Application of targeted metabolomics to reveal associations between carbohydrate quality and cardiometabolic risk factors

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Abstract

Background: The health impact of carbohydrate (CHO) food sources varies considerably based on CHO structure and function. Emerging research shows that the physiological effects of different fiber sources and simple sugars vary substantially between individuals through mechanisms that have not yet been fully elucidated. Identifying metabolite responses to different sugars or fiber sources will enhance our understanding of CHO intake on cardiometabolic health. The **overall objective** of this research was to investigate the impact of different sugars and food sources of dietary fiber on metabolite profiles and consider the relationship of these metabolites with cardiometabolic risk factors.

Methods:

Aim 1: To identify metabolite changes in response to the consumption of two sugar moieties, glucose and fructose, and to correlate these metabolite changes with phenotypic responses to intake. In a parallel trial of 31 adults with overweight or obesity, metabolite (97 metabolites) and phenotypic data (sex, metabolic syndrome and insulin resistance (HOMA-IR)) were collected at baseline and after consumption of fructose- or glucose-sweetened beverages for 10 weeks. Orthogonal Partial Least Square Discriminatory Analysis (OPLS-DA) was used to identify metabolites that could discriminate the glucose from fructose intervention groups. Paired and independent sample 2-sided t-tests were used to assess within-group and between-group differences in selected metabolites, respectively, with a False Discovery Rate (FDR)-adjusted p-value.

Aim 2: To identify novel dietary fiber-related metabolites reflecting different fiber food sources. Using a systematic approach, we conducted a scoping review of intervention and observational studies reporting associations between dietary fiber intake and metabolites. Data on biofluid, fiber source, and direction of the association (positive, negative, or nonsignificant) were summarized in a dietary fiber–metabolite (DFM) database with metabolites grouped by metabolite class. A unique dietary fiber scoring system (DFSS) was developed and applied to assess the validity and replicability of these metabolites as potential biomarkers of dietary fiber intake. We applied the DFSS to metabolites identified as positively associated with high-fiber foods (cereal, fruit, vegetable, legume, or total fiber) in two or more studies. **Aim 3a: To develop and validate metabolomic signatures of recent dietary fiber intake (total, cereal, fruit, vegetable, and nut and legume). Aim 3b: To examine the relationship between dietary fiber intakes and their metabolite signatures with longitudinal changes in cardiometabolic risk factors [waist circumference (WC, in), diastolic blood pressure (DBP, mmHg), systolic blood pressure (SBP, mmHg), fasting plasma HDL cholesterol (HDL-C, mg/dL), fasting plasma triglycerides (mg/dL), fasting serum glucose (mg/dL), and hemoglobin A1c (HbA1c, %)].** We used prospective data from the Framingham Offspring study (N= 5,124). Intake of dietary fiber was assessed using the previously validated Harvard semiquantitative Food Frequency Questionnaire (FFQ), and targeted metabolomic profiling was performed on fasting plasma samples collected at the same visit. An elastic net regression model was used to develop distinct metabolomic signatures predicting each fiber type. Multiple linear regression models were used to examine the associations

between self-reported fiber intakes and their corresponding metabolomic signatures with 4-year changes in cardiometabolic risk factors.

Results:
Aim 1: Nominal differences by intervention group were observed in the change of sixteen individual metabolites (five in the branched-chain amino acid catabolic pathway) and a branched-chain keto acid (BCKA) composite score. A significant increase in ketoisoleucine was observed after glucose supplementation (+ 2.19 μM, P_{FDR} = 0.04) compared to a null change after fructose supplementation. **Aim 2:** A total of 4,173 abstracts were screened, 198 articles were selected for full-text review, and data was extracted from 80 articles. A total of 662 unique metabolites from 30 different metabolite classes were included in the DFM database. Out of the 34 metabolites identified in 2 or more studies, DFSS scores ranged between 0.8 – 5, where 7 metabolites received a score ≥ 3. Ferulic acid derivatives, attached to grains and released by the gut microbiome, scored highly for cereal fiber. **Aim 3:** We identified metabolomic signatures that significantly predicted intakes of total fiber (r = 0.39, p-value < 0.001), cereal fiber (r = 0.33, p-value < 0.001), fruit fiber (r = 0.32, p-value < 0.001), vegetable fiber (r = 0.20, p-value < 0.001), and nut and legume fiber (r = 0.12, p-value < 0.001). Notably, cereal fiber and its metabolomic signature were significantly associated with more favorable changes (β [SE]) in fasting glucose (-1.01 [0.31] mg/dL, P_{adj} = 0.005; and -0.80 [0.17] mg/dL, P_{adj} < 0.001), HbA1c (-0.05 [0.02] %, P_{adj} = 0.005; and -0.03 [0.01] %, P= 0.01), and HDL-C levels (0.98 [0.26] mg/dL, P_{adj} < 0.001; and 0.37 [0.15] mg/dL, P_{adj}= 0.04), suggesting that cereal fiber intake may have a particularly beneficial impact on cardiometabolic health.

Conclusion: Our aim 1 findings suggest that, in people with overweight/obesity, consuming glucose versus fructose sweetened beverages differentially affects the metabolism of branched-chain amino acids, including within the isoleucine catabolic pathway. Our aims 2 and 3 suggest that dietary fiber from cereal sources, as well as metabolites associated with cereal fiber intake, show a consistent inverse association with fasting glucose, HbA1c, and a positive association with HDL-C compared to intake of dietary fiber from other food sources. These findings provide a foundation for future research aiming to explore molecular mechanisms underlying CHO metabolism. Replications of our findings in larger, well-powered studies are warranted to further identify potential inter-individual differences in response to CHO consumption.

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Introduction: General Statement of Problem and Significance

The quality of carbohydrate (CHO) foods varies considerably based on CHO structure, ranging from simple to complex; and degree of digestibility, ranging from quick absorbing to non-digestible and fermentable. The numerous pathways of CHO metabolism contribute to the complexity of defining CHO health effects. For example, for type 2 diabetes (T2D) control and prevention, emphasis is placed on consuming nutritionally superior CHO-quality foods, i.e., high in dietary fiber and low in added sugars, to improve blood sugar control. However, the physiological effects of different dietary fiber sources and simple sugars vary substantially among individuals through mechanisms that have not yet been fully elucidated.

In nutritional epidemiology, targeted metabolomics is increasingly being applied to identify food intake biomarkers and discover biological processes influenced by our diet that affect disease risk. Metabolomics is a promising tool to advance precision nutrition, as it may allow for the exploration of the large interindividual variability in response to diet, which has been poorly understood to date. Thus, research examining changes in metabolite concentrations by CHO consumption may help elucidate potential mechanisms of CHO metabolism on cardiometabolic risk in humans.

We proposed to study the impact of two types of CHOs – sugar and dietary fiber, reflecting opposite sides of the CHO quality spectrum, on metabolite profiles and the relationship of these metabolites with cardiometabolic risk factors.

Hypotheses to be tested

Our central hypothesis is that carbohydrates — differing by their structure (glucose vs fructose), and dietary sources (fiber from cereal, fruit, vegetables, and legumes) — have differential effects on cardiometabolic pathways linked to metabolic risk factors that can be identified by metabolomics.

To test our hypothesis, this thesis project aimed to identify metabolite profiles associated with both carbohydrate consumption and metabolic risk factors in feeding studies and prospective cohort studies.

Aim 1: Examine metabolic and phenotypic differences after short-term intake of fructose-sweetened or glucose-sweetened beverages in the Inpatient, Outpatient (IPOP) trial (n=31).

- **Aim 1a. Study the difference in 10-week changes in fasting candidate metabolites (n=97) between the glucose-sweetened and the fructose-sweetened beverage arms. Hypothesis**: We will identify differences in 10-week metabolic changes between the individuals assigned to consume glucose-sweetened versus fructose-sweetened beverages. Particularly, we expect to see larger increases in the branched-chain amino acids (BCAA) in the glucose arm and larger increases in branched-chain keto acids (BCKA) in the fructose arm.
- **Aim 1b. Identify metabolites that interact with phenotypic and clinical biomarkers [sex, metabolic syndrome (MetSyn), and insulin resistance (IR)]. Hypothesis**: We expect significant interactions between the intervention and MetSyn on BCAA pathway metabolite changes, particularly in the fructose intervention.

Aim 2: Conduct a scoping review of intervention and observational studies that aimed to identify metabolites associated with intake of high fiber foods. Hypotheses: We expect to find unique metabolites associated with different high fiber foods replicated in the literature. We expect some metabolites will be replicated in our Aim 3.

Aim 3. To develop and validate metabolomic signatures predicting short-term dietary fiber intake and to examine their association with longitudinal changes in cardiometabolic risk factors in the Framingham Offspring Study (FOS).

- **Aim 3a. To develop and validate metabolomic signatures of recent dietary fiber intake (total, cereal, fruit, vegetable, and nut and legume).** We predict that unique metabolomic signatures can be derived that reflect the intake of cereal, fruit, vegetables, nut & legumes, and total dietary fiber. We further hypothesize that individual metabolites will be associated with specific fiber intakes.
- **Aim 3b: To examine the relationship between dietary fiber intakes and their metabolite signatures with longitudinal changes in cardiometabolic risk factors [waist circumference (WC, in), diastolic blood pressure (DBP, mmHg), systolic blood pressure (SBP, mmHg), fasting plasma HDL cholesterol (HDL-C, mg/dL), fasting plasma triglycerides (mg/dL), fasting circulating serum glucose (mg/dL), and hemoglobin A1c (HbA1c, %)].** We hypothesize that our fiber intake and metabolomic exposures are associated with favorable changes in cardiometabolic risk factors. We expect cereal fiber intake and its corresponding metabolomic signature will have the strongest favorable association, although different results may reveal novel mechanistic pathways.

Chapter 1: Review of Literature

Metabolomics in Nutrition Research

The metabolome is the complete collection of metabolites in a biosample, which can be tissue, cells, or biofluids. Metabolomics is a branch of analytical chemistry aiming to identify and annotate the metabolome, generally characterized as small molecules (molecular weight <1,500 Da)[1] that include endogenous compounds such as amino acids, peptides, nucleic acids, sugars, lipids, organic acids, and fatty acids, as well as exogenous chemicals such as toxins and xenobiotics.[2] The advent of metabolomics can be attributed to the recent advances in the instruments that quantify metabolites, which include nuclear magnetic resonance (NMR) spectroscopy[3], gas chromatography-mass spectrometry (GC-MS)[4], and liquid chromatography MS (LC-MS).[5, 6] While NMR best quantifies abundant metabolites by providing absolute quantification, GC-MS and, LC-MS are better at detecting lower abundance metabolites by providing relative quantification. Metabolite quantification can be carried out by either untargeted or targeted metabolomics. Untargeted metabolomics can detect up to 10,000 independent spectral features in a single specimen but can only identify approximately 1/3 of these features.[6] Untargeted metabolomics is used in hypothesis generation to discover novel compounds and obtain a complete, unbiased profile of the metabolome. On the other hand, targeted metabolomics can quantify known metabolites with identified chemical standards, generally, at higher sensitivity and specificity than untargeted and is more commonly used in hypothesis testing to identify or confirm candidate biomarkers.[7] Study designs for biomarker discovery using metabolomics can include the combination of interventions and observational studies, where short to medium-term interventions identify biomarkers that are then assessed in observational studies. In these studies, the crossover design is favored over parallel design because of its ability to account for inter-subject variation.[8]

The Human Metabolome Database (HMDB) has identified 217,920 annotated metabolites so far.[9] These metabolites can be used to approximate metabotypes (metabolic phenotypes) and study their interaction with genetics, the microbiome, drug therapies, and nutrition. Targeted metabolomics is increasingly applied to food and

nutrition science for food composition analysis[10, 11], identifying biomarkers of food intake[12,13], and discovering biological processes influenced by our diet in nutritional epidemiology.[14,15] Examples of major initiatives for each of these metabolomics applications include FooDB, the largest food metabolome database[16]; the Food Biomarker Alliance (FoodBall) to identify biomarkers of food intake[17]; and the Trans-Omics for Precision Medicine (TOPMed) program, led by the NIH under the Precision Medicine Initiative to provide disease treatments tailored to an individual's unique OMICs background.[18]

The potential of metabolomics in the nutrition field has been demonstrated in both experimental and observational studies. For example, a recent study out of the United Kingdom successfully used metabolic phenotyping from urine to categorize dietary patterns of individuals in a controlled crossover trial.[19] Metabolomics presents a technology for advancing precision nutrition, as it may allow for the identification of metabotypes that could be used for individual stratification and the elucidation of the large interindividual variability in response to diet.[20,21]As an example, a landmark study carried out by Zeevi et al (2015) used metabolomics and metagenomics to develop individualized dietary recommendations for blood glucose control for 800 participants[22] and led to the development of the first precision nutrition company, DayTwo.[23] Nutrition metabolomics can also be applied to study associations between dietary intake and disease outcomes. Dysregulated metabolism is a hallmark of chronic diseases such as obesity, type 2 diabetes, and cardiovascular disease and identifying specific biomarkers of disease may improve prediction and prevention among high-risk individuals.

This thesis investigated mechanisms by which CHO metabolism may contribute to cardiometabolic risk outcomes by examining the associations between the metabolomics of two types of CHOs at opposite sides of the CHO quality spectrum - added sugar and dietary fiber. It first studied the metabolic disruptions that can be attributed to the consumption of glucose vs fructose-sweetened beverages (GSB and FSB), particularly with respect to branched-chain amino acids (BCAA) metabolism, as recent preclinical data suggests a connection between fructose metabolism and deranged BCAA metabolism.[24]

Second, this thesis studied the distinct metabolomic signatures that predict intake of different types and sources of dietary fiber , and the associations of those signatures with cardiometabolic risk. Consequently, pertinent topics on sugar, BCAA, and dietary fiber metabolism are elaborated in the following sections.

Differential metabolism of Fructose versus Glucose

Glucose and fructose, both monosaccharides, are digested and absorbed through different mechanisms in the human body. Glucose is present in various foods such as grains, fruits, vegetables, honey, and as part of disaccharides like sucrose and polysaccharides like starch. The digestion of glucose begins in the mouth with the action of salivary amylase, continues in the stomach where gastric acid partially hydrolyzes these carbohydrates, and primarily occurs in the small intestine with pancreatic amylase and brush border enzymes like maltase breaking them down into monosaccharides.[25] Glucose is then absorbed in the small intestine via active transport through the sodium-glucose co-transporter 1 (SGLT1) and facilitated diffusion via glucose transporter 2 (GLUT2) into the bloodstream.[26] Fructose, mostly found in fruits, honey, and high-fructose corn syrup,[27] undergoes a different absorption process. Unlike glucose, fructose does not require digestion and is absorbed directly in the small intestine through facilitated diffusion via glucose transporter 5 (GLUT5).[28] Once inside the enterocytes, some fructose is converted to glucose or lactate, while the rest enters the bloodstream through GLUT2.[28]

The metabolic pathways of glucose and fructose also differ. Glucose is primarily utilized for energy through glycolysis, the Krebs cycle, and oxidative phosphorylation, while excess glucose is stored as glycogen in the liver and muscles.[29] Fructose metabolism occurs mainly in the liver, where it is phosphorylated by fructokinase and enters the glycolytic pathway.[28] Excessive intake of fructose has been associated with various cardiometabolic risk factors, including increased lipogenesis leading to higher levels of triglycerides, insulin resistance (IR), and non-alcoholic fatty liver disease (NAFLD).[30] In contrast, while excessive glucose intake can also contribute to obesity and IR,[31] the body's regulation and utilization pathways for glucose are more robust compared to fructose,

which is why fructose is considered more harmful in the context of metabolic diseases.[32] Both monosaccharides, however, when consumed in high amounts, can lead to detrimental health outcomes, underscoring the importance of a balanced dietary intake.

The intake of sugars, particularly fructose, may also be associated with alterations in circulating branched-chain amino acids (BCAAs), which include leucine, isoleucine, and valine. Specifically, Wang et al. demonstrated that dietary patterns high in sugars and fats are strongly associated with elevated plasma BCAAs, further emphasizing the role of diet in modulating amino acid metabolism and its impact on metabolic health.[33] Furthermore, Zucker fatty rats fed a high fructose diet demonstrated reduced BCAA catabolism. However, the effect of fructose intake in humans has not yet been fully explored. As demonstrated in the next section, BCAAs are linked to IR, type 2 diabetes, and obesity, highlighting a potential mechanistic pathway through which high sugar intake exacerbates metabolic disorders.

Circulating Branch Chain Amino Acids and Health

Cumulating evidence suggests that high levels of circulating BCAAs are predictive of T2D.[21] Indeed, prospective cohort studies demonstrate robust positive associations of circulating BCAA's with IR and T2D.[21,34,35,36] Although the underlying mechanisms of these associations remain unclear, several pathways have been proposed (Figure 1). Two Mendelian randomization studies explore a causal link between high circulating BCAAs and IR.[37,38] Circulating BCAAs levels were found to be altered among obese versus lean humans and contributed to IR in experimental studies. Further, BCAAs significantly decreased in two large diet and lifestyle interventions.[3940] Newgard *et al.* have also shown that metabolic profiling of obese individuals often reveals increased BCAA levels, which correlates with IR and metabolic syndrome (MetSyn).[41] Additionally, BCAAs levels are higher in obese microbiomes.[42,43] Furthermore, leucine interacts with mTOR kinase, which can lead to increased insulin secretion and pancreatic exhaustion.[44] Finally, circulating BCAA's have been linked to increased lipid accumulation in skeletal muscle via glycine

depletion and increased 3-hydroxyisobutyrate.[45,46] Supporting this notion, decreased BCAA oxidation was observed in the liver and adipose tissue of individuals with IR.[41,37, 40]

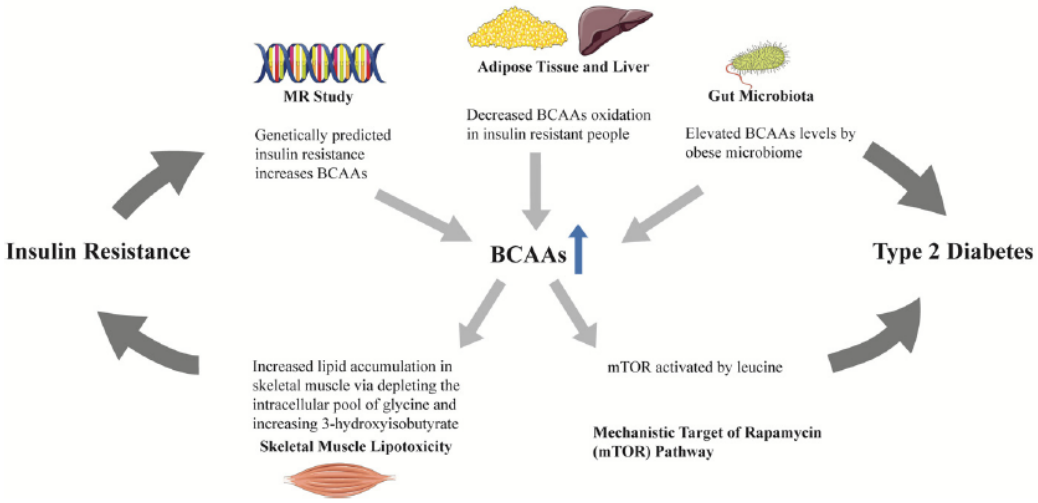

Figure 1. Multifunctional role of BCAAs in the progression of IR and T2D. Figure from Jin et al.[47]

Although the three BCAAs (Valine, Isoleucine, and Leucine) are often lumped together in analyses as a "BCAA score", it is important to understand that their synthesis and catabolism are sets of unique pathways. BCAA synthesis occurs in plants, fungi, and bacteria, but not animals. Circulating BCAAs in humans primarily arise from dietary or tissue protein, with a smaller contribution from the microbiome. These circulating BCAAs are then either taken up by tissues and oxidized or included in newly synthesized protein, and no appreciable BCAAs are lost in urine.[48]

BCAA catabolism occurs in all life-forms[48,49] in three major steps. The first step is transamination of the BCAAs by Branched Chain Amino Transferase (BCAT) to their equivalent branched chain α-keto acids (BCKAs) – which are α-ketoisocaproic acid (α-KIC) for leucine, α-ketomethylvaleric acid (α-KMV) for isoleucine, and α-ketoisovaleric acid (α-KIV) for valine.[50] The most common nitrogen acceptor from this transamination is α-ketoglutarate, forming glutamate.[51] Two genes encode for BCAT – BCAT1, which is primarily expressed in the brain; and BCAT2, which is ubiquitously expressed in muscle and adipose tissue.[51,52]

The second step is the irreversible decarboxylation of the BCKAs, where their carboxylate groups are removed, and a Coenzyme A (CoA) group is attached. Oxidation occurs by the branched-chain α-keto acid dehydrogenase (BCKDH) complex, which is primarily in the liver.[53] The activity of this complex is inhibited by phosphorylation catalyzed by the branched chain keto acid dehydrogenase kinase (BCKDK)[54,55] and promoted by dephosphorylation catalyzed by the phosphatase PPM1K (also known as Protein Phosphatase 2Cm, PP2Cm).[56] BCKAs promote their own oxidation by inhibiting BCKDK, with α-KIC having the greatest potency.[57,58] Before the final step, BCKAs can release their bound CoA and form their corresponding hydroxy acids (BCHA) - which are α-hydroxyisocaproate (α-HIC) for leucine, α-hydroxy-3-methylvalerate (α-HMV) for isoleucine, and α-hydroxyisovalerate (α-HIV) for valine.[59,60]

The third step of BCAA catabolism is a series of enzymatic reactions similar to fatty acid oxidation, which ultimately ends in the tricarboxylic acid cycle (TCA cycle) in the liver.[61] These catabolic pathways are unique to each BCKA derivative and lead to different side-products/signaling cascades. Valine loses 2 carbons to CO_2 and contributes 3 carbons to the TCA as succinyl-CoA, making it glucogenic. Leucine loses 1 carbon to CO_2 and contributes 5 carbons to the TCA as acetyl-CoA, making it ketogenic. Finally, isoleucine loses 1 carbon to CO_2 and contributes 5 carbons to the TCA as succinyl-CoA and acetyl-CoA, making it both glucogenic and ketogenic. The literature suggests that a large portion of BCKAs produced in muscle (or adipose) are released to circulation where they are taken up by liver for complete oxidation.[48]

3-hydroxyisobutyrate (3-HIB) and β-amino-isobutyric acid (BAIBA) are intermediates of the valine catabolic pathway, and can be released into plasma from skeletal muscle. BAIBA further promotes fatty acid β-oxidation in the liver, adipocyte thermogenesis, and osteocyte survival.[62,63] Plasma 3-HIB can report rates of mitochondrial BCAA catabolic flux and has been linked to free fatty acid transport into the skeletal muscle.[63] Leucine and α-KIC, but not the other BCAAs, promote insulin release by activating glutamate dehydrogenase.[64,65]

Circulating BCAAs in humans can be regulated via intake and rate of catabolism.[66,67] However, emerging research suggests that carbohydrates may also regulate circulating BCAAs by interacting with enzymes in their catabolic pathway. Zucker fatty rats that were fed a high fructose diet demonstrated reduced BCAA catabolism by means of activating the hepatic transcription factor Carbohydrate-Responsive Element-Binding Protein (CHREBP), which in turn promotes BCKDK activity which phosphorylates and inhibits BCKDH.[24] Additionally, another pathway has been proposed whereby glucose inhibits BCAA degradation by downregulating KLF15, ultimately resulting in downregulation of BCAT in cardiomyocytes.[68] These mechanisms were observed in both in vitro and rat models, so the specific pathways that tie sugar intake with circulating BCAAs in humans have not been completely elucidated.

For aim 1, we hypothesize that the pathways regulating BCAA catabolism demonstrated in non-human animal models may also be present in human BCAA catabolism. According to the proposed pathway, we hypothesized that increased fructose consumption compared to increased glucose consumption would inhibit BCKDH activity and potentially increase circulating BCAAs and BCKAs.

Dietary Fiber Metabolism and Health

Physiological responses to dietary fiber vary depending on the combination of its properties (solubility, viscosity, fermentability)[69, 70], which are different in cereal, legumes, fruits, and vegetables. Briefly, solubility is defined as fiber's ability to dissolve in water; viscosity occurs when a soluble fiber with a high water-holding capacity forms a gel-like substance that travels through the gastrointestinal track; and fermentability refers to fiber's rate at which it is broken down and used as a food source by the large intestine's microbiome. Both soluble and insoluble fibers lead to an improved satiety[71-73], weight loss[74, 75], and inflammation.[76] Whereas insoluble fiber is predominantly non-viscous and poorly fermentable, soluble fiber can come in different combinations of viscosity and fermentability, which leads to differential physiological health benefits. Soluble, gel forming fibers decrease postprandial glycemic index[77-80], and both total and LDL cholesterol.[81-85] Fermentable fibers, which can be gel-forming or not, lead to increased

bacterial production of beneficial short chain fatty acids (SCFA), which has consequently been shown to lower LDL cholesterol and postprandial glycemic responses.[86-89] Finally, it is only the fibers that resist fermentation and directly affect stool water content that normalize bowel movements. Specifically, coarse insoluble fibers mechanically irritate the large bowel mucosa, stimulating secretion of mucous to speed transit time and prevent constipation, while fine wheat bran can have a constipation effect. Soluble, viscous fiber has high water-holding capacity that can resist dehydration in the large bowel, thereby both softening stool when it is too dry or providing it bulk when it is too liquid (e.g. Psyllium).[70] In summary, current literature suggests that dietary fibers elicit largely different, sometimes contrasting, human responses depending on their source, particle size, solubility, viscosity, and fermentability. The complexity of fiber's mechanism of action underlines the limitations of studying total fiber intake, as it may confound physiological responses and underlying mechanisms (Figure 2).

One of the consistent and mechanistically intriguing observations from large, prospective cohorts is that higher intake of cereal fibers[69, 90, 91] or whole grain products[92-94], predominantly insoluble, have a strong protective association against risk of developing T2D.[95, 96] Indeed, whereas greater consumption of whole grain, cereal, and legume fibers consistently shows a reduced risk in type 2 diabetes (T2D) [90, 91, 97] and improved insulin sensitivity [98], the associated health benefit is not as consistent with fibers obtained from fruits and vegetables.[69, 99] These discrepancies may be explained by the different nutrient profiles of food categories[100], or by its interference in dietary protein absorption.[101] However, these hypotheses lack molecular evidence and consistency with existing literature.

Literature Gap in Fiber Metabolomics

As previously mentioned, dietary fiber is recognized for its numerous health benefits, many of which are mediated through the metabolites produced by the gut microbiota. The fermentation of dietary fibers in the colon results in the production of short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate. These SCFAs are readily absorbed into the bloodstream and have been linked to various beneficial

effects on metabolism and overall health.[102] Acetate can travel to peripheral tissues and act as a substrate for cholesterol synthesis and lipogenesis, propionate has been shown to inhibit cholesterol synthesis in the liver, and butyrate is a primary energy source for colonic epithelial cells and has anti-inflammatory properties.[103] However, the evidence of SCFAs and fermentable fibers on metabolic benefits remains inconsistent due to variations in the type and amounts of dietary fiber consumed, as well as the composition and activity of the gut microbiota.[104, 105]

In addition to SCFAs, other metabolites, such as phenolic acids and secondary bile acids have been associated with dietary fiber intake. Phenolic acids are derived from the breakdown of polyphenols, often bound to dietary fiber.[106] These metabolites possess antioxidant properties and contribute to the modulation of oxidative stress and inflammation.[107, 108] Secondary bile acids, which are modified by gut bacteria, can be influenced by fiber-induced changes in gut microbiota composition[109] and have implications for lipid metabolism and gut health.[110]

Despite these associations, establishing a direct link between dietary fiber intake and specific circulating metabolites is challenging due to their heterogeneous nature. As mentioned, fibers vary widely in their properties, such as solubility, fermentability, and viscosity, and are sourced from different foods like fruits, vegetables, grains, and legumes.[70] This heterogeneity affects their interaction with gut microbes and subsequent fermentation processes, leading to diverse metabolic outputs.[111] Moreover, since dietary fibers are not absorbed in the gastrointestinal tract, their health effects are indirectly mediated by microbial metabolism. The complexity of the gut microbiota composition, influenced by numerous factors, including diet, genetics, and the environment, adds another layer of variability in the metabolic response to fiber intake. Despite the growing body of research, there is still a lack of comprehensive evidence linking specific dietary fibers to distinct circulating metabolites. To date, only a few studies have identified metabolite signatures of dietary fiber intake. In a post-hoc data analysis, 18-58 plasma metabolites - including choline, cotinine, and γ-butyrobetaine - were positively associated with dietary glycemic load (GL), glycemic index (GI), and overall carbohydrate quality

index.[112] More recently, studies have examined links between plasma acylcarnitines, bile acids and signaling lipids and whole grain intake and liver fat; [113] plasma metabolites associated with fiber-related bacteria;[114] and have developed metabolite profiles of plant-based diets and cardiometabolic risk.[115] However, a comprehensive plasma metabolic signature of total dietary fiber and dietary fiber by food sources, and their association with changes in cardiometabolic risk factors has not been studied. The current gaps in knowledge underscore the need for more robust research to further elucidate the metabolic pathways influenced by dietary fiber intake and their health implications.

This thesis' second and third aims address existing knowledge gaps in two key ways. Aim 2 involves reviewing the current evidence in the literature on the associations between dietary fiber from different sources and circulating plasma, urine, and fecal metabolites. Aim 3 focuses on developing a comprehensive metabolomic signature to predict dietary fiber intake from various food sources and examining the associations of these signatures with metabolic health outcomes.

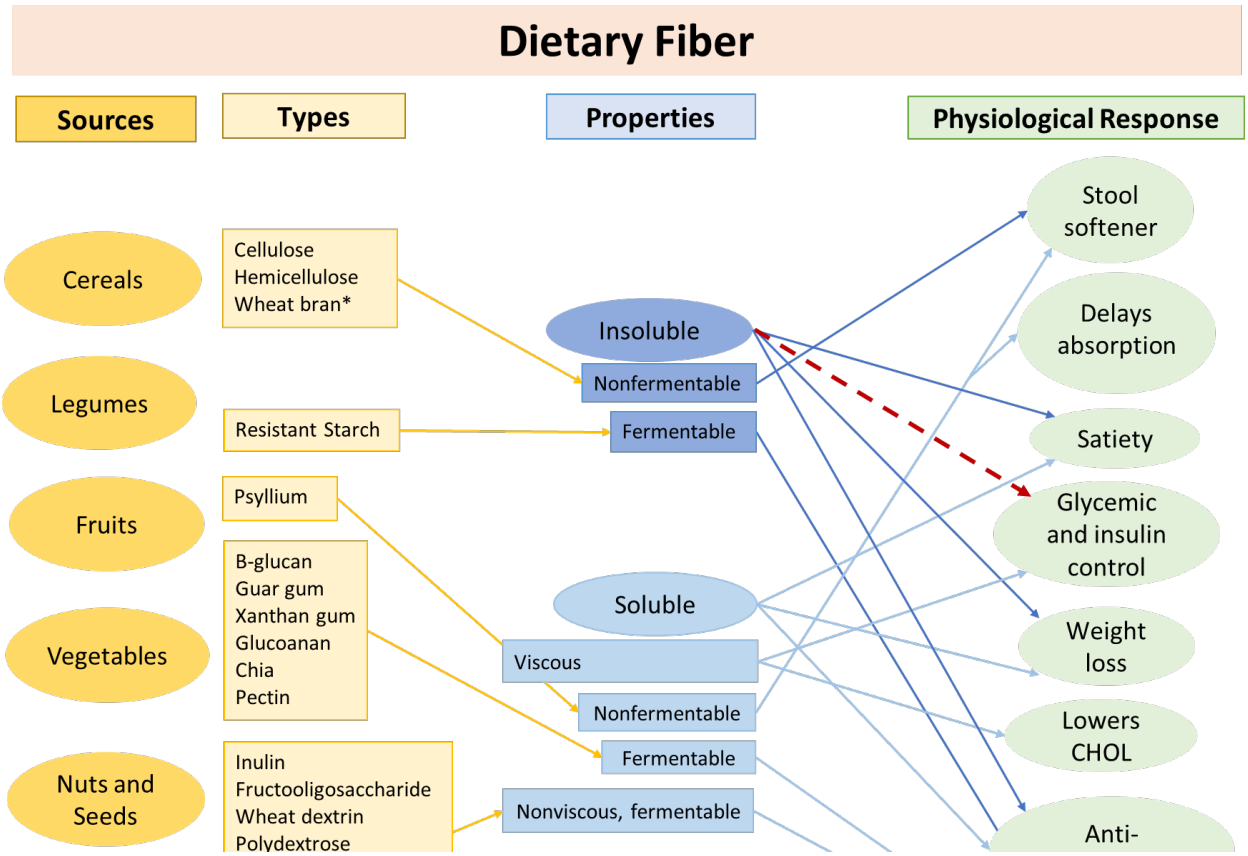

Figure 2 Complicated relationships between sources, properties, and physiological responses of dietary fiber. Cereal, legumes, fruits, vegetables, and nuts/seeds contain a variety of insoluble and soluble fibers, therefore no further subcategories are shown for simplicity. Orange arrows tie the fiber types to their corresponding combination of properties (solubility, viscosity, and fermentability). Blue arrows connect dietary fiber properties with observed physiological reactions in humans, dark blue for insoluble dietary fibers and light blue for soluble dietary fibers. Red, dashed arrow shows relationship between insoluble fibers and insulin sensitivity which will be explored in this thesis. *Wheat bran has differential effects on GI where coarse particles promote laxative effect, fine particles show delay absorption.

Chapter 2: Changes in plasma metabolites after a 10-week exposure to glucose vs fructose-sweetened beverages in subjects with overweight/obesity

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Abstract

To identify metabolites as potential biomarkers of fructose-induced metabolic disease, we performed metabolomic profiling in fasting blood samples from participants in a double-blind, parallel arm trial carried out among thirty-one male and female subjects with overweight/obesity, before and after glucose- vs. fructose-sweetened beverage supplementation. Orthogonal Partial Least Square Discriminatory Analysis (OPLS-DA) was used to identify metabolites that could discriminate the two intervention groups. Changes in sixteen metabolites (five of which are branched-chain amino acid catabolic pathway metabolites) and the branched chain keto acid (BCKA) composite score showed nominal (FDR adjusted p-value < 0.2, unadjusted p-value ≤ 0.08) differences in response to the two interventions. We observed 2.19 μM (or 13%) increase in the BCKA, ketomethylvaleric acid (P_{FDR} =0.04, P = 0.001), after glucose supplementation compared to a null change after fructose supplementation was observed. These data suggest that in people with overweight/obesity, consumption of glucose versus fructose sweetened beverages differentially affects the metabolism of branched-chain amino acids, particularly? within the isoleucine catabolic pathway.

Keywords: branched-chain amino acids; BCAA catabolic pathway; fructose metabolism; metabolomics; sugar-sweetened beverage

1. Introduction

High levels of circulating branch chained amino acids (BCAAs), attributed to impaired BCAA catabolism, are predictive of insulin resistance (IR) and type 2 diabetes (T2D). [21, 34, 35, 41, 116] Moreover, in Mendelian randomization (MR) studies, genetic variations that causally contribute to development of T2D also associate with increased BCAAs. [47] BCAAs are essential amino acids that cannot be synthesized by animals, and circulating BCAAs in humans have primarily been attributed to dietary intake.[48] Gut microbiota have the capacity for de novo synthesis of BCAA and may make a contribution to host BCAA levels. [117 118] However, whether other specific dietary factors can impact BCAA catabolic pathways and circulating BCAAs remains to be explored.

Circulating BCAAs are regulated by rates of protein degradation and rates of BCAA catabolism.[119] The first step in BCAA catabolism occurs primarily in the skeletal muscle, where BCAAs are transaminated by Branched Chain Amino Transferase (BCAT) to their corresponding branched chain a-keto acids (BCKAs) – α-ketoisocaproic acid (α-KIC) for leucine, α-ketomethylvaleric acid (α-KMV) for isoleucine, and α-ketoisovaleric acid (α-KIV) for valine.[61] The second step is the irreversible decarboxylation of the BCKAs by the branched-chain α-keto acid dehydrogenase (BCKDH) complex, which may occur in liver, skeletal muscle, and multiple other tissues.[61] The activity of this complex is inhibited by phosphorylation catalyzed by the branched-chain keto acid dehydrogenase kinase (BCKDK) and promoted by dephosphorylation catalyzed by the phosphatase PPM1K [48]. The subsequent steps of BCAA catabolism are catalyzed by a series of enzymatic reactions similar to fatty acid oxidation, which ultimately produce succinyl-CoA or acetyl-CoA which can enter the tricarboxylic acid (TCA cycle).[61]These catabolic pathways are unique to each BCKA derivative and lead to different side-products. The literature suggests that a large portion of BCKAs produced in the skeletal muscle (or adipose tissue) are released to circulation, where they are taken up by the liver for complete oxidation (Figure S1).[48]

Emerging research suggests that simple carbohydrates may impact circulating BCAAs by regulating the early steps in the BCAA catabolic pathway. Feeding a high-fructose diet to Zucker fatty rats activated the hepatic transcription factor Carbohydrate-Responsive

Element-Binding Protein (CHREBP), which in turn increased the expression and activity of BCKDK.[24] BCKDK-mediated inhibition of BCKDH should increase the accumulation of BCKAs. However, the effects of high fructose intake on circulating BCAAs/BCKAs and how those effects may be different from high glucose intake have not been previously examined.

To test the effects of different sugars on circulating BCAA metabolites and to identify additional metabolites as potential biomarkers of fructose-ingestion or fructose-induced metabolic disease, we examined changes in circulating metabolomic profiles in adults before and after a 10-week supplementation with fructose- vs. glucose-sweetened beverage (FSB and GSB) in the Inpatient Outpatient (IPOP) Study.[120] Previous results from this intervention pertinent to our exploratory study include significantly larger increases in dyslipidemia,[120-122] inflammation,[123] and insulin insensitivity[120] in the FSB group compared to the GSB group. Here, we hypothesized that increased fructose consumption compared to increased glucose consumption would inhibit BCKDH activity and potentially increase circulating BCAAs and BCKAs. In addition to assessing the effects of fructose vs. glucose on BCAA and related metabolites, we conducted an exploratory analysis to identify other metabolites regulated by fructose versus glucose consumption and whether these changes were modified by underlying metabolic dysregulation.

2. Materials and Methods

2.1. Study Design and Intervention Beverages

These analyses used the data collected on participants of the IPOP double blind, 12-week parallel arm intervention conducted at UC Davis Clinical and Translational Science Center's Clinical Research Center (CCRC) and completed in 2009. Recruitment, study design, and subject profiles were described elsewhere.[120] Briefly, fifteen subjects were assigned to the GSB arm and seventeen subjects to the FSB arm. One subject from the FSB arm was removed from the analyses due to their extreme levels across multiple metabolites (> 2 SD deviation from the mean for the FSB group in 15 metabolites at baseline or 10 weeks). The first two weeks of the study were an inpatient baseline period at the CCRC, where participants consumed an energy-balanced diet (55% of total energy

provided from complex CHO; 15% PRO; 30% FAT). This was followed by 8 weeks of outpatient intervention and two weeks of inpatient intervention periods. During the baseline inpatient intervention, participants received an energy-balanced diet consisting of a 4-day rotating menu of three daily meals providing 25% of energy at breakfast (0900 hours), 35% at lunch (1300 hours), and 40% at dinner (1800 hours). During the outpatient period (Weeks 3-10), participants were instructed to consume their usual diet with three daily servings of either FSB or GSB flavored with an unsweetened drink mix (Kool-Aid; Kraft). During the second prolonged inpatient period (Weeks 11-12), participants consumed an energy-balanced diet, matched as closely as possible to the baseline diet excepting the substitution of their assigned intervention drinks for complex carbohydrate (30% of total energy provided from complex CHO; 25% from intervention beverage; 15% PRO; 30% FAT). The beverages contained riboflavin, a compliance biomarker that was measured fluorometrically in urine samples at the time of beverage pickup (twice weekly) to measure compliance. A summary of the study timeline and diet schedule is illustrated in Figure 1.

2.2. Anthropometric measurements

Anthropometric and clinical measures from fasting plasma samples were taken throughout the intervention. Measures of waist circumference (WC, cm), blood pressure (mm Hg), triglyceride (TG, mg/dL), high-density lipoprotein (HDL) cholesterol (mg/dL), glucose levels (mg/dL), and insulin levels (μU/mL) captured at baseline were used to characterize metabolic syndrome (MetSyn) and IR risk. Individuals were classified with the presence of MetSyn if they met three or more of the following criteria: large WC (>102 cm for men, > 88cm for women), high TG levels (≥150 mg/dL), low HDL cholesterol (<40 mg/dL for men, <50 mg/dL for women), high blood pressure (≥130/≥85 mm Hg), or elevated fasting glucose levels (≥110 mg/dL) as defined by the American Heart Association/National Heart, Lung, and Blood Institute.[124] Individuals were classified with the presence of IR if their HOMA-IR was greater than 2.5, which was calculated using glucose and insulin concentrations.[125]

2.3. Sample collection and metabolite profiling

Plasma metabolite data were measured from fasting pooled samples drawn at 8:00, 8:30, and 9:00 hours from the 24-hr blood collection obtained at Weeks 1 and 12. These blood collections were performed after the subjects had consumed energy-balanced, weight-maintaining diets in the CCRC for 10 days. Stored plasma samples were aliquoted and analyzed by mass spectrometry in the metabolomics core laboratory at the Duke Molecular Physiology Institute (Supplemental Appendix 1). A total of 94 targeted metabolites were captured at baseline and Week 12 using previously published methods.[41] Metabolites were imputed and transformed using the inverse normal to fit a normal distribution. Composite scores for the branched-chain acids were created by adding their corresponding derivatives. As such, the BCAA score is the summed concentrations of valine, leucine, and isoleucine; and the BCKA score is the summed concentrations of KIV, KIC, and KMV.

2.4. Statistical Analysis

In this exploratory analysis, to alleviate the burden of multiple comparisons, we chose to use Orthogonal Partial Least Square Discriminatory Analysis (OPLS-DA) for metabolite selection [126]. This analysis was carried out on the change in metabolite levels from fasting samples drawn before and after the intervention. We selected metabolites with a Variable Importance in Projection (VIP) score ≥ 1, in addition to the metabolites known to be involved in the BCAA catabolic pathway for further evaluation. We tested within-group and between-group differences for these selected metabolites using paired and independent sample 2-sided t-tests, respectively, with an FDR adjusted p-value. We used a threshold of $P < 0.05$ for changes in the composite scores and an exploratory FDR threshold of 0.2 for nominal significance in changes of individual metabolites due to the small sample size of participants (N = 31).

We also examined the potential interaction with the selected metabolites and composite scores in a 3-way factor ANOVA adjusting for the intervention (FSB vs GSB), sex, presence of MetSyn, or presence of IR. Post hoc, stratified analyses by either sex, MetSyn, or IR were then run on the significant corresponding interaction terms. Similarly, we used a $P < 0.05$

and a $P_{FDR} < 0.2$ for the composite scores and individual metabolites, respectively, as threshold for our significance level for the interaction tests and $P < 0.05$ for the post-hoc tests. All statistical analyses were conducted in R (version 2023.03.0+386)[127] and OPLS-DA visualization was obtained using MetaboAnalyst 6.0 (www.metaboanalyst.ca).

3. Results

3.1. Study Participants

We analyzed the data of 15 participants who completed the GSB intervention and 16 participants who completed the FSB intervention. Participants had a mean age of 53.8 (± 1.43 SE) and mean BMI of 29.0 (± 0.5). There were no significant anthropometric nor metabolic differences between the two arms at baseline, nor between males and females assigned to different intervention arms (Table 1).

3.2. Network Analyses: OPLS-DA

To reduce the burden of multiple comparisons associated with testing a large number of metabolites, OPLS-DA was first used for metabolite selection.[126] The full OPLS-DA model captured 24% of the variance in the metabolite data ($R^2X = 0.24$) and 96% of the total variance in the intervention groups ($R^2Y = 0.96$). The model showed reasonable ability to predict intervention group based on metabolite changes ($Q^2 = 0.36$) (Figure 2A). Figure 2B depicts the most important metabolites selected by the model (VIP score ≥ 1), and if they were present in higher or lower concentrations for each intervention arm. The higher the metabolite's VIP score, the more influential that metabolite is at distinguishing glucose vs fructose consumers. A total of 31 metabolites (out of 94 measured metabolites) had a VIP score ≥ 1, six of which are part of the BCAA catabolic pathway (KMV, valine, KIC, KIV, 3-HIB, and C3 acylcarnitine). Notably, valine, the BCKAs and 3-HIB were among the metabolites contributing the most power to discriminate glucose versus fructose supplementation.

3.3. Metabolite Changes

We tested between group differences of within group changes during the intervention in 31 metabolites selected from the OPLS-DA, 5 additional candidate metabolites from BCAA pathway (leucine, isoleucine, glutamic acid, C5 acylcarnitine, and β-amino isobutyric acid), and the BCAA and BCKA scores. Changes in sixteen metabolites and the BCKA score

displayed nominal differences ($P_{FDR} < 0.2$) between the two intervention arms (Table 2). Only KMV reached an FDR adjusted p-value < 0.05 significance level ($P_{FDR} = 0.04$) with a 2.19 μM (13%) increase following the GSB intervention compared to a 0.17 μM (1%) decrease in the FSB intervention. The other two BCKAs, KIC and KIV, and the BCKA score, increased by 0.98 μM (3%), 1.43 μM (10%), and 4.51 μM (7%) respectively in the GSB arm compared to a -2.81 μM (-7%, $P_{FDR} = 0.18$), -0.10 μM (-1%, $P_{FDR} = 0.12$), and -3.08 μM (-4%, P = 0.003) change in the FSB arm.

Valine was the only BCAA that had a larger increase in the FSB group compared to the GSB group, by 27.11 μM (11%) and 5.32 μM (2%) respectively ($P_{FDR} = 0.18$). The metabolite 2-HB demonstrated a 10.17 μM (25%) increase in the GSB group and a -0.23 μM (-1%) change in the FSB group ($P_{FDR} = 0.11$). The analysis also identified three short (C3, C4/Ci4, and C5-DC), one medium (C8:1-DC), two long (C14-1-OH and C18:1-DC) chain acylcarnitines (ACs), and five amines (cystine, ornithine, tryptophan, 1-methylhisdine, and homocysteine) with a differential response to fructose versus glucose (Table 2). The metabolites with significant within group changes before and after the interventions are presented in supplementary Table S1 for the FSB arm and Table S2 for the GSB arm. Notably, mean isoleucine and 1-methylhistidine levels increased in both the GSB (isoleucine: $P_{FDR} = 0.05$; 1-methylhistidine: $P_{FDR} < 0.0001$) and FSB (isoleucine: $P_{FDR} = 0.07$; 1-methylhistidine: $P_{FDR} = 0.12$) arms after the intervention.

3.4. Intervention Interaction with Sex, MetSyn, and Insulin Resistance

Changes in five metabolites and the BCKA score significantly differed between interventions when stratified by sex, MetSyn, or IR (Table 3). In those with MetSyn, changes in KIC, KIV, KMV and the BCKA score were negatively amplified in the FSB group and positively amplified in the GSB group (P_{FDR} for interaction 0.02 to 0.2; post-hoc P 0.0003 to 0.02). A similar trend was detected in changes in 2-HB among males (P_{FDR} for interaction 0.20; post-hoc P = 0.0003). Finally, for those without IR, the decrease in C4/Ci4 was doubled in the FSB group and the null change increased in the GSB group (P_{FDR} for interaction = 0.22; post-hoc P = 0.04).

4. Discussion

A reasonable portion of the variation in the metabolite data captured a substantial portion of variation in the intervention groups, and was able to discriminate between the intervention groups. The 95% CI ellipses for GSB and FSB consumers were non-overlapping, illustrating the ability of the model to discriminate between the two interventions. This included overrepresentation of the metabolites involved in BCAA metabolism. Additionally, we observed less inter-individual metabolic response to glucose consumption compared to fructose consumption. This may reflect differences in fructose versus glucose metabolism, whereby fructose may have a higher heterogeneity in metabolomic responses than glucose.[128]

To our knowledge, this is the first study in humans to examine differences in plasma metabolites after glucose or fructose supplementation. The observation that BCKAs increased with glucose but not fructose is inconsistent with the original hypothesis. However, it is well established that fructose is primarily metabolized in the small intestine and liver, while glucose is ubiquitously metabolized, including a large contribution by skeletal muscle mediated by insulin action. Here, the increases in the BCKAs after glucose supplementation are potentially consistent with an effect of glucose to enhance BCAA transamination in peripheral, non-hepatic tissues such as skeletal muscle which is enriched in branched-chain amino acid transaminase (BCAT) activity. Since BCKAs are known to be exported from muscle to the circulation, this could have been the primary contributor to the increase in BCKA levels in the glucose-fed group.

Whereas glucose supplementation had a dominant effect on BCKAs, fructose had a dominant effect over glucose to increase valine. The fructose mediated increase in valine could be consistent with our initial hypothesis that fructose activates BCKDK to inhibit BCKDH, suppressing BCAA catabolism. Fructose also reduced C3 (propionyl) acylcarnitines more than glucose. As C3 acylcarnitine reports on levels of propionyl CoA, a metabolite that can be generated from Val and Ile catabolism, this finding is also consistent with a model of fructose-mediated inhibition of BCAA catabolism. The main impact of fructose feeding may have been in the liver, a tissue with minimal BCAT activity,

where a fructose driven increase in BCKDK and inhibition of BCKDH would have a small impact on the circulating pool of BCKA relative to muscle, but a larger impact on valine and C3 acylcarnitine levels.

KMV was the leading metabolite to discriminate the two interventions and was the only metabolite significantly different between the two interventions after adjusting for multiple comparisons ($P_{FDR} < 0.05$). Further, in an exploratory analysis, KMV significantly interacted with presence of MetSyn, where individuals with MetSyn display larger increases in KMV in the glucose group compared to those without MetSyn. Similar interactions were also observed for KIC, KIV, and the BCKA score. These results suggest mechanisms seem to be preferentially affecting the isoleucine catabolic pathway over that of leucine and valine. These trends in isoleucine metabolites are observed even though the paired *t*-test showed isoleucine significantly increased by similar magnitudes after both interventions. A recent meta-analysis of prospective cohort studies identified increased isoleucine concentrations, but not valine and leucine, in daily sugar-sweetened beverage consumers [Haslam 2024, in preparation]. Further investigation into interactions between different sugar consumption and the isoleucine catabolic pathway may be worth pursuing.

There were additional observed trends that are worth noting. The high VIP scores and increased levels of 3-hydroxytetradecanoyl carnitine (C14:1-OH, VIP = 1.90), dicarboxyoleyl carnitine (C18:1-DC, VIP = 1.0), and dehydrosuberylcarnitine (C8:1-DC, VIP = 2.23) in the FSB arm compared to the GSB arm may stem from fructose's role in downregulation of hepatic fatty acid oxidation[129] and upregulation of de novo lipogenesis (DNL).[130, 131] This observation aligns with the previously observed increased DNL of the FSB arm in this study.[120]

2-HB, a catabolite produced by threonine catabolism or as a byproduct of glutathione synthesis,[132] had the second strongest VIP score (VIP = 2.15) in metabolites that increased in GSB compared to FSB after KMV, and demonstrated a significant increase in the GSB arm, which was greater in males. A recent review identified 2-HB as a potential early biomarker of IR and its role in glucose homeostasis.[132] This metabolite was also recently identified as strongly reduced by an incretin diagonist agent (terzepatide)

compared to a monoagonist (dulaglutide), in concert with stronger effects of the former to improve glucose and lipid homeostasis.[133] Interestingly, 2-HB equilibrates readily with 2-ketobutyrate (2-KB), an alternate substrate of BCKDH, potentially linking the change in 2-HB to alterations in the BCAA catabolic pathway. This analysis confirms 2-HB's recently observed trends and points further to sex-differentiated effects, where males may be more prone to increases in 2-HB following high glucose intake.

Another interesting finding is with 1-methylhistidine (VIP = 1.43), which increased by 15% in the FSB arm and 31% in the GSB arm. These results are complemented by a pilot case control study, which observed higher levels of glucose and 1-methylhistidine in children with obesity compared to those with normal weight.[134] The ratio of 3-methylhistidine:1-methylhistidine was also higher in those with obesity and T2D.[135] The relationship between 1-methylhistidine and simple sugar consumption requires further exploration.

5. Conclusions and Future Perspectives

Although our analysis remains exploratory, it successfully identified metabolic pathways differentially affected after large increases in intake of glucose or fructose. The changes in metabolites related to BCAA catabolism, fatty acid synthesis, and insulin resistance demonstrate differences in glucose vs fructose effects on metabolism. The results demonstrate an increase in the immediate BCAA-derived metabolites, the BCKAs, in response to glucose compared to fructose feeding, versus an increase in Val and a decrease in the Val-derived metabolite C3 acylcarnitine elicited by fructose feeding. The differences in these responses may relate to tissue-specific impact of the two sugars on BCAA metabolism, with glucose exerting a dominant effect in muscle, while fructose acts mainly in the liver. Consistent with this idea, the increase in BCKA levels in response to glucose supplementation was more prominent in exploratory analyses among those with MetSyn. Larger, well powered studies could examine these relationships further to elucidate the intricacies of glucose vs fructose on different metabolic pathways in association with metabolic dysregulation.

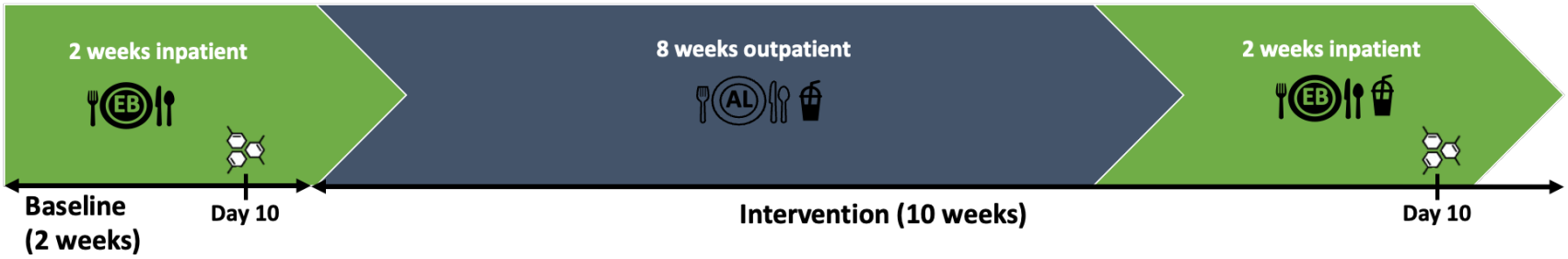
Tables and Figures

Table 1. Baseline anthropometric parameters of the IPOP study participants

Parameter (unit)	GSB			FSB		
	Male (n=7)	**Female (n=8)**	**Total (n=15)**	**Male (n=9)**	**Female (n=7)**	**Total (n=16)**
Age (yr)	54 ± 3	56 ± 2	55.3 ± 1.8	52 ± 4	53 ± 3	52.5 ± 2.2
Weight (kg)	88.4 ± 2.9	84.0 ± 4.5	86.0 ± 2.7	89.3 ± 2.9	80.5 ± 4.6	85.5 ± 2.7
BMI (kg/m2)	28.3 ± 1.0	29.4 ± 1.3	28.9 ± 0.8	28.3 ± 0.6	30.0 ± 1.2	29.1 ± 0.6
Wait circumference (cm)	98.8 ± 2.7	91.0 ± 4.1	94.6 ± 2.6	97.3 ± 3.3	91.9 ± 5.1	94.9 ± 2.9
Body fat (%)	28.0 ± 1.5	42.2 ± 1.1	35.6 ± 2.1	26.8 ± 2.0	40.5 ± 2.1	32.8 ± 2.2
TG (mg/dl)	148 ± 28	145 ± 23	146.4 ± 17.4	131 ± 21	161 ± 35	144.3 ± 18.8
Total cholesterol (mg/dl)	179 ± 14	193 ± 10	186.5 ± 8.2	177 ± 6	191 ± 15	182.8 ± 7.3
HDL (mg/dl)	36 ± 3	41 ± 3	38.9 ± 2.3	39 ± 4	41 ± 4	40.2 ± 2.8
LDL (mg/dl)	124 ± 5	123 ± 11	123.4 ± 5.9	107 ± 7	115 ± 13	110.6 ± 6.9
Glucose (mg/dl)	87 ± 2	89 ± 2	87.6 ± 1.5	88 ± 1	90 ± 2	88.6 ± 1.1
Insulin (µU/ml)	15.6 ± 2.7	14.6 ± 2.9	15.0 ± 2.0	12.0 ± 1.6	17.1 ± 2.7	14.2 ± 1.6

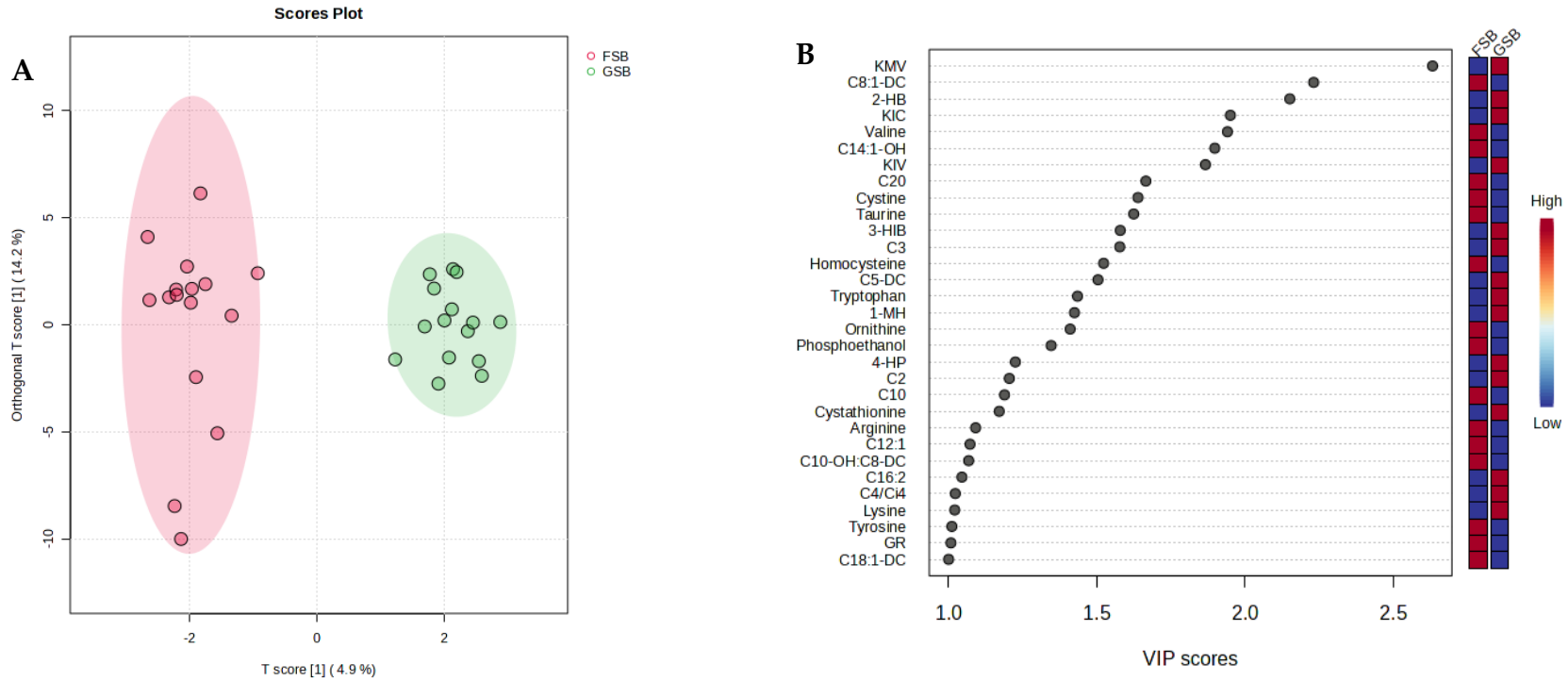
Data represented as Mean ± Standard Error.

Figure 1. IPOP study timeline



Metabolites were measured from pooled fasting plasma samples collected on day 10 of the first and last 2-week inpatient period. Intervention beverage provided at the start of the first outpatient period through the end of the study.
Abbreviations: AL: Ad Libitum diet; EB: Energy Balanced meal

Figure 2. (A) OPLS-DA Score Plot and (B) VIP scores of the top serum metabolites for discriminating the changes in fasting metabolites between the glucose-sweetened beverage (GSB) and fructose-sweetened beverage (FSB) intervention



(A) t1 holds group separation where 5% of response variance is explained by the predictor component only; to1 holds 14% orthogonal separation not related to group separation. Ellipses show the 95% of multivariate normal distributions with samples covariance for each class. **(B)** Only metabolites with VIP > 1 are included. Relative changes in the metabolite concentrations between GSB and FSB are shown on the right, where blue depicts a relative decrease and red depicts a relative increase in metabolite concentration. Abbreviations: 1-MH: 1-methylhistidine; 2-HB: 2-hydroxybutyrate; 3-HIB: 3-hydroxyisobutyrate; 4-HP: 4-hydroxyproline; GR: glutathione reduced; KIC: ketoisocaproic acid; KIV: ketoisovaleric acid; KMV: ketomethylvaleric acid.

Table 2. Mean changes in metabolites (μM) with nominal between-group differences

Metabolite	Type	HMDB ID	Mean Change in GSB	Mean Change in FSB	P	P_{FDR}
BCKA score†	BCKA		4.51	-3.08	0.003	
KMV*†	BCKA	253791	2.19	-0.17	0.001	0.04
2-HB*	Polymer	0000008	10.17	-0.23	0.008	0.11
C8:1-DC*	Acylcarnitine	241733	-0.0042	0.00038	0.009	0.11
KIV*†	BCKA	0000019	1.43	-0.10	0.01	0.12
KIC*†	BCKA	0000695	0.89	-2.81	0.03	0.18
C14:1-OH*	Acylcarnitine	0061640	-0.00041	0.0016	0.03	0.18
Valine*†	Amine	0000883	5.32	27.11	0.04	0.18
Ornithine*	Amine	0000214	-1.75	1.24	0.04	0.18
C18:1-DC*	Acylcarnitine		-0.0008	0.0002	0.05	0.18
Cystine*	Amine	0000192	-0.50	2.36	0.05	0.18
C5-DC*	Acylcarnitine	0013130	-0.00087	-0.018	0.06	0.19
C3*†	Acylcarnitine	0000824	-0.0020	-0.043	0.07	0.19
1-Methylhistidine*	Amine	0000001	1.14	0.60	0.07	0.19
Tryptophan*	Amine	0000929	1.87	-1.90	0.07	0.19
C4/Ci4*	Acylcarnitine	0002013/0000736	-0.01	-0.03	0.08	0.19
Homocysteine*	Amine	0000742	-0.04	0.03	0.08	0.19

Only metabolites with an FDR adjusted P-value < 0.2 or composite scores with a P-value < 0.05 are reported here.

* Metabolites with a VIP score ≥ 1

† Metabolites on the BCAA catabolic pathway

Table 3. Mean changes (SD) in metabolites (μM) with a significant intervention effect and interaction with presence of MetSyn, sex, or absence of IR

Metabolite	Effect and Interaction Terms		P-value	
			P	P_{FDR}
	Intervention		0.00009	0.001
	GSB (n=15)	FSB (n=16)		
	4.51 (7.56)	-3.08 (5.38)		
BCