentarius (CODEX) Guidelines on Nutrition Labeling CAC/GL 2-1985 as last amended 2010. Rome, Italy: FAO. 2010.
83. Food and Drug Administration H. Food Labeling: Revision of the Nutrition and Supplement Facts Labels. Maryland, United States.May 27,, 2016. RIN 0910-AF22.
84. Fuller S, Beck E, Salman H, Tapsell L. New Horizons for the Study of Dietary Fiber and Health: A Review. *Plant Foods Hum Nutr.* 2016;71(1):1-12.
85. Tungland BC, Meyer D. Nondigestible Oligo- and Polysaccharides (Dietary Fiber): Their Physiology and Role in Human Health and Food. *Compr Rev Food Sci Food Saf.* 2002;1(3):90-109.
86. Raigond P, Ezekiel R, Raigond B. Resistant starch in food: a review. *J Sci Food Agr.* 2015;95(10):1968-78.
87. Mudgil D, Barak S. Composition, properties and health benefits of indigestible carbohydrate polymers as dietary fiber: a review. *Int J Biol Macromol.* 2013;61:1-6.
88. Guillona F, Champ M. Structural and physical properties of dietary fibres, and consequences of processing on human physiology. *Food Res Int.* 2000;33(3-4):233–45.
89. Vrieze A, Van Nood E, Holleman F*, et al.* Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology.* 2012;143(4):913-16 e7.
90. Hehemann J-H, Correc G, Barbeyron T, Helbert W, Czjzek M, Michel G. Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota. *Nature.* 2010;464(7290):908-12.
91. Chassard C, Delmas E, Robert C, Bernalier-Donadille A. The cellulose-degrading microbial community of the human gut varies according to the presence or absence of methanogens. *FEMS Microbiol Ecol.* 2010;74(1):205-13.
92. Walter J. Murine Gut Microbiota—Diet Trumps Genes. *Cell Host Microbe.* 2015;17(1):3-5.
93. Kovatcheva-Datchary P, Egert M, Maathuis A*, et al.* Linking phylogenetic identities of bacteria to starch fermentation in an *in vitro* model of the large intestine by RNA-based stable isotope probing. *Environ Microbiol.* 2009;11(4):914-26.
94. Leitch ECM, Walker AW, Duncan SH, Holtrop G, Flint HJ. Selective colonization of insoluble substrates by human faecal bacteria. *Environ Microbiol.* 2007;9(3):667-79.
95. Rakoff-Nahoum S, Coyne MJ, Comstock LE. An ecological network of polysaccharide utilization among human intestinal symbionts. *Curr Biol.* 2014;24(1):40-49.
96. Flint HJ, Scott KP, Duncan SH, Louis P, Forano E. Microbial degradation of complex carbohydrates in the gut. *Gut Microbes.* 2012;3(4):289-306.
97. Belenguer A, Duncan SH, Calder AG*, et al.* Two routes of metabolic cross-feeding between *Bifidobacterium adolescentis* and butyrate-producing anaerobes from the human gut. *Appl Environ Microbiol.* 2006;72(5):3593-99.

98. Coyte KZ, Schluter J, Foster KR. The ecology of the microbiome: Networks, competition, and stability. *Science.* 2015;350(6261):663-66.
99. Koropatkin NM, Cameron EA, Martens EC. How glycan metabolism shapes the human gut microbiota. *Nat Rev Microbiol.* 2012;10(5):323-35.
100. Rey FE, Faith JJ, Bain J*, et al.* Dissecting the in vivo metabolic potential of two human gut acetogens. *J Biol Chem.* 2010;285(29):22082-90.
101. Fischbach MA, Sonnenburg JL. Eating for two: how metabolism establishes interspecies interactions in the gut. *Cell Host Microbe.* 2011;10(4):336-47.
102. Barcenilla A, Pryde SE, Martin JC*, et al.* Phylogenetic relationships of butyrate-producing bacteria from the human gut. *Appl Environ Microbiol.* 2000;66(4):1654-61.
103. Chassard C, Bernalier-Donadille A. H2 and acetate transfers during xylan fermentation between a butyrate-producing xylanolytic species and hydrogenotrophic microorganisms from the human gut. *FEMS Microbiol Lett.* 2006;254(1):116-22.
104. Duncan SH, Louis P, Flint HJ. Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. *Appl Environ Microbiol.* 2004;70(10):5810-17.
105. Lozupone CA, Hamady M, Cantarel BL*, et al.* The convergence of carbohydrate active gene repertoires in human gut microbes. *Proc Natl Acad Sci USA.* 2008;105(39):15076-81.
106. Vandeputte D, Falony G, Vieira-Silva S*, et al.* Prebiotic inulin-type fructans induce specific changes in the human gut microbiota. *Gut.* 2017;66(11):1968-74.
107. Heiman ML, Greenway FL. A healthy gastrointestinal microbiome is dependent on dietary diversity. *Mol Metab.* 2016;5(5):317-20.
108. Clayton JB, Vangay P, Huang H*, et al.* Captivity humanizes the primate microbiome. *Proc Natl Acad Sci USA.* 2016;113(37):10376-81.
109. Kong LC, Holmes BA, Cotillard A*, et al.* Dietary patterns differently associate with inflammation and gut microbiota in overweight and obese subjects. *PLoS One.* 2014;9(10):e109434.
110. Upadhyaya B, McCormack L, Fardin-Kia AR*, et al.* Impact of dietary resistant starch type 4 on human gut microbiota and immunometabolic functions. *Sci Rep.* 2016;6:28797.
111. West NP, Christophersen CT, Pyne DB*, et al.* Butyrylated starch increases colonic butyrate concentration but has limited effects on immunity in healthy physically active individuals. *Exerc Immunol Rev.* 2013;19:102-19.
112. Hooda S, Boler BMV, Serao MCR*, et al.* 454 pyrosequencing reveals a shift in fecal microbiota of healthy adult men consuming polydextrose or soluble corn fiber. *J Nutr.* 2012;142(7):1259-65.
113. Wong JM, de Souza R, Kendall CW, Emam A, Jenkins DJ. Colonic health: fermentation and short chain fatty acids. *J Clin Gastroenterol.* 2006;40(3):235-43.
114. Cummings JH, Pomare E, Branch W, Naylor C, Macfarlane GT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut.* 1987;28(10):1221-27.
115. Boets E, Gomand SV, Deroover L*, et al.* Systemic availability and metabolism of colonic-derived short-chain fatty acids in healthy subjects - a stable isotope study. *J Physiol.* 2017;595(2):541-55.
116. Yang J, Martínez I, Walter J, Keshavarzian A, Rose DJ. *In vitro* characterization of the impact of selected dietary fibers on fecal microbiota composition and short chain fatty acid production. *Anaerobe.* 2013;23:74-81.

117. Yang J, Rose DJ. Long-term dietary pattern of fecal donor correlates with butyrate production and markers of protein fermentation during in vitro fecal fermentation. *Nutr Res.* 2014;34(9):749-59.
118. Roediger W. Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. *Gut.* 1980;21(9):793-98.
119. Cummings JH, Macfarlane GT. The control and consequences of bacterial fermentation in the human colon. *J Appl Bacteriol.* 1991;70(6):443-59.
120. Duncan SH, Belenguer A, Holtrop G, Johnstone AM, Flint HJ, Lobley GE. Reduced dietary intake of carbohydrates by obese subjects results in decreased concentrations of butyrate and butyrate-producing bacteria in feces. *Appl Environ Microbiol.* 2007;73(4):1073-78.
121. Russell WR, Gratz SW, Duncan SH*, et al.* High-protein, reduced-carbohydrate weight-loss diets promote metabolite profiles likely to be detrimental to colonic health. *Am J Clin Nutr.* 2011;93(5):1062-72.
122. Windey K, De Preter V, Verbeke K. Relevance of protein fermentation to gut health. *Mol Nutr Food Res.* 2012;56(1):184-96.
123. Sonnenburg JL, Xu J, Leip DD*, et al.* Glycan foraging *in vivo* by an intestine-adapted bacterial symbiont. *Science.* 2005;307(5717):1955-59.
124. Earle KA, Billings G, Sigal M*, et al.* Quantitative Imaging of Gut Microbiota Spatial Organization. *Cell Host Microbe.* 2015;18(4):478-88.
125. Desai Mahesh S, Seekatz Anna M, Koropatkin Nicole M*, et al.* A Dietary Fiber-Deprived Gut Microbiota Degrades the Colonic Mucus Barrier and Enhances Pathogen Susceptibility. *Cell.* 2016;167(5):1339-53.e21.
126. Lambeau KV, McRorie JWJ. Fiber supplements and clinically proven health benefits: How to recognize and recommend an effective fiber therapy. *J Am Acad Nurse Pract.* 2017;29(4):216-23.
127. Schwiertz A, Taras D, Schäfer K*, et al.* Microbiota and SCFA in Lean and Overweight Healthy Subjects. *Obesity.* 2010;18(1):190-95.
128. Belcheva A, Irrazabal T, Robertson SJ*, et al.* Gut microbial metabolism drives transformation of MSH2-deficient colon epithelial cells. *Cell.* 2014;158(2):288-99.
129. Lupton JR. Microbial degradation products influence colon cancer risk: the butyrate controversy. *J Nutr.* 2004;134(2):479-82.
130. Du H, van der A DL, Boshuizen HC*, et al.* Dietary fiber and subsequent changes in body weight and waist circumference in European men and women. *Am J Clin Nutr.* 2010;91(2):329-36.
131. Ben Q, Sun Y, Chai R, Qian A, Xu B, Yuan Y. Dietary fiber intake reduces risk for colorectal adenoma: a meta-analysis. *Gastroenterology.* 2014;146(3):689-99 e6.
132. Kunzmann AT, Coleman HG, Huang W-Y, Kitahara CM, Cantwell MM, Berndt SI. Dietary fiber intake and risk of colorectal cancer and incident and recurrent adenoma in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. *Am J Clin Nutr.* 2015;102(4):881-90.
133. Tan J, McKenzie C, Potamitis M, Thorburn AN, Mackay CR, Macia L. The role of short-chain fatty acids in health and disease. *Adv Immunol.* 2014;121:91-119.
134. Tazoe H, Otomo Y, Karaki S-i*, et al.* Expression of short-chain fatty acid receptor GPR41 in the human colon. *Biomedical Research.* 2009;30(3):149-56.

135. Tazoe H, Otomo Y, Kaji I, Tanaka R, Karaki S-i, Kuwahara A. Roles of short-chain fatty acids receptors, GPR41 and GPR43 on colonic functions. *J Physiol Pharmacol.* 2008;59(Suppl 2):251-62.
136. Brown AJ, Goldsworthy SM, Barnes AA*, et al.* The Orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *J Biol Chem.* 2003;278(13):11312-19.
137. Le Poul E, Loison C, Struyf S*, et al.* Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. *J Biol Chem.* 2003;278(28):25481-89.
138. Thangaraju M, Cresci GA, Liu K*, et al.* GPR109A is a G-protein-coupled receptor for the bacterial fermentation product butyrate and functions as a tumor suppressor in colon. *Cancer Research.* 2009;69(7):2826-32.
139. Wanders D, Graff EC, Judd RL. Effects of high fat diet on GPR109A and GPR81 gene expression. *Biochem Biophys Res Commun.* 2012;425(2):278-83.
140. Cresci GA, Thangaraju M, Mellinger JD, Liu K, Ganapathy V. Colonic gene expression in conventional and germ-free mice with a focus on the butyrate receptor GPR109A and the butyrate transporter SLC5A8. *J Gastrointest Surg.* 2010;14(3):449-61.
141. Blundell JE, De Graaf C, Hulshof T*, et al.* Appetite control: methodological aspects of the evaluation of foods. *Obes Rev.* 2010;11(3):251-70.
142. Samuel BS, Shaito A, Motoike T*, et al.* Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. *Proc Natl Acad Sci USA.* 2008;105(43):16767-72.
143. Tolhurst G, Heffron H, Lam YS*, et al.* Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. *Diabetes.* 2012;61(2):364-71.
144. Savage A, Adrian TE, Carolan G, Chatterjee V, Bloom S. Effects of peptide YY (PYY) on mouth to caecum intestinal transit time and on the rate of gastric emptying in healthy volunteers. *Gut.* 1987;28(2):166-70.
145. Batterham RL, Cowley MA, Small CJ*, et al.* Gut hormone PYY(3-36) physiologically inhibits food intake. *Nature.* 2002;418(6898):650-54.
146. Wei Y, Mojsov S. Tissue-specific expression of the human receptor for glucagon-like peptide-I: brain, heart and pancreatic forms have the same deduced amino acid sequences. *FEBS Lett.* 1995;358(3):219-24.
147. Merchenthaler I, Lane M, Shughrue P. Distribution of pre-pro-glucagon and glucagon-like peptide-1 receptor messenger RNAs in the rat central nervous system. *J Comp Neurol.* 1999;403(2):261-80.
148. Schjoldager B, Mortensen P, Christiansen J, Ørskov C, Holst JJ. GLP-1 (glucagon-like peptide 1) and truncated GLP-1, fragments of human proglucagon, inhibit gastric acid secretion in humans. *Dig Dis Sci.* 1989;34(5):703-08.
149. Näslund E, Bogefors J, Skogar S*, et al.* GLP-1 slows solid gastric emptying and inhibits insulin, glucagon, and PYY release in humans. *Am J Physiol.* 1999;277(3 Pt 2):R910-R16.
150. Xiong Y, Miyamoto N, Shibata K*, et al.* Short-chain fatty acids stimulate leptin production in adipocytes through the G protein-coupled receptor GPR41. *Proc Natl Acad Sci USA.* 2004;101(4):1045-50.

151. Zaibi MS, Stocker CJ, O'Dowd J*, et al.* Roles of GPR41 and GPR43 in leptin secretory responses of murine adipocytes to short chain fatty acids. *FEBS Lett.* 2010;584(11):2381-86.
152. Frost G, Sleeth ML, Sahuri-Arisoylu M*, et al.* The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nat Commun.* 2014;5:3611.
153. De Vadder F, Kovatcheva-Datchary P, Goncalves D*, et al.* Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. *Cell.* 2014;156(1-2):84-96.
154. Delaere F, Duchampt A, Mounien L*, et al.* The role of sodium-coupled glucose co-transporter 3 in the satiety effect of portal glucose sensing. *Mol Metab.* 2013;2(1):47-53.
155. Kimura I, Inoue D, Maeda T*, et al.* Short-chain fatty acids and ketones directly regulate sympathetic nervous system via G protein-coupled receptor 41 (GPR41). *Proc Natl Acad Sci USA.* 2011;108(19):8030-35.
156. Gao Z, Yin J, Zhang J*, et al.* Butyrate improves insulin sensitivity and increases energy expenditure in mice. *Diabetes.* 2009;58(7):1509-17.
157. Freitag J, Berod L, Kamradt T, Sparwasser T. Immunometabolism and autoimmunity. *Immunol Cell Biol.* 2016;94(10):925-34.
158. Hajer GR, van Haeften TW, Visseren FLJ. Adipose tissue dysfunction in obesity, diabetes, and vascular diseases. *Eur Heart J.* 2008;29(24):2959-71.
159. Beserra BTS, Fernandes R, do Rosario VA, Mocellin MC, Kuntz MGF, Trindade EBSM. A systematic review and meta-analysis of the prebiotics and synbiotics effects on glycaemia, insulin concentrations and lipid parameters in adult patients with overweight or obesity. *Clin Nutr.* 2015;34(5):845-58.
160. Whitehead A, Beck EJ, Tosh S, Wolever TMS. Cholesterol-lowering effects of oat β-glucan: a meta-analysis of randomized controlled trials. *Am J Clin Nutr.* 2014;100(6):1413-21.
161. Kellow NJ, Coughlan MT, Reid CM. Metabolic benefits of dietary prebiotics in human subjects: a systematic review of randomised controlled trials. *Br J Nutr.* 2014;111(7):1147-61.
162. Ning H, Van Horn L, Shay CM, Lloyd-Jones DM. Associations of dietary fiber intake with long-term predicted cardiovascular disease risk and C-reactive protein levels (from the National Health and Nutrition Examination Survey Data [2005-2010]). *Am J Cardiol.* 2014;113(2):287-91.
163. Yao B, Fang H, Xu W*, et al.* Dietary fiber intake and risk of type 2 diabetes: a dose-response analysis of prospective studies. *Eur J Epidemiol.* 2014;29(2):79-88.
164. Kreymann B, Ghatei MA, Williams G, Bloom SR. Glucagon-Like Peptide-1 7-36: A Physiological Incretin in Man. *Lancet.* 1987;330(8571):1300-04.
165. Komatsu R, Matsuyama T, Namba M*, et al.* Glucagonostatic and insulinotropic action of glucagonlike peptide I-(7-36)-amide. *Diabetes.* 1989;38(7):902-05.
166. Farilla L, Bulotta A, Hirshberg B*, et al.* Glucagon-like peptide 1 inhibits cell apoptosis and improves glucose responsiveness of freshly isolated human islets. *Endocrinology.* 2003;144(12):5149-58.
167. Holz GGI, Kühtreiber WM, Habener JF. Pancreatic beta-cells are rendered glucose-competent by the insulinotropic hormone glucagon-like peptide-1(7-37). *Nature.* 1993;361(6410):362-65.

168. Pingitore A, Chambers ES, Hill T, *et al.* The diet-derived short chain fatty acid propionate improves beta-cell function in humans and stimulates insulin secretion from human islets in vitro. *Diabetes Obes Metab.* 2017;19(2):257-65.
169. Roder PV, Wu B, Liu Y, Han W. Pancreatic regulation of glucose homeostasis. *Exp Mol Med.* 2016;48:e219.
170. Clore JN, Stillman J, Sugerman H. Glucose-6-phosphatase flux in vitro is increased in type 2 diabetes. *Diabetes.* 2000;49(6):969-74.
171. Magnusson I, Rothman DL, Katz LD, Shulman RG, Shulman GI. Increased rate of gluconeogenesis in type II diabetes mellitus. A 13C nuclear magnetic resonance study. *J Clin Invest.* 1992;90(4):1323-27.
172. den Besten G, Bleeker A, Gerding A, *et al.* Short-Chain Fatty Acids Protect Against High-Fat Diet–Induced Obesity via a PPARγ-Dependent Switch From Lipogenesis to Fat Oxidation. *Diabetes.* 2015;64(7):2398-408.
173. Wolever TM, Spadafora P, Eshuis H. Interaction between colonic acetate and propionate in humans. *Am J Clin Nutr.* 1991;53(3):681-87.
174. Ge H, Li X, Weiszmann J, *et al.* Activation of G protein-coupled receptor 43 in adipocytes leads to inhibition of lipolysis and suppression of plasma free fatty acids. *Endocrinology.* 2008;149(9):4519-26.
175. Al-Lahham Sa, Roelofsen H, Rezaee F, *et al.* Propionic acid affects immune status and metabolism in adipose tissue from overweight subjects. *Eur J Clin Invest.* 2012;42(4):357-64.
176. Gregor MF, Hotamışlıgil GS. Inflammatory mechanisms in obesity. *Annu Rev Immunol.* 2011;29:415-45.
177. Jiao J, Xu J-Y, Zhang W, Han S, Qin L-Q. Effect of dietary fiber on circulating C-reactive protein in overweight and obese adults: a meta-analysis of randomized controlled trials. *Int J Food Sci Nutr.* 2015;66(1):114-19.
178. North CJ, Venter CS, Jerling JC. The effects of dietary fibre on C-reactive protein, an inflammation marker predicting cardiovascular disease. *Eur J Clin Nutr.* 2009;63(8):921-33.
179. Piya MK, Harte AL, McTernan PG. Metabolic endotoxaemia: is it more than just a gut feeling? *Curr Opin Lipidol.* 2013;24(1):78-85.
180. Lassenius MI, Pietiläinen KH, Kaartinen K, *et al.* Bacterial endotoxin activity in human serum is associated with dyslipidemia, insulin resistance, obesity, and chronic inflammation. *Diabetes Care.* 2011;34(8):1809-15.
181. Hawkesworth S, Moore SE, Fulford AJ, *et al.* Evidence for metabolic endotoxemia in obese and diabetic Gambian women. *Nutr Diabetes.* 2013;3:e83.
182. Amar J, Burcelin R, Ruidavets JB, *et al.* Energy intake is associated with endotoxemia in apparently healthy men. *Am J Clin Nutr.* 2008;87(5):1219-23.
183. Cani PD, Amar J, Iglesias MA, *et al.* Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes.* 2007;56(7):1761-72.
184. Everard A, Lazarevic V, Derrien M, *et al.* Responses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic obese and diet-induced leptin-resistant mice. *Diabetes.* 2011;60(11):2775-86.
185. Neyrinck AM, Possemiers S, Druart C, *et al.* Prebiotic Effects of Wheat Arabinoxylan Related to the Increase in *Bifidobacteria*, *Roseburia* and *Bacteroides*/*Prevotella* in Diet-Induced Obese Mice. *PLoS One.* 2011;6(6):e20944.

186. Cani PD, Possemiers S, Van de Wiele T, *et al.* Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut.* 2009;58(8):1091-103.
187. Kelly CJ, Zheng L, Campbell EL, *et al.* Crosstalk between Microbiota-Derived Short-Chain Fatty Acids and Intestinal Epithelial HIF Augments Tissue Barrier Function. *Cell Host Microbe.* 2015;17(5):662-71.
188. Saeedi BJ, Kao DJ, Kitzenberg DA, *et al.* HIF-dependent regulation of claudin-1 is central to intestinal epithelial tight junction integrity. *Mol Biol Cell.* 2015;26(12):2252-62.
189. Peng L, He Z, Chen W, Holzman IR, Lin J. Effects of butyrate on intestinal barrier function in a Caco-2 cell monolayer model of intestinal barrier. *Pediatr Res.* 2007;61(1):37-41.
190. Chen H, Wang W, Degroote J, *et al.* Arabinoxylan in wheat is more responsible than cellulose for promoting intestinal barrier function in weaned male piglets. *J Nutr.* 2015;145(1):51-58.
191. Gaudier E, Jarry A, Blottière HM, *et al.* Butyrate specifically modulates MUC gene expression in intestinal epithelial goblet cells deprived of glucose. *Am J Physiol Gastrointest Liver Physiol.* 2004;287(6):G1168-G74.
192. Burger-van Paassen N, Vincent A, Puiman PJ, *et al.* The regulation of intestinal mucin MUC2 expression by short-chain fatty acids: implications for epithelial protection. *Biochem J.* 2009;420(2):211-19.
193. Singh N, Gurav A, Sivaprakasam S, *et al.* Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. *Immunity.* 2014;40(1):128-39.
194. Nowarski R, Jackson R, Gagliani N, *et al.* Epithelial IL-18 Equilibrium Controls Barrier Function in Colitis. *Cell.* 2015;163(6):1444-56.
195. Cipolletta D. Adipose tissue-resident regulatory T cells: phenotypic specialization, functions and therapeutic potential. *Immunology.* 2014;142(4):517-25.
196. Zeng H, Chi H. Metabolic control of regulatory T cell development and function. *Trends Immunol.* 2015;36(1):3-12.
197. Smith PM, Howitt MR, Panikov N, *et al.* The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science.* 2013;341(6145):569-73.
198. Arpaia N, Campbell C, Fan X, *et al.* Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature.* 2013;504(7480):451-55.
199. Furusawa Y, Obata Y, Fukuda S, *et al.* Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature.* 2013;504(7480):446-50.
200. Park J, Kim M, Kang SG, *et al.* Short-chain fatty acids induce both effector and regulatory T cells by suppression of histone deacetylases and regulation of the mTOR-S6K pathway. *Mucosal Immunol.* 2015;8(1):80-93.
201. Schwarz A, Bruhs A, Schwarz T. The Short-Chain Fatty Acid Sodium Butyrate Functions as a Regulator of the Skin Immune System. *J Invest Dermatol.* 2017;137(4):855-64.
202. Trompette A, Gollwitzer ES, Yadava K, *et al.* Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat Med.* 2014;20(2):159-66.

203. Chang PV, Hao L, Offermanns S, Medzhitov R. The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition. *Proc Natl Acad Sci USA.* 2014;111(6):2247-52.
204. Kamp ME, Shim R, Nicholls AJ*, et al.* G Protein-Coupled Receptor 43 Modulates Neutrophil Recruitment during Acute Inflammation. *PLoS One.* 2016;11(9):e0163750.
205. Vinolo MAR, Rodrigues HG, Hatanaka E, Sato FT, Sampaio SC, Curi R. Suppressive effect of short-chain fatty acids on production of proinflammatory mediators by neutrophils. *J Nutr Biochem.* 2011;22(9):849-55.
206. Maslowski KM, Vieira AT, Ng A*, et al.* Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature.* 2009;461(7268):1282-86.
207. Wu W, Sun M, Chen F*, et al.* Microbiota metabolite short-chain fatty acid acetate promotes intestinal IgA response to microbiota which is mediated by GPR43. *Mucosal Immunol.* 2016;10(4):946-56.
208. Kim M, Qie Y, Park J, Kim CH. Gut Microbial Metabolites Fuel Host Antibody Responses. *Cell Host Microbe.* 2016;20(2):202-14.
209. Sun J, Furio L, Mecheri R*, et al.* Pancreatic β-Cells Limit Autoimmune Diabetes via an Immunoregulatory Antimicrobial Peptide Expressed under the Influence of the Gut Microbiota. *Immunity.* 2015;43(2):304-17.
210. Cani PD, Neyrinck AM, Fava F*, et al.* Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. *Diabetologia.* 2007;50(11):2374-83.
211. He B, Nohara K, Ajami NJ*, et al.* Transmissible microbial and metabolomic remodeling by soluble dietary fiber improves metabolic homeostasis. *Sci Rep.* 2015;5:10604.
212. Priyadarshini M, Villa SR, Fuller M*, et al.* An Acetate-Specific GPCR, FFAR2, Regulates Insulin Secretion. *Mol Endocrinol.* 2015;29(7):1055-66.
213. Psichas A, Sleeth ML, Murphy KG*, et al.* The short chain fatty acid propionate stimulates GLP-1 and PYY secretion via free fatty acid receptor 2 in rodents. *Int J Obes.* 2015;39(3):424-29.
214. Brooks L, Viardot A, Tsakmaki A*, et al.* Fermentable carbohydrate stimulates FFAR2-dependent colonic PYY cell expansion to increase satiety. *Mol Metab.* 2017;6(1):48-60.
215. Natarajan N, Hori D, Flavahan S*, et al.* Microbial short chain fatty acid metabolites lower blood pressure via endothelial G-protein coupled receptor 41. *Physiol Genomics.* 2016;48(11):826-34.
216. Macia L, Tan J, Vieira AT*, et al.* Metabolite-sensing receptors GPR43 and GPR109A facilitate dietary fibre-induced gut homeostasis through regulation of the inflammasome. *Nat Commun.* 2015;6:6734.
217. Coates ME. Gnotobiotic animals in research: their uses and limitations. *Lab Anim.* 1975;9(4):275-82.
218. Belkaid Y, Hand T. Role of the Microbiota in Immunity and Inflammation. *Cell.* 2014;157(1):121–41.
219. Bäckhed F, Manchester JK, Semenkovich CF, Gordon JI. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proc Natl Acad Sci USA.* 2007;104(3):979-84.
220. Schwabe RF, Jobin C. The microbiome and cancer. *Nat Rev Cancer.* 2013;13(11):800-12.

221. Sonnenburg JL, Bäckhed F. Diet-microbiota interactions as moderators of human metabolism. *Nature.* 2016;535(7610):56-64.
222. Buyken AE, Goletzke J, Joslowski G*, et al.* Association between carbohydrate quality and inflammatory markers: systematic review of observational and interventional studies. *Am J Clin Nutr.* 2014;99(4):813-33.
223. Food and Drug Administration H. Subpart E-Specific Requirements for Health Claims. Maryland, United States. April 1, 2016.
224. EFSA Panel on Dietetic Products Nutrition and Allergies. Scientific Opinion on the substantiation of a health claim related to "native chicory inulin" and maintenance of normal defecation by increasing stool frequency pursuant to Article 13.5 of Regulation (EC) No 1924/2006. *EFSA J.* 2015;13(1):3951.
225. Lambert JE, Parnell JA, Tunnicliffe JM, Han J, Sturzenegger T, Reimer RA. Consuming yellow pea fiber reduces voluntary energy intake and body fat in overweight/obese adults in a 12-week randomized controlled trial. *Clin Nutr.* 2017;36(1):126-33.
226. Vanegas SM, Meydani M, Barnett JB*, et al.* Substituting whole grains for refined grains in a 6-wk randomized trial has a modest effect on gut microbiota and immune and inflammatory markers of healthy adults. *Am J Clin Nutr.* 2017;105(3):635-50.
227. Clarke ST, Green-Johnson JM, Brooks SPJ*, et al.* β2-1 Fructan supplementation alters host immune responses in a manner consistent with increased exposure to microbial components: results from a double-blinded, randomised, cross-over study in healthy adults. *Br J Nutr.* 2016;115(10):1748-59.
228. Collado Yurrita L, San Mauro Martin I, Ciudad-Cabanas MJ, Calle-Puron ME, Hernandez Cabria M. Effectiveness of inulin intake on indicators of chronic constipation; a meta-analysis of controlled randomized clinical trials. *Nutr Hosp.* 2014;30(2):244-52.
229. Liu F, Prabhakar M, Ju J, Long H, Zhou H-W. Effect of inulin-type fructans on blood lipid profile and glucose level: a systematic review and meta-analysis of randomized controlled trials. *Eur J Clin Nutr.* 2017;71(1):9-20.
230. Brighenti F. Dietary Fructans and Serum Triacylglycerols: A Meta-Analysis of Randomized Controlled Trials. *J Nutr.* 2007;137(11 Suppl):2552S-56S.
231. Fechner A, Kiehntopf M, Jahreis G. The formation of short-chain fatty acids is positively associated with the blood lipid-lowering effect of lupin kernel fiber in moderately hypercholesterolemic adults. *J Nutr.* 2014;144(5):599-607.
232. Freeland KR, Wolever TMS. Acute effects of intravenous and rectal acetate on glucagon-like peptide-1, peptide YY, ghrelin, adiponectin and tumour necrosis factor-alpha. *Br J Nutr.* 2010;103(3):460-66.
233. van der Beek CM, Canfora EE, Lenaerts K*, et al.* Distal, not proximal, colonic acetate infusions promote fat oxidation and improve metabolic markers in overweight/obese men. *Clin Sci.* 2016;130(22):2073-82.
234. Livingston KA, Chung M, Sawicki CM*, et al.* Development of a Publicly Available, Comprehensive Database of Fiber and Health Outcomes: Rationale and Methods. *PLoS One.* 2016;11(6):e0156961.
235. Saura-Calixto F. Dietary fiber as a carrier of dietary antioxidants: an essential physiological function. *J Agric Food Chem.* 2011;59(1):43-49.
236. Vitaglione P, Mennella I, Ferracane R*, et al.* Whole-grain wheat consumption reduces inflammation in a randomized controlled trial on overweight and obese subjects with

unhealthy dietary and lifestyle behaviors: role of polyphenols bound to cereal dietary fiber. *Am J Clin Nutr.* 2015;101(2):251-61.

237. Quiros-Sauceda A, Palafox-Carlos H, Sayago-Ayerdi S*, et al.* Dietary fiber and phenolic compounds as functional ingredients: interaction and possible effect after ingestion. *Food Funct.* 2014;5(6):1063-72.
238. Jacobs DR, Tapsell LC. Food, Not Nutrients, Is the Fundamental Unit in Nutrition. *Nutr Rev.* 2007;65(10):439-50.
239. Cardona F, Andres-Lacueva C, Tulipani S, Tinahones FJ, Queipo-Ortuno MI. Benefits of polyphenols on gut microbiota and implications in human health. *J Nutr Biochem.* 2013;24(8):1415-22.
240. Jenkins DJ, Kendall CW, Popovich DG*, et al.* Effect of a very-high-fiber vegetable, fruit, and nut diet on serum lipids and colonic function. *Metabolism.* 2001;50(4):494-503.
241. Kendall CW, Esfahani A, Jenkins DJ. The link between dietary fibre and human health. *Food Hydrocoll.* 2010;24(1):42-48.
242. Delcour JA, Aman P, Courtin CM, Hamaker BR, Verbeke K. Prebiotics, Fermentable Dietary Fiber, and Health Claims. *Adv Nutr.* 2016;7(1):1-4.
243. Martínez I, Perdicaro DJ, Brown AW*, et al.* Diet-induced alterations of host cholesterol metabolism are likely to affect the gut microbiota composition in hamsters. *Appl Environ Microbiol.* 2013;79(2):516-24.
244. Carding S, Verbeke K, Vipond DT, Corfe BM, Owen LJ. Dysbiosis of the gut microbiota in disease. *Microb Ecol Health Dis.* 2015;26:26191.
245. Bindels LB, Segura Munoz RR, Gomes-Neto JC*, et al.* Resistant starch can improve insulin sensitivity independently of the gut microbiota. *Microbiome.* 2017;5(1):12.
246. Burkitt DP, Walker ARP, Painter NS. Effect of dietary fibre on stools and the transit-times, and its role in the causation of disease. *Lancet.* 1972;2(7792):1408-12.
247. Falony G, Joossens M, Vieira-Silva S*, et al.* Population-level analysis of gut microbiome variation. *Science.* 2016;352(6285):560-64.
248. Cameron-Smith D, Collier G, O'Dea K. Effect of soluble dietary fibre on the viscosity of gastrointestinal contents and the acute glycaemic response in the rat. *Br J Nutr.* 1994;71(4):563-71.
249. Dikeman CL, Murphy MR, Fahey GCJ. Dietary fibers affect viscosity of solutions and simulated human gastric and small intestinal digesta. *J Nutr.* 2006;136(4):913-19.
250. Ferguson LR, Harris PJ. Adsorption of Carcinogens by Dietary Fiber. In: Cho SS, Dreher ML, eds. *Hand-book of Dietary Fiber*. New York: Marcel Dekker; 2001:207–18.
251. Gunness P, Gidley MJ. Mechanisms underlying the cholesterol-lowering properties of soluble dietary fibre polysaccharides. *Food Funct.* 2010;1(2):149-55.
252. Li T, Chiang JYL. Bile acid signaling in metabolic disease and drug therapy. *Pharmacol Rev.* 2014;66(4):948-83.
253. Shepherd R. Resistance to changes in diet. *Proc Nutr Soc.* 2002;61(2):267-72.
254. Ng M, Fleming T, Robinson M*, et al.* Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet.* 2014;384(9945):766-81.
255. NCD Risk Factor Collaboration (NCD-RisC). Worldwide Trends in Diabetes Since 1980: A Pooled Analysis of 751 Population-Based Studies With 4.4 Million Participants *Lancet.* 2016;387(10027):1513-30.

256. Tremmel M, Gerdtham U-G, Nilsson PM, Saha S. Economic Burden of Obesity: A Systematic Literature Review. *Int J Environ Res Public Health.* 2017;14(4):435.
257. Fock KM, Khoo J. Diet and exercise in management of obesity and overweight. *J Gastroenterol Hepatol.* 2013;28(S4):59-63.
258. Hall KD, Ayuketah A, Brychta R*, et al.* Ultra-Processed Diets Cause Excess Calorie Intake and Weight Gain: An Inpatient Randomized Controlled Trial of Ad Libitum Food Intake. *Cell Metab.* 2019;30(1):67-77.e3.
259. Hotamışlıgil GS. Foundations of Immunometabolism and Implications for Metabolic Health and Disease. *Immunity.* 2017;47(3):406-20.
260. Saltiel AR, Olefsky JM. Inflammatory mechanisms linking obesity and metabolic disease. *J Clin Invest.* 2017;127(1):1-4.
261. United States Department of Health and Human Services, United States Department of Agriculture. Dietary guidelines for Americans 2015-2020. December, 2015. Available from: https://health.gov/dietaryguidelines/2015/resources/2015-2020_Dietary_Guidelines.pdf.
262. Health Canada. Canada's Dietary Guidelines for Health Professionals and Policy Makers. January, 2019. Available from: https://food-guide.canada.ca/static/assets/pdf/CDG-EN-2018.pdf.
263. Bermudez OI, Dwyer JT, Yu W, Tolstoi LG. Dietary Guidelines around the World: An Update. In: Berdanier CD, Dwyer JT, Feldman EB, eds. *Handbook of Nutrition and Food*. 2nd ed. Boca Raton (USA): CRC Press; 2007:429-49.
264. Rose DJ. Impact of whole grains on the gut microbiota: the next frontier for oats? *Br J Nutr.* 2014;112(Suppl 2):S44-S49.
265. Abuajah CI, Ogbonna AC, Osuji CM. Functional components and medicinal properties of food: a review. *J Food Sci Technol.* 2015;52(5):2522-29.
266. Makki K, Deehan EC, Walter J, Bäckhed F. The Impact of Dietary Fiber on Gut Microbiota in Host Health and Disease. *Cell Host Microbe.* 2018;23(6):705-15.
267. Reynolds A, Mann J, Cummings J, Winter N, Mete E, Te Morenga L. Carbohydrate quality and human health: a series of systematic reviews and meta-analyses. *Lancet.* 2019;393(10170):434-45.
268. AlEssa HB, Bhupathiraju SN, Malik VS*, et al.* Carbohydrate quality and quantity and risk of type 2 diabetes in US women. *Am J Clin Nutr.* 2015;102(6):1543-53.
269. Veronese N, Solmi M, Caruso MG*, et al.* Dietary fiber and health outcomes: an umbrella review of systematic reviews and meta-analyses. *Am J Clin Nutr.* 2018;107(3):436–44.
270. Yang Y, Zhao L-G, Wu Q-J, Ma X, Xiang Y-B. Association between dietary fiber and lower risk of all-cause mortality: a meta-analysis of cohort studies. *Am J Epidemiol.* 2015;181(2):83-91.
271. Kim Y, Je Y. Dietary fiber intake and total mortality: a meta-analysis of prospective cohort studies. *Am J Epidemiol.* 2014;180(6):565-73.
272. Kaczmarczyk MM, Miller MJ, Freund GG. The health benefits of dietary fiber: beyond the usual suspects of type 2 diabetes mellitus, cardiovascular disease and colon cancer. *Metabolism.* 2012;61(8):1058-66.
273. Deehan EC, Duar RM, Armet AM, Perez-Muñoz ME, Jin M, Walter J. Modulation of the Gastrointestinal Microbiome with Nondigestible Fermentable Carbohydrates To Improve Human Health. *Microbiol Spectr.* 2017;5(5).

274. Mobley AR, Jones JM, Rodriguez J, Slavin JL, Zelman KM. Identifying practical solutions to meet America's fiber needs: proceedings from the Food & Fiber Summit. *Nutrients.* 2014;6(7):2540-51.
275. Clemens R, Kranz S, Mobley AR*, et al.* Filling America's fiber intake gap: summary of a roundtable to probe realistic solutions with a focus on grain-based foods. *J Nutr.* 2012;142(7):1390S-401S.
276. Sonnenburg ED, Sonnenburg JL. The ancestral and industrialized gut microbiota and implications for human health. *Nat Rev Microbiol.* 2019;17(6):383-90.
277. Thompson SV, Hannon BA, An R, Holscher HD. Effects of isolated soluble fiber supplementation on body weight, glycemia, and insulinemia in adults with overweight and obesity: a systematic review and meta-analysis of randomized controlled trials. *Am J Clin Nutr.* 2017;106(6):1514-28.
278. Sharma SK, Bansal S, Mangal M, Dixit AK, Gupta RK, Mangal AK. Utilization of Food Processing By-products as Dietary, Functional and Novel Fibre: A Review. *Crit Rev Food Sci Nutr.* 2016;56(10):1647-61.
279. Meijnikman AS, Gerdes VE, Nieuwdorp M, Herrema H. Evaluating Causality of Gut Microbiota in Obesity and Diabetes in Humans. *Endocr Rev.* 2018;39(2):133-53.
280. Zhao L, Zhang F, Ding X*, et al.* Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes. *Science.* 2018;359(6380):1151-56.
281. Birkett A, Muir J, Phillips J, Jones G, O'Dea K. Resistant starch lowers fecal concentrations of ammonia and phenols in humans. *Am J Clin Nutr.* 1996;63(5):766-72.
282. Boler BMV, Serao MCR, Bauer LL*, et al.* Digestive physiological outcomes related to polydextrose and soluble maize fibre consumption by healthy adult men. *Br J Nutr.* 2011;106(12):1864-71.
283. Reichardt N, Vollmer M, Holtrop G*, et al.* Specific substrate-driven changes in human faecal microbiota composition contrast with functional redundancy in short-chain fatty acid production. *ISME J.* 2018;12(2):610-22.
284. Alexander C, Swanson KS, Fahey GC, Garleb KA. Perspective: Physiologic Importance of Short-Chain Fatty Acids from Nondigestible Carbohydrate Fermentation. *Adv Nutr.* 2019;10(4):576-89.
285. Tuncil YE, Thakkar RD, Arioglu-Tuncil S, Hamaker BR, Lindemann SR. Subtle Variations in Dietary-Fiber Fine Structure Differentially Influence the Composition and Metabolic Function of Gut Microbiota. *mSphere.* 2020;5(3):e00180-20.
286. Cantu-Jungles TM, Hamaker BR. New View on Dietary Fiber Selection for Predictable Shifts in Gut Microbiota. *mBio.* 2020;11(1):e02179-19.
287. Hamaker BR, Cantu-Jungles TM. Discrete Fiber Structures Dictate Human Gut Bacteria Outcomes. *Trends Endocrinol Metab.* 2020;epub ahead of print.
288. Coffin CS, Shaffer EA. The hot air and cold facts of dietary fibre. *Can J Gastroenterol.* 2006;20(4):255-56.
289. Papas MA, Giovannucci E, Platz EA. Fiber from Fruit and Colorectal Neoplasia. *Cancer Epidemiol Biomarkers Prev.* 2004;13(8):1267-70.
290. Dwyer JT, Ausman LM. Fiber: unanswered questions. *J Natl Cancer Inst.* 1992;84(24):1851-53.
291. Fardet A. New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre? *Nutr Res Rev.* 2010;23(1):65-134.

292. Mozaffarian D, Rosenberg I, Uauy R. History of modern nutrition science-implications for current research, dietary guidelines, and food policy. *BMJ.* 2018;361:k2392.
293. Aberg S, Mann J, Neumann S, Ross AB, Reynolds AN. Whole-Grain Processing and Glycemic Control in Type 2 Diabetes: A Randomized Crossover Trial. *Diabetes Care.* 2020.
294. Jenkins DJ, Wesson V, Wolever TM*, et al.* Wholemeal versus wholegrain breads: proportion of whole or cracked grain and the glycaemic response. *BMJ.* 1988;297(6654):958-60.
295. Kohn JB. Is Dietary Fiber Considered an Essential Nutrient? *J Acad Nutr Diet.* 2016;116(2):360.
296. Raninen K, Lappi J, Mykkanen H, Poutanen K. Dietary fiber type reflects physiological functionality: comparison of grain fiber, inulin, and polydextrose. *Nutr Rev.* 2011;69(1):9-21.
297. Jones JM. Dietary fibre's co-passengers: is it the fibre or the co-passengers? In: van der Kamp JW, Jones JM, McCleary BV, Topping DL, eds. *Dietary fibre: new frontiers for food and health*. Wageningen, Netherlands: Wageningen Academic Publishers; 2010:365-78.
298. Poutanen K. Effect of Processing on the Properties of Dietary Fibre. In: McCleary BV, Prosky L, eds. *Advanced Dietary Fibre Technology*. Oxford, UK: Blackwell Science Ltd.; 2001:277-82.
299. Grundy MM-L, Fardet A, Tosh SM, Rich GT, Wilde PJ. Processing of oat: the impact on oat's cholesterol lowering effect. *Food Funct.* 2018;9(3):1328-43.
300. Tannock GW, Liu Y. Guided dietary fibre intake as a means of directing short-chain fatty acid production by the gut microbiota. *J Roy Soc New Zeal.* 2019.
301. O'Grady J, O'Connor EM, Shanahan F. Review article: dietary fibre in the era of microbiome science. *Aliment Pharmacol Ther.* 2019;49(5):506-15.
302. O'Keefe SJ. The association between dietary fibre deficiency and high-income lifestyle-associated diseases: Burkitt's hypothesis revisited. *Lancet Gastroenterol Hepatol.* 2019;4(12):984-96.
303. O'Keefe SJD. The Need to Reassess Dietary Fiber Requirements in Healthy and Critically Ill Patients. *Gastroenterol Clin North Am.* 2018;47(1):219-29.
304. Ludwig DS, Hu FB, Tappy L, Brand-Miller J. Dietary carbohydrates: role of quality and quantity in chronic disease. *BMJ.* 2018;361:k2340.
305. Chen J-P, Chen G-C, Wang X-P, Qin L, Bai Y. Dietary Fiber and Metabolic Syndrome: A Meta-Analysis and Review of Related Mechanisms. *Nutrients.* 2018;10(1):24.
306. Wei B, Liu Y, Lin X, Fang Y, Cui J, Wan J. Dietary fiber intake and risk of metabolic syndrome: A meta-analysis of observational studies. *Clin Nutr.* 2018;37(6 Pt A):1935-42.
307. InterAct Consortium. Dietary fibre and incidence of type 2 diabetes in eight European countries: the EPIC-InterAct Study and a meta-analysis of prospective studies. *Diabetologia.* 2015;58(7):1394-408.
308. Pereira MA, O'Reilly E, Augustsson K*, et al.* Dietary fiber and risk of coronary heart disease: a pooled analysis of cohort studies. *Arch Intern Med.* 2004;164(4):370-76.
309. McRorie JWJ, McKeown NM. Understanding the Physics of Functional Fibers in the Gastrointestinal Tract: An Evidence-Based Approach to Resolving Enduring Misconceptions about Insoluble and Soluble Fiber. *J Acad Nutr Diet.* 2017;117(2):251-64.

310. Moher D, Shamseer L, Clarke M, *et al.* Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev.* 2015;4:1.
311. Higgins JP, Green S, (editors). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. *The Cochrane Collaboration.* 2011: Available from: www.handbook.cochrane.org.
312. de Craen AJ, Kaptchuk TJ, Tijssen JG, Kleijnen J. Placebos and placebo effects in medicine: historical overview. *J R Soc Med.* 1999;92(10):511-5.
313. Staudacher HM, Irving PM, Lomer MCE, Whelan K. The challenges of control groups, placebos and blinding in clinical trials of dietary interventions. *Proc Nutr Soc.* 2017;76(3):203-12.
314. Jenkins DJ, Kendall CW, Augustin LS, *et al.* Glycemic index: overview of implications in health and disease. *Am J Clin Nutr.* 2002;76(1):266S-73S.
315. Ludwig DS. The glycemic index: physiological mechanisms relating to obesity, diabetes, and cardiovascular disease. *JAMA.* 2002;287(18):2414-23.
316. Sugawara M, Suzuki T, Totsuka A, Takeuchi M, Ueki K. Composition of Corn Hull Dietary Fiber. *Starch/Stärke.* 1994;46(9):335-37.
317. Goel V, Cheema SK, Agellon LB, Ooraikul B, McBurney MI, Basu TK. *In vitro* binding of bile salt to rhubarb stalk powder. *Nutr Res.* 1998;18(5):893-903.
318. Office of Nutrition and Food Labeling. The Declaration of Certain Isolated or Synthetic Non-Digestible Carbohydrates as Dietary Fiber on Nutrition and Supplement Facts Labels: Guidance for Industry. Maryland, United States: Center for Food Safety and Applied Nutrition, Food and Drug Administration. June, 2018. Docket No: FDA-2018-D-1323.
319. Poutanen KS, Fiszman S, Marsaux CFM, Pentikainen SP, Steinert RE, Mela DJ. Recommendations for characterization and reporting of dietary fibers in nutrition research. *Am J Clin Nutr.* 2018;108(3):437-44.
320. Suez J, Korem T, Zeevi D, *et al.* Artificial sweeteners induce glucose intolerance by altering the gut microbiota. *Nature.* 2014;514(7521):181-86.
321. Ruiz-Ojeda FJ, Plaza-Díaz J, Sáez-Lara MJ, Gil A. Effects of Sweeteners on the Gut Microbiota: A Review of Experimental Studies and Clinical Trials. *Adv Nutr.* 2019;10(suppl_1):S31-S48.
322. Heijnen M-L, van Amelsvoort JM, Deurenberg P, Beynen AC. Neither raw nor retrograded resistant starch lowers fasting serum cholesterol concentrations in healthy normolipidemic subjects. *Am J Clin Nutr.* 1996;64(3):312-18.
323. Landin K, Holm G, Tengborn L, Smith U. Guar gum improves insulin sensitivity, blood lipids, blood pressure, and fibrinolysis in healthy men. *Am J Clin Nutr.* 1992;56(6):1061-65.
324. Effertz M, Denman P, Slavin JL. The effect of soy polysaccharide on body weight, serum lipids, blood glucose, and fecal parameters in moderately obese adults. *Nutr Res.* 1991;11(8):849-59.
325. Pasman WJ, Wils D, Saniez M-HH, Kardinaal AF. Long-term gastrointestinal tolerance of NUTRIOSE® FB in healthy men. *Eur J Clin Nutr.* 2006;60(8):1024-34.
326. Gower BA, Bergman R, Stefanovski D, *et al.* Baseline insulin sensitivity affects response to high-amylose maize resistant starch in women: a randomized, controlled trial. *Nutr Metab.* 2016;13:2.

327. Morel FB, Dai Q, Ni J, Thomas D, Parnet P, Fanca-Berthon P. α-Galacto-oligosaccharides Dose-Dependently Reduce Appetite and Decrease Inflammation in Overweight Adults. *J Nutr.* 2015;145(9):2052-59.
328. Robertson MD, Wright JW, Loizon E*, et al.* Insulin-sensitizing effects on muscle and adipose tissue after dietary fiber intake in men and women with metabolic syndrome. *J Clin Endocrinol Metab.* 2012;97(9):3326-32.
329. Cherbut C, Aube A, Mekki N, Dubois C, Lairon D, Barry J. Digestive and metabolic effects of potato and maize fibres in human subjects. *Br J Nutr.* 1997;77(1):33-46.
330. Robertson MD, Bickerton AS, Dennis AL, Vidal H, Frayn KN. Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism. *Am J Clin Nutr.* 2005;82(3):559-67.
331. Bodinham CL, Smith L, Wright J, Frost GS, Robertson MD. Dietary fibre improves first-phase insulin secretion in overweight individuals. *PLoS One.* 2012;7(7):e40834.
332. Braaten J, Wood P, Scott F*, et al.* Oat β-glucan reduces blood cholesterol concentration in hypercholesterolemic subjects. *Eur J Clin Nutr.* 1994;48(7):465-74.
333. Arvill A, Bodin L. Effect of short-term ingestion of konjac glucomannan on serum cholesterol in healthy men. *Am J Clin Nutr.* 1995;61(3):585-89.
334. Kohl A, Gogebakan O, Möhlig M*, et al.* Increased interleukin-10 but unchanged insulin sensitivity after 4 weeks of (1, 3)(1, 6)-β-glycan consumption in overweight humans. *Nutr Res.* 2009;29(4):248-54.
335. Cloetens L, Broekaert WF, Delaedt Y*, et al.* Tolerance of arabinoxylan-oligosaccharides and their prebiotic activity in healthy subjects: a randomised, placebo-controlled cross-over study. *Br J Nutr.* 2010;103(5):703-13.
336. Solà R, Bruckert E, Valls R-M*, et al.* Soluble fibre (*Plantago ovata* husk) reduces plasma low-density lipoprotein (LDL) cholesterol, triglycerides, insulin, oxidised LDL and systolic blood pressure in hypercholesterolaemic patients: A randomised trial. *Atherosclerosis.* 2010;211(2):630-37.
337. Russo F, Riezzo G, Chiloiro M*, et al.* Metabolic effects of a diet with inulin-enriched pasta in healthy young volunteers. *Curr Pharm Des.* 2010;16(7):825-31.
338. Pouteau E, Ferchaud-Roucher V, Zair Y*, et al.* Acetogenic fibers reduce fasting glucose turnover but not peripheral insulin resistance in metabolic syndrome patients. *Clin Nutr.* 2010;29(6):801-07.
339. Reimer RA, Pelletier X, Carabin IG*, et al.* Increased plasma PYY levels following supplementation with the functional fiber PolyGlycopleX in healthy adults. *Eur J Clin Nutr.* 2010;64(10):1186-91.
340. Li S, Guerin-Deremaux L, Pochat M, Wils D, Reifer C, Miller LE. NUTRIOSE dietary fiber supplementation improves insulin resistance and determinants of metabolic syndrome in overweight men: a double-blind, randomized, placebo-controlled study. *Appl Physiol Nutr Metab.* 2010;35(6):773-82.
341. Ruiz-Roso B, Quintela JC, de la Fuente E, Haya J, Pérez-Olleros L. Insoluble carob fiber rich in polyphenols lowers total and LDL cholesterol in hypercholesterolemic sujects. *Plant Foods Hum Nutr.* 2010;65(1):50-56.
342. Pal S, Khossousi A, Binns C, Dhaliwal S, Ellis V. The effect of a fibre supplement compared to a healthy diet on body composition, lipids, glucose, insulin and other metabolic syndrome risk factors in overweight and obese individuals. *Br J Nutr.* 2011;105(1):90-100.

343. Hashizume C, Kishimoto Y, Kanahori S, Yamamoto T, Okuma K, Yamamoto K. Improvement effect of resistant maltodextrin in humans with metabolic syndrome by continuous administration. *J Nutr Sci Vitaminol.* 2012;58(6):423-30.
344. Jackson K, Taylor G, Clohessy A, Williams C. The effect of the daily intake of inulin on fasting lipid, insulin and glucose concentrations in middle-aged men and women. *Br J Nutr.* 1999;82(1):23-30.
345. de Luis DA, de la Fuente B, Izaola O, Aller R, Gutiérrez S, Morillo M. Double blind randomized clinical trial controlled by placebo with a FOS enriched cookie on saciety and cardiovascular risk factors in obese patients. *Nutr Hosp.* 2013;28(1):78-85.
346. Reimer RA, Yamaguchi H, Eller LK*, et al.* Changes in visceral adiposity and serum cholesterol with a novel viscous polysaccharide in Japanese adults with abdominal obesity. *Obesity.* 2013;21(9):E379-E87.
347. Childs CE, Roytio H, Alhoniemi E*, et al.* Xylo-oligosaccharides alone or in synbiotic combination with *Bifidobacterium animalis* subsp. *lactis* induce bifidogenesis and modulate markers of immune function in healthy adults: a double-blind, placebo-controlled, randomised, factorial cross-over study. *Br J Nutr.* 2014;111(11):1945-56.
348. Tripkovic L, Muirhead N, Hart K, Frost GS, Lodge J. The effects of a diet rich in inulin or wheat fibre on markers of cardiovascular disease in overweight male subjects. *J Hum Nutr Diet.* 2015;28(5):476-85.
349. Brahe LK, Le Chatelier E, Prifti E*, et al.* Dietary modulation of the gut microbiota--a randomised controlled trial in obese postmenopausal women. *Br J Nutr.* 2015;114(3):406-17.
350. Stenman LK, Lehtinen MJ, Meland N*, et al.* Probiotic With or Without Fiber Controls Body Fat Mass, Associated With Serum Zonulin, in Overweight and Obese Adults-Randomized Controlled Trial. *EBioMedicine.* 2016;13:190-200.
351. Dainty SA, Klingel SL, Pilkey SE*, et al.* Resistant Starch Bagels Reduce Fasting and Postprandial Insulin in Adults at Risk of Type 2 Diabetes. *J Nutr.* 2016;146(11):2252-59.
352. Guess ND, Dornhorst A, Oliver N, Frost GS. A Randomised Crossover Trial: The Effect of Inulin on Glucose Homeostasis in Subtypes of Prediabetes. *Ann Nutr Metab.* 2016;68(1):26-34.
353. Canfora EE, van der Beek CM, Hermes GDA*, et al.* Supplementation of Diet With Galacto-oligosaccharides Increases *Bifidobacteria*, but Not Insulin Sensitivity, in Obese Prediabetic Individuals. *Gastroenterology.* 2017;153(1):87-97.e3.
354. Zunft H, Luder W, Harde A*, et al.* Carob pulp preparation rich in insoluble fibre lowers total and LDL cholesterol in hypercholesterolemic patients. *Eur J Nutr.* 2003;42(5):235-42.
355. Parnell JA, Klancic T, Reimer RA. Oligofructose decreases serum lipopolysaccharide and plasminogen activator inhibitor-1 in adults with overweight/obesity. *Obesity.* 2017;25(3):510-13.
356. Alfa MJ, Strang D, Tappia PS*, et al.* A Randomized Placebo Controlled Clinical Trial to Determine the Impact of Digestion Resistant Starch *MSPrebiotic*® on Glucose, Insulin, and Insulin Resistance in Elderly and Mid-Age Adults. *Front Med.* 2018;4:260.
357. Krumbeck JA, Rasmussen HE, Hutkins RW*, et al.* Probiotic *Bifidobacterium* strains and galactooligosaccharides improve intestinal barrier function in obese adults but show no synergism when used together as synbiotics. *Microbiome.* 2018;6(1):121.

358. Peterson CM, Beyl RA, Marlatt KL, *et al.* Effect of 12 wk of resistant starch supplementation on cardiometabolic risk factors in adults with prediabetes: a randomized controlled trial. *Am J Clin Nutr.* 2018;108(3):492-501.
359. Lampe J, Slavin JL, Baglien K, Thompson W, Duane W, Zavoral J. Serum lipid and fecal bile acid changes with cereal, vegetable, and sugar-beet fiber feeding. *Am J Clin Nutr.* 1991;53(5):1235-41.
360. Marett R, Slavin JL. No long-term benefits of supplementation with arabinogalactan on serum lipids and glucose. *J Am Diet Assoc.* 2004;104(4):636-39.
361. King DE, Mainous AGr, Egan BM, Woolson RF, Geesey ME. Effect of psyllium fiber supplementation on C-reactive protein: the trial to reduce inflammatory markers (TRIM). *Ann Fam Med.* 2008;6(2):100-06.
362. Maki KC, Carson ML, Kerr Anderson WH, *et al.* Lipid-altering effects of different formulations of hydroxypropylmethylcellulose. *J Clin Lipidol.* 2009;3(3):159-66.
363. Reppas C, Swidan S, Tobey S, Turowski M, Dressman J. Hydroxypropylmethylcellulose significantly lowers blood cholesterol in mildly hypercholesterolemic human subjects. *Eur J Clin Nutr.* 2009;63(1):71-77.
364. Giacco R, Clemente G, Luongo D, *et al.* Effects of short-chain fructo-oligosaccharides on glucose and lipid metabolism in mild hypercholesterolaemic individuals. *Clin Nutr.* 2004;23(3):331-40.
365. Maki KC, Pelkman CL, Finocchiaro ET, *et al.* Resistant starch from high-amylose maize increases insulin sensitivity in overweight and obese men. *J Nutr.* 2012;142(4):717-23.
366. François IEJA, Lescroart O, Veraverbeke WS, *et al.* Effects of a wheat bran extract containing arabinoxylan oligosaccharides on gastrointestinal health parameters in healthy adult human volunteers: a double-blind, randomised, placebo-controlled, cross-over trial. *Br J Nutr.* 2012;108(12):2229-42.
367. Gato N, Kadowaki A, Hashimoto N, Yokoyama S-i, Matsumoto K. Persimmon fruit tannin-rich fiber reduces cholesterol levels in humans. *Ann Nutr Metab.* 2013;62(1):1-6.
368. Savastano DM, Hodge RJ, Nunez DJ, Walker A, Kapikian R. Effect of two dietary fibers on satiety and glycemic parameters: a randomized, double-blind, placebo-controlled, exploratory study. *Nutr J.* 2014;13:45.
369. Pal S, Ho S, Gahler RJ, Wood S. Effect on Insulin, Glucose and Lipids in Overweight/Obese Australian Adults of 12 Months Consumption of Two Different Fibre Supplements in a Randomised Trial. *Nutrients.* 2017;9(2):91.
370. Salden BN, Troost FJ, Wilms E, *et al.* Reinforcement of intestinal epithelial barrier by arabinoxylans in overweight and obese subjects: A randomized controlled trial: Arabinoxylans in gut barrier. *Clin Nutr.* 2018;37(2):471-80.
371. Lyon M, Wood S, Pelletier X, Donazzolo Y, Gahler R, Bellisle F. Effects of a 3-month supplementation with a novel soluble highly viscous polysaccharide on anthropometry and blood lipids in nondieting overweight or obese adults. *J Hum Nutr Diet.* 2011;24(4):351-59.
372. Keogh GF, Cooper GJS, Mulvey TB, *et al.* Randomized controlled crossover study of the effect of a highly β-glucan-enriched barley on cardiovascular disease risk factors in mildly hypercholesterolemic men. *Am J Clin Nutr.* 2003;78(4):711-18.
373. Sandström B, Hansen LT, Sørensen A. Pea fiber lowers fasting and postprandial blood triglyceride concentrations in humans. *J Nutr.* 1994;124(12):2386-96.

374. Park OJ, Kang NE, Chang MJ, Kim WK. Resistant starch supplementation influences blood lipid concentrations and glucose control in overweight subjects. *J Nutr Sci Vitaminol.* 2004;50(2):93-99.
375. Pérez-Jiménez J, Serrano J, Tabernero M*, et al.* Effects of grape antioxidant dietary fiber in cardiovascular disease risk factors. *Nutrition.* 2008;24(7-8):646-53.
376. Urquiaga I, D'Acuña S, Pérez D*, et al.* Wine grape pomace flour improves blood pressure, fasting glucose and protein damage in humans: a randomized controlled trial. *Biol Res.* 2015;48:49.
377. Martínez-Maqueda D, Zapatera B, Gallego-Narbón A, Vaquero MP, Saura-Calixto F, Pérez-Jiménez J. A 6-week supplementation with grape pomace to subjects at cardiometabolic risk ameliorates insulin sensitivity, without affecting other metabolic syndrome markers. *Food Funct.* 2018;9(11):6010-19.
378. Jensen C, Spiller G, Gates J, Miller A, Whittam J. The effect of acacia gum and a water-soluble dietary fiber mixture on blood lipids in humans. *J Am Coll Nutr.* 1993;12(2):147-54.
379. Hall R, Johnson S, Baxter A, Ball M. Lupin kernel fibre-enriched foods beneficially modify serum lipids in men. *Eur J Clin Nutr.* 2005;59(3):325-33.
380. Yoshida M, Vanstone C, Parsons W, Zawistowski J, Jones P. Effect of plant sterols and glucomannan on lipids in individuals with and without type II diabetes. *Eur J Clin Nutr.* 2006;60(4):529-37.
381. Garcia AL, Steiniger J, Reich S-C*, et al.* Arabinoxylan fibre consumption improved glucose metabolism, but did not affect serum adipokines in subjects with impaired glucose tolerance. *Horm Metab Res.* 2006;38(11):761-66.
382. Queenan KM, Stewart ML, Smith KN, Thomas W, Fulcher RG, Slavin JL. Concentrated oat β-glucan, a fermentable fiber, lowers serum cholesterol in hypercholesterolemic adults in a randomized controlled trial. *Nutr J.* 2007;6:6.
383. Brighenti F, Casiraghi MC, Canzi E, Ferrari A. Effect of consumption of a ready-to-eat breakfast cereal containing inulin on the intestinal milieu and blood lipids in healthy male volunteers. *Eur J Clin Nutr.* 1999;53(9):726-33.
384. Urquiaga I, Troncoso D, Mackenna MJ*, et al.* The Consumption of Beef Burgers Prepared with Wine Grape Pomace Flour Improves Fasting Glucose, Plasma Antioxidant Levels, and Oxidative Damage Markers in Humans: A Controlled Trial. *Nutrients.* 2018;10(10).
385. Kapoor MP, Ishihara N, Okubo T. Soluble dietary fibre partially hydrolysed guar gum markedly impacts on postprandial hyperglycaemia, hyperlipidaemia and incretins metabolic hormones over time in healthy and glucose intolerant subjects. *J Funct Foods.* 2016;24:207-20.
386. Gallaher DD, Gallaher CM, Mahrt GJ*, et al.* A glucomannan and chitosan fiber supplement decreases plasma cholesterol and increases cholesterol excretion in overweight normocholesterolemic humans. *J Am Coll Nutr.* 2002;21(5):428-33.
387. Ganji V, Kuo J. Serum lipid responses to psyllium fiber: differences between pre- and post-menopausal, hypercholesterolemic women. *Nutr J.* 2008;7:22.
388. Vidal-Quintanar RL, Mendívil RL, Peña M, Fernandez ML. Lime-treated maize husks lower plasma LDL-cholesterol levels in normal and hypercholesterolaemic adult men from northern Mexico. *Br J Nutr.* 1999;81(4):281-88.

389. Hanai H, Ikuma M, Sato Y, *et al.* Long-term effects of water-soluble corn bran hemicellulose on glucose tolerance in obese and non-obese patients: improved insulin sensitivity and glucose metabolism in obese subjects. *Biosci Biotechnol Biochem.* 1997;61(8):1358-61.
390. Pedersen C, Lefevre S, Peters V, *et al.* Gut hormone release and appetite regulation in healthy non-obese participants following oligofructose intake. A dose-escalation study. *Appetite.* 2013;66:44-53.
391. Glover DA, Ushida K, Phillips AO, Riley SG. Acacia(sen) SUPERGUM™ (Gum arabic): An evaluation of potential health benefits in human subjects. *Food Hydrocoll.* 2009;23(8):2410-15.
392. Kawatra A, Kapoor A, Seghal S. Hypocholesterolemic effect of guargum in overweight adults. *Plant Foods Hum Nutr.* 1991;41(3):241-45.
393. Nasir O, Babiker S, Salim A-MM. Protective effect of gum arabic supplementation for type 2 diabetes mellitus and its complications. *Int J Multidiscip Curr Res.* 2016;4:288-94.
394. Sundell I, Rånby M. Oat husk fiber decreases plasminogen activator inhibitor type 1 activity. *Haemostasis.* 1993;23(1):45-50.
395. Zunft H, Luder W, Harde A, Haber B, Graubaum H, Gruenwald J. Carob pulp preparation for treatment of hypercholesterolemia. *Adv Ther.* 2001;18(5):230-36.
396. Cossack Z, Musaiger AO. Effect on lipid metabolism of beet fibre in desert nomads with low habitual fibre intake. *Eur J Clin Nutr.* 1991;45(2):105-10.
397. Goel V, Ooraikul B, Basu T. Cholesterol lowering effects of rhubarb stalk fiber in hypercholesterolemic men. *J Am Coll Nutr.* 1997;16(6):600-04.
398. Nicolosi R, Bell S, Bistrian B, Greenberg I, Forse R, Blackburn G. Plasma lipid changes after supplementation with β-glucan fiber from yeast. *Am J Clin Nutr.* 1999;70(2):208-12.
399. Robinson RR, Feirtag J, Slavin JL. Effects of dietary arabinogalactan on gastrointestinal and blood parameters in healthy human subjects. *J Am Coll Nutr.* 2001;20(4):279-85.
400. Smith KN, M QK, Thomas W, Fulcher RG, Slavin JL. Physiological effects of concentrated barley β-glucan in mildly hypercholesterolemic adults. *J Am Coll Nutr.* 2008;27(3):434-40.
401. Liu F, Li P, Chen M, *et al.* Fructooligosaccharide (FOS) and Galactooligosaccharide (GOS) Increase *Bifidobacterium* but Reduce Butyrate Producing Bacteria with Adverse Glycemic Metabolism in healthy young population. *Sci Rep.* 2017;7(1):11789.
402. Worthley DL, Le Leu RK, Whitehall VL, *et al.* A human, double-blind, placebo-controlled, crossover trial of prebiotic, probiotic, and synbiotic supplementation: effects on luminal, inflammatory, epigenetic, and epithelial biomarkers of colorectal cancer. *Am J Clin Nutr.* 2009;90(3):578-86.
403. King DE, Egan BM, Woolson RF, Mainous AG, Al-Solaiman Y, Jesri A. Effect of a high-fiber diet vs a fiber-supplemented diet on C-reactive protein level. *Arch Intern Med.* 2007;167(5):502-06.
404. Spiller G, Farquhar J, Gates J, Nichols S. Guar gum and plasma cholesterol. Effect of guar gum and an oat fiber source on plasma lipoproteins and cholesterol in hypercholesterolemic adults. *Arterioscler Thromb.* 1991;11(5):1204-08.
405. Haskell WL, Spiller GA, Jensen CD, Ellis BK, Gates JE. Role of water-soluble dietary fiber in the management of elevated plasma cholesterol in healthy subjects. *Am J Cardiol.* 1992;69(5):433-39.

406. Blackwood A, Salter J, Dettmar P, Chaplin M. Dietary fibre, physicochemical properties and their relationship to health. *J R Soc Promot Health.* 2000;120(4):242-47.
407. Hartley L, May MD, Loveman E, Colquitt JL, Rees K. Dietary fibre for the primary prevention of cardiovascular disease. *Cochrane Database Syst Rev.* 2016(1):CD011472.
408. Onakpoya IJ, Heneghan CJ. Effect of the novel functional fibre, polyglycoplex (PGX), on body weight and metabolic parameters: A systematic review of randomized clinical trials. *Clin Nutr.* 2015;34(6):1109-14.
409. Ho HV, Sievenpiper JL, Zurbau A*, et al.* The effect of oat β-glucan on LDL-cholesterol, non-HDL-cholesterol and apoB for CVD risk reduction: a systematic review and meta-analysis of randomised-controlled trials. *Br J Nutr.* 2016;116(8):1369-82.
410. Ho HVT, Jovanovski E, Zurbau A*, et al.* A systematic review and meta-analysis of randomized controlled trials of the effect of konjac glucomannan, a viscous soluble fiber, on LDL cholesterol and the new lipid targets non-HDL cholesterol and apolipoprotein B. *Am J Clin Nutr.* 2017;105(5):1239-47.
411. Jovanovski E, Yashpal S, Komishon A*, et al.* Effect of psyllium (*Plantago ovata*) fiber on LDL cholesterol and alternative lipid targets, non-HDL cholesterol and apolipoprotein B: a systematic review and meta-analysis of randomized controlled trials. *Am J Clin Nutr.* 2018;108(5):922-32.
412. Ho HV, Sievenpiper JL, Zurbau A*, et al.* A systematic review and meta-analysis of randomized controlled trials of the effect of barley β-glucan on LDL-C, non-HDL-C and apoB for cardiovascular disease risk reduction. *Eur J Clin Nutr.* 2016;70(11):1239-45.
413. Jovanovski E, Khayyat R, Zurbau A*, et al.* Should Viscous Fiber Supplements Be Considered in Diabetes Control? Results From a Systematic Review and Meta-analysis of Randomized Controlled Trials. *Diabetes Care.* 2019;42(5):755-66.
414. Smith CE, Tucker KL. Health benefits of cereal fibre: a review of clinical trials. *Nutr Res Rev.* 2011;24(1):118-31.
415. Bourquin LD, Titgemeyer EC, Garleb KA, Fahey GCJ. Short-chain fatty acid production and fiber degradation by human colonic bacteria: effects of substrate and cell wall fractionation procedures. *J Nutr.* 1992;122(7):1508-20.
416. van Dam R, Seidell J. Carbohydrate intake and obesity. *Eur J Clin Nutr.* 2007;61 Suppl 1:S75-S99.
417. Johansson-Persson A, Ulmius M, Cloetens L, Karhu T, Herzig K-H, Önning G. A high intake of dietary fiber influences C-reactive protein and fibrinogen, but not glucose and lipid metabolism, in mildly hypercholesterolemic subjects. *Eur J Nutr.* 2014;53(1):39-48.
418. National Research Council. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients). Washington, D.C.: The National Academies Press; 2005.
419. Chou S-Y, Chien P-J, Chau C-F. Particle size reduction effectively enhances the cholesterol-lowering activities of carrot insoluble fiber and cellulose. *J Agric Food Chem.* 2008;56(22):10994-98.
420. Chau C-F, Huang Y-L. Effects of the insoluble fiber derived from *Passiflora edulis* seed on plasma and hepatic lipids and fecal output. *Mol Nutr Food Res.* 2005;49(8):786-90.
421. Hamauzu Y, Mizuno Y. Non-extractable procyanidins and lignin are important factors in the bile acid binding and radical scavenging properties of cell wall material in some fruits. *Plant Foods Hum Nutr.* 2011;66(1):70-77.

422. Ngamukote S, Makynen K, Thilawech T, Adisakwattana S. Cholesterol-lowering activity of the major polyphenols in grape seed. *Molecules.* 2011;16(6):5054-61.
423. Ellegård L, Andersson H. Oat bran rapidly increases bile acid excretion and bile acid synthesis: an ileostomy study. *Eur J Clin Nutr.* 2007;61(8):938-45.
424. Roager HM, Vogt JK, Kristensen M*, et al.* Whole grain-rich diet reduces body weight and systemic low-grade inflammation without inducing major changes of the gut microbiome: a randomised cross-over trial. *Gut.* 2019;68(1):83-93.
425. Hajihashemi P, Azadbakht L, Hashemipor M, Kelishadi R, Esmaillzadeh A. Whole-grain intake favorably affects markers of systemic inflammation in obese children: a randomized controlled crossover clinical trial. *Mol Nutr Food Res.* 2014;58(6):1301-08.
426. Nicolucci AC, Hume MP, Martinez I, Mayengbam S, Walter J, Reimer RA. Prebiotic Reduces Body Fat and Alters Intestinal Microbiota in Children With Overweight or Obesity. *Gastroenterology.* 2017;153:711–22.
427. Capuano E. The behavior of dietary fiber in the gastrointestinal tract determines its physiological effect. *Crit Rev Food Sci Nutr.* 2017;57(16):3543-64.
428. Gemen R, de Vries JF, Slavin JL. Relationship between molecular structure of cereal dietary fiber and health effects: focus on glucose/insulin response and gut health. *Nutr Rev.* 2011;69(1):22-33.
429. Kim Y, Je Y. Dietary fibre intake and mortality from cardiovascular disease and all cancers: A meta-analysis of prospective cohort studies. *Arch Cardiovasc Dis.* 2016;109(1):39-54.
430. Ze X, Duncan SH, Louis P, Flint HJ. *Ruminococcus bromii* is a keystone species for the degradation of resistant starch in the human colon. *ISME J.* 2012;6(8):1535-43.
431. Leach JD. Evolutionary perspective on dietary intake of fibre and colorectal cancer. *Eur J Clin Nutr.* 2007;61(1):140-42.
432. Freeland KR, Wilson C, Wolever TMS. Adaptation of colonic fermentation and glucagon-like peptide-1 secretion with increased wheat fibre intake for 1 year in hyperinsulinaemic human subjects. *Br J Nutr.* 2010;103(1):82-90.
433. Harvard University. The Healthy Eating Plate. 2011. Available at: Available from: https://www.hsph.harvard.edu/nutritionsource/healthy-eating-plate/.
434. Willett WC, Skerrett PJ. Eat, drink, and be healthy: the Harvard Medical School guide to healthy eating. New York, New York: Free Press; 2017.
435. Broekaert WF, Courtin CM, Verbeke K, Van de Wiele T, Verstraete W, Delcour JA. Prebiotic and other health-related effects of cereal-derived arabinoxylans, arabinoxylan-oligosaccharides, and xylooligosaccharides. *Crit Rev Food Sci Nutr.* 2011;51(2):178-94.
436. Rimm EB, Ascherio A, Giovannucci E, Spiegelman D, Stampfer MJ, Willett WC. Vegetable, fruit, and cereal fiber intake and risk of coronary heart disease among men. *JAMA.* 1996;275(6):447-51.
437. Armet AM, Deehan EC, Thöne JV, Hewko SJ, Walter J. The Effect of Isolated and Synthetic Dietary Fibers on Markers of Metabolic Diseases in Human Intervention Studies: A Systematic Review. *Adv Nutr.* 2020;11(2):420–38.
438. Healey GR, Murphy R, Brough L, Butts CA, Coad J. Interindividual variability in gut microbiota and host response to dietary interventions. *Nutr Rev.* 2017;75(12):1059-80.
439. Deehan EC, Yang C, Perez-Muñoz ME*, et al.* Precision Microbiome Modulation with Discrete Dietary Fiber Structures Directs Short-Chain Fatty Acid Production. *Cell Host Microbe.* 2020;27(3):389-404.e6.

440. Kjølbæk L, Benítez-Páez A, Gómez Del Pulgar EM*, et al.* Arabinoxylan oligosaccharides and polyunsaturated fatty acid effects on gut microbiota and metabolic markers in overweight individuals with signs of metabolic syndrome: A randomized cross-over trial. *Clin Nutr.* 2020;39(1):67-79.
441. Flint HJ, Duncan SH, Louis P. The impact of nutrition on intestinal bacterial communities. *Curr Opin Microbiol.* 2017;38:59-65.
442. Cockburn DW, Koropatkin NM. Polysaccharide Degradation by the Intestinal Microbiota and Its Influence on Human Health and Disease. *J Mol Biol.* 2016;428(16):3230-52.
443. De Filippis F, Pasolli E, Tett A*, et al.* Distinct Genetic and Functional Traits of Human Intestinal *Prevotella copri* Strains Are Associated with Different Habitual Diets. *Cell Host Microbe.* 2019;25(3):444-53.e3.
444. ClinicalTrials.gov. National Library of Medicine (US). Identifier NCT02322112, The Alberta FYBER (Feed Your gut Bacteria morE fibeR) Study. 2015, July 3. Available at: Retrieved April 25, 2020 from: https://clinicaltrials.gov/ct2/history/NCT02322112?V_2=View#StudyPageTop.
445. Tuncil YE, Nakatsu CH, Kazem AE*, et al.* Delayed utilization of some fast-fermenting soluble dietary fibers by human gut microbiota when presented in a mixture. *J Funct Foods.* 2017;32:347-57.
446. Rose DJ, Patterson JA, Hamaker BR. Structural differences among alkali-soluble arabinoxylans from maize (*Zea mays*), rice (*Oryza sativa*), and wheat (*Triticum aestivum*) brans influence human fecal fermentation profiles. *J Agric Food Chem.* 2010;58(1):493-99.
447. Rumpagaporn P, Reuhs BL, Kaur A, Patterson JA, Keshavarzian A, Hamaker BR. Structural features of soluble cereal arabinoxylan fibers associated with a slow rate of *in vitro* fermentation by human fecal microbiota. *Carbohydr Polym.* 2015;130:191-97.
448. Saulnier L, Vigouroux J, Thibault J-F. Isolation and partial characterization of feruloylated oligosaccharides from maize bran. *Carbohydr Res.* 1995;272(2):241-53.
449. Csizmadi I, Boucher BA, Lo Siou G*, et al.* Using national dietary intake data to evaluate and adapt the US Diet History Questionnaire: the stepwise tailoring of an FFQ for Canadian use. *Public Health Nutr.* 2016;19(18):3247-55.
450. McInerney M, Csizmadi I, Friedenreich CM*, et al.* Associations between the neighbourhood food environment, neighbourhood socioeconomic status, and diet quality: An observational study. *BMC Public Health.* 2016;16:984.
451. Kipnis V, Subar AF, Midthune D*, et al.* Structure of Dietary Measurement Error: Results of the OPEN Biomarker Study. *Am J Epidemiol.* 2003;158(1):14-21.
452. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. *Am J Epidemiol.* 1986;124(1):17-27.
453. Winter J, Young GP, Hu Y, Gratz SW, Conlon MA, Le Leu RK. Accumulation of promutagenic DNA adducts in the mouse distal colon after consumption of heme does not induce colonic neoplasms in the western diet model of spontaneous colorectal cancer. *Mol Nutr Food Res.* 2014;58(3):550-58.
454. Jin M, Kalainy S, Baskota N*, et al.* Faecal microbiota from patients with cirrhosis has a low capacity to ferment non-digestible carbohydrates into short-chain fatty acids. *Liver Int.* 2019;39(8):1437-47.

455. Krumbeck JA, Maldonado-Gomez MX, Martínez I*, et al. In vivo* selection to identify bacterial strains with enhanced ecological performance in synbiotic applications. *Appl Environ Microbiol.* 2015;81(7):2455-65.
456. Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics.* 2010;26(19):2460-61.
457. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol.* 2007;73(16):5261-67.
458. Quast C, Pruesse E, Yilmaz P*, et al.* The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 2013;41(Database issue):D590-D96.
459. Yoon S-H, Ha S-M, Kwon S*, et al.* Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol.* 2017;67(5):1613-17.
460. Markowitz VM, Chen I-MA, Palaniappan K*, et al.* IMG: the Integrated Microbial Genomes database and comparative analysis system. *Nucleic Acids Res.* 2012;40(D1):D115-D22.
461. Chen I-MA, Chu K, Palaniappan K*, et al.* IMG/M v.5.0: an integrated data management and comparative analysis system for microbial genomes and microbiomes. *Nucleic Acids Res.* 2019;47(D1):D666-D77.
462. Aitchison J. The Statistical Analysis of Compositional Data. *J R Statist Soc B.* 1982;44(2):139-60.
463. vegan: Community Ecology Package [computer program]. Version R-package version 2.5-52019.
464. Wickham H. ggplot2: Elegant Graphics for Data Analysis. 2 ed: Springer International Publishing; 2016.
465. Højsgaard S, Halekoh U, Yan J. The R Package geepack for Generalized Estimating Equations. *J Stat Softw.* 2005;15(2).
466. Tong X, Xu J, Lian F*, et al.* Structural Alteration of Gut Microbiota during the Amelioration of Human Type 2 Diabetes with Hyperlipidemia by Metformin and a Traditional Chinese Herbal Formula: a Multicenter, Randomized, Open Label Clinical Trial. *mBio.* 2018;9(3):e02392-17.
467. Gu Z, Eils R, Schlesner M. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics.* 2016;32(18):2847-28479.
468. Faust K, Raes J. CoNet app: inference of biological association networks using Cytoscape. *F1000Res.* 2016;5:1519.
469. Cai C, Zhang Z, Morales M, Wang Y, Khafipour E, Friel J. Feeding practice influences gut microbiome composition in very low birth weight preterm infants and the association with oxidative stress: A prospective cohort study. *Free Radic Biol Med.* 2019;142:146-54.
470. factoextra: Extract and Visualize the Results of Multivariate Data Analyses [computer program]. Version R-package version 1.0.52017.
471. Lê S, Josse J, Husson F. FactoMineR: An R package for multivariate analysis. *J Stat Softw.* 2008;25(1).
472. leaps: Regression Subset Selection [computer program]. Version R-package version 3.02017.

473. AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c) [computer program]. Version R-package versioin 2.2-22019.
474. Millet S, Van Oeckel MJ, Aluwe M, Delezie E, De Brabander DL. Prediction of *In Vivo* Short-Chain Fatty Acid Production in Hindgut Fermenting Mammals: Problems and Pitfalls. *Crit Rev Food Sci Nutr.* 2010;50(7):605-19.
475. McOrist AL, Miller RB, Bird AR*, et al.* Fecal butyrate levels vary widely among individuals but are usually increased by a diet high in resistant starch. *J Nutr.* 2011;141(5):883-89.
476. Krumholz LR, Bryant M. *Eubacterium oxidoreducens* sp. nov. requiring H_2 or formate to degrade gallate, pyrogallol, phloroglucinol and quercetin. *Arch Microbiol.* 1986;144(1):8-14.
477. Louis P, Flint HJ. Formation of propionate and butyrate by the human colonic microbiota. *Environ Microbiol.* 2017;19(1):29-41.
478. Van den Abbeele P, Gérard P, Rabot S*, et al.* Arabinoxylans and inulin differentially modulate the mucosal and luminal gut microbiota and mucin-degradation in humanized rats. *Environ Microbiol.* 2011;13(10):2667-80.
479. Van den Abbeele P, Venema K, Van de Wiele T, Verstraete W, Possemiers S. Different human gut models reveal the distinct fermentation patterns of Arabinoxylan versus inulin. *J Agric Food Chem.* 2013;61(41):9819-27.
480. Crittenden R, Karppinen S, Ojanen S*, et al. In vitro* fermentation of cereal dietary fibre carbohydrates by probiotic and intestinal bacteria. *J Sci Food Agr.* 2002;82(8):781-89.
481. Rivière A, Moens F, Selak M, Maes D, Weckx S, De Vuyst L. The ability of bifidobacteria to degrade arabinoxylan oligosaccharide constituents and derived oligosaccharides is strain dependent. *Appl Environ Microbiol.* 2014;80(1):204-17.
482. Komeno M, Hayamizu H, Fujita K, Ashida H. Two Novel α-l-Arabinofuranosidases from *Bifidobacterium longum* subsp. *longum* Belonging to Glycoside Hydrolase Family 43 Cooperatively Degrade Arabinan. *Appl Environ Microbiol.* 2019;85(6):e02582-18.
483. Fehlner-Peach H, Magnabosco C, Raghavan V*, et al.* Distinct Polysaccharide Utilization Profiles of Human Intestinal *Prevotella copri* Isolates. *Cell Host Microbe.* 2019;26(5):680-90 e5.
484. Tan H, Zhao J, Zhang H, Zhai Q, Chen W. Isolation of Low-Abundant Bacteroidales in the Human Intestine and the Analysis of Their Differential Utilization Based on Plant-Derived Polysaccharides. *Front Microbiol.* 2018;9:1319.
485. La Rosa SL, Kachrimanidou V, Buffetto F*, et al.* Wood-Derived Dietary Fibers Promote Beneficial Human Gut Microbiota. *mSphere.* 2019;4(1):e00554-18.
486. Zhang M, Chekan JR, Dodd D*, et al.* Xylan utilization in human gut commensal bacteria is orchestrated by unique modular organization of polysaccharide-degrading enzymes. *Proc Natl Acad Sci USA.* 2014;111(35):E3708-17.
487. Centanni M, Hutchison JC, Carnachan SM*, et al.* Differential growth of bowel commensal *Bacteroides* species on plant xylans of differing structural complexity. *Carbohydr Polym.* 2017;157:1374-82.
488. Pareek S, Kurakawa T, Das B*, et al.* Comparison of Japanese and Indian intestinal microbiota shows diet-dependent interaction between bacteria and fungi. *NPJ Biofilms Microbi.* 2019;5:37.
489. Dodd D, Mackie RI, Cann IK. Xylan degradation, a metabolic property shared by rumen and human colonic Bacteroidetes. *Mol Microbiol.* 2011;79(2):292-304.

490. Benítez-Páez A, Kjølbæk L, Gómez Del Pulgar EM*, et al.* A Multi-omics Approach to Unraveling the Microbiome-Mediated Effects of Arabinoxylan Oligosaccharides in Overweight Humans. *mSystems.* 2019;4(4):e00209-19.
491. Pastell H, Westermann P, Meyer AS, Tuomainen P, Tenkanen M. *In vitro* fermentation of arabinoxylan-derived carbohydrates by bifidobacteria and mixed fecal microbiota. *J Agric Food Chem.* 2009;57(18):8598-606.
492. Rogowski A, Briggs JA, Mortimer JC*, et al.* Glycan complexity dictates microbial resource allocation in the large intestine. *Nat Commun.* 2015;6:7481.
493. Lugli GA, Mancino W, Milani C*, et al.* Reconstruction of the Bifidobacterial Pan-Secretome Reveals the Network of Extracellular Interactions between Bifidobacteria and the Infant Gut. *Appl Environ Microbiol.* 2018;84(16):e00796-18.
494. Milani C, Lugli GA, Duranti S*, et al.* Bifidobacteria exhibit social behavior through carbohydrate resource sharing in the gut. *Sci Rep.* 2015;5:15782.
495. Holmstrøm K, Collins MD, Moller T, Falsen E, Lawson PA. *Subdoligranulum variabile* gen. nov., sp. nov. from human feces. *Anaerobe.* 2004;10(3):197-203.
496. Lawson PA, Finegold SM. Reclassification of *Ruminococcus obeum* as *Blautia obeum* comb. nov. *Int J Syst Evol Microbiol.* 2015;65(Pt 3):789-93.
497. Hopkins MJ, Englyst HN, Macfarlane S, Furrie E, Macfarlane GT, McBain AJ. Degradation of Cross-Linked and Non-Cross-Linked Arabinoxylans by the Intestinal Microbiota in Children. *Appl Environ Microbiol.* 2003;69(11):6354-60.
498. Rumpagaporn P, Reuhs BL, Cantu-Jungles TM*, et al.* Elevated propionate and butyrate in fecal ferments of hydrolysates generated by oxalic acid treatment of corn bran arabinoxylan. *Food Funct.* 2016;7(12):4935-43.
499. Chen T, Long W, Zhang C, Liu S, Zhao L, Hamaker BR. Fiber-utilizing capacity varies in *Prevotella*- versus *Bacteroides*-dominated gut microbiota. *Sci Rep.* 2017;7(1):2594.
500. Reichardt N, Duncan SH, Young P*, et al.* Phylogenetic distribution of three pathways for propionate production within the human gut microbiota. *ISME J.* 2014;8(6):1323-35.
501. Watanabe Y, Nagai F, Morotomi M. Characterization of *Phascolarctobacterium succinatutens* sp. nov., an asaccharolytic, succinate-utilizing bacterium isolated from human feces. *Appl Environ Microbiol.* 2012;78(2):511-18.
502. Rothschild D, Weissbrod O, Barkan E*, et al.* Environment dominates over host genetics in shaping human gut microbiota. *Nature.* 2018;555(7695):210-15.
503. Wang J, Thingholm LB, Skieceviciene J*, et al.* Genome-wide association analysis identifies variation in vitamin D receptor and other host factors influencing the gut microbiota. *Nat Genet.* 2016;48(11):1396-406.
504. Subar AF, Freedman LS, Tooze JA*, et al.* Addressing Current Criticism Regarding the Value of Self-Report Dietary Data. *J Nutr.* 2015;145(12):2639-45.
505. Louca S, Polz MF, Mazel F*, et al.* Function and functional redundancy in microbial systems. *Nat Ecol Evol.* 2018;2(6):936-43.
506. Hatzenpichler R, Scheller S, Tavormina PL, Babin BM, Tirrell DA, Orphan VJ. *In situ* visualization of newly synthesized proteins in environmental microbes using amino acid tagging and click chemistry. *Environ Microbiol.* 2014;16(8):2568-90.
507. Wong CB, Odamaki T, Xiao J-z. Beneficial effects of *Bifidobacterium longum* subsp. *longum* BB536 on human health: Modulation of gut microbiome as the principal action. *J Funct Foods.* 2019;54:506-19.

508. Colombel J, Cortot A, Neut C, Romond C. Yoghurt with *Bifidobacterium longum* Reduces Erythromycin-Induced Gastrointestinal Effects. *Lancet.* 1987;330(8549):43.
509. Tamaki H, Nakase H, Inoue S*, et al.* Efficacy of probiotic treatment with *Bifidobacterium longum* 536 for induction of remission in active ulcerative colitis: A randomized, double-blinded, placebo-controlled multicenter trial. *Dig Endosc.* 2016;28(1):67-74.
510. McCarville J, Dong J, Caminero A*, et al.* A Commensal *Bifidobacterium longum* Strain Prevents Gluten-Related Immunopathology in Mice through Expression of a Serine Protease Inhibitor. *Appl Environ Microbiol.* 2017;83(19):e01323-17.
511. Xiao J-Z, Kondo S, Yanagisawa N*, et al.* Probiotics in the treatment of Japanese cedar pollinosis: a double-blind placebo-controlled trial. *Clin Exp Allergy.* 2006;36(11):1425-35.
512. Pinto-Sanchez MI, Hall GB, Ghajar K*, et al.* Probiotic *Bifidobacterium longum* NCC3001 Reduces Depression Scores and Alters Brain Activity: A Pilot Study in Patients With Irritable Bowel Syndrome. *Gastroenterology.* 2017;153(2):448-59.e8.
513. Bercik P, Park AJ, Sinclair D*, et al.* The anxiolytic effect of *Bifidobacterium longum* NCC3001 involves vagal pathways for gut-brain communication. *Neurogastroenterol Motil.* 2011;23(12):1132-39.
514. Scher JU, Sczesnak A, Longman RS*, et al.* Expansion of intestinal *Prevotella copri* correlates with enhanced susceptibility to arthritis. *eLife.* 2013;2:e01202.
515. Cani PD. Human gut microbiome: hopes, threats and promises. *Gut.* 2018;67(9):1716-25.
516. Christensen L, Vuholm S, Roager HM*, et al. Prevotella* Abundance Predicts Weight Loss Success in Healthy, Overweight Adults Consuming a Whole-Grain Diet Ad Libitum: A Post Hoc Analysis of a 6-Wk Randomized Controlled Trial. *J Nutr.* 2019;149(12):2174-81.
517. Hjorth MF, Roager HM, Larsen TM*, et al.* Pre-treatment microbial *Prevotella*-to-*Bacteroides* ratio, determines body fat loss success during a 6-month randomized controlled diet intervention. *Int J Obes.* 2018;42(3):580-83.
518. Chambers ES, Viardot A, Psichas A*, et al.* Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults. *Gut.* 2015;64(11):1744-54.
519. Chambers ES, Byrne CS, Morrison DJ*, et al.* Dietary supplementation with inulin-propionate ester or inulin improves insulin sensitivity in adults with overweight and obesity with distinct effects on the gut microbiota, plasma metabolome and systemic inflammatory responses: a randomised cross-over trial. *Gut.* 2019;68(8):1430-38.
520. Venter C, Vorster H, Cummings JH. Effects of dietary propionate on carbohydrate and lipid metabolism in healthy volunteers. *Am J Gastroenterol.* 1990;85(5):549-53.
521. Kalantar-Zadeh K, Berean KJ, Burgell RE, Muir JG, Gibson PR. Intestinal gases: influence on gut disorders and the role of dietary manipulations. *Nat Rev Gastroenterol Hepatol.* 2019;16(12):733-47.
522. Livesey G. Tolerance of low-digestible carbohydrates: a general view. *Br J Nutr.* 2001;85(Suppl 1):S7-S16.
523. Corsetti M, Gevers A-M, Caenepeel P, Tack J. The role of tension receptors in colonic mechanosensitivity in humans. *Gut.* 2004;53(12):1787-93.
524. Mendis M, Leclerc E, Simsek S. Arabinoxylans, gut microbiota and immunity. *Carbohydr Polym.* 2016;139:159-66.

525. Koecher KJ, Noack JA, Timm DA, Klosterbuer AS, Thomas W, Slavin JL. Estimation and interpretation of fermentation in the gut: coupling results from a 24 h batch *in vitro* system with fecal measurements from a human intervention feeding study using fructo-oligosaccharides, inulin, gum acacia, and pea fiber. *J Agric Food Chem.* 2014;62(6):1332-37.
526. Kaur A, Rose DJ, Rumpagaporn P, Patterson JA, Hamaker BR. *In vitro* batch fecal fermentation comparison of gas and short-chain fatty acid production using "slowly fermentable" dietary fibers. *J Food Sci.* 2011;76(5):H137-H42.
527. Erickson JM, Carlson JL, Stewart ML, Slavin JL. Fermentability of Novel Type-4 Resistant Starches in *In Vitro* System. *Foods.* 2018;7(2):E18.
528. Grabitske HA, Slavin JL. Gastrointestinal effects of low-digestible carbohydrates. *Crit Rev Food Sci Nutr.* 2009;49(4):327-60.
529. Cherbut C, Michel C, Raison V, Kravtchenko T, Severine M. Acacia Gum is a Bifidogenic Dietary Fibre with High Digestive Tolerance in Healthy Humans. *Microb Ecol Health Dis.* 2003;15(1):43-50.
530. Mego M, Manichanh C, Accarino A*, et al.* Metabolic adaptation of colonic microbiota to galactooligosaccharides: a proof-of-concept-study. *Aliment Pharmacol Ther.* 2017;45(5):670-80.
531. Kruse HP, Kleessen B, Blaut M. Effects of inulin on faecal bifidobacteria in human subjects. *Br J Nutr.* 1999;82(5):375-82.
532. Babiker R, Merghani TH, Elmusharaf K, Badi RM, Lang F, Saeed AM. Effects of Gum Arabic ingestion on body mass index and body fat percentage in healthy adult females: two-arm randomized, placebo controlled, double-blind trial. *Nutr J.* 2012;11:111.
533. Okubo T, Ishihara N, Takahashi H*, et al.* Effects of Partially Hydrolyzed Guar Gum Intake on Human Intestinal Microflora and Its Metabolism. *Biosci Biotechnol Biochem.* 1994;58(8):1364-69.
534. Mego M, Accarino A, Tzortzis G*, et al.* Colonic gas homeostasis: Mechanisms of adaptation following HOST-G904 galactooligosaccharide use in humans. *Neurogastroenterol Motil.* 2017;29(9):e13080.
535. Davis LMG, Martínez I, Walter J, Hutkins RW. A dose dependent impact of prebiotic galactooligosaccharides on the intestinal microbiota of healthy adults. *Int J Food Microbiol.* 2010;144(2):285-92.
536. Deehan EC, Zhang Z, Riva A*, et al.* Elucidation of the role of microbial fermentation in the physiological effects of dietary fiber in adults with overweight or obesity. *In Preparation*.
537. Nguyen NK, Deehan EC, Zhang Z*, et al.* Gut microbiota modulation with long-chain corn bran arabinoxylan in adults with overweight and obesity is linked to an individualized temporal increase in fecal propionate. *Microbiome.* 2020;8:118.
538. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods.* 2016;13(7):581-3.
539. Voreades N, Kozil A, Weir TL. Diet and the development of the human intestinal microbiome. *Front Microbiol.* 2014;5:494.
540. Lam YY, Zhang C, Zhao L. Causality in dietary interventions-building a case for gut microbiota. *Genome Med.* 2018;10(1):62.

541. Lee J-H, O'Sullivan DJ. Genomic Insights into Bifidobacteria. *Microbiol Mol Biol Rev.* 2010;74(3):378-416.
542. Holscher HD, Doligale JL, Bauer LL*, et al.* Gastrointestinal tolerance and utilization of agave inulin by healthy adults. *Food Funct.* 2014;5(6):1142-49.
543. Hernot DC, Boileau TW, Bauer LL*, et al. In vitro* fermentation profiles, gas production rates, and microbiota modulation as affected by certain fructans, galactooligosaccharides, and polydextrose. *J Agric Food Chem.* 2009;57(4):1354-61.
544. Smith NW, Shorten PR, Altermann EH, Roy NC, McNabb WC. Hydrogen cross-feeders of the human gastrointestinal tract. *Gut Microbes.* 2019;10(3):270-88.
545. Jalanka-Tuovinen J, Salonen A, Nikkila J*, et al.* Intestinal microbiota in healthy adults: temporal analysis reveals individual and common core and relation to intestinal symptoms. *PLoS One.* 2011;6(7):e23035.
546. Guyonnet D, Chassany O, Ducrotte P*, et al.* Effect of a fermented milk containing *Bifidobacterium animalis* DN-173 010 on the health-related quality of life and symptoms in irritable bowel syndrome in adults in primary care: a multicentre, randomized, double-blind, controlled trial. *Aliment Pharmacol Ther.* 2007;26(3):475-86.
547. Whorwell PJ, Altringer L, Morel J*, et al.* Efficacy of an encapsulated probiotic *Bifidobacterium infantis* 35624 in women with irritable bowel syndrome. *Am J Gastroenterol.* 2006;101(7):1581-90.
548. O'Mahony L, McCarthy J, Kelly P*, et al. Lactobacillus* and *Bifidobacterium* in Irritable Bowel Syndrome: Symptom Responses and Relationship to Cytokine Profiles. *Gastroenterology.* 2005;128(3):541-51.
549. Perman J, Modler S, Olson A. Role of pH in production of hydrogen from carbohydrates by colonic bacterial flora. Studies *in vivo* and *in vitro*. *J Clin Invest.* 1981;67(3):643-50.
550. Lomax AE, Pradhananga S, Sessenwein JL, O'Malley D. Bacterial modulation of visceral sensation: mediators and mechanisms. *Am J Physiol Gastrointest Liver Physiol.* 2019;317(3):G363-G72.
551. Hiel S, Bindels LB, Pachikian BD*, et al.* Effects of a diet based on inulin-rich vegetables on gut health and nutritional behavior in healthy humans. *Am J Clin Nutr.* 2019;109(6):1683-95.
552. Manichanh C, Eck A, Varela E*, et al.* Anal gas evacuation and colonic microbiota in patients with flatulence: effect of diet. *Gut.* 2014;63(3):401-08.
553. Valdes AM, Walter J, Segal E, Spector TD. Role of the gut microbiota in nutrition and health. *BMJ.* 2018;361:k2179.
554. Baxter NT, Schmidt AW, Venkataraman A, Kim KS, Waldron C, Schmidt TM. Dynamics of Human Gut Microbiota and Short-Chain Fatty Acids in Response to Dietary Interventions with Three Fermentable Fibers. *mBio.* 2019;10(1):e02566-18.
555. Rivera-Chavez F, Zhang LF, Faber F*, et al.* Depletion of Butyrate-Producing *Clostridia* from the Gut Microbiota Drives an Aerobic Luminal Expansion of *Salmonella*. *Cell Host Microbe.* 2016;19(4):443-54.
556. van der Beek CM, Dejong CHC, Troost FJ, Masclee AAM, Lenaerts K. Role of short-chain fatty acids in colonic inflammation, carcinogenesis, and mucosal protection and healing. *Nutr Rev.* 2017;75(4):286-305.
557. Moya A, Ferrer M. Functional Redundancy-Induced Stability of Gut Microbiota Subjected to Disturbance. *Trends Microbiol.* 2016;24(5):402-13.

558. DeMartino P, Cockburn DW. Resistant starch: impact on the gut microbiome and health. *Curr Opin Biotechnol.* 2020;61:66-71.
559. Calame W, Weseler AR, Viebke C, Flynn C, Siemensma AD. Gum arabic establishes prebiotic functionality in healthy human volunteers in a dose-dependent manner. *Br J Nutr.* 2008;100(6):1269-75.
560. So D, Whelan K, Rossi M*, et al.* Dietary fiber intervention on gut microbiota composition in healthy adults: a systematic review and meta-analysis. *Am J Clin Nutr.* 2018;107(6):965-83.
561. van den Heuvel EG, Wils D, Pasman WJ, Bakker M, Saniez M-HH, Kardinaal AF. Short-term digestive tolerance of different doses of NUTRIOSE® FB, a food dextrin, in adult men. *Eur J Clin Nutr.* 2004;58(7):1046-55.
562. Brumovsky JO, Thompson DB. Production of Boiling-Stable Granular Resistant Starch by Partial Acid Hydrolysis and Hydrothermal Treatments of High-Amylose Maize Starch. *Cereal Chem.* 2001;78(6):680-89.
563. Chen Q, Yu H, Wang L*, et al.* Recent progress in chemical modification of starch and its applications. *RSC Adv.* 2015;5(83):67459-74.
564. Thompson FE, Dixit-Joshi S, Potischman N*, et al.* Comparison of Interviewer-Administered and Automated Self-Administered 24-Hour Dietary Recalls in 3 Diverse Integrated Health Systems. *Am J Epidemiol.* 2015;181(12):970-78.
565. Csizmadi I, Kahle L, Ullman R*, et al.* Adaptation and evaluation of the National Cancer Institute's Diet History Questionnaire and nutrient database for Canadian populations. *Public Health Nutr.* 2007;10(1):88-96.
566. Mäder U, Martin BW, Schutz Y, Marti B. Validity of four short physical activity questionnaires in middle-aged persons. *Med Sci Sports Exerc.* 2006;38(7):1255-66.
567. Cohen S, Williamson GM. Perceived stress in a probability sample of the United States. In: Spacapan S, Oskamp S, eds. *The Social Psychology of Health. The Claremont Symposium on Applied Social Psychology.* Thousand Oaks, CA: Sage Publications, Inc.; 1988.
568. Maki KC, Rains TM, Kelley KM, Cook CM, Schild AL, Gietl E. Fibermalt is well tolerated in healthy men and women at intakes up to 60 g/d: a randomized, double-blind, crossover trial. *Int J Food Sci Nutr.* 2013;64(3):274-81.
569. Stewart ML, Wilcox ML, Bell M, Buggia MA, Maki KC. Type-4 Resistant Starch in Substitution for Available Carbohydrate Reduces Postprandial Glycemic Response and Hunger in Acute, Randomized, Double-Blind, Controlled Study. *Nutrients.* 2018;10(2):129.
570. Costea PI, Zeller G, Sunagawa S*, et al.* Towards standards for human fecal sample processing in metagenomic studies. *Nat Biotechnol.* 2017;35(11):1069-76.
571. Eren AM, Vineis JH, Morrison HG, Sogin ML. A filtering method to generate high quality short reads using illumina paired-end technology. *PLoS One.* 2013;8(6):e66643.
572. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods.* 2013;10(10):996-98.
573. Westram R, Bader K, Prüsse E*, et al.* ARB: a software environment for sequence data. In: de Bruijn FJ, ed. *Handbook of Molecular Microbial Ecology, Volume I: Metagenomics and Complementary Approaches*. 1 ed: John Wiley & Sons, Inc; 2011:399-406.
574. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *J Mol Biol.* 1990;215(3):403-10.

575. Michigan State University. RDP (Ribosomal Database Project). *RDP Release 11, Update 5.* 2016. September 30:Available at: www.rdp.cme.msu.edu/seqmatch/seqmatch_intro.jsp;jsessionid=900158E5787CA0B0CFB591C737B90E9B.radian.
576. Caporaso JG, Kuczynski J, Stombaugh JI*, et al.* QIIME allows analysis of high-throughput community sequencing data. *Nat Methods.* 2010;7(5):335-36.
577. Hall M, Beiko RG. 16S rRNA Gene Analysis with QIIME2. *Methods Mol Biol.* 2018;1849:113-29.
578. Sakamoto M, Benno Y. Reclassification of *Bacteroides distasonis*, *Bacteroides goldsteinii* and *Bacteroides merdae* as *Parabacteroides distasonis* gen. nov., comb. nov., *Parabacteroides goldsteinii* comb. nov. and *Parabacteroides merdae* comb. nov. *Int J Syst Evol Microbiol.* 2006;56(Pt 7):1599-605.
579. Streiner D, Geddes J. Intention to treat analysis in clinical trials when there are missing data. *Evid Based Ment Health.* 2001;4(3):70-71.
580. Lenth R, Singmann H, Love J, Buerkner P, Herve M. Estimated Marginal Means, aka Least-Squares Means. 2019. Available at: www.cran.r-project.org/web/packages/emmeans/emmeans.pdf.
581. Christensen RHB. Cumulative Link Models for Ordinal Regression with the R Package ordinal. 2018. Available at: www.cran.r-project.org/web/packages/ordinal/vignettes/clm_article.pdf.
582. Schmider E, Ziegler M, Danay E, Beyer L, Bühner M. Is It Really Robust? Reinvestigating the Robustness of ANOVA Against Violations of the Normal Distribution Assumption. *Methodology.* 2010;6(4):147-51.
583. Harwell MR, Rubinstein EN, Hayes WS, Olds CC. Summarizing Monte Carlo Results in Methodological Research: The One- and Two-Factor Fixed Effects ANOVA Cases. *J Educ Stat.* 1992;17(4):315-39.
584. Stewart ML, Zimmer JP. A High Fiber Cookie Made with Resistant Starch Type 4 Reduces Post-Prandial Glucose and Insulin Responses in Healthy Adults. *Nutrients.* 2017;9(3):237.
585. Coulon DB, Page R, Raggio AM*, et al.* Novel Resistant Starch Type 4 Products of Different Starch Origins, Production Methods, and Amounts are not Equally Fermented when Fed to Sprague-Dawley Rats. *Mol Nutr Food Res.* 2020;64(2):e1900901.
586. Wang Y, Chen J, Song Y-H*, et al.* Effects of the resistant starch on glucose, insulin, insulin resistance, and lipid parameters in overweight or obese adults: a systematic review and meta-analysis. *Nutr Diabetes.* 2019;9(1):19.
587. Guilhot E, Alou MT, Lagier J-C*, et al.* Genome sequence and description of *Anaeromassilibacillus senegalensis* gen. nov., sp. nov., isolated from the gut of patient with kwashiorkor. *New Microbes New Infect.* 2017;17:54-64.
588. Duranti S, Turroni F, Lugli GA*, et al.* Genomic characterization and transcriptional studies of the starch-utilizing strain *Bifidobacterium adolescentis* 22L. *Appl Environ Microbiol.* 2014;80(19):6080-90.
589. Cerqueira FM, Photenhauer AL, Pollet RM, Brown HA, Koropatkin NM. Starch Digestion by Gut Bacteria: Crowdsourcing for Carbs. *Trends Microbiol.* 2019;28(2):95-108.
590. Korpela K. Diet, Microbiota, and Metabolic Health: Trade-Off Between Saccharolytic and Proteolytic Fermentation. *Annu Rev Food Sci Technol.* 2018;9:65-84.

591. Wang K, Liao M, Zhou N, *et al.* *Parabacteroides distasonis* Alleviates Obesity and Metabolic Dysfunctions via Production of Succinate and Secondary Bile Acids. *Cell Rep.* 2019;26(1):222-35 e5.
592. Xu J, Mahowald MA, Ley RE, *et al.* Evolution of symbiotic bacteria in the distal human intestine. *PLoS Biol.* 2007;5(7):e156.
593. Cockburn DW, Orlovsky NI, Foley MH, *et al.* Molecular details of a starch utilization pathway in the human gut symbiont *Eubacterium rectale*. *Mol Microbiol.* 2015;95(2):209-30.
594. Abell GCJ, Cooke CM, Bennett CN, Conlon MA, McOrist AL. Phylotypes related to *Ruminococcus bromii* are abundant in the large bowel of humans and increase in response to a diet high in resistant starch. *FEMS Microbiol Ecol.* 2008;66(3):505-15.
595. Vital M, Howe A, Bergeron N, Krauss RM, Jansson JK, Tiedje JM. Metagenomic Insights into the Degradation of Resistant Starch by Human Gut Microbiota. *Appl Environ Microbiol.* 2018;84(23):e01562-18.
596. Clarke JM, Topping DL, Christophersen CT, *et al.* Butyrate esterified to starch is released in the human gastrointestinal tract. *Am J Clin Nutr.* 2011;94(5):1276-83.
597. Le Leu RK, Winter JM, Christophersen CT, *et al.* Butyrylated starch intake can prevent red meat-induced O^6-methyl-2-deoxyguanosine adducts in human rectal tissue: a randomised clinical trial. *Br J Nutr.* 2015;114(2):220-30.
598. Dahl WJ, Ford AL, Ukhanova M, *et al.* Resistant potato starches (type 4 RS) exhibit varying effects on laxation with and without phylum level changes in microbiota: A randomised trial in young adults. *J Funct Foods.* 2016;23:1-11.
599. Tester R, Qi X, Karkalas J. Hydrolysis of native starches with amylases. *Anim Feed Sci Technol.* 2006;130(1-2):39-54.
600. Mani-López E, García HS, López-Malo A. Organic acids as antimicrobials to control *Salmonella* in meat and poultry products. *Food Res Int.* 2012;45(2):713-21.
601. Oh J-H, Alexander LM, Pan M, *et al.* Dietary Fructose and Microbiota-Derived Short-Chain Fatty Acids Promote Bacteriophage Production in the Gut Symbiont *Lactobacillus reuteri*. *Cell Host Microbe.* 2019;25(2):273-84 e6.
602. Martínez I, Muller CE, Walter J. Long-term temporal analysis of the human fecal microbiota revealed a stable core of dominant bacterial species. *PLoS One.* 2013;8(7):e69621.
603. Crittenden R, Laitila A, Forssell P, *et al.* Adhesion of bifidobacteria to granular starch and its implications in probiotic technologies. *Appl Environ Microbiol.* 2001;67(8):3469-75.
604. Kolodziejczyk AA, Zheng D, Elinav E. Diet-microbiota interactions and personalized nutrition. *Nat Rev Microbiol.* 2019;17:742–53.
605. Qin J, Li Y, Cai Z, *et al.* A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature.* 2012;490(7418):55-60.
606. Del Chierico F, Nobili V, Vernocchi P, *et al.* Gut microbiota profiling of pediatric nonalcoholic fatty liver disease and obese patients unveiled by an integrated meta-omics-based approach. *Hepatology.* 2017;65(2):451-64.
607. Verdam FJ, Fuentes S, de Jonge C, *et al.* Human intestinal microbiota composition is associated with local and systemic inflammation in obesity. *Obesity.* 2013;21(12):E607-E15.

608. Guerin-Deremaux L, Pochat M, Reifer C, Wils D, Cho S, Miller LE. The soluble fiber NUTRIOSE induces a dose-dependent beneficial impact on satiety over time in humans. *Nutr Res.* 2011;31(9):665-72.
609. Vermorel M, Coudray C, Wils D*, et al.* Energy value of a low-digestible carbohydrate, NUTRIOSE® FB, and its impact on magnesium, calcium and zinc apparent absorption and retention in healthy young men. *Eur J Nutr.* 2004;43(6):344-52.
610. Huaman J-W, Mego M, Manichanh C*, et al.* Effects of Prebiotics vs a Diet Low in FODMAPs in Patients With Functional Gut Disorders. *Gastroenterology.* 2018;155(4):1004-07.
611. Dikeman CL, Fahey GC. Viscosity as related to dietary fiber: a review. *Crit Rev Food Sci Nutr.* 2006;46(8):649-63.
612. Lattimer JM, Haub MD. Effects of dietary fiber and its components on metabolic health. *Nutrients.* 2010;2(12):1266-89.
613. Elleuch M, Bedigian D, Roiseux O, Besbes S, Blecker C, Attia H. Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. *Food Chem.* 2011;124(2):411-21.
614. Lefranc-Millot C, Wils D, Roturier J-M, Le Bihan C, Saniez-Degrave M-H. NUTRIOSE® Soluble Fiber. In: Cho SS, Samuel P, eds. *Fiber Ingredients: Food Applications and Health Benefits*. 1st ed. Boca Raton (USA): CRC Press; 2009:19-40.
615. Ahmed W, Rashid S. Functional and therapeutic potential of inulin: A comprehensive review. *Crit Rev Food Sci Nutr.* 2019;59(1):1-13.
616. Housez B, Cazaubiel M, Vergara C*, et al.* Evaluation of digestive tolerance of a soluble corn fibre. *J Hum Nutr Diet.* 2012;25(5):488-96.
617. Berry SE, Valdes AM, Drew DA*, et al.* Human postprandial responses to food and potential for precision nutrition. *Nat Med.* 2020;26(6):964-73.
618. Rowland I, Gibson G, Heinken A*, et al.* Gut microbiota functions: metabolism of nutrients and other food components. *Eur J Nutr.* 2018;57(1):1-24.
619. Holscher HD, Bauer LL, Gourineni V, Pelkman CL, Fahey GCJ, Swanson KS. Agave Inulin Supplementation Affects the Fecal Microbiota of Healthy Adults Participating in a Randomized, Double-Blind, Placebo-Controlled, Crossover Trial. *J Nutr.* 2015;145(9):2025-32.
620. Vulevic J, Juric A, Tzortzis G, Gibson GR. A mixture of trans-galactooligosaccharides reduces markers of metabolic syndrome and modulates the fecal microbiota and immune function of overweight adults. *J Nutr.* 2013;143(3):324-31.
621. Cekanaviciute E, Yoo BB, Runia TF*, et al.* Gut bacteria from multiple sclerosis patients modulate human T cells and exacerbate symptoms in mouse models. *Proc Natl Acad Sci USA.* 2017;114(42):E8943.
622. Duscha A, Gisevius B, Hirschberg S*, et al.* Propionic Acid Shapes the Multiple Sclerosis Disease Course by an Immunomodulatory Mechanism. *Cell.* 2020;180(6):1067-80 e16.
623. ClinicalTrials.gov. National Library of Medicine (US). Identifier NCT02322112, The Alberta FYBER (Feed Your gut Bacteria morE fibeR) Study. 2019, Sep 25. Available at: https://clinicaltrials.gov/ct2/show/NCT02322112.
624. BCD Bioscience Incorporated. 2020. Available at: https://www.bcdbio.com/.
625. Kaleido Biosciences Incorporated. 2020.

626. Tham Y-L, Sexton K, Weiss HL, Elledge RM, Friedman LC, Kramer RM. The adherence to practice guidelines in the assessment of bone health in women with chemotherapy-induced menopause *J Support Oncol.* 2006;4(6):295-304.
627. Schiavo L, Scalera G, Pilone V, De Sena G, Ciorra FR, Barbarisi A. Patient adherence in following a prescribed diet and micronutrient supplements after laparoscopic sleeve gastrectomy: our experience during 1 year of follow-up. *J Hum Nutr Diet.* 2017;30(1):98-104.
628. Pfister AK, Welch CA, WuLu JTJ, Hager KA, Saville PD. An assessment of postmenopausal women's adherence to calcium with vitamin D supplements. *J Appl Res.* 2008;8(2):143-50.
629. Deehan EC, Sizemore M, Walter J, Stewart ML. Characterization of two structurally distinct resistant starch type IV in a blueberry muffin batter and a pretzel bun dough application. Poster presented at 7th International Dietary Fiber Conference 2018; June 4th-6th, 2018; Rotterdam, Netherlands.
630. Stewart ML, Zimmer JP. Postprandial glucose and insulin response to a high-fiber muffin top containing resistant starch type 4 in healthy adults: a double-blind, randomized, controlled trial. *Nutrition.* 2018;53:59-63.
631. Du Y, Wu Y, Xiao D*, et al.* Food prototype containing resistant starch type 4 on postprandial glycemic response in healthy adults. *Food Funct.* 2020;11(3):2231-37.
632. Singh V, Yeoh BS, Chassaing B*, et al.* Dysregulated Microbial Fermentation of Soluble Fiber Induces Cholestatic Liver Cancer. *Cell.* 2018;175(3):679-94 e22.
633. Akbulut G. Nutrition in Stoma Patients: A Practical View of Dietary Therapy. *Int J Hematol Oncol.* 2011;21(1):61-66.
634. Cozma-Petrut A, Loghin F, Miere D, Dumitrascu DL. Diet in irritable bowel syndrome: What to recommend, not what to forbid to patients! *World J Gastroenterol.* 2017;23(21):3771-83.
635. Louis P, Scott KP, Duncan SH, Flint HJ. Understanding the effects of diet on bacterial metabolism in the large intestine. *J Appl Microbiol.* 2007;102(5):1197-208.
636. Sartor RB. Gut microbiota: Optimal sampling of the intestinal microbiota for research. *Nat Rev Gastroenterol Hepatol.* 2015;12(5):253-54.
637. Gurry T, Nguyen LTT, Yu X, Alm EJ. Functional heterogeneity in the fermentation capabilities of the healthy human gut microbiota. *bioRxiv.* 2020.
638. Gorzelak MA, Gill SK, Tasnim N, Ahmadi-Vand Z, Jay M, Gibson DL. Methods for Improving Human Gut Microbiome Data by Reducing Variability through Sample Processing and Storage of Stool. *PLoS One.* 2015;10(8):e0134802.
639. Tedjo DI, Jonkers DM, Savelkoul PH*, et al.* The effect of sampling and storage on the fecal microbiota composition in healthy and diseased subjects. *PLoS One.* 2015;10(5):e0126685.
640. Cunningham JL, Bramstang L, Singh A*, et al.* Impact of time and temperature on gut microbiota and SCFA composition in stool samples. *PLoS One.* 2020;15(8):e0236944.
641. Salem MB, Aiche G, Rubbert L, Renaud P, Haddab Y. Design of a Microbiota Sampling Capsule using 3D-Printed Bistable Mechanism. *Conf Proc IEEE Eng Med Biol Soc.* 2018;2018:4868-71.
642. Tang Q, Jin G, Wang G*, et al.* Current Sampling Methods for Gut Microbiota: A Call for More Precise Devices. *Front Cell Infect Microbiol.* 2020;10:151.

Appendix A: Elucidation of the role of microbial fermentation in the physiological effects of dietary fiber in adults with overweight and obesity

Title: Elucidation of the role of microbial fermentation in the physiological effects of dietary fiber in adults with overweight or obesity

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ABSTRACT

Objective: Dietary fibers promote beneficial physiological effects that vary based on their physicochemical properties. However, the mechanisms are insufficiently understood, and the extent to which fiber fermentability contributes to these effects remains unclear. Here we compared the health effects of fermentable arabinoxylan (AX) with non-fermentable microcrystalline cellulose (MCC) in adults with overweight or obesity. Then, we determined if obesity-related surrogate endpoints could be predicted by microbiota compositional features and mechanistic endpoints that reflect host-microbiota interactions involved in the pathophysiology of obesity.

Design: Six-week randomized controlled exploratory trial where adults consumed 25 g/day (females) or 35 g/day (males) of AX (*n*=15) or MCC (*n*=16). Surrogate endpoints, microbiota compositional features, including bioorthogonal non-canonical amino acid tagging (BONCAT), and mechanistic endpoints were assessed.

Results: AX enhanced satiety after a meal and decreased homeostatic model assessment of insulin resistance (HOMA-IR), while MCC reduced tumor necrosis factor-α and fecal calprotectin. Effects on satiety were predicted by the bacterial taxa involved in AX fermentation as identified by BONCAT. While fecal bile acid shifts predicted both AX- and MCC-induced reductions in HOMA-IR and calprotectin, the benefits were not explained by the bile acid responses. Other mechanistic endpoints were not linked to physiological benefits of the fibers.

Conclusion: This study provides evidence for the role of the microbiome in the satiating effect of AX, while the metabolic benefits of AX and the immunological benefits of MCC are likely microbiota independent. Our results inform the targeted use of physicochemically-distinct fibers to achieve specific health outcomes.

Trial registration number NCT02322112.

What is already known about this subject?

- Dietary fibers are considered important for the prevention of obesity and related comorbidities, and physiological effects vary based on their physicochemical properties.
- Fermentation of fibers, the generation of bioactive metabolites, and enrichment of health-promoting bacterial taxa are some of the mechanisms hypothesized to underlie the physiological effects of fiber.
- Arabinoxylan (AX), which is moderately viscous and fermentable, has been shown to enhance satiety and attenuate insulin resistance.

What are the new findings?

- Effects of AX on satiety were predicted by the bacterial consortia that utilized AX, but not fiber-induced compositional shifts or fecal output of microbiota-generated metabolites.
- Improvements in the homeostatic model assessment of insulin resistance (HOMA-IR) by AX were linked to shifts in fecal concentrations of specific secondary bile acids.
- Non-fermentable microcrystalline cellulose (MCC) improved measures of intestinal and systemic inflammation that were also linked to shifts in bile acids, although the bile acids involved differed from AX-associated effects.
- While fecal bile acid shifts predicted both AX-induced HOMA-IR improvements and MCC-induced reductions in calprotectin, the benefits were not linked to the bile acid responses.
- Effects of AX and MCC were not predicted by other commonly hypothesized mechanistic endpoints that reflect host-microbiota interactions, such as short-chain fatty acids, gut hormones, or barrier function measures.

How might it impact on clinical practice in the foreseeable future?

- Our findings support the use of particular fibers for achieving targeted improvements in specific health outcomes relevant to obesity.
- Mechanistic insight on how dietary fibers benefit human health can inform the development of fiber supplements that target specific clinical efficacy.

INTRODUCTION

Obesity and related comorbidities such as type II diabetes have reached epidemic proportions worldwide.[1] One dietary component that has been linked in observational research with reduced prevalence of chronic diseases is dietary fiber[2,3], with putative mechanisms further established in animal models[4,5]. The physiological outcomes of fiber supplementation are dependent on their physicochemical properties,[6] with evidence of clinical efficacy mainly shown for viscous fibers and improved lipid and glucose metabolism.[7,8] For instance, the European Food Safety Authority (EFSA) has approved a health claim for moderately viscous wheat endosperm arabinoxylans (AXs) and improved postprandial glycemic control.[9] Therefore, fiber supplementation could be an effective treatment or preventive strategy for obesity-related chronic diseases.

The mechanistic foundations for the beneficial effects of physicochemically distinct fibers remain insufficiently understood in humans. Viscous fibers are presumed to increase digesta viscosity, prolonging satiety and lowering postprandial metabolic responses by delaying gastric emptying and intestinal nutrient absorption.[10] Fermentable fibers are hypothesized to favorably modulate the gut microbiota,[4,11] a highly diverse community of microorganisms that inhabits the gastrointestinal tract and has been linked to the pathophysiology of obesity and related comorbidities.[12] Fermentable fibers can alter compositional features of the gut microbiota in a structure-dependent manner,[13,14] selecting for well-adapted primary degraders and secondary fermenters that further associate with metabolic effects in humans (*e.g. Prevotella copri*[15] and *Eubacterium rectale*[16]). Moreover, fiber fermentation generates metabolites such as short-chain fatty acids (SCFAs), which may act as signaling molecules to maintain intestinal barrier integrity and immune homeostasis along with regulate satiety and glucose metabolism.[5,17] The intestinal bile acid pool is also influenced by the gut microbiota through the biotransformation of primary bile acids into structurally-distinct secondary bile acids, which display immunomodulatory and metabolic properties that are structure- and receptor-dependent.[18,19] Since some fiber structures can bind bile acids, facilitating microbial biotransformation, metabolic effects of fiber might also arise from the reconfiguration of the bile acid pool.[11] Although a few studies have reported correlations between physiological effects of fiber supplementation and specific bacterial taxa[20,21] or metabolite shifts[22,23], physiological effects may also be independent of the gut microbiota[24]. As causal relationships are challenging to establish in humans[25], it remains unclear

whether the gut microbiota is mechanistically implicated in the effects of fiber consumption. Human studies that systematically compare physiochemically-distinct fibers could determine if health outcomes are predictable by fiber fermentation and the resulting shifts in gut microbiota composition, its production of bioactive metabolites, and molecular markers of host-microbiota interactions.

In our preceding study,[26] we compared the effects of high doses of a moderately viscous[27] and fermentable[28] AX with an insoluble, non-fermentable microcrystalline cellulose (MCC)[26] on compositional and functional features of the fecal microbiota. Here, we extended this research to the human host and compared the effects of the fibers on perceived satiety and obesity-related surrogate endpoints in the same individuals. We then investigated whether these effects associated with fecal microbiota compositional features and mechanistic endpoints hypothesized to reflect host-microbiota interactions (SCFAs, bile acids, trimethylamine *N*-oxide [TMAO], gut hormones, cytokines, and barrier function measures). To determine the role of bacterial taxa involved in AX fermentation, we employed an *ex vivo* anaerobic incubation that combined bioorthogonal non-canonical amino acid tagging (BONCAT) with fluorescence-activated cell sorting (FACS) to identify the metabolically active bacterial consortia.[29] Finally, we integrated data using random forest analysis to determine whether effects on satiety or surrogate endpoints could be predicted by compositional features of the gut microbiota or related mechanistic endpoints.

METHODS

Study Design

This single-blinded, parallel-armed, six-week randomized controlled exploratory trial was registered at ClinicalTrials.gov (NCT02322112; see online supplementary methods for registration information), and the study procedures have been described elsewhere.[26] Briefly, 38 individuals with a body mass index (BMI) between 25–35 kg/m^2 were enrolled and randomly assigned to consume either AX or MCC at 25 g/day (females) or 35 g/day (males) fiber doses (**Figure 1**). AX was BIOFIBER GUM, a fermentable, long-chain corn bran isolated AX (Agrifiber Holdings LLC, USA), while MCC was MICROCEL MC-12, a non-fermentable, large particle (160-micron average) wood-derived cellulose (Blanver Farmoquimica LTDA, Brazil).[26]

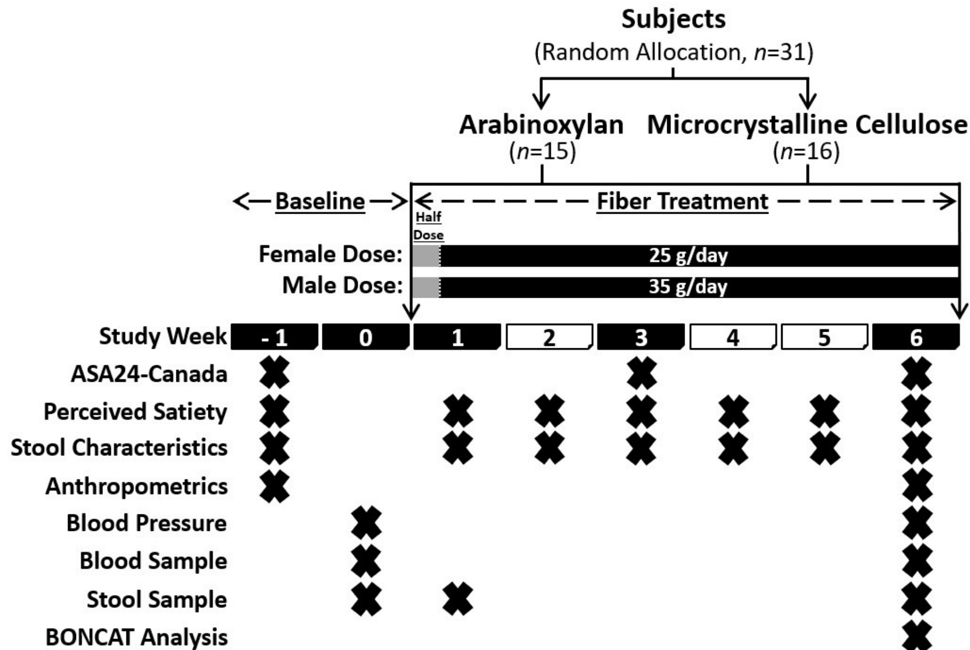


Figure 1. Study design of the randomized controlled trial. BONCAT, bioorthogonal non-canonical amino acid tagging; ASA24-Canada, Canadian version of the web-based Automated Self-Administered 24-hour Dietary Assessment Tool; stool characteristics, self-reported stool consistency and bowel movement frequency.

Dietary Assessment

The Canadian version of the web-based Automated Self-Administered 24-hour Dietary Assessment Tool was used to assess dietary intake at baseline and during the intervention (**Figure 1**).[30] See online supplementary methods for dietary analysis methodology.

Blood and Fecal Sampling

Blood samples were collected at baseline and week 6 after a 12-hour overnight fast using serum separation, K_2EDTA-coated, and P800 tubes (BD Biosciences, USA). After centrifugation, serum, plasma, and inhibitor-treated plasma aliquots were stored at −80°C. P800 tubes are pre-coated with K_2EDTA plus a proprietary cocktail of protease, esterase, and DPP-IV inhibitors to prevent hormone degradation. Fecal samples were collected at baseline, week 1, and week 6, as previously described.[26]

Perceived Satiety and Anthropometrics

To evaluate perceived satiety, the validated Satiety Labeled Intensity Magnitude (SLIM) questionnaire[31] was completed at baseline and during each intervention week within 30 mins of

waking and 30-60 mins of consuming a meal with AX or MCC added (**Figure 1**). Anthropometric and body composition measurements were obtained at baseline and week 6. See online supplementary methods for a detailed description of the SLIM scale and anthropometric measurements.

Obesity-Related Surrogate Endpoints

Blood pressure was measured at baseline and week 6. For assessment of lipid and glucose metabolism, total cholesterol, high-density lipoprotein cholesterol, triglycerides, and glucose were quantified in serum, while insulin was measured in inhibitor-treated plasma. Then, homeostatic model assessment of insulin resistance (HOMA-IR), quantitative insulin sensitivity check index (QUICKI), and low-density lipoprotein cholesterol values were calculated. For nonspecific clinical assessment of intestinal and systemic inflammation, fecal calprotectin and plasma C-reactive protein (CRP) were quantified, respectively. Finally, whole blood was collected for immediate quantification of complete blood count parameters. See online supplementary methods for a detailed description of surrogate endpoint characterization.

Fecal Microbiota Compositional Features and Mechanistic Endpoints Hypothesized to Reflect Host-Microbiota Interactions Implicated in the Pathophysiology of Obesity

Fecal Microbiota Compositional Features

Findings from the fecal microbiota profiling by 16S rRNA gene amplicon sequencing are published elsewhere.[26] To identify bacterial taxa involved in AX utilization within the fecal microbiota, we applied BONCAT,[29] a fluorescence-based single-cell labeling of cellular activity, to week 6 fecal samples from participants that consumed AX. Fecal slurries were incubated for 6-hours under anaerobic conditions with AX and *L*-azidohomoalanine, a marker of cellular activity. Activated bacterial cells were recovered using FACS and profiled using 16S rRNA gene amplicon sequencing. Sequence data were analysed using a bioinformatic approach based on amplicon sequence variants (ASVs)[32] (see online supplementary methods and **Figures S1 and S2** for details). The 16S rRNA gene amplicon sequencing data were deposited in the NCBI Sequence Read Archive and are available for download under BioProjects PRJNA564636 (fecal) and PRJNA630848 (*ex vivo*).

Mechanistic Endpoints

Fecal Microbiota Functional Features

TMAO, a metabolite generated in the liver from the oxidation of bacterial trimethylamine, was measured in serum, as previously described.[33] Fecal bile acids were quantified by targeted metabolomics of 61 bile acids with ultrahigh performance liquid chromatography/multiple-reaction monitoring-mass spectrometry at the University of Victoria Genome British Columbia Proteomics Centre, as previously described.[34] Bile acids detected in ≥90% of fecal samples (31 compounds) were then used in further analyses. Methodologies for the characterization of fecal SCFAs, stool consistency, and bowel frequency were published previously.[26]

Molecular Markers

To gain mechanistic insight into the molecular interplay between gut microbiota and surrogate endpoints, we characterized molecular markers of host-microbiota interactions hypothesized to influence immune and metabolic pathologies. Inhibitor-treated plasma was used to quantify hormonal regulators of appetite and glucose metabolism: ghrelin, peptide tyrosine tyrosine (PYY), leptin, active glucagon-like peptide-1 (GLP-1), and glucagon. In addition, the adipocyte-derived hormone adiponectin, and cytokines tumor necrosis factor-α (TNF-α), interleukin (IL)-6, IL-8, and IL-10 were measured in plasma. Plasma lipopolysaccharide-binding protein and fecal albumin and zonulin were further quantified as measures of gut barrier function recently validated by our group.[35] See online supplementary methods for a detailed description of mechanistic endpoint characterization.

Statistical Analyses

Prior to statistical analysis, outlying data were identified and removed based on a mean ± 5 standard deviation cut-off (≤2 participants per endpoint).[36] To assess the overall effects of fiber supplementation on perceived satiety and surrogate endpoints, data were ordinated using principal component analysis (PCA). Then, between-group differences (AX-vs-MCC) were assessed by permutational multivariate analysis of variance (PERMANOVA) based on Manhattan distances.[37] For perceived satiety, surrogate endpoints, mechanistic endpoints (apart from SCFAs[26]), and diet variables, repeated measures one-way ANOVA and paired *t*-tests with permutations (n=1000) were applied to compare within-group differences relative to baseline.

Between-group differences were assessed by unpaired permutational *t*-tests (n=1000). For surrogate and mechanistic endpoints, $p<0.01$ was considered statistically significant to account for multiple comparisons, while $p<0.05$ was considered significant for remaining analyses. Analysis of covariance (ANCOVA) models were then used to adjust for potential confounding effects. Due to limitations in statistical power (modest sample size), separate ANCOVA models were performed for each covariate. Data are reported as means ± standard deviations.

Differentially abundant taxa between the bacterial consortia recovered by FACS after a 6-hour incubation in the presence of AX and the total fecal bacterial communities after 0-hour and 6-hour incubations were identified using the R package DEseq2,[38] where false discovery rate (FDR) adjusted q values <0.05 were considered significant. To identify potential determinants of host-microbiota interactions that are predictive of surrogate endpoint changes, separate random forest classifiers (RFCs) were independently trained on microbiota compositional features, mechanistic endpoints, and macronutrient intake datasets. Area under the receiver operating characteristic curves (AUC-ROCs) were then used to evaluate RFC performance, where AUC-ROCs ≥0.70 were considered significant. Spearman's correlations, followed by multivariate generalized linear models (GLMs) to adjust for relevant covariates, were performed between the endpoint and its best predictors, where FDR adjust q values <0.1 and p values <0.05 were considered significant.

RESULTS

Baseline Characteristics of Study Participants

To compare the effects of AX and MCC supplements at high doses (females: 25 g/day; males: 35 g/day) and high purity (>80% fiber) on human health, we performed a six-week randomized controlled exploratory trial in adults with overweight or class-I obesity. A total of 31 participants (AX: 10F and 5M; MCC: 11F and 5M) aged 32.9±8.5 years with a BMI of 28.7±2.3 kg/m^2 completed the study protocol and were included in statistical analyses. No differences in age, sex, surrogate endpoints, and other study variables were detected between the treatment groups at baseline (see **Table S1** for baseline characteristics).

Dietary Intake

Dietary fiber intake, assessed by two 24-hour recalls, significantly increased by 142% and 171% during fiber supplementation as compared to baseline (p=0.0002 for both AX and MCC, permutational t-test) with no significant difference between groups. This corresponded to an increase from 21±6 and 19±8 g/day to 46±12 and 44±8 g/day for AX and MCC, respectively (**Table S2**). Sugar consumption also increased by 35% and 46% during AX (p=0.04) and MCC (p=0.03) supplementation. This observation can potentially be explained by the subjects incorporating the powdered supplements into foods and drinks that contained sugar, such as yogurt and smoothies. No significant differences were detected between-groups (p>0.1), suggesting similar dietary changes were made by both groups.

AX and MCC Differ Markedly in their Effects on Health

While PCA ordination of perceived satiety and surrogate endpoints revealed no differences at baseline (p=0.77, PERMANOVA; **Figure 2A**), AX induced percentage changes in the endpoints that were distinguishable from those of MCC (p=0.006; **Figure 2B**). We observed that subjects that consumed AX reported higher SLIM scores (*i.e.* more perceived satiety) 30-60 mins after consuming a meal (henceforth referred to as 'satiety after a meal') with AX compared to subjects that consumed a meal with MCC (p=0.035, permutational t-test) (**Figure 2C**). Between-group differences were detected during weeks 2 (p=0.04), 4 (p=0.007), and 6 (p=0.03). AX also consistently, though not significantly, increased satiety after awakening over the entire treatment period compared to MCC (p=0.12; **Figure 2D**), with a significant between-group difference detected during week 5 (p=0.03).

For surrogate endpoints related to diabetes, AX consumption reduced HOMA-IR (insulin-resistance index; p=0.006, permutational t-tests; **Figure 2E**) and increased QUICKI (insulin-sensitivity index, p=0.006; **Figure 2F**) compared to the percent change in the MCC group. Within the AX group, reductions of 10% and 12% were detected in insulin (p=0.046) and HOMA-IR (p=0.04), respectively, that approached significance when compared to baseline.

MCC reduced fecal calprotectin, a surrogate endpoint of intestinal inflammation, by 39% relative to baseline (p=0.004) and the percent change in the AX group (p=0.002) (**Figure 2G**). Further evaluation of the molecular markers revealed a 7% reduction from baseline in TNF-α by MCC

(p=0.004; **Figure 2H**). Molecular markers related to satiety and glucose homeostasis (*e.g.* ghrelin, PYY, GLP-1, glucagon), CRP, or blood pressure were not significantly altered by fiber supplementation (**Table S3**).

To confirm significant effects were independent of potential confounders, ANCOVA models were performed using age, sex, and changes in total fiber and sugar consumption as covariates (**Table S4**). Stool characteristic variables were also included as covariates since obesity has been associated with altered bowel habits[39] and, in our preceding study,[26] AX and MCC promoted more frequent bowel movements (p<0.05, generalized estimating equation model) while AX promoted softer stool consistencies compared to MCC (p=0.049) (**Table S3**). These analyses suggest that the effects of AX on satiety and insulin resistance and MCC on inflammation were not confounded by host factors, dietary changes, or stool characteristics (p<0.05; **Table S4**).

Fecal Bile Acids were Altered by MCC Supplementation

Our preceding study showed that, while AX consumption directed microbial output of SCFAs in favor of propionate, fecal SCFAs were not affected by MCC.[26] Since bile acid derivatives also possess immunomodulatory and metabolic properties,[18,19] we applied targeted metabolomics to determine fecal bile acids. Analysis of the 31 dominant bile acids (present in ≥90% of samples) revealed that MCC decreased fecal concentrations of apocholic acid (p=0.009, permutational *t*-test; **Table 1**) and hyodeoxycholic acid (p=0.009) relative to baseline. Reductions in fecal concentrations of total bile acids and the four secondary bile acids deoxycholic acid, isolithocholic acid (ILCA), glycodeoxycholic acid (GDCA), and taurolithocholic acid (TLCA) also approached statistical significance (p<0.05). By contrast, AX did not reduce bile acid concentrations relative to baseline but, when compared to MCC, increased 7αOH-3-oxo-4-cholestenoic acid (p=0.0096). Changes induced by both treatments also showed large standard deviations, indicating that bile acid shifts were highly individualised. Overall, our findings suggest that consumption of large particle MCC alters the fecal bile acid profile by reducing secondary bile acid concentrations.

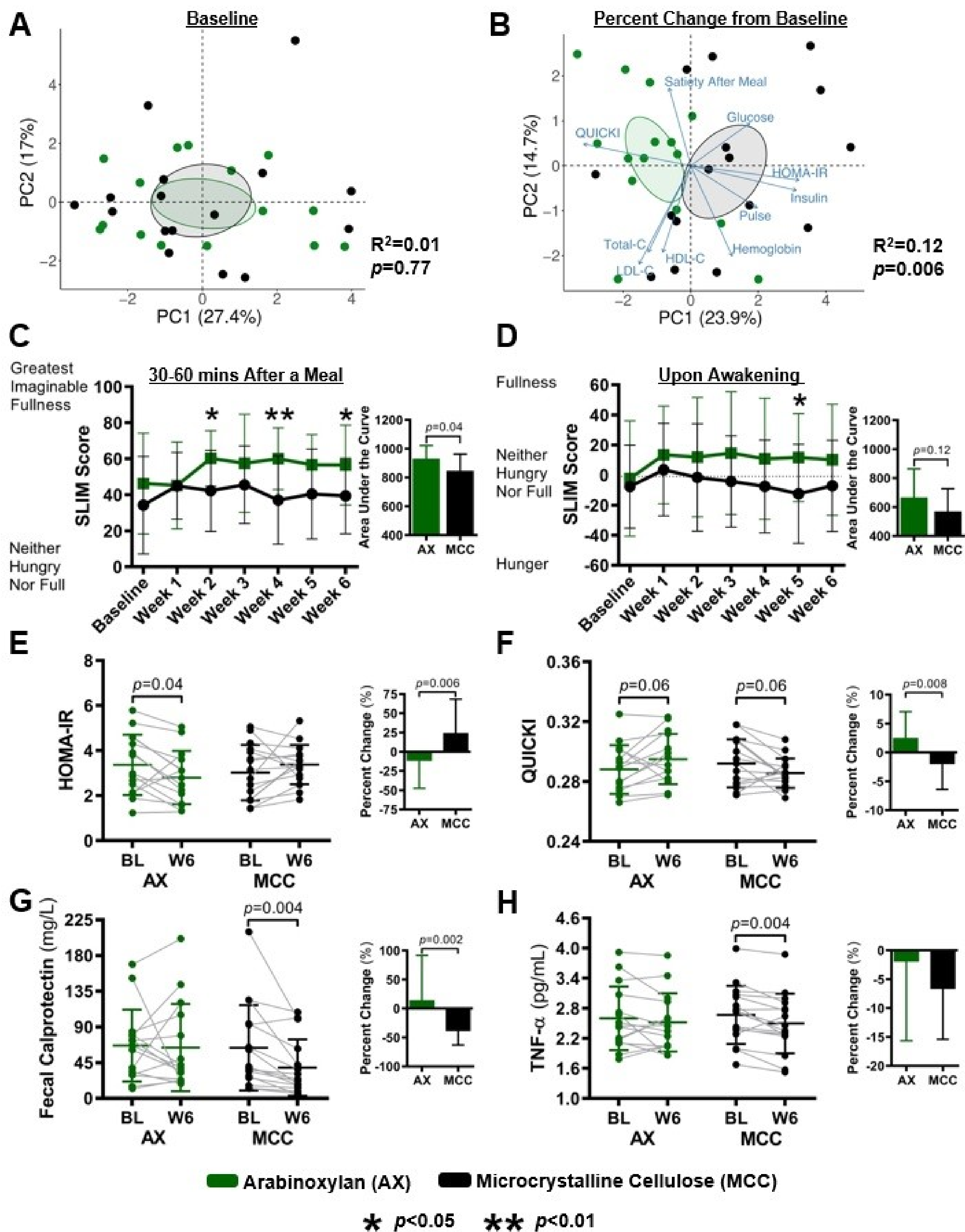


Figure 2. Effects of AX and MCC supplementation on satiety and surrogate endpoints. *Legend continued on the next page.*

Principal component analysis (PCA) plots show **(A)** perceived satiety and surrogate endpoints at baseline and **(B)** their percentage change from baseline per AX and MCC groups. Line graphs show weekly SLIM scale ratings **(C)** 30-60 mins after consuming a meal with AX or MCC and **(D)** upon awakening. Bars (insets) represent the area under the SLIM score curve (AUC_{BL-W6}). Scatter plots show **(E)** HOMA-IR, **(F)** QUICKI, **(G)** fecal calprotectin, and **(H)** TNF-α at baseline and week 6 of AX or MCC supplementation. Bars (insets) represent the percent change from baseline values per group. To assess within-group changes relative to baseline, data were analyzed for (C and D) using repeated measures one-way ANOVA with permutations and for **(E to H)** using paired permutational *t*-tests. To assess between-group differences, data were analyzed for **(A and B)** using PERMANOVA based on Manhattan distance and for **(C to H)** using unpaired permutational *t*-tests. Statistical significance was set for **(A to D)** at $p<0.05$ and for **(E to H)** at $p<0.01$. Data for **(C to H)** presented as mean ± SD; for **(E to H)** symbols represent individual samples. AX, arabinoxylan; HOMA-IR, homeostatic model assessment of insulin resistance; MCC, microcrystalline cellulose; QUICKI, quantitative insulin sensitivity check index; SLIM, satiety labeled intensity magnitude; TNF-α, tumor necrosis factor-α.

Table 1. Fecal Concentrations of Bile Acids at Baseline and Six Weeks of Arabinoxylan or Microcrystalline Cellulose Supplementation.

Fecal Bile Acids (nmol/g)	Arabinoxylan (*n*=15)				Microcrystalline Cellulose (*n*=16)				Between Group Change *p* value
	Baseline	Week 6	Within Group *p* value	Change (W6-BL)	Baseline	Week 6	Within Group *p* value	Change (W6-BL)	
Total bile acids	90140.7± 52067.3	75570.8± 36138.7	0.13	-14569.9±35493.8	76748.0±45152.6	53400.4± 42156.7	**0.03**	-23347.6±41145.7	0.88
Cholic acid	1289.4±4663.3 [a]	623.9±1541.4 [a]	0.77	-665.5± 4094.5 [a]	49.9±79.3	781.0±2150.8	0.45	731.1±2154.9	0.64
Chenodeoxycholic acid	1392.3±2752.4 [a]	2339.4±6773.5 [a]	0.70	947.1±6328.0 [a]	78.1±96.4	640.2±1739.7	0.46	562.1±1748.3	1.00
Deoxycholic acid	46951.5±31821.9	39324.1±24346.5	0.28	-7627.4±26367.7	38305.5±24802.5	25727.3±21327.7	**0.04**	-12578.2±23022.2	0.88
Lithocholic acid	32898.7±20028.4	24456.2±14586.5	0.06	-8442.5±15869.9	31183.8±15926.9	21373.9±16591.2	0.06	-9809.9±18874.8	0.92
Allocholic acid	58.6±81.7	56.5±79.8	0.93	-2.2±106.5	21.7±26.2	18.5±26.0	0.58	-3.2±22.6	1.00
Dehydrolithocholic acid	215.6±123.4	173.1±108.2	0.16	-42.5±112.5	425.7±759.4 [a]	187.1±171.9 [a]	0.05	-238.6±636.0 [a]	0.26
7-Ketodeoxycholic acid	23.7±67.6 [a]	19.3±32.4 [a]	0.88	-4.4±57.1 [a]	5.1±4.4	58.1±188.1	0.45	53.0±188.0	0.23
7-Ketolithocholic acid	144.8±422.4 [a]	30.7±35.8 [a]	0.27	-114.1±393.6 [a]	11.0±9.7	31.1±79.6	0.46	20.2±80.8	**0.047**
Alloisolithocholic acid	124.0±103.4	110.0±90.0	0.43	-14.0±65.7	187.2±140.3 [a]	118.3±99.5 [a]	0.07	-68.9±135.2 [a]	0.26
Apocholic acid	742.1±681.7	493.9±246.7	0.15	-248.2±626.0	836.2±1074.2 [a]	318.4±177.8 [a]	**0.009 ***	-517.8±1009.5 [a]	0.88
Hyodeoxycholic acid	145.7±120.9	136.7±119.8	0.80	-9.1±133.9	207.6±192.2 [a]	96.0±81.5 [a]	**0.009 ***	-111.6±185.4 [a]	0.08
Murocholic acid	18.8±19.8	12.1±10.8	0.18	-6.7±18.7	13.5±15.7	7.4±6.4	0.07	-6.1±12.9	0.92
Isolithocholic acid	1867.0±1020.8	1524.4±987.1	0.28	-342.6±1183.0	3133.7±2649.2	1669.6±2070.6	**0.016**	-1464.1±2298.5	0.11
12-Ketochenodeoxycholic acid	9.7±4.1	8.4±5.5	**0.03**	-1.4±2.4	7.3±3.1	6.7±3.5	0.61	-0.6±4.2	0.75
Nordeoxycholic acid	1.7±1.2	1.4±1.2	0.18	-0.3±0.8	2.5±2.1 [a]	1.6±1.4 [a]	0.06	-0.8±1.1 [a]	0.11
Glycochenodeoxycholic acid	16.3±14.7 [a]	16.9±12.3 [a]	0.90	0.6±15.6 [a]	26.8±32.9	13.1±14.6	0.11	-13.6±34.2	0.15
Glycocholic acid	16.2±13.9	17.3±13.8	0.78	1.1±15.6	15.9±18.8 [a]	9.0±7.1 [a]	0.07	-6.8±14.6 [a]	0.18
Glycodeoxycholic acid	18.5±14.8	15.0±11.1	0.45	-3.5±17.8	24.1±24.4 [a]	8.3±6.1 [a]	**0.014**	-15.8±24.4 [a]	0.20
Glycolithocholic acid	2.4±2.1	1.6±1.3	0.30	-0.8±2.7	2.0±1.6	1.4±1.1	0.16	-0.7±1.8	1.00
Taurochenodeoxycholic acid	11.9±27.2	7.6±13.5	0.69	-4.3±30.7	7.8±10.1 [a]	6.7±18.2 [a]	0.88	-1.1±21.0 [a]	0.76
Taurocholic acid	6.5±6.0	15.5±37.4	0.61	9.0±38.1	5.4±5.7 [a]	8.5±22.4 [a]	0.92	3.1±21.2 [a]	0.59
Taurolithocholic acid	0.8±1.1	2.1±4.6	0.20	1.3±4.3	3.3±5.9	1.1±2.8	**0.03**	-2.2±4.0	**0.018**
Ursocholic acid	295.3±642.2	307.1±849.4	0.96	11.8±1074.5	30.0±51.2	259.7±800.7	0.26	229.7±804.7	0.66
Norcholic acid	9.5±4.3	8.4±4.7	0.23	-1.1±3.4	8.0±5.1	6.2±5.5	0.15	-1.8±4.8	0.39
3βOH-5-cholestenoic acid	227.1±154.4	225.5±184.5	0.96	-1.6±145.9	120.5±82.4	86.9±33.3	0.07	-33.6±67.9	0.84
7αOH-3-oxo-4-cholestenoic acid	1.7±1.3 [a]	4.0±4.0 [a]	**0.04**	2.3±4.3 [a]	1.6±1.3	1.4±1.6	0.39	-0.2±0.9	**0.0096 ***
Lithocholic acid 3-SO_4^{2-}	113.9±263.0	83.1±257.5	0.59	-30.8±275.2	34.4±65.8 [a]	98.3±301.0 [a]	0.39	63.9±315.5 [a]	0.54
Deoxycholic acid 3-SO_4^{2-}	33.7±96.7 [a]	616.9±2293.9 [a]	0.78	583.3±2290.2 [a]	274.3±745.5	128.6±428.1	0.39	-145.7±857.7	0.19
Cholic acid 3-SO_4^{2-}	65.5±172.7	38.9±147.1	0.96	-26.6±237.8	74.5±202.6	0.8±1.0	0.39	-73.7±202.4	0.90
Glycolithocholic acid 3-SO_4^{2-}	5.0±8.2	2.6±1.3	0.27	-2.4±8.1	6.7±8.2 [a]	4.4±8.0 [a]	0.39	-2.2±12.1 [a]	0.94
Glycodeoxycholic acid 3-SO_4^{2-}	1.0±1.0	0.6±0.5	0.20	-0.4±1.1	1.2±1.6 [a]	0.4±0.4 [a]	0.39	-0.8±1.5 [a]	0.76

Statistical significances of within-group shifts were determined by paired permutational *t*-tests, while between-group differences (AX vs MCC; week 6 - baseline) were determined by unpaired permutational *t*-tests. Data are means ± SD. * Statistical significance was set at $p<0.01$, bolded *p* values without an asterisk (*) are approaching statistical significance ($p<0.05$). [a] One outlier >5*SD from the mean was excluded.

Identification of Bacterial Consortia Involved in AX Degradation

Considering that membership of the bacterial consortia involved in fiber fermentation likely dictates the production of health-related metabolites,[40] we aimed to identify which bacterial taxa utilize AX or its break-down products within the fecal microbiota of each subject.[29] We used BONCAT to label metabolically active bacterial cells within the fecal microbiota after a 6-hour *ex vivo* anaerobic incubation with AX. Active cells were then sorted by FACS and profiled by 16S rRNA gene amplicon sequencing (**Figures S1 and S2**).

Compared to the total bacterial community, the active consortia had lower α-diversity (Shannon index: q=0.0008, one-way ANOVA with permutations) and richness (Chao1 index: q<0.0001) with, on average, 28% fewer ASV numbers (q=0.0002; **Table S5**). To compare membership of individual taxa between the active consortia and fecal bacterial community, a differential abundance test (DESeq2[38]) was applied. This analysis revealed that members of the families *Bacteroidaceae*, *Lactobacillaceae,* and *Enterobacteriaceae* and ASVs classified as *Bacteroides koreensis*, *Bacteroides plebeius*, *Bacteroides xylanisolvens*, *Lactobacillus* spp., and *Escherichia/Shigella* spp. were more abundant in the active consortia (q<0.05; **Table S5**). Several putative secondary fermenters were also metabolically active during incubation with AX but less abundant in the active consortia (q<0.05). This included ASVs related to the SCFA producers *Coprococcus eutactus*, *Faecalibacterium prausnitzii*, and *Dialister invisus*,[41] which might be utilizing sugars and metabolic by-products (*e.g.* acetate and lactate) released during AX degradation.[26] Overall, these results suggest that degradation of AX is not limited to a few cooperative species but extends to numerous members of the broader bacterial community and involves several secondary fermenters that may contribute to SCFA output.

Identification of Microbiota-Related Predictors of Satiety and Surrogate Endpoints

To gain mechanistic insight into the role of the gut microbiota in the physiological effects of the dietary fibers, we integrated the significant findings on perceived satiety and surrogate endpoints with microbiota compositional features (fiber-responsive bacterial taxa in feces[26] and active consortia assessed by BONCAT), mechanistic endpoints (fecal SCFAs,[26] bile acids, TMAO, and molecular markers [gut hormones, cytokines, and barrier function measures]), and calorie-adjusted macronutrient intake datasets using a machine learning approach. For each endpoint

affected by fiber consumption, high- and low-responders were first identified according to the study cohort median, and then datasets were used as predictor variables for the training of independent RFCs to rank microbiota-related predictors that discriminate high-responders from low-responders (**Figures S3A and S3B**).

For satiety after a meal, only RFCs trained on the metabolically active taxa (as established by BONCAT) could predict self-reported satiety during AX consumption, with the 14 differentially abundant ASVs (AUC-ROC=0.95; **Figure 3A**) having better prediction accuracy than all active ASVs (AUC-ROC=0.82; **Figure 3B**). Of the ASVs important for this classification, at least half were likely SCFA producers.[41] A positive correlation was detected between satiety and *D. invisus* (ASV6pygnt; r_s=0.63, q=0.09, Spearman's correlation), which is a propionate producer (**Figure 3A**).[41] Further, formate-producing *Dorea formicigenerans* (ASV2xmw96; r_s=-0.81, q=0.009) and butyrate-producing *Eubacterium ramulus* (ASV56kx74; r_s=-0.60, q=0.09) were highly predictive but inversely associated with satiety (**Figure 3B**). Although AX induced propionate,[26] which is implicated in satiety, RFCs based on SCFA shifts in fecal samples could not predict satiety after a meal (OOB error>0.6). Other variables could not predict significant results (**Figure S3A**).

The only RFCs that predicted HOMA-IR were AX-induced shifts in secondary bile acids (AUC-ROC=0.77; **Figure 3C**). Fecal calprotectin responses were also only predicted by MCC-induced shifts in secondary bile acids (AUC-ROC=0.72; **Figure 3D**). However, different secondary bile acids were important. HOMA-IR responses associated inversely with reductions in the concentrations of lithocholic acid (LCA) (r_s=-0.62, q=0.09) and its derivative ILCA (r_s=-0.60, q=0.09) (**Figure 3C**), while fecal calprotectin responses showed negative correlations with reductions in conjugated bile acids TLCA (r_s=-0.68, q=0.05) and GDCA (r_s=-0.63, q=0.07) (**Figure 3D**). Despite these significant associations, fiber-induced reductions in bile acids were only detected in low-responders but not high-responders (**Figures 3C and 3D**). This analysis suggests that, although bile acid shifts were linked to both insulin resistance and intestinal inflammation and potentially involved in the regulation of these endpoints, the immune and metabolic benefits detected in our study were not driven by fiber-induced changes in fecal bile acid concentrations.

For the TNF-α response, only the RFC based on calorie-adjusted intakes of macronutrients at baseline could predict the effect of MCC (AUC-ROC=0.73; **Figure 3E**). Although the most discriminative nutrient was saturated fat, consumption at baseline did not significantly associate with TNF-α responses (r_s=0.46, *q*=0.16). Effects on TNF-α, together with satiety, HOMA-IR, and fecal calprotectin, were not predicted by fiber-induced shifts in fecal microbiota composition or other mechanistic endpoints that reflect host-microbiota interactions (SCFAs, TMAO, gut hormones, cytokines, and barrier function measures), as prediction accuracies were poor (AUC-ROCs <0.70).

To assess whether associations detected by RFCs were independent of potential confounders, we used multivariate GLMs to control for sex, age, changes in total fiber and sugar intake, stool consistency, and bowel frequency as covariates (**Table S6**). These analyses suggested that the associations observed with AX-induced changes in satiety and HOMA-IR and MCC-induced changes in fecal calprotectin were not majorly confounded by host factors, dietary changes, or stool characteristics (*p*<0.1).

Finally, as health-relevant features of the gut microbiota were significantly altered by AX and MCC supplementation, additional univariate GLMs were performed to confirm whether these responses associated with effects on perceived satiety and surrogate endpoints (**Figure S4**). This analysis reaffirmed that the dominant fiber-induced shifts in fecal microbiota composition, propionate, and bile acids were not linked to the physiological benefits of fiber supplementations (*q*>0.1).

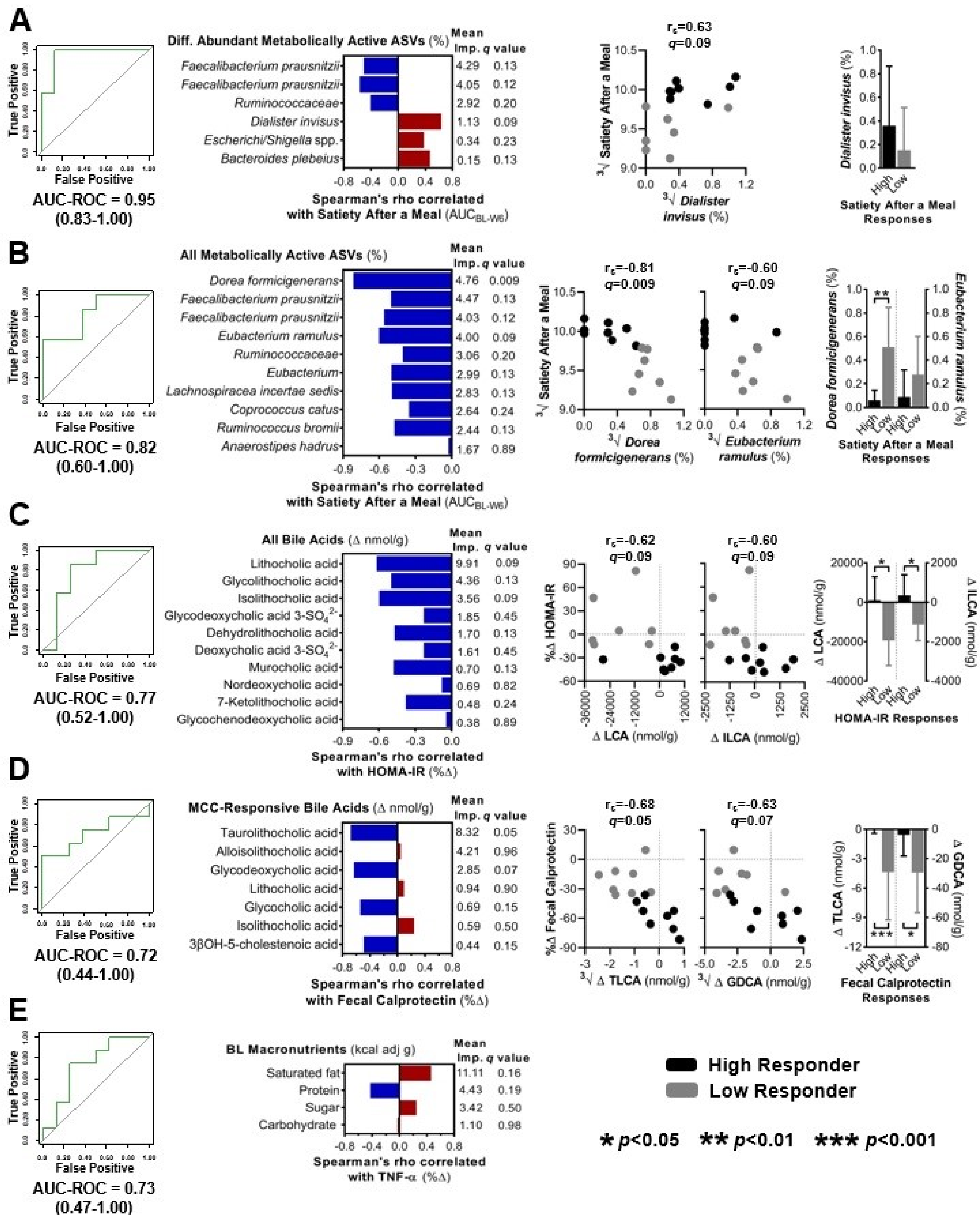


Figure 3. Identification of gut microbiota compositional features and mechanistic endpoints that predict satiety and surrogate endpoint responses by machine learning. *Legend continued on the next page.*

(left) AUC-ROC curves show the performance accuracy of random forest classifiers trained to predict high-vs-low responders for: **(A and B)** perceived satiety after a meal with AX using the relative abundance of bacterial taxa activated during *ex vivo* incubation with AX; **(C)** HOMA-IR and **(D)** fecal calprotectin for AX and MCC, respectively, using fecal bile acid shifts; and **(E)** TNF-α for MCC using baseline intakes of calorie-adjusted macronutrients. (center) Horizontal bars represent Spearman's correlation coefficients between endpoints and **(A and B)** metabolically active ASVs, **(C and D)** fecal bile acids, or **(E)** macronutrients shown to be important for predicting responses. Mean importance values were determined by random forest, which identifies factors that contribute the most to the model. (right) Scatter plots show the association between endpoints and the most discriminative microbiota-related factors that correlate with AX-induced **(A and B)** satiety after a meal and **(C)** HOMA-IR attenuation, and **(D)** MCC-induced fecal calprotectin attenuation. Vertical bar graphs show the most discriminative microbiota-related factors grouped by high- and low-responders. High-responders (black) and low-responders (gray) were defined according to the study cohort median. Statistical significance was set at $p<0.05$ and FDR corrected q values <0.1. Δ, absolute change from baseline to week 6; %Δ, percent change from baseline to week 6; $^3\surd$, cube root transformed prior to analysis; All ASVs, amplicon sequence variants with average relative abundances ≥0.15%; AX, arabinoxylan; AUC-ROC, area under the receiver operating characteristic curve; BL, baseline; Diff. Abundant ASVs, differentially abundant amplicon sequence variants among the bacterial consortia recovered by fluorescence-activated cell sorting; GDCA, glycodeoxycholic acid; HOMA-IR, homeostatic model assessment of insulin resistance; ILCA, isolithocholic acid; LCA, lithocholic acid; MCC, microcrystalline cellulose; TLCA, taurolithocholic acid; TNF-α, tumor necrosis factor-α.

DISCUSSION

This study revealed fiber fermentation by the gut microbiota mediates some of its health effects in humans, as the ability of AX to improve satiety was predicted by bacterial taxa involved in its utilization as established by BONCAT. In contrast, AX-induced attenuation of insulin resistance was linked to changes in the fecal bile acid pool, specifically concentrations of LCA and its derivatives. Interestingly, MCC, which was meant to function as a fermentation-negative placebo, promoted anti-inflammatory effects in the gut by reducing fecal calprotectin levels. This response was also predicted by bile acid shifts, but the bile acids differed from those linked to AX, with highest correlations among TLCA and GDCA. Overall, our study provides novel mechanistic hints as to how physicochemically-distinct fibers affect the gut microbiota, perceived satiety, and surrogate endpoints that underlie the pathophysiology of obesity in humans.

The ability of AX to induce satiety is in agreement with previous research,[42] as both long-chain AX supplements[43,44] and whole grains rich in AXs[45-47] have been reported to enhance satiety.

Due to its viscous properties, AX may delay gastric emptying thereby prolonging post-prandial satiety.[10] Our results indicate that microbial fermentation of AX is also a determinant, as satiety effects were predicted by personalised bacterial taxa shown to utilize AX. While we can only speculate on these associations, *D. invisus*, a propionate producer,[41] was positively associated with satiety, and propionate can induce satiety in humans.[48,49] Associations between satiety and fecal propionate were not detected, even though fecal propionate levels were elevated by AX.[26] In addition, associations with AX-induced shifts in the relative fecal abundance of bacterial taxa were not observed. Fecal measurements of SCFAs are confounded,[50,51] and not every species that contributes to fiber fermentation becomes enriched in feces.[5,26] By directly detecting primary degraders and secondary fermenters of AX, our findings suggest that BONCAT has increased sensitivity for the identification of potential health-relevant taxa that utilise fiber. Although taxa detected by BONCAT were excellent predictors of satiety, with an AUC-ROC of 0.95, we acknowledge that exact mechanisms cannot be elucidated with our data. Nevertheless, our findings, together with reports in the literature[48,52,53], suggest that microbial fermentation and subsequent production of propionate might be one mechanism underlying the satiety-enhancing properties of AX.

AX further reduced HOMA-IR, a surrogate endpoint of insulin resistance, which aligns with previous research[42,43,54-56] and an EFSA health claim,[9] indicating positive outcomes of long-chain AX on glucose metabolism are reproducible. Effect sizes were comparable with other strategies aimed to attenuate insulin resistance in individuals with obesity, such as a plant-based diet[57] or fecal microbiota transplantation.[58] However, consuming AXs as supplements or within foods would be comparatively more cost-effective and less burdensome for individuals. Contrary to AX, HOMA-IR increased in the MCC group, an effect likely attributable to the elevated sugar consumption that was detectable in both treatment groups. Our findings, therefore, indicate that AX may have counteracted the detrimental effects of sugar on insulin sensitivity.

MCC induced local and systemic anti-inflammatory effects by reducing fecal calprotectin and plasma TNF-α; effects that - to our knowledge - have yet to be reported in humans. These findings are, however, in agreement with research in mice, where very high-cellulose diets mitigated chemically-induced colitis[59,60] and improved LPS-induced intestinal permeability.[61] As

our findings with MCC are novel in humans, future research should confirm whether anti-inflammatory properties are specific to large particle, highly crystalline cellulose. In summary, our study provides evidence that these two physicochemically-distinct fibers could be used in isolation or perhaps as a fiber blend to target and regress specific biological processes that underlie the pathophysiology of obesity and related comorbidities.

Although the physiological effects of AX and MCC differed, both responses were predictable, exclusively, by fecal shifts in secondary bile acids. For AX, HOMA-IR responses were inversely associated with shifts in LCA and ILCA, which have been shown to regulate glucose homeostasis through activation of FXR- and TGR5-mediated signaling pathways.[19] Alternatively, fecal calprotectin responses associated with MCC-induced reductions in TLCA and GDCA. While immunoregulatory properties of TLCA and GDCA remain poorly defined, TLCA may exert anti-inflammatory effects by inhibiting inflammasome signaling.[62] Differing from MCC, hemicelluloses such as AX can also bind bile acids.[63,64] These findings are relevant given the emerging role of bile acids in the regulation of metabolism and immunology.[18,19] While secondary bile acid shifts may contribute to individualized responses, they are unlikely to provide primary mechanistic explanations for the actions of AX and MCC, as only negative correlations were detected with immune and metabolic effects and these shifts were observed primarily in low-responders. Given that connections to other mechanistic endpoints were not detected, our findings do not provide evidence for microbiome-dependent effects of fiber on HOMA-IR and fecal calprotectin. In accordance with this, prior work in germ-free mice showed that attenuation of insulin resistance by resistant starch was microbiota-independent but correlated with fiber-induced changes to fecal bile acids.[24] Although reduced secondary bile acid concentrations may not provide primary mechanistic explanations, our findings for MCC are still relevant as secondary bile acids are generally considered to be cytotoxic.[65] Therefore, their reductions might underlie the protective effects of dietary fiber in colon cancer prevention.[66]

In this study, we attempted to test several hypothesized mechanistic links between the gut microbiota, dietary fiber, and human health. For instance, we assessed whether *B. longum*[67] or SCFAs[17] influenced systemic inflammation by altering barrier function or whether *P. copri*[15,68] or SCFAs[17,52,53] influenced gut hormones and, as a result, satiety and insulin sensitivity.

However, we did not detect such associations. Despite convincing evidence for mechanistic connections between SCFAs, the assessed molecular markers, and physiological effects of fermentable fibers in animal models,[4,5] our findings do not support such hypothesized links in humans as effects of AX supplementation were not associated with changes in fecal SCFAs, gut hormones, or barrier function measures. To our knowledge, such associations with improved surrogate endpoints have not been consistently reported, if at all, in humans consuming fermentable fibers. In addition, since the anti-inflammatory effects of the non-fermentable MCC are independent of SCFA production, it remains unclear whether immunomodulatory effects of fiber are caused by increased SCFA production in humans. Similarly, even though *B. longum* and *P. copri* were the numerically dominant AX-responders[26] and previously linked to improved inflammation,[67] satiety,[68] and insulin sensitivity[15] in humans, we detected no associations with these species in our study. While establishing such mechanistic explanations are challenging in humans,[25] particularly when sample sizes are modest and mechanistic endpoints are inherently confounded,[50,51] our findings do support the role of fiber fermentation in some physiological effects of fiber.

Overall, this study provides critical evidence for the tailored use of specific, physicochemically-distinct fibers at relevant doses to elicit targeted health outcomes in adults with obesity. Our findings further serve as a basis for a conceptual framework on the intelligent application of purified fibers for the prevention or treatment of chronic diseases, such as AX for type II diabetes and MCC for inflammatory bowel disease. Our integration analysis of perceived satiety, surrogate endpoints, gut microbiota compositional features, and mechanistic endpoints provides compelling evidence for potential mechanistic links between satiety and the human gut microbiota, while other important metabolic and immunological effects of fiber appear to be primarily microbiota independent. Further establishment of a mechanistic basis for the associations detected in this study might allow for optimized clinical efficacy through the development of distinct fiber structures or designer carbohydrates with clinically relevant physicochemical properties, such as viscosity, fermentability, or the ability to manipulate intestinal bile acid profiles.

SUPPLEMENTARY MATERIAL

Supplementary Methods

Clinical Trial Registration

This randomized controlled trial (RCT) was prospectively registered on July 2015 at ClinicalTrials.gov (NCT02322112) as part of a large four-arm parallel RCT that aimed to compare the effects of four structurally-distinct fibers (arabinoxylan [AX], acacia gum, resistant starch type-IV, and microcrystalline cellulose [MCC]) on the gut microbiota and human health, referred to as The Alberta FYBER (Feed Your gut Bacteria morE fibeR) Study (for original registration refer to Ref.[69]). In response to requests by reviewers of a grant application, which advised against including a premarket ingredient in a large human trial, the AX arm was separated from the original RCT on October 2016, and data from the 15 subjects that completed the protocol were analyzed independently (**Figure 1**). The study was approved by the University of Alberta Health Research Ethics Board (Pro00050274) and conducted at the University of Alberta Human Nutrition Research Unit (Alberta, Canada). All participants provided written informed consent prior to study enrollment.

Dietary Intake Assessment

To assess whether dietary fiber (DF) supplementation influenced the dietary intake of participants, two non-consecutive 24-hour dietary recalls were completed at baseline, and weeks 3 and 6 (**Figure 1**). Dietary recalls were completed using the Canadian version of the web-based Automated Self-Administered 24-hour Dietary Assessment Tool (ASA24-Canada-2014),[30] a method shown to be less burdensome than an interviewer-administered, Automated Multiple-Pass Method 24-hour dietary recall.[70] Output files from the ASA24-Canada-2014 include free text fields that report “unfound food” and “other” responses when the specific description of the food or drink could not be found by subjects. These entries were reviewed and inaccurately assigned default food codes were replaced with a more accurate food code.[71] Mean values of baseline (two recalls) and of weeks 3 and 6 (four recalls) were used in statistical analyses. Prior to assessing associations between AX- and MCC-induced changes in surrogate endpoints and both baseline diet and dietary changes, ASA24-Canada-2014 obtained diet data were first calorie-adjusted using methods described by Willett and Stampfer.[72]

Assessment of Perceived Satiety

Perceived satiety was evaluated at baseline and weekly during the intervention using the validated Satiety Labeled Intensity Magnitude (SLIM) questionnaire (**Figure 1**), which was a 100 mm, bidirectional hunger-fullness scale anchored by "greatest imaginable fullness" (50 mm) and "greatest imaginable hunger" (-50 mm), with "neither hungry nor full" in the center (0 mm).[31] Each week, a SLIM scale was completed within i) 30 mins of waking and ii) 30-60 mins of consuming a meal with AX or MCC added. For between-group comparisons, the area under the SLIM curve (AUC_{BL-W6}) was calculated by the linear trapezoidal method. When applicable, missing data points were imputed using the mean of the participants' known values, as previously described.[73]

Anthropometry, Body Composition, and Blood Pressure Measurements

Anthropometric and blood pressure measurements were obtained during baseline and week 6 visits, with height only assessed at baseline. To calculate body mass index (BMI), body weight was measured to the nearest 0.1 kg (Health o meter® Professional 752KL, Pelstar LLC, Illinois, USA) and height was measured to the nearest 0.1 cm (Digi-kit digital stadiometer, Measurement Concepts, Quick Medical, Washington, USA). Both measurements were taken twice in light clothing, with empty pockets, and shoes removed, and then mean values were used in statistical analyses. Waist circumference was also measured twice to the nearest 0.5 cm using a Gulick II plus tape measure (Country Technology Inc., Wisconsin, USA) according to the National Institutes of Health guidelines.[74] Body fat percentage was estimated in triplicate by bioelectrical impedance analysis (Tanita TBF-300A Body Composition Analyzer, Illinois, USA) using a proprietary equation. Systolic and diastolic blood pressure and heart rate were measured with an automatic sphygmomanometer (Welch Allyn, Hill-Rom Inc., Indiana, USA) after the participant had been sitting for a least 10 min.

Biochemical Analyses of Blood Samples

Apart from the complete blood count (CBC), all blood sample analyses were performed at the end of the study. In cases where analyte concentrations were below the lower limit of detection (LLOD), half-LLOD concentrations were used for statistical analyses. Whole blood CBC, serum glucose, triglycerides, total cholesterol, low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol were quantified at the University of Alberta, Alberta

Health Services laboratory (Alberta, Canada). CBC data were obtained on a Sysmex XN-10 analyzer (Sysmex Corporation, Kobe, Japan), and included red blood cells, white blood cells, hemoglobin, hematocrit, and platelets. Serum glucose and lipid data were obtained on a Beckman Coulter DxC 80 (Beckman Coulter Inc., California, USA), in which serum concentrations of LDL cholesterol were calculated using the Friedewald equation.

$$LDL\ Cholesterol = Total\ Cholesterol\ - \left(\frac{Triglycerides}{5}\right) - HDL\ Cholesterol$$

To determine active glucagon-like peptide-1 (GLP-1), insulin, glucagon, leptin, total ghrelin, and total peptide tyrosine tyrosine (PYY) concentrations, plasma samples were obtained from blood collected in BD™ P800 tubes (BD Biosciences, California, USA), which contain K_2EDTA and a proprietary cocktail of protease, esterase and DPP-IV inhibitors that prevented analyte degradation. The MesoScale Discovery® Human Active GLP-1/Insulin/Glucagon/Leptin MULTI-SPOT® Assay (Maryland, USA) was used to measure GLP-1 (LLOD 1.0 pg/mL, mean intra-assay coefficient of variation [CV] 6.6%), insulin (LLOD 11.0 pg/mL, CV 4.5%), glucagon (LLOD 21.0 pg/mL, CV 6.0%) and, leptin (LLOD 17.0 pg/mL, CV 5.1%). The MesoScale Discovery® U-PLEX® Metabolic Assay was used to measure ghrelin (LLOD 1.7 pg/mL, CV 3.2%) and PYY (LLOD 2.7 pg/mL, CV 2.4%). Fasting glucose and insulin data were used to estimate insulin resistance using the homeostatic model assessment of insulin resistance (HOMA-IR) and insulin sensitivity using the quantitative insulin sensitivity check index (QUICKI) as previously described.[75,76]

$$HOMA\ IR = (insulin\ [\mu IU/mL] * glucose\ [mmol/L])/22.5$$

$$QUICKI = 1/(log_{10}\, insulin + log_{10}\, glucose)$$

The remaining biochemical analytes were quantified in EDTA-treated plasma by MesoScale Discovery® electrochemiluminescence immunoassays using the SECTOR® Imager 6000 (MesoScale Discovery®), according to the manufacturer's protocol. Single-plex assays were used to measure high-sensitivity C-reactive protein (LLOD 1.33 pg/mL, CV 2.1%) and adiponectin (LLOD 0.005 ng/mL, CV 3.3%). The V-Plex Proinflammatory MULTI-SPOT® Assay was used to measure interleukin (IL)-6 (LLOD 0.06 pg/mL, CV 4.3%), IL-8 (LLOD 0.04 pg/mL, CV

3.7%), IL-10 (LLOD 0.03 pg/mL, CV 5.8%), and tumor necrosis factor-α (TNF-α; LLOD 0.04 pg/mL, CV 3.0%).

Markers of Gut Barrier Function

Lipopolysaccharide-binding protein was quantified in EDTA-treated plasma diluted 1:1300 in phosphate-buffered saline (PBS) using a sandwich enzyme-linked immunosorbent assay (ELISA; as previously described;[16] USCN Life Science and Technology, Texas, USA), with a LLOD of 1.2 ng/mL and CV of 12.6%. Fecal concentrations of calprotectin, albumin, and zonulin were measured in singles using ELISA assays (Catalog Numbers K6927, K6330, and K5600, Immundiagnostik AG, Bensheim, Germany), according to the manufacturer's protocol. Prior to ELISA assays, fecal samples were prepared using the Stool Sample Application System (Catalog Number K6998SAS, Immundiagnostik AG), which diluted fecal samples 1:100 in a proprietary extraction buffer.

Serum Trimethylamine *N*-Oxide (TMAO) Detection

Methodologies for the quantification of TMAO in serum by high-performance liquid chromatography - tandem mass spectrometry (HPLC-MS/MS) have been described in detail by Mi and colleagues with minor modifications.[33] Briefly, for the extraction of TMAO from serum, 50 µL of thawed serum was spiked with 50 µL of internal standard solution (TMAO-d9 and TMA-d9) and 150 µL of methanol with 0.1% formic acid. The mixture was then vortexed for 1 min and centrifuged at 10,000 rpm for 15 mins at 15°C. The supernatant was collected and stored frozen at -20°C until analysis. For sample derivatization, 25 µL of supernatant was reacted with 50 µL of ethyl bromoacetate (4mg/ml in acetonitrile) in the presence of 3 µL concentrated ammonium hydroxide for 40 mins at room temperature. Then, HPLC-grade water containing 0.5% formic acid was added to obtain the final volume of 500 µL and then stored at -20°C until HPLC-MS/MS analysis.

An Agilent 1200 series HPLC system (Agilent Technologies Inc., California, USA) coupled to a 3200 QTRAP mass spectrometer (AB SCIEX, Ontario, Canada) was used under turbospray positive mode to analyze standard and sample solutions. An Ascentis Express HILIC column (15 cm × 2.1 mm, 2.7 µm particle size; Sigma, Missouri, USA) was used at room temperature for LC

separation. Composition of the mobile phase used for isocratic elution was (solvent A) 0.1% formic acid in acetonitrile and (solvent B) 10 mM ammonium formate (70:30, v/v). The run time was set as 6 min with a flow rate of 0.25mL/min. Electrospray ionization (ESI) was used under positive with multiple-reaction monitoring (MRM) scans. All other instrumental parameters used were as follows: curtain gas at 20 arbitrary units; gas 1 at 50; gas 2 at 60; ion spray voltage at 5200V. The dwell time for each transition ion was 300 ms and the ion source temperature was 400˚C. The MRM transitions used were as follows: TMA derivative: 146.1 > 118.1; TMAO: 76.1 > 58.1; TMA-d9 derivative: 155.1 > 127.1; TMAO-d9: 85.1 > 68.1. Samples were analysed in duplicate with an intra-assay CV of 5.0%.

Fecal Bile acid Detection

Methodologies for the quantification of the 61 bile acids in feces by ultrahigh performance liquid chromatography/multiple-reaction monitoring-mass spectrometry (UPLC-MRM/MS) have been described in detail by Han and colleagues.[34] Prior to quantification by UPLC-MRM/MS, fecal samples were first lyophilized and then homogenized to a fine powder by using two 5-mm stainless steel beads. Bile acids were then extracted by adding 1 mL of 75% acetonitrile to 10 mg of sample, followed by 20 secs of vortexing at 3,000 rpm, 5 mins of sonication in an ice water bath, and 5 secs of additional vortexing. The samples were centrifuged at 15,000 rpm and 10°C for 15 mins, and then 20 µL of the supernatant was mixed with 60 µL of 50% methanol and 40 µL of the internal standard solution (contained 14 D-labeled bile acids). Finally, 10 µL of the mixture was injected onto the UPLC-MRM/MS for bile acid quantitation. Linear calibration curves were constructed by using analyte-to-internal standard peak area ratios versus molar concentrations (nmol/mL) of standard solutions for each bile acid.

An Agilent 1290 series UHPLC system (Agilent Technologies Inc., California, USA) coupled to a SCIEX 4000 QTRAP mass spectrometer (AB SCIEX, Ontario, Canada) was used for quantification, with the MS instrument operated in the MRM mode with negative-ion detection. A Waters BEH C_{18} UPLC column (2.1 mm x 150 mm, 1.7 µm particle size; Waters Corp., Massachusetts, USA) was used for chromatographic separation. Composition of the mobile phase used for binary-solvent gradient elution was 0.01% formic acid in water (solvent A) and

0.01% formic acid in acetonitrile (solvent B). The flow rate was 0.35mL/min with the column temperature maintained at 45°C.

Ex Vivo Detection of Arabinoxylan (AX)-Utilizing Bacteria

Anaerobic Incubations

Fecal samples were collected at 6-weeks of AX supplementation as previously described,[26] diluted 1:10 in phosphate-buffered saline with 10% glycerol, aliquoted, and stored at -80ºC. Thawed fecal homogenates were filtered (40 µm filter, Corning, Germany) to remove particles and washed twice in 1X PBS by centrifugation to remove residual glycerol. Samples were then added to sterile Hungate tubes with 1 mM of cellular activity marker *L*-azidohomoalanine (AHA, Baseclick GmbH, Germany) and 2 mg/mL of AX (consistent with dietary intakes of AX[77,78]), and then incubated in an anaerobic tent at 37°C for 6-hours. Prior to use, the AX was subjected to an *in vitro* pre-digestion as described.[79] For each sample, a no-amendment negative control, wherein only 1 mM of AHA was added, was also incubated to account for potential basal activity without the presence of AX. After 6-hours of incubation, biomasses from both amendment and non-amendment samples were washed with 1X PBS, fixed in ethanol, and stored at -20°C in 1:1 ethanol/PBS until labeling. Biomasses were also collected from amendment samples at 0-hour and 6-hour incubations and stored at -80°C for additional DNA extractions. Refer to **Figure S1** for a schematic representation of the *ex vivo* detection assay.

Bioorthogonal Non-Canonical Amino Acid Tagging (BONCAT) of AX-Utilizing Bacterial Cells

Immediately before being sorted by flow cytometry, Cu(I)-catalyzed click labeling of chemically-fixed microbial cells was performed in solution according to Hatzenpichler *et al.* (**Figure S1**).[29] Briefly, 300-500 µl of fixed microbial cells were centrifuged (10,000 rpm) at room temperature (RT) for 10 min, the supernatant was then removed, and cells were resuspended in 96% EtOH. After 3 min of incubation at RT, microbial cells were centrifuged for an additional 10 mins. Next, a dye pre-mix consisting of 1.25 µl of 20 mM $CuSO_4$, 2.50 µl of 50 mM THPTA (Baseclick, Germany), and 0.30 µl of Cy5 alkyne dye (Jena Bioscience, Germany) was prepared. After being left in the dark for 3 min at RT to react, the dye pre-mix was added to 221 µl of 1X PBS, 12.5 µl of 100 mM sodium ascorbate (Sigma-Aldrich, Austria), and 12.5 µl of 100mM aminoguanidine hydrochloride (Sigma-Aldrich, Austria). Finally, 60-100 µl of the dye

solution was added to the microbial cells, incubated in the dark at RT for 30 min, and then washed three times by centrifugation with 1X PBS. Samples were then filtered with 35 µm nylon mesh using 12 x 75 mm BD tubes (BD, Germany) immediately before being sorted by flow cytometry. For a representative BONCAT image of a fecal sample incubated with AX for 6-hours and a no-amendment control, refer to **Figure S1.**

Fluorescence-Activated Cell Sorting (FACS) of AX-Utilizing Bacterial Cells

Flow cytometry FACS of Cy5-labeled bacterial cells was done with an ultra-high-speed cell sorter MoFlo Astrios EQ (Beckman Coulter, California, USA) using the software Summit v6.2 (Beckman Coulter). To standardize measurements and assess bacterial size, silica calibration beads (100, 500, and 1000 nm, Kisker Biotech, Germany) with refractive indexes close to that of biological material were recorded. The sorting of Cy5-labeled bacteria was performed as followed: background noise of the machine was first detected using the parameters forward scatter (FSC) and side scatter (SSC). 488nm FSC1-Height-Log vs 488nm SSC-Height-Log was then used to show the different sizes of silica beads in the first measurement and the scattering of the bacteria in subsequent measurements. Bacteria were pre-gated and displayed on a third scatter plot with 488nm SSC-Area-Log vs 640nm 671/30-Area-Log axes. Cy5-positive bacteria were then sorted out into tubes with a maximum event rate of 50,000 events/sec. Reanalysis of the samples showed a purity of more than 99%. For a representation of the gating strategy applied, refer to **Figure S2**.

DNA Extraction, 16S rRNA Gene Illumina Libraries Preparation, and Sequence Pre-Processing

Bacterial DNA from both stool incubations (0-hours and 6-hours) and FACS-sorted cells (6-hours) were extracted using QIAamp DNA Mini Kit (Qiagen, Germany) following the manufacturer instructions with an additional lysozyme step (Sigma-Aldrich, Austria). The V3-V4 region of the 16S rRNA gene was amplified and barcoded in a unique dual setup by a 2-step PCR approach previously described by Herbold *et al*.[80] and by using 16S rRNA gene primers S-D-Bact-0341-b-S-17 (5'-CCTACGGGNGGCWGCAG-3') and S-D-Bact-0785-a-A-21 (5'-GACTACHVGGGTATCTAATCC-3').[81] Barcoded samples were then purified and normalized over a SequalPrep™ Normalization Plate Kit (Invitrogen) using a Biomek® NXP Span-8 pipetting robot (Beckman Coulter, California, USA), then pooled and concentrated on columns

(Anlaytik Jena). Next, sequence libraries were prepared with the Illumina TruSeq Nano Kit as previously described[80] by sequencing in paired-end mode (2×300 nt; v3 chemistry) on an Illumina MiSeq. For every 90 samples sequenced, four negative controls (PCR-grade water blanks) were included in the workflow. After sequencing, amplicon pools were extracted from the raw sequencing data using the FASTQ workflow in BaseSpace (Illumina) with default parameters, and then sequences were demultiplexed with the python package demultiplex[82] by permitting one mismatch each for barcodes, linkers, and primers. Sequencing data are available for download at the NCBI Sequence Read Archive under BioProject: PRJNA630848.

Inference of Bacterial Amplicon Sequence Variants (ASVs)

Bacterial ASVs were inferred using DADA2[32] by applying the previously recommended pipeline.[83] FASTQ reads 1 and 2 were trimmed at 230 nt with allowed expected errors of 15. ASV sequences were then classified using RDP Classifier v2.0.2[84] with a confidence cut-off of 0.5.

Filtering and Contamination Removal

Sequences from contaminants were removed using the R package decontam v1.6.0[85] with the prevalence method and a threshold setting of 0.01. To account for the variance in sequencing depths, the input data for decontam were rarefied to the median read count of the four negative controls (two extraction and two PBS controls; 229 reads), resulting in 12 ASVs being removed. Mitochondria and chloroplast sequences were also removed.

Statistical Analyses

R Packages for the Permutation Analyses

Statistical analyses were performed using R v3.5.1, Stata v15.0, and GraphPad Prism v8.3.1. For analyses in R, a number of packages were applied. Permutation analyses were performed using the permuco[86] package for paired *t*-tests, repeated measures one-way analysis of variance (ANOVA), and repeated measures analysis of covariance (ANCOVA), whereas the lmPerm[87] package was used for unpaired *t*-tests and ANCOVA. Principal component analysis (PCA) plots were generated using factoextra[88] and FactoMineR[89] packages, while permutational multivariate analysis of variance (PERMANOVA) was performed using the Adonis function in the vegan[90]

package. Differentially abundant taxa between the bacterial consortia recovered by FACS after a 6-hour incubation in the presence of AX and the total fecal bacterial communities after 0-hour and 6-hour incubations were further identified using negative binomial generalized linear models (GLMs) and Wald tests in the DEseq2[38] package.

Evaluation of Confounding Effects by ANCOVA

ANCOVA models were performed to evaluate whether the significant effects of fiber supplementation on perceived satiety and surrogate endpoints were independent of potentially confounding factors. Based on previously described principles of confounder selection,[91] the following eight variables were considered as covariates in the ANCOVA analyses: sex, age, absolute changes in total dietary fiber and sugar consumption (W6-BL), and differences in stool consistency and bowel movement frequency during the study (AUC_{BL-W6}). Due to limitations of statistical power (modest sample size), separate ANCOVA models were performed for each covariate.

Machine Learning and Statistical Assessment of Associations

Random forests are supervised tree-based machine learning algorithms previously shown to be a robust approach for the discriminant analysis of high dimensional, low sample size data.[92,93] Therefore, we performed random forest classifications using the randomForest[94] package in R to identify gut microbiota compositional features and mechanistic endpoints indicative of host-microbiota interactions that predicted the effects of fiber supplementation on perceived satiety and surrogate endpoints. Prior to analysis, subjects were categorized as high- and low-responders for each endpoint according to the study cohort median, as in satiety after a meal (AUC_{BL-W6}), HOMA-IR, fecal calprotectin, and TNF-α (percentage change). Since HOMA-IR and QUICKI indexes showed significant collinearity (r_s=-0.97 and p<0.0001, Pearson's correlation) with subject classifications being the same for both indexes, random forest classifier (RFC) analyses were only performed on HOMA-IR as the surrogate endpoint of insulin resistance. The predictor variables used for training the RFCs consisted of separate microbiota compositional features (fecal microbiota composition and metabolically active consortia), mechanistic endpoints (fecal short-chain fatty acids [SCFAs], bile acids, and molecular markers [gut hormones, cytokines, and

barrier function measures]), and calorie-adjusted macronutrient intake datasets. Independent RFCs were then performed for each endpoint using the following predictor datasets:

- % Δ Molecular markers: Percentage change from baseline to week 6 in the 14 mechanistic endpoints of molecular processes that are indicative of host-microbiota interactions (**Table S3**).
- Δ W1-BL SCFAs: Shorter-term shifts from baseline to week 1 in the fecal concentrations of acetate, propionate, butyrate, valerate, isobutyrate, and isovalerate and the relative proportions of acetate, propionate, and butyrate (refer to our preceding study[26]).
- Δ W6-BL SCFAs: Longer-term shifts from baseline to week 6 in the fecal concentrations of acetate, propionate, butyrate, valerate, isobutyrate, and isovalerate and the relative proportions of acetate, propionate, and butyrate (refer to our preceding study[26]).
- Δ All Bile Acids: Shifts from baseline to week 6 in the 31 bile acids most frequently detected among fecal samples (**Table 1**).
- Δ Responsive Bile Acids: Shifts from baseline to week 6 in the 14 bile acids that showed a tendency to respond to MCC supplementation when compared to baseline or to AX ($p<0.1$, paired permutational *t*-tests; **Table 1**).
- Δ Responsive OTUs: Shifts in the fecal relative abundance of the 18 bacterial operational taxonomic units (OTUs) that significantly changed by week 6 of AX consumption when compared to baseline or MCC ($q<0.15$; refer to our preceding study[26]).
- BL Responsive OTUs: Baseline fecal relative abundance of the 18 bacterial OTUs AX-responsive OTUs ($q<0.15$; refer to our preceding study[26]).
- Δ All OTUs: Shifts in the fecal relative abundance of the 100 bacterial OTUs with an average relative abundance ≥0.15% (refer to our preceding study[26]).
- BL All OTUs: Baseline fecal relative abundance of the 100 bacterial OTUs with an average relative abundance ≥0.15% (refer to our preceding study[26]).
- Diff. Abundant Metabolically Active ASVs: Relative abundance of the 14 bacterial ASVs shown to be differentially abundant in the BONCAT-labeled, FACS-recovered consortia at 6-hours incubation with AX when compared to the total fecal bacterial community at 0-hours incubation ($q<0.05$, DESeq2; **Table S5**).

- <u>All Metabolically Active ASVs:</u> Relative abundance of the 90 bacterial ASVs shown to have an average relative abundance ≥0.15% among the BONCAT-labeled, FACS-recovered consortia detected at 6-hours incubation with AX.
- <u>Δ Macronutrients:</u> Change from baseline to week 6 in the calorie-adjusted intake of macronutrients assessed by ASA24-Canada-2014, as in total carbohydrates, sugar, dietary fiber, protein, total fat, saturated fat, unsaturated fat, and cholesterol (**Table S2**).
- <u>BL Macronutrients:</u> Baseline calorie-adjusted intake of macronutrients assessed by ASA24-Canada-2014 (**Table S2**).

The generalization error of each RFC was estimated across 100 replicates using the leave-one-out cross-validation as previously described,[95] where each group was predicted by a model trained on data from the study participants. RFCs were performed using the default settings in the randomForest[94] package. The number of variables available for splitting at each tree node was determined by cross-validation. To evaluate the performance of each RFC, area under the receiver operating characteristic curves (AUC-ROCs) were generated using the true possibilities from cross-validation results (pROC[96] package in R), and average out-of-bag error rates (OOBs) were estimated across 100 replicates to calculate prediction error. RFCs with AUC-ROC values >0.7 and OOBs <0.6 were considered to have good prediction accuracy. A confusion matrix was further generated to evaluate subgroup prediction accuracy.

To determine the importance of each individual variable for the classification of high-vs-low responders, average mean importance scores were calculated by 100 replicates estimation. Spearman's correlation analyses were performed as an additional statistical approach to assess monotonic relationships between perceived satiety and surrogate endpoints and the most important variables for discriminating high- and low-responders, where FDR corrected *q* values <0.1 were considered statistically significant. Variables identified by RFC and Spearman's analyses were selected as potential determinants of the host-microbial dialogue that predict the effects of fiber supplementation on perceived satiety and surrogate endpoints. To adjust for potential confounding effects, we applied GLMs in Stata to test the association between perceived satiety, HOMA-IR, fecal calprotectin and the most discriminative variables when controlling for the following covariates: sex, age, absolute changes in total dietary fiber and

sugar consumption (W6-BL), and differences in stool consistency and bowel movement frequency during the study ($AUC_{BL\text{-}W6}$). Data distributions were visually assessed by inspection of residual and histogram plots. Non-normally distributed data were cubed-root transformed prior to analysis by Gaussian-distribution GLM with the identity link. Binominal-distribution GLMs with the logistic link were alternatively applied for HOMA-IR, as the percentage change data showed a clear binominal distribution.

Supplementary Tables

Supplementary Table S1. Baseline Clinical Measurements.

	Arabinoxylan (*n*=15)	Microcrystalline Cellulose (*n*=16)	Between Group *p* value
Sex (F/M)	10/5	11/5	
Age (y)	33.7 ± 9.7	32.1 ± 7.4	1.00
Anthropometric Measurements			
Body weight (kg)	84.8 ± 12.3	81.9 ± 10.5	0.61
BMI (kg/m^2)	28.7 ± 2.7	28.7 ± 2.0	0.98
Waist circumference (cm)	95.7 ± 8.7	92.9 ± 6.0	0.34
Body fat % - females	36.4 ± 2.9	38.0 ± 6.1	0.42
Body fat % - males	22.5 ± 3.6	23.0 ± 5.3	0.87
Surrogate Endpoints			
Systolic (mm Hg)	118.5 ± 13.6	120.6 ± 15.7	0.59
Diastolic (mm Hg)	71.4 ± 9.0	74.9 ± 12.3	0.32
Pulse (beats/min)	73.1 ± 12.8	74.2 ± 12.6	0.84
Glucose (mmol/L)	5.1 ± 0.4	5.0 ± 0.4	0.84
Insulin (pg/mL)	649.9 ± 253.5	593.7 ± 254.3	0.55
HOMA-IR	3.37 ± 1.34	3.02 ± 1.22	0.36
QUICKI	0.288 ± 0.2	0.292 ± 0.2	0.84
Total cholesterol (mmol/L)	4.4 ± 0.8	4.6 ± 1.0	0.57
LDL cholesterol (mmol/L)	2.5 ± 0.6	2.7 ± 0.7	0.94
HDL cholesterol (mmol/L)	1.3 ± 0.3	1.3 ± 0.3	1.00
Triglycerides (mmol/L)	1.2 ± 0.8	1.4 ± 0.7	0.96
Hemoglobin (g/L)	140.7 ± 11.6	134.2 ± 14.9	0.22
White blood cells (10^9/L)	6.3 ± 1.1	6.3 ± 1.5	0.88
hs-CRP (mg/L)	4.0 ± 4.6	3.6 ± 3.5 [b]	0.84
f-Calprotectin (mg/kg)	66.4 ± 45.2	63.4 ± 53.7	0.90
Molecular Markers of Host-Microbiota Interactions			
Glucagon (pg/mL)	137.0 ± 40.2	137.6 ± 61.0	0.98
GLP-1 (pg/mL)	13.9 ± 4.2	13.5 ± 6.3	0.76
PYY (pg/mL)	62.6 ± 25.4	48.7 ± 15.4	0.10
Ghrelin (pg/mL)	583.6 ± 291.0	687.4 ± 223.5	0.18
Leptin (ng/mL)	28.5 ± 25.0	30.2 ± 27.5 [a]	0.80
Adiponectin (mg/L)	15.0 ± 5.2	18.7 ± 10.9	0.22
TNF-α (pg/mL)	2.6 ± 0.6	2.7 ± 0.6	0.94
IL-6 (pg/mL)	0.69 ± 0.45	0.66 ± 0.25	0.98
IL-8 (pg/mL)	3.2 ± 1.0 [a]	3.3 ± 0.8	0.96
IL-10 (pg/mL)	0.25 ± 0.07	0.22 ± 0.07	0.28
LBP (µg/mL)	52.1 ± 17.9	53.9 ± 14.7	0.82
f-Albumin (mg/L)	5.1 ± 6.8	3.1 ± 4.7	0.29
f-Zonulin (µg/mL)	0.41 ± 0.44 [a]	0.21 ± 0.34	0.18
TMAO (µM)	3.7 ± 1.8	2.9 ± 1.3	0.19

Statistical significances of between-group differences at baseline (AX vs MCC) were determined by unpaired permutational *t*-tests. Data are means ± SD. Statistical significance was set at $p<0.01$.

[a] One outlier >5*SD from the mean was excluded; [b] Two outliers >5*SD from the mean were excluded.

Abbreviations: BMI, body mass index; f-, quantified in feces; GLP-1, active glucagon-like peptide-1; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; HOMA-IR, homeostatic model assessment of insulin resistance; IL, interleukin; LBP, lipopolysaccharide-binding protein; LDL, low-density lipoprotein; PYY, total peptide tyrosine tyrosine; QUICKI, quantitative insulin sensitivity check index; TMAO, trimethylamine *N*-oxide; TNF-α, tumor necrosis factor-α.

Supplementary Table S2. Macronutrient Consumption at Baseline and During Arabinoxylan or Microcrystalline Cellulose Supplementation.

	Arabinoxylan (*n*=15)				Microcrystalline Cellulose (*n*=16)				Between
	Baseline	During Intervention	Within Group *p* value	Change (%)	Baseline	During Intervention	Within Group *p* value	Change (%)	Group Change *p* value
Energy (kcal)	2344 ± 1230	2331 ± 978	0.95	9 ± 38	1939 ± 505	2220 ± 648	0.06	17 ± 27	0.63
Carbohydrates (g)	262 ± 133	273 ± 130	0.66	11 ± 35	221 ± 76	257 ± 89	**0.046**	20 ± 31	0.21
Sugar (g)	114 ± 58	138 ± 72	**0.04**	35 ± 62	90 ± 35	118 ± 54	**0.03**	46 ± 80	0.55
Background dietary fiber (g) [a]	21 ± 6	19 ± 8	0.56	0.3 ± 46	19 ± 8	18 ± 5	0.41	3 ± 39	0.92
Background dietary fiber plus fiber supplement (g) [b]	21 ± 6	46 ± 12	**0.0002**	142 ± 84	19 ± 8	44 ± 8	**0.0002**	171 ± 113	0.50
Females (g) [b]	19 ± 4	40 ± 5	**0.0002**	128 ± 81	19 ± 6	40 ± 6	**0.0002**	140 ± 101	0.69
Males (g) [b]	24 ± 9	58 ± 13	**0.006**	169 ± 93	19 ± 13	53 ± 6	**0.0004**	239 ± 116	0.15
Proteins (g)	99 ± 62	97 ± 35	0.82	19 ± 67	81 ± 37	94 ± 25	0.17	26 ± 39	0.68
Total fats (g)	98 ± 60	96 ± 42	0.83	21 ± 68	76 ± 25	87 ± 33	0.15	19 ± 43	0.98
Saturated fat (g)	32 ± 23	32 ± 16	0.94	43 ± 126	26 ± 12	33 ± 14	0.09	37 ± 65	1.00
Unsaturated fat (g)	58 ± 34	56 ± 24	0.73	16 ± 61	43 ± 12	47 ± 17	0.26	11 ± 35	0.82
Cholesterol (mg)	358 ± 236	419 ± 183	**0.047**	56 ± 88	335 ± 166	376 ± 128	0.31	34 ± 68	0.46

Statistical significances of changes within-group were determined by paired permutational *t*-test, while between-group differences of percent change (AX vs MCC; during intervention – baseline/baseline*100) were determined by unpaired permutational *t*-test. Data are means ± SD. Statistical significance was set at $p<0.05$ (bolded *p* values).

[a] Total dietary fiber provided by the background diet without consideration of the supplemented fiber.

[b] Total dietary fiber provided by the background diet plus supplemental fiber when considering adherence to the intervention protocol.

Supplementary Table S3. Clinical Measurements at Baseline and Six Weeks of Arabinoxylan or Microcrystalline Cellulose Supplementation.

	Arabinoxylan (*n*=15)				Microcrystalline Cellulose (*n*=16)				Between Group Change *p* value
	Baseline	Week 6	Within Group *p* value	Change (%)	Baseline	Week 6	Within Group *p* value	Change (%)	
Anthropometric Measurements									
Body weight (kg)	84.8±12.3	85.2±12.9	0.45	0.4±1.9	81.9±10.5	81.3±10.2	0.12	-0.8±1.9	0.17
BMI (kg/m^2)	28.7±2.7	28.8±2.8	0.47	0.4±1.9	28.7±2.0	28.5±2.1	0.13	-0.8±1.9	0.11
WC (cm)	95.7±8.7	95.5±8.1	0.81	-0.1±3.2	92.9±6.0	91.9±6.8	0.16	-1.2±3.0	0.43
Body fat % - females	36.4±2.9	36.6±3.6	0.22	0.7±3.9	38.0±6.1	38.5±6.1	0.50	1.5±3.4	0.82
Body fat % - males	22.5±3.6	23.0±3.2	0.61	2.3±4.1	23.0±5.3	23.2±5.6	0.30	1.1±4.3	1.00
Surrogate Endpoints									
Systolic (mm Hg)	118.5±13.6	116.6±13.6	0.28	-1.5±5.5	120.6±15.7	120.3±17.8	0.89	-0.2±6.4	0.60
Diastolic (mm Hg)	71.4±9.0	71.4±8.7	1.00	0.4±7.4	74.9±12.3	75.3±12.1	0.77	0.8±7.0	0.92
Pulse (beats/min)	73.1±12.8	70.6±10.3	0.15	-2.6±8.6	74.2±12.6	77.3±11.2	0.18	5.4±13.9	0.12
Glucose (mmol/L)	5.1±0.4	5.0±0.4	0.21	-1.8±5.5	5.0±0.4	5.1±0.5	0.63	0.7±6.0	0.69
Insulin (pg/mL)	649.9±253.5	544.4±200.1	**0.046**	-10.2±36.3	593.7±254.3	658.7±177.5	0.21	22.5±38.1	**0.02**
HOMA-IR	3.4±1.3	2.8±1.2	**0.04**	-11.8±35.5	3.0±1.2	3.4±0.9	0.23	24.6±44.0	**0.006 ***
QUICKI	0.288±0.02	0.295±0.02	0.06	2.5±4.6	0.292±0.02	0.286±0.01	0.06	-2.0±4.4	**0.008 ***
Total-C (mmol/L)	4.4±0.8	4.6±0.8	**0.02**	4.9±7.1	4.6±1.0	4.7±1.0	0.42	2.2±9.9	0.43
LDL-C (mmol/L)	2.5±0.6	2.7±0.7	**0.03**	9.3±14.6	2.7±0.7	2.7±0.9	0.63	1.7±18.1	0.39
HDL-C (mmol/L)	1.3±0.3	1.3±0.3	0.65	2.8±12.9	1.3±0.3	1.2±0.3	0.12	-3.9±9.1	0.09
Triglycerides (mmol/L)	1.2±0.8	1.1±0.5	0.59	9.8±43.2	1.4±0.7	1.6±0.6	0.23	30.0±57.7	0.33
Hemoglobin (g/L)	140.7±11.6	141.3±9.7	0.63	0.6±4.1	134.2±14.9	136.6±15.1	**0.03**	1.8±3.0	0.33
WBC (10^9/L)	6.3±1.1	6.2±1.1	0.82	0.1±13.4	6.3±1.5	6.3±1.2	0.91	2.6±16.6	0.45
hs-CRP (mg/L)	4.0±4.6	3.1±3.2	0.25	5.7±66.1	3.6±3.5 [b]	3.5±3.4 [b]	0.71	-0.6±37.4 [b]	0.82
f-Calprotectin (mg/kg)	66.4±45.2	63.8±55.1	0.85	14.3±77.5	63.4±53.7	38.5±35.5	**0.004 ***	-39.1±24.0	**0.002 ***
Molecular Markers of Host-Microbiota Interactions									
Glucagon (pg/mL)	137.0±40.2	129.6±34.6	0.09	-3.7±12.7	137.6±61.0	132.3±57.1	0.28	-2.9±11.6	0.88
GLP-1 (pg/mL)	13.9±4.2	12.3±5.5	**0.047**	-15.6±27.7	13.5±6.3	13.6±6.2	0.96	5.1±58.3	0.22
PYY (pg/mL)	62.6±25.4	56.9±21.8	0.13	-6.4±23.9	48.7±15.4	49.6±19.1	0.82	3.1±32.5	0.35
Ghrelin (pg/mL)	583.6±291.0	539.8±427.1	0.57	-7.8±33.9	687.4±223.5	670.7±262.6	0.65	-2.8±20.2	0.66
Leptin (ng/mL)	28.5±25.0	27.6±18.0	0.73	21.7±42.4	30.2±27.5 [a]	31.0±25.6 [a]	0.69	9.7±29.2 [a]	0.38
Adiponectin (mg/L)	15.0±5.2	16.6±6.7	0.10	11.1±19.0	18.7±10.9	18.6±11.4	0.93	1.7±14.8	0.12
TNF-α (pg/mL)	2.6±0.6	2.5±0.6	0.36	-2.0±13.6	2.7±0.6	2.5±0.6	**0.004 ***	-6.7±8.7	0.67
IL-6 (pg/mL)	0.69±0.45	0.67±0.36	0.86	11.0±49.0	0.66±0.25	0.61±0.27	0.32	-2.8±33.1	0.51
IL-8 (pg/mL)	3.2±1.0 [a]	3.2±1.2 [a]	0.91	0.7±19.8 [a]	3.3±0.8	3.4±0.7	0.52	4.8±18.0	0.64
IL-10 (pg/mL)	0.25±0.07	0.25±0.12	0.82	-1.0±34.9	0.22±0.07	0.24±0.07	0.23	12.7±30.9	0.15
LBP (µg/mL)	52.1±17.9	50.7±17.1	0.80	3.1±39.1	53.9±14.7	48.1±14.3	0.09	-7.6±26.7	0.76
f-Albumin (mg/L)	5.1±6.8	5.1±5.2	0.96	78.8±207.9	3.1±4.7	2.7±3.9	0.71	48.7±197.4	1.00
f-Zonulin (µg/mL)	0.41±0.44 [a]	0.43±0.60 [a]	0.85	48.9±116.1 [a]	0.21±0.34	0.25±0.43	0.42	28.2±103.4	0.80
TMAO (µM)	3.7±1.8	3.1±1.4	0.35	3.2±62.1	2.9±1.3	2.8±1.4	0.93	16.1±78.0	0.58
Stool characteristics [c]				**AUC_{BL-W6}** [c]				**AUC_{BL-W6}** [c]	
Stool consistency	1.9±1.1	2.3±0.5	0.65	12.9±3.3	1.8±0.9	1.8±0.9	1.00	10.9±2.2	0.06
Bowel frequency	1.7±0.8	2.1±0.7	0.47	13.0±4.5	2.1±0.9	2.1±1.0	1.00	13.3±5.4	0.81

Statistical significances of changes within-group were determined by paired permutational *t*-tests, while between-group differences (AX vs MCC; week 6 – baseline/baseline*100) were determined by unpaired permutational *t*-tests. Data are means ± SD.

* Statistical significance was set at $p<0.01$, bolded *p* values without an asterisk (*) are approaching statistical significance ($p<0.05$).

[a] One outlier >5*SD from the mean was excluded; [b] Two outliers >5*SD from the mean were excluded; [c] referred to our preceding study[26], changes within-group were determined by GEE models, while between-group differences in the area under the curve from baseline to week 6 (AUC_{BL-W6}) were determined by Mann-Whitney tests.

Abbreviations: BMI, body mass index; f-, quantified in feces; GLP-1, active glucagon-like peptide-1; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; HOMA-IR, homeostatic model assessment of insulin resistance; IL, interleukin; LBP, lipopolysaccharide-binding protein; LDL-C, low-density lipoprotein cholesterol; PYY, total peptide tyrosine tyrosine; QUICKI, quantitative insulin sensitivity check index; Total-C, total cholesterol; TMAO, trimethylamine *N*-oxide; TNF-α, tumor necrosis factor-α; WC, waist circumference; WBC, white blood cells.

Supplementary Table S4. Covariate-Adjustment of Dietary Fiber Treatment Effects by ANCOVA.

	Within AX Group (Baseline vs Week 6; *n*=15)						Within MCC Group (Baseline vs Week 6; *n*=16)						Between Group (AX vs MCC)					
	M1	M2	M3	M4	M5	M6	M1	M2	M3	M4	M5	M6	M1	M2	M3	M4	M5	M6
Satiety after a meal [a]	--	--	--	--	--	--	--	--	--	--	--	--	**0.03**	**0.04**	**0.03**	**0.03**	**0.04**	**0.04**
HOMA-IR [b]	**0.04**	**0.04**	0.33	0.18	0.11	**0.03**	0.23	0.23	0.06	0.57	0.21	0.22	**0.02**	**0.005**	**0.02**	**0.01**	**0.03**	**0.03**
QUICKI [b]	0.07	0.06	0.50	0.29	0.13	0.05	0.06	0.06	**0.01**	0.20	0.06	0.06	**0.005**	**0.005**	**0.01**	**0.005**	**0.006**	**0.01**
Fecal calprotectin [b]	0.85	0.85	0.18	0.96	0.85	0.94	**0.002**	**0.004**	0.98	**0.007**	**0.006**	**0.005**	**0.01**	**0.01**	**0.007**	**0.007**	**0.005**	**0.005**
Tumor necrosis factor-α [b]	0.37	0.36	0.06	0.21	0.38	0.18	**0.006**	**0.005**	**<0.001**	**0.007**	**0.03**	**0.006**	0.51	0.32	0.24	0.47	0.42	0.20

M1: model adjusted for age. **M2**: model adjusted for sex. **M3**: model adjusted for changes in total dietary fiber intake, which considers the amount of supplemental fiber. **M4**: model adjusted for changes in total dietary sugar intake. **M5**: model adjusted for differences in stool consistency. **M6**: model adjusted for differences in bowel movement frequency. Statistical significances of changes within-group were determined by repeated measures permutational ANCOVA models, while between-group differences of either ([a]) area under the curve or ([b]) percent change from baseline (during intervention – baseline/baseline*100) were determined by unpaired permutational ANCOVA. Data presented as *p* values with significance set at $p<0.05$ (bolded *p* values).

Abbreviations: AX, arabinoxylan; ANCOVA, analysis of covariance; HOMA-IR, homeostatic model assessment of insulin resistance; MCC, microcrystalline cellulose; QUICKI, quantitative insulin sensitivity check index.

Supplementary Table S5. Relative Abundance of Bacterial Taxa Activated through *ex vivo* Incubation with Arabinoxylan and Recovered by FACS.

α-Diversity Index or Taxonomic Group	Arabinoxylan (*n*=15)						
	Time 0	Time 6	FACS Sorted	FACS vs Time 0 Log_2 Fold Difference	FACS vs Time 0 *q* value	FACS vs Time 6 Log_2 Fold Difference	FACS vs Time 6 *q* value
α-Diversity Index [a]							
Shannon Index	3.60±0.26	3.66±0.23	2.75±0.89	-0.50	**0.0008**	-0.52	**0.002**
Inverse Simpson Index	20.35±8.61	22.09±7.99	11.82±9.13	-1.15	**0.0005**	-1.30	**0.0002**
Chao1 Index	215.59±48.53	210.46±40.21	159.06±23.88	-0.42	**<0.0001**	-0.39	**<0.0001**
Total Number of ASVs	167.73±39.9	168.00±32.22	115.27±17.97	-0.51	**0.0002**	-0.53	**0.0002**
Phyla [b]							
Proteobacteria	1.45±1.50	4.78±5.90	15.70±28.72	2.07	**0.006**	0.79	0.48
Family [b]							
Enterobacteriaceae	0.83±1.31	4.36±5.89	15.57±28.71	5.34	**<0.0001**	3.48	**0.002**
Lactobacillaceae	0.03±0.06	0.09±0.14	1.02±1.42	6.54	**<0.0001**	5.38	**0.0001**
Bacteroidaceae	28.27±14.89	31.40±13.34	39.58±27.12	1.55	**0.005**	1.42	**0.02**
Rikenellaceae	3.09±4.56	3.09±4.82	0.34±0.45	-2.67	**0.001**	-2.75	**0.007**
Ruminococcaceae	12.64±5.05	11.32±5.60	5.43±6.74	-0.85	**0.006**	-0.62	0.23
Streptococcaceae	1.88±1.74	1.97±2.31	0.30±0.26	-1.81	**0.01**	-1.78	0.06
Genera [b]							
Escherichia/Shigella	0.83±1.31	4.36±5.89	15.57±28.71	3.13	**0.0004**	3.04	**0.009**
Lactobacillus	0.03±0.06	0.09±0.14	1.02±1.42	5.90	**<0.0001**	4.96	**0.0003**
Alistipes	3.09±4.56	3.09±4.82	0.34±0.45	-3.20	**<0.0001**	-3.12	**0.001**
Faecalibacterium	5.34±2.99	4.38±2.62	1.34±1.58	-2.21	**<0.0001**	-1.55	**0.02**
Roseburia	3.01±2.18	3.29±1.99	2.16±2.84	-1.23	**0.01**	-0.14	0.87
Clostridium XVIII	0.75±0.53	0.67±0.55	0.15±0.18	-1.90	**0.03**	-1.38	0.26
Oscillibacter	1.61±0.97	1.55±1.11	0.47±0.57	-1.75	**0.001**	-1.49	**0.04**
Streptococcus	1.57±1.11	1.83±2.28	0.30±0.26	-2.16	**0.003**	-1.97	**0.04**
Amplicon sequence variant (ASV Number, closest hit in database, % identity) [b]							
3ur8h3, *Escherichia fergusonii/Shigella sonnei*, 100%	0.90±1.55	4.50±6.39	15.59±28.82	3.20	**<0.0001**	1.19	0.32
pgue8q, *Lactobacillus crispatus/gallinarum*, 100%	0.02±0.05	0.09±0.14	0.97±1.41	7.03	**<0.0001**	5.10	**0.0001**
5mi71s, *Bacteroides koreensis*, 99.7%	0.19±0.36	0.40±0.87	1.88±4.17	3.68	**0.005**	3.31	**0.0495**
tmdvof, *Bacteroides plebeius*, 100%	3.74±9.70	2.71±7.32	3.07±8.09	3.31	**0.008**	1.28	0.28
4c03f6, *Bacteroides xylanisolvens*, 100%	0.61±1.10	1.44±2.58	4.82±9.40	2.96	**0.02**	2.06	0.19
bdmzc3, *Faecalibacterium prausnitzii*, 99%	1.44±1.70	0.76±0.80	0.21±0.36	-3.66	**<0.0001**	-1.98	**0.03**
eb999b, *Faecalibacterium prausnitzii*, 99.7%	1.35±1.48	1.22±1.19	0.47±0.78	-2.20	**0.006**	-1.21	0.32
3rw028, *Faecalibacterium prausnitzii*, 98.76%	0.99±0.61	1.02±1.08	0.27±0.34	-2.40	**0.0003**	-1.74	0.05
19n89a, *Coprococcus eutactus*, 99.75%	0.28±0.64	0.26±0.67	0.18±0.66	-3.85	**0.006**	-2.62	0.35
6pygnt, *Dialister invisus*, 100%	1.64±2.72	1.27±1.85	0.26±0.44	-2.80	**0.005**	-2.35	0.05
dx5pax, *Faecalibacillus intestinalis*, 100%	0.82±0.56	0.72±0.58	0.17±0.16	-1.76	**0.03**	-1.03	0.35
rfal2b, *Fusicatenibacter saccharivorans*, 100%	2.88±1.48	2.49±1.34	1.90±1.50	-1.40	**0.001**	-0.37	0.58
49kgjb, *Ruminococcaceae*	0.87±1.09	0.75±0.97	0.43±1.17	-2.26	**0.04**	-1.28	0.43
gh9q6u, *Streptococcus thermophiles*, 100%	0.83±0.68	1.14±1.64	0.18±0.19	-2.52	**0.0002**	-2.29	**0.009**

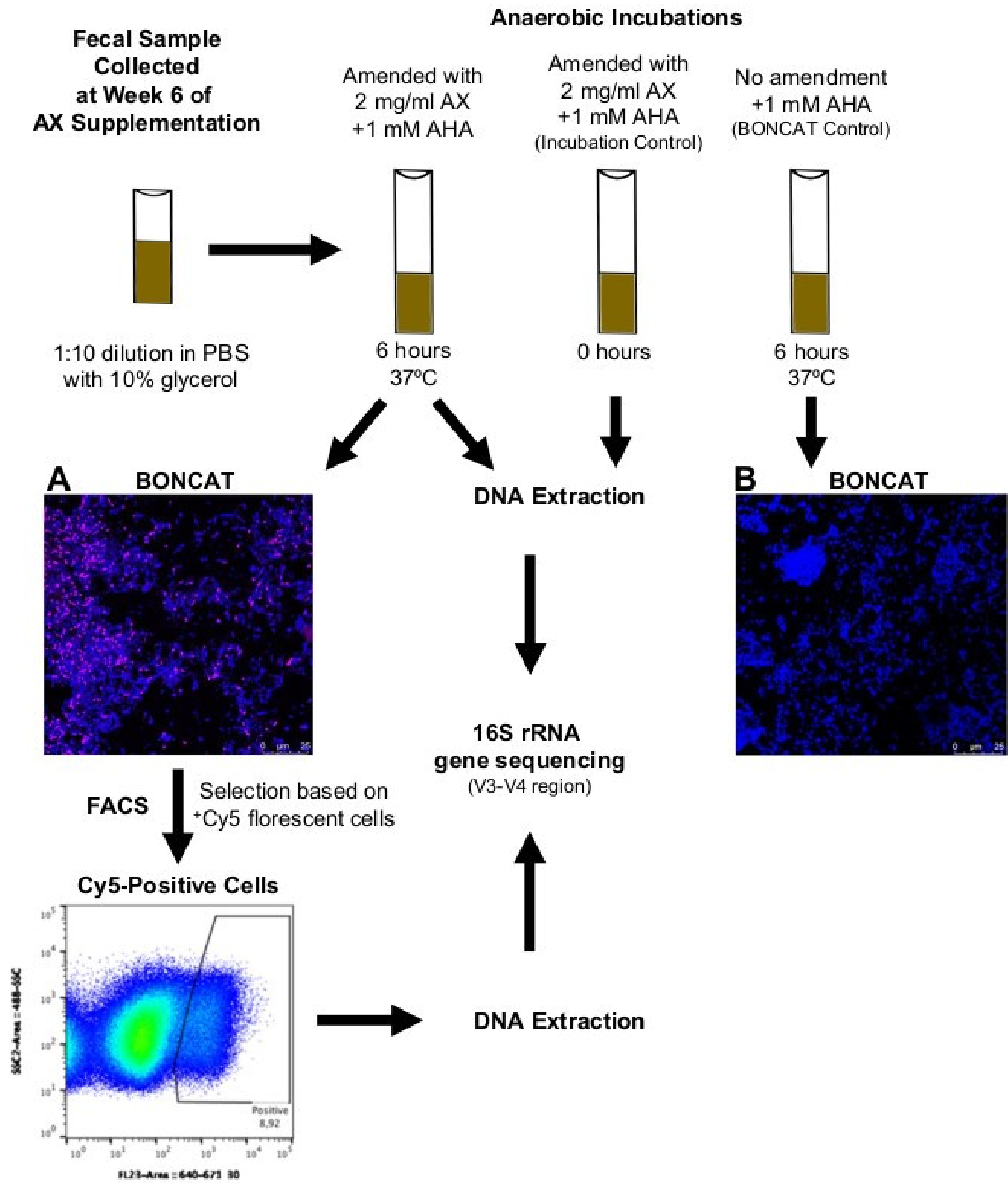
Statistically significant differences between the bacterial consortia activated at 6-hours incubation with arabinoxylan and recovered by fluorescence-activated cell sorting (FACS) and the fecal bacterial community at 0-hours and 6-hours incubation with arabinoxylan were determined by either ([a]) one-way ANOVA with permutations or ([b]) testing for differences in the differential abundance of taxa using the R package DEseq2. *p* values were adjusted by FDR, and FDR significance was set at q<0.05 (bolded *q* values). Data are presented as mean relative abundance ± SD.

Supplementary Table S6. Univariate and Covariate-Adjusted GLMs Assessing Fecal Microbiota-Related Factors that Associate with Surrogate Endpoints of Dietary Fiber Supplementation.

	Within AX Group (*n*=15)							Within MCC Group (*n*=16)						
	M1	M2	M3	M4	M5	M6	M7	M1	M2	M3	M4	M5	M6	M7
Satiety After a Meal (AUC) [a]														
ASV6pygnt_*Dialister invisus* (%) [3]	**0.03**	**0.04**	**0.03**	**0.01**	**0.02**	**0.03**	**0.03**	--	--	--	--	--	--	--
ASV2xmw96_*Dorea formicigenerans* (%) [3]	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	--	--	--	--	--	--	--
ASV56kx74_*Eubacterium ramulus* (%) [3]	**0.01**	**0.01**	**0.02**	**0.03**	**0.02**	**0.006**	**0.02**	--	--	--	--	--	--	--
HOMA-IR (%Δ) [b]														
Lithocholic acid (Δ)	**0.02**	**0.03**	**0.02**	**0.01**	**0.02**	**0.04**	**0.02**	0.84	0.85	0.76	0.83	0.96	0.80	0.89
Isolithocholic acid (Δ)	**0.046**	0.09	0.05	**0.049**	**0.046**	0.06	0.05	0.53	0.53	0.45	0.49	0.57	0.41	0.56
Fecal Calprotectin (%Δ) [c]														
Taurolithocholic acid (Δ) [3]	0.15	0.17	0.15	0.22	0.27	0.22	0.09	**0.001**	**0.001**	**0.001**	**<0.001**	**0.001**	**<0.001**	**<0.001**
Glycodeoxycholic acid (Δ)	0.18	0.16	0.20	0.31	0.16	0.55	0.29	**0.002**	**0.002**	**0.003**	**0.003**	**0.001**	**0.003**	**<0.001**

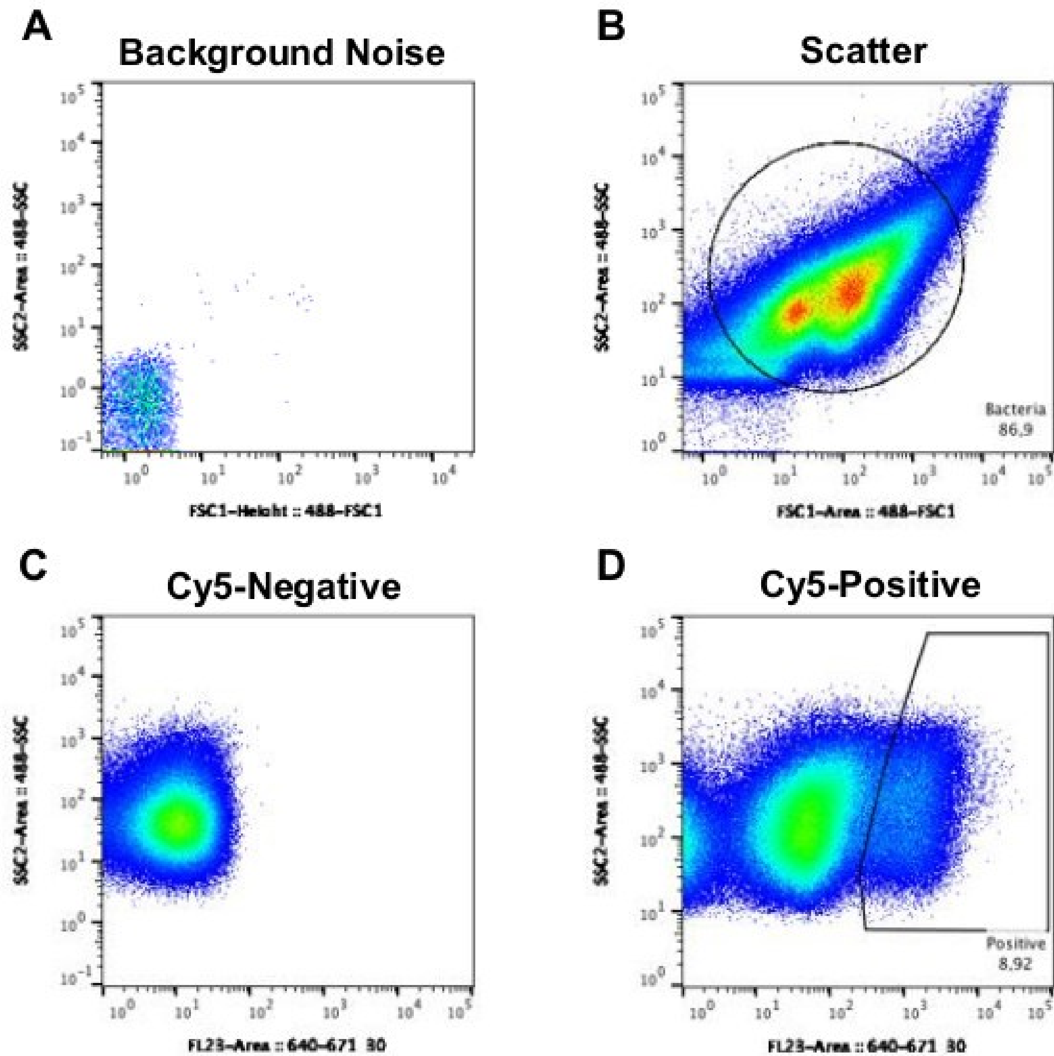
[a] Satiety after a meal (AUC_{BL-W6}) was the dependent variable in gaussian-distributed GLM with an identity link and the relative abundance of fluorescence-activated cell sorting (FACS) sorted amplicon sequence variants (ASV) as predictors.
[b] HOMA-IR (percent change) was the dependent variable in binomial-distributed GLM with a probit link and shifts in the concentration of fecal bile acids (absolute change) as predictors.
[c] Fecal calprotectin (percent change) was the dependent variable in gaussian-distributed GLM with an identity link and shifts in the concentration of fecal bile acids (absolute change) as predictors.
M1: univariate model without adjustment. **M2**: model adjusted for age. **M3**: model adjusted for sex. **M4**: model adjusted for changes in total dietary fiber intake, which considers the amount of supplemental fiber (W6-BL). **M5**: model adjusted for changes in total dietary sugar intake (W6-BL). **M6**: model adjusted for differences in stool consistency during the intervention (AUC_{BL-W6}). **M7**: model adjusted for differences in bowel movement frequency during the intervention (AUC_{BL-W6}). Data presented as *p* values with significance set at $p<0.05$ (bolded *p* values).
Abbreviations: [3], cube root transformed prior to analysis; ASV, amplicon sequence variant; AX, arabinoxylan; AUC, area under the curve; BL, baseline; GLM, generalized linear model; HOMA-IR, homeostatic model assessment of insulin resistance; MCC, microcrystalline cellulose; W6, week 6.

Supplementary Figures

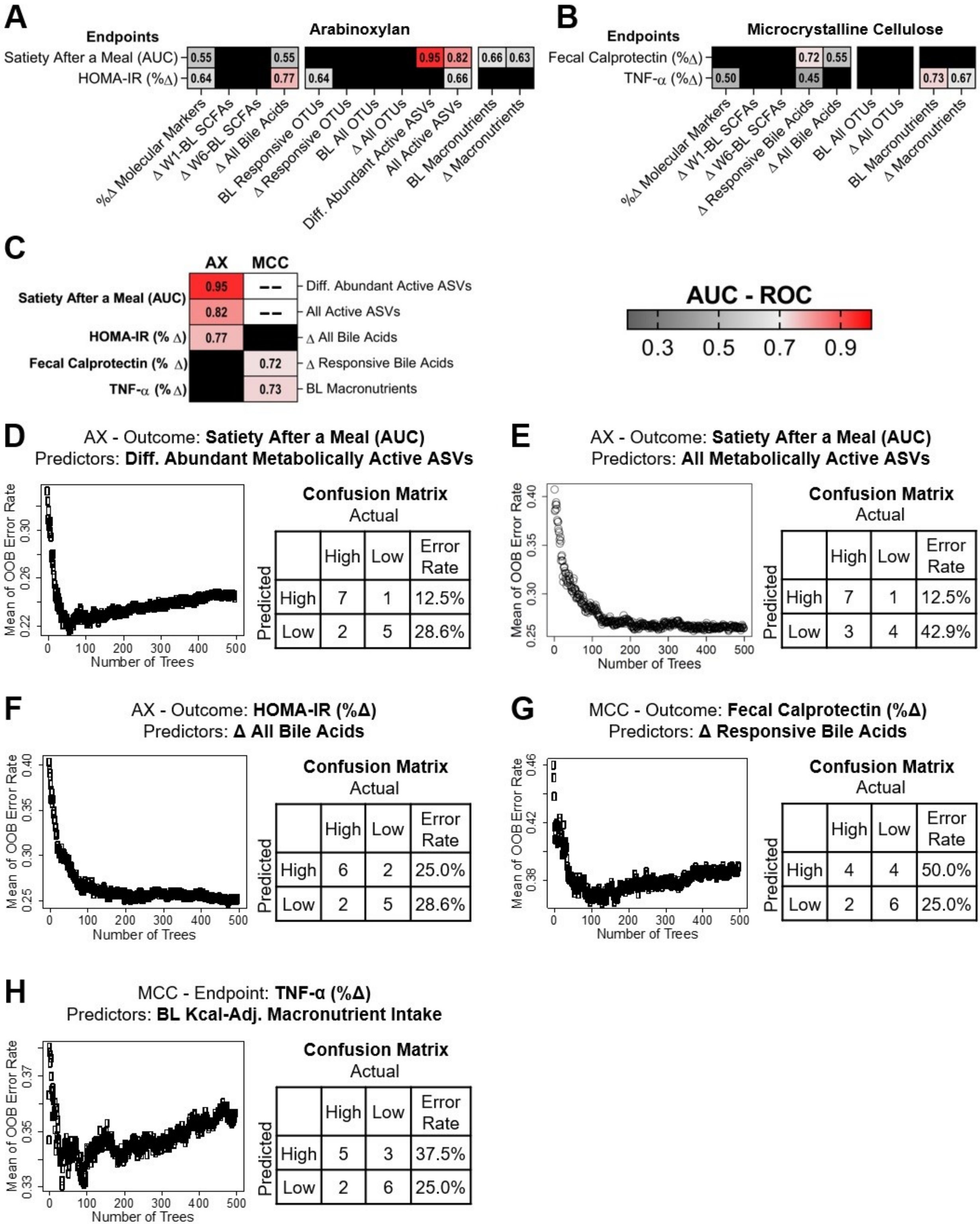


Supplementary Figure 1. Schematic representation of the *ex vivo* detection assay. *Legend continued on the next page.*

Stool samples stored frozen in phosphate-buffered saline (PBS) with 10% glycerol were thawed, filtered, and washed in PBS and then incubated in the presence of AX and the cellular activity marker *L*-azidohomoalanine (AHA) to detect AX-stimulated bacterial cells. A no-amendment control, containing only AHA, was incubated to detect possible basal activity in the absence of AX. Microscopic inspection showed no BONCAT signal for all controls; thus, no basal activity was detected. AX-incubated samples were then fixed in ethanol and active cells were stained using a Cu(I)-catalyzed click reaction (refer to supplementary methods for details). **(A and B)** A representative picture of fecal microbiota incubated for 6-hours **(A)** with AX and **(B)** without AX (BONCAT control). Stimulated cells are shown in pink as a Cy5-positive BONCAT signal were sorted by FACS, with all microbial cells shown in blue (DAPI stained). Cy5-negative bacterial cells (samples incubated with AHA but not submitted to the Cu(I)-catalyzed click reaction) were used to define the gate. Cy5-positive bacterial cells (samples incubated with AHA and submitted to Cu(I)-catalyzed click reaction) were gated and sorted out. DNA was extracted from both sorted cells and samples at 0-hour and 6-hour anaerobic incubations. The 16S rRNA gene was amplified by PCR and amplicons were sequenced using the Illumina Miseq platform. AX, arabinoxylan; BONCAT, bioorthogonal non-canonical amino acid tagging; FACS, fluorescence-activated cell sorting.

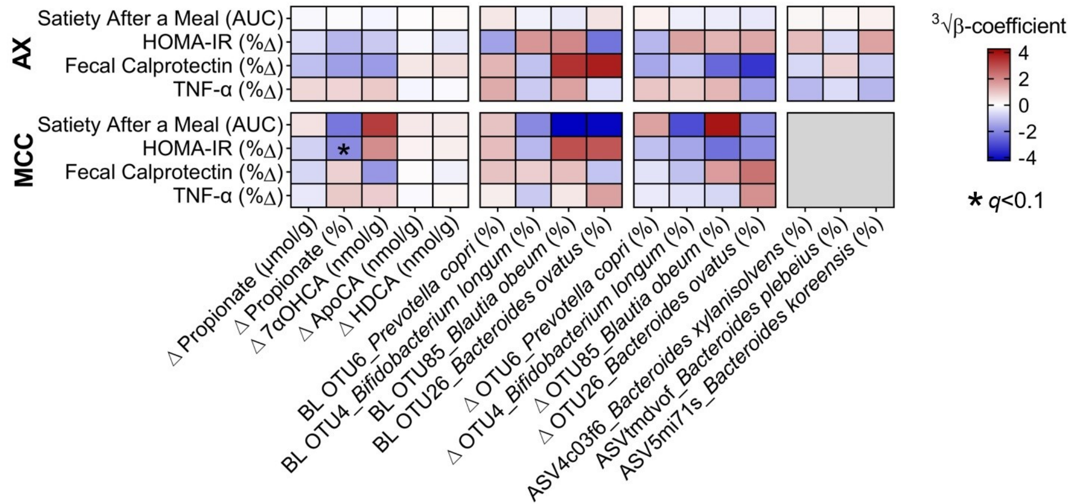


Supplementary Figure 2. Sorting of AX-stimulated bacterial cells by FACS on a MoFlow Astrios EQ cell sorter. As shown in the dot plots, **(A)** background noise of the machine was detected using FSC and SSC parameters. **(B)** Bacterial cells were measured in the same setting and pre-gated. **(C)** An example of Cy5-negative cells is presented in the dot plot showing the Cy5 channel via the SSC channel. **(D)** An example of Cy5-positive fluorescent cells (activated by AX) that were gated and sorted out by FACS. AX, arabinoxylan; FACS, fluorescence-activated cell sorting; FSC, forward scatter; SSC, side scatter.



Supplementary Figure 3. Confirmation of gut microbiota compositional features and mechanistic endpoints that predict clinical responses. *Legend continued on the next page.*

AUC-ROC values show the performance accuracy of random forest classifiers for predicting high-vs-low responders in **(A)** AX-induced perceived satiety after a meal and HOMA-IR attenuation, **(B)** MCC-induced fecal calprotectin and TNF-α attenuation, and **(C)** AX and MCC induced changes in HOMA-IR, fecal calprotectin, and TNF-α. High and low responders were defined according to the study cohort median. Black cells denote OOB error rates ≥0.6. Prediction performance of random forest classifiers trained to predict high-vs-low responders in AX-induced **(D to E)** satiety after a meal and **(F)** HOMA-IR attenuation, and MCC-induced **(G)** fecal calprotectin and **(H)** TNF-α attenuation. OOB shows the mean prediction error of the random forests model with boosted decision trees (n=500). The confusion matrix shows subgroup prediction accuracy, where row i and column j indicates the number of subjects predicted as i but were actually classified as j. Error rates indicate the percentage of incorrect classifications. Δ, absolute change from baseline to week 6; %Δ, percent change from baseline to week 6; ASV, amplicon sequence variant; AX, arabinoxylan; AUC-ROC, area under the receiver operating characteristic curve; HOMA-IR, homeostatic model assessment of insulin resistance; MCC, microcrystalline cellulose; OTU, operational taxonomic unit; OOB: out-of-bag; TNF-α, tumor necrosis factor-α.



Supplementary Figure 4. Associations between the effects on perceived satiety and surrogate endpoints and the dominant fecal microbiota features affected by fiber supplementation. Heatmap shows cubed-root transformed β-coefficients of univariate generalized linear models performed on the compositional (dominant AX-responsive taxa at baseline, shifts, and *ex vivo*) and functional (fecal propionate and bile acid shifts) features of the gut microbiota. Statistical significance was considered at FDR corrected q values <0.1. Δ, absolute change from baseline to week 6; %Δ, percent change from baseline to week 6; 7αOHCA; 7αOH-3-oxo-4-cholestenoic acid; ApoCA; apocholic acid; ASV, amplicon sequence variant; AUC, area under the curve; AX, arabinoxylan; BL, baseline; HDCA, hyodeoxycholic acid; HOMA-IR, homeostatic model assessment of insulin resistance; MCC, microcrystalline cellulose; OTU, operational taxonomic unit; TNF-α, tumor necrosis factor-α.

REFERENCES

1.) Ng M, Fleming T, Robinson M, *et al.* Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2014;384:766-81.

2.) Reynolds A, Mann J, Cummings J, *et al.* Carbohydrate quality and human health: a series of systematic reviews and meta-analyses. *Lancet* 2019;393:434-45.

3.) AlEssa HB, Bhupathiraju SN, Malik VS, *et al.* Carbohydrate quality and quantity and risk of type 2 diabetes in US women. *Am J Clin Nutr* 2015;102:1543-53.

4.) Kaczmarczyk MM, Miller MJ, Freund GG. The health benefits of dietary fiber: beyond the usual suspects of type 2 diabetes mellitus, cardiovascular disease and colon cancer. *Metabolism* 2012;61:1058-66.

5.) Deehan EC, Duar RM, Armet AM, *et al.* Modulation of the Gastrointestinal Microbiome with Nondigestible Fermentable Carbohydrates To Improve Human Health. *Microbiol Spectr* 2017;5.

6.) McRorie JWJ, McKeown NM. Understanding the Physics of Functional Fibers in the Gastrointestinal Tract: An Evidence-Based Approach to Resolving Enduring Misconceptions about Insoluble and Soluble Fiber. *J Acad Nutr Diet* 2017;117:251-64.

7.) Armet AM, Deehan EC, Thöne JV, *et al.* The Effect of Isolated and Synthetic Dietary Fibers on Markers of Metabolic Diseases in Human Intervention Studies: A Systematic Review. *Adv Nutr* 2020;11:420–38.

8.) Jovanovski E, Khayyat R, Zurbau A, *et al.* Should Viscous Fiber Supplements Be Considered in Diabetes Control? Results From a Systematic Review and Meta-analysis of Randomized Controlled Trials. *Diabetes Care* 2019;42:755-66.

9.) EFSA Panel on Dietetic Products Nutrition and Allergies. Scientific Opinion on the substantiation of health claims related to arabinoxylan produced from wheat endosperm and reduction of post-prandial glycaemic responses (ID 830) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *EFSA J* 2011;9:2205.

10.) Capuano E. The behavior of dietary fiber in the gastrointestinal tract determines its physiological effect. *Crit Rev Food Sci Nutr* 2017;57:3543-64.

11.) Makki K, Deehan EC, Walter J, *et al.* The Impact of Dietary Fiber on Gut Microbiota in Host Health and Disease. *Cell Host Microbe* 2018;23:705-15.

12.) Meijnikman AS, Gerdes VE, Nieuwdorp M, *et al.* Evaluating Causality of Gut Microbiota in Obesity and Diabetes in Humans. *Endocr Rev* 2018;39:133-53.

13.) Tuncil YE, Thakkar RD, Arioglu-Tuncil S, *et al.* Subtle Variations in Dietary-Fiber Fine Structure Differentially Influence the Composition and Metabolic Function of Gut Microbiota. *mSphere* 2020;5:e00180-20.

14.) Deehan EC, Yang C, Perez-Muñoz ME, *et al.* Precision Microbiome Modulation with Discrete Dietary Fiber Structures Directs Short-Chain Fatty Acid Production. *Cell Host Microbe* 2020;27:389-404.e6.

15.) Kovatcheva-Datchary P, Nilsson A, Akrami R*, et al.* Dietary Fiber-Induced Improvement in Glucose Metabolism Is Associated with Increased Abundance of *Prevotella*. *Cell Metab* 2015;22:971-82.

16.) Martínez I, Lattimer JM, Hubach KL*, et al.* Gut microbiome composition is linked to whole grain-induced immunological improvements. *ISME J* 2013;7:269-80.

17.) Blaak EE, Canfora EE, Theis S*, et al.* Short chain fatty acids in human gut and metabolic health. *Benef Microbes* 2020;11:411-55.

18.) Winston JA, Theriot CM. Diversification of host bile acids by members of the gut microbiota. *Gut Microbes* 2020;11:158-71.

19.) Chiang JY, Ferrell JM. Bile Acids as Metabolic Regulators and Nutrient Sensors. *Annu Rev Nutr* 2019;39:175-200.

20.) Dewulf EM, Cani PD, Claus SP*, et al.* Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. *Gut* 2013;62:1112-21.

21.) Zhao L, Zhang F, Ding X*, et al.* Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes. *Science* 2018;359:1151-6.

22.) Fechner A, Kiehntopf M, Jahreis G. The formation of short-chain fatty acids is positively associated with the blood lipid-lowering effect of lupin kernel fiber in moderately hypercholesterolemic adults. *J Nutr* 2014;144:599-607.

23.) Nicolucci AC, Hume MP, Martinez I*, et al.* Prebiotic Reduces Body Fat and Alters Intestinal Microbiota in Children With Overweight or Obesity. *Gastroenterology* 2017;153:711–22.

24.) Bindels LB, Segura Munoz RR, Gomes-Neto JC*, et al.* Resistant starch can improve insulin sensitivity independently of the gut microbiota. *Microbiome* 2017;5:12.

25.) Walter J, Armet AM, Finlay BB*, et al.* Establishing or Exaggerating Causality for the Gut Microbiome: Lessons from Human Microbiota-Associated Rodents. *Cell* 2020;180:221-32.

26.) Nguyen NK, Deehan EC, Zhang Z*, et al.* Gut microbiota modulation with long-chain corn bran arabinoxylan in adults with overweight and obesity is linked to an individualized temporal increase in fecal propionate. *Microbiome* 2020;8:118.

27.) Kale MS, Yadav MP, Hicks KB*, et al.* Concentration and shear rate dependence of solution viscosity for arabinoxylans from different sources. *Food Hydrocoll* 2015;47:178-83.

28.) Rumpagaporn P, Reuhs BL, Kaur A*, et al.* Structural features of soluble cereal arabinoxylan fibers associated with a slow rate of *in vitro* fermentation by human fecal microbiota. *Carbohydr Polym* 2015;130:191-7.

29.) Hatzenpichler R, Scheller S, Tavormina PL*, et al. In situ* visualization of newly synthesized proteins in environmental microbes using amino acid tagging and click chemistry. *Environ Microbiol* 2014;16:2568-90.

30.) Kirkpatrick SI, Subar AF, Douglass D*, et al.* Performance of the Automated Self-Administered 24-hour Recall relative to a measure of true intakes and to an interviewer-administered 24-h recall. *Am J Clin Nutr* 2014;100:233-40.

31.) Cardello AV, Schutz HG, Lesher LL, *et al.* Development and testing of a labeled magnitude scale of perceived satiety. *Appetite* 2005;44:1-13.

32.) Callahan BJ, McMurdie PJ, Rosen MJ, *et al.* DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* 2016;13:581-3.

33.) Mi S, Zhao Y-Y, Jacobs RL, *et al.* Simultaneous determination of trimethylamine and trimethylamine *N*-oxide in mouse plasma samples by hydrophilic interaction liquid chromatography coupled to tandem mass spectrometry. *J Sep Sci* 2017;40:688-96.

34.) Han J, Liu Y, Wang R, *et al.* Metabolic profiling of bile acids in human and mouse blood by LC-MS/MS in combination with phospholipid-depletion solid-phase extraction. *Anal Chem* 2015;87:1127-36.

35.) Seethaler B, Nguyen NK, Basrai M, *et al.* A Mediterranean diet improves intestinal barrier integrity in women at risk of breast cancer (LIBRE study). *In Preparation*. 2020.

36.) Wolever TMS, van Klinken BJ-W, Spruill SE, *et al.* Effect of serving size and addition of sugar on the glycemic response elicited by oatmeal: A randomized, cross-over study. *Clin Nutr ESPEN* 2016;16:48-54.

37.) Brito IL, Gurry T, Zhao S, *et al.* Transmission of human-associated microbiota along family and social networks. *Nat Microbiol* 2019;4:964-71.

38.) Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 2014;15:550.

39.) Ballou S, Singh P, Rangan V, *et al.* Obesity is associated with significantly increased risk for diarrhoea after controlling for demographic, dietary and medical factors: a cross-sectional analysis of the 2009-2010 National Health and Nutrition Examination Survey. *Aliment Pharmacol Ther* 2019;50:1019-24.

40.) Tannock GW, Liu Y. Guided dietary fibre intake as a means of directing short-chain fatty acid production by the gut microbiota. *J Roy Soc New Zeal* 2019.

41.) Louis P, Flint HJ. Formation of propionate and butyrate by the human colonic microbiota. *Environ Microbiol* 2017;19:29-41.

42.) Delzenne NM, Olivares M, Neyrinck AM, *et al.* Nutritional interest of dietary fiber and prebiotics in obesity: Lessons from the MyNewGut consortium. *Clin Nutr* 2020;39:414-24.

43.) Hartvigsen ML, Gregersen S, Laerke HN, *et al.* Effects of concentrated arabinoxylan and β-glucan compared with refined wheat and whole grain rye on glucose and appetite in subjects with the metabolic syndrome: a randomized study. *Eur J Clin Nutr* 2014;68:84-90.

44.) Brum JM, Gibb RD, Peters JC, *et al.* Satiety effects of psyllium in healthy volunteers. *Appetite* 2016;105:27-36.

45.) Isaksson H, Sundberg B, Åman P, *et al.* Whole grain rye porridge breakfast improves satiety compared to refined wheat bread breakfast. *Food Nutr Res* 2008;52:1809.

46.) Cioffi I, Ibrugger S, Bache J, *et al.* Effects on satiation, satiety and food intake of wholegrain and refined grain pasta. *Appetite* 2016;107:152-8.

47.) Hartvigsen ML, Laerke HN, Overgaard A*, et al.* Postprandial effects of test meals including concentrated arabinoxylan and whole grain rye in subjects with the metabolic syndrome: a randomised study. *Eur J Clin Nutr* 2014;68:567-74.

48.) Chambers ES, Viardot A, Psichas A*, et al.* Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults. *Gut* 2015;64:1744-54.

49.) Ruijschop RM, Boelrijk AE, te Giffel MC. Satiety effects of a dairy beverage fermented with propionic acid bacteria. *Int Dairy J* 2008;18:945-50.

50.) Gurry T, Nguyen LTT, Yu X*, et al.* Functional heterogeneity in the fermentation capabilities of the healthy human gut microbiota. *bioRxiv* 2020.

51.) Müller M, Hernández MAG, Goossens GH*, et al.* Circulating but not faecal short-chain fatty acids are related to insulin sensitivity, lipolysis and GLP-1 concentrations in humans. *Sci Rep* 2019;9:12515.

52.) Parnell JA, Reimer RA. Weight loss during oligofructose supplementation is associated with decreased ghrelin and increased peptide YY in overweight and obese adults. *Am J Clin Nutr* 2009;89:1751-9.

53.) Cani PD, Lecourt E, Dewulf EM*, et al.* Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite sensation and glucose response after a meal. *Am J Clin Nutr* 2009;90:1236-43.

54.) Lu ZX, Walker KZ, Muir JG*, et al.* Arabinoxylan fiber, a byproduct of wheat flour processing, reduces the postprandial glucose response in normoglycemic subjects. *Am J Clin Nutr* 2000;71:1123-8.

55.) Lu ZX, Walker KZ, Muir JG*, et al.* Arabinoxylan fibre improves metabolic control in people with Type II diabetes. *Eur J Clin Nutr* 2004;58:621-8.

56.) Garcia AL, Otto B, Reich S-C*, et al.* Arabinoxylan consumption decreases postprandial serum glucose, serum insulin and plasma total ghrelin response in subjects with impaired glucose tolerance. *Eur J Clin Nutr* 2007;61:334-41.

57.) Kahleova H, Tura A, Hill M*, et al.* A Plant-Based Dietary Intervention Improves Beta-Cell Function and Insulin Resistance in Overweight Adults: A 16-Week Randomized Clinical Trial. *Nutrients* 2018;10.

58.) Kootte RS, Levin E, Salojarvi J*, et al.* Improvement of Insulin Sensitivity after Lean Donor Feces in Metabolic Syndrome Is Driven by Baseline Intestinal Microbiota Composition. *Cell Metab* 2017;26:611-9 e6.

59.) Nagy-Szakal D, Hollister EB, Luna RA*, et al.* Cellulose supplementation early in life ameliorates colitis in adult mice. *PLoS One* 2013;8:e56685.

60.) Kim Y, Hwang SW, Kim S*, et al.* Dietary cellulose prevents gut inflammation by modulating lipid metabolism and gut microbiota. *Gut Microbes* 2020;11:944-61.

61.) Di Caro V, Alcamo AM, Cummings JL*, et al.* Effect of dietary cellulose supplementation on gut barrier function and apoptosis in a murine model of endotoxemia. *PLoS One* 2019;14:e0224838.

62.) Guo C, Xie S, Chi Z*, et al.* Bile Acids Control Inflammation and Metabolic Disorder through Inhibition of NLRP3 Inflammasome. *Immunity* 2016;45:802-16.

63.) Dongowski G. Interactions between dietary fibre-rich preparations and glycoconjugated bile acids *in vitro*. *Food Chem* 2007;104:390-7.

64.) Zacherl C, Eisner P, Engel K-H. *In vitro* model to correlate viscosity and bile acid-binding capacity of digested water-soluble and insoluble dietary fibres. *Food Chem* 2011;126:423-8.

65.) Zeng H, Umar S, Rust B*, et al.* Secondary Bile Acids and Short Chain Fatty Acids in the Colon: A Focus on Colonic Microbiome, Cell Proliferation, Inflammation, and Cancer. *Int J Mol Sci* 2019;20:1214.

66.) Ocvirk S, O'Keefe SJ. Influence of Bile Acids on Colorectal Cancer Risk: Potential Mechanisms Mediated by Diet - Gut Microbiota Interactions. *Curr Nutr Rep* 2017;6:315-22.

67.) Wong CB, Odamaki T, Xiao J-z. Beneficial effects of *Bifidobacterium longum* subsp. *longum* BB536 on human health: Modulation of gut microbiome as the principal action. *J Funct Foods* 2019;54:506-19.

68.) Sandberg J, Kovatcheva-Datchary P, Bjorck I*, et al.* Abundance of gut *Prevotella* at baseline and metabolic response to barley prebiotics. *Eur J Nutr* 2018;58:2365-76.

69.) ClinicalTrials.gov. National Library of Medicine (US). Identifier NCT02322112, The Alberta FYBER (Feed Your gut Bacteria morE fibeR) Study. 2015, July 3. Retrieved April 25, 2020 from: https://clinicaltrials.gov/ct2/history/NCT02322112?V_2=View#StudyPageTop.

70.) Thompson FE, Dixit-Joshi S, Potischman N*, et al.* Comparison of Interviewer-Administered and Automated Self-Administered 24-Hour Dietary Recalls in 3 Diverse Integrated Health Systems. *Am J Epidemiol* 2015;181:970-8.

71.) Zimmerman TP, Potischman N, Douglass D*, et al.* The Effect of Editing Open-Ended Text Responses on Nutrient and Food Group Estimates from the Automated Self-Administered 24-Hour Dietary Recall (ASA24). *Procedia Food Sci* 2015;4:160-72.

72.) Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. *Am J Epidemiol* 1986;124:17-27.

73.) Engels JM, Diehr P. Imputation of missing longitudinal data: a comparison of methods. *J Clin Epidemiol* 2003;56:968-76.

74.) National Heart Lung and Blood Institute. The Practical Guide: Identification, Evaluation, and Treatment of Overweight and Obesity in Adults. Bethesda, MD: National Institutes of Health, 2000:94.

75.) Matthews DR, Hosker JP, Rudenski AS*, et al.* Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412-9.

76.) Katz A, Nambi SS, Mather K*, et al.* Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab* 2000;85:2402-10.

77.) Slavin JL. Fiber and prebiotics: mechanisms and health benefits. *Nutrients* 2013;5:1417-35.

78.) Lockyer S, Stanner S. Prebiotics – an added benefit of some fibre types. *Nutr Bull* 2019;44:74-91.

79.) Jin M, Kalainy S, Baskota N*, et al.* Faecal microbiota from patients with cirrhosis has a low capacity to ferment non-digestible carbohydrates into short-chain fatty acids. *Liver Int* 2019;39:1437-47.

80.) Herbold CW, Pelikan C, Kuzyk O*, et al.* A flexible and economical barcoding approach for highly multiplexed amplicon sequencing of diverse target genes. *Front Microbiol* 2015;6:731.

81.) Klindworth A, Pruesse E, Schweer T*, et al.* Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res* 2013;41:e1.

82.) Laros JF. demultiplex. 2020. version 1.1.0. https://github.com/jfjlaros/demultiplex.

83.) Callahan BJ, Sankaran K, Fukuyama JA*, et al.* Bioconductor Workflow for Microbiome Data Analysis: from raw reads to community analyses. *F1000Res* 2016;5:1492.

84.) Wang Q, Garrity GM, Tiedje JM*, et al.* Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* 2007;73:5261-7.

85.) Davis NM, Proctor DM, Holmes SP*, et al.* Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. *Microbiome* 2018;6:226.

86.) Frossard J, Renaud O. permuco: Permutation Tests for Regression, (Repeated Measures) ANOVA/ANCOVA and Comparison of Signals. 2019. R-package version 1.1.0. https://CRAN.R-project.org/package=permuco.

87.) Wheeler B, Torchiano M. lmPerm: Permutation Tests for Linear Models. 2016. R-package version 2.1.0. https://CRAN.R-project.org/package=lmPerm.

88.) Kassambara A, Mundt F. factoextra: Extract and Visualize the Results of Multivariate Data Analyses. 2017. R-package version 1.0.5. https://CRAN.R-project.org/package=factoextra.

89.) Lê S, Josse J, Husson F. FactoMineR: An R package for multivariate analysis. *J Stat Softw* 2008;25.

90.) Oksanen J, Blanchet GF, Friendly M*, et al.* vegan: Community Ecology Package. 2019. R-package version 2.5-5. https://CRAN.R-project.org/package=vegan.

91.) VanderWeele TJ. Principles of confounder selection. *Eur J Epidemiol* 2019;34:211-9.

92.) Knights D, Costello EK, Knight R. Supervised classification of human microbiota. *FEMS Microbiol Rev* 2011;35:343-59.

93.) Chen X, Ishwaran H. Random forests for genomic data analysis. *Genomics* 2012;99:323-9.

94.) Liaw A, Wiener M. Classification and Regression by randomForest. *R News* 2002;2:18-22.

95.) Maldonado-Gómez MX, Martínez I, Bottacini F*, et al.* Stable Engraftment of *Bifidobacterium longum* AH1206 in the Human Gut Depends on Individualized Features of the Resident Microbiome. *Cell Host Microbe* 2016;20:515–26.

96.) Robin X, Turck N, Hainard A*, et al.* pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinformatics* 2011;12:77.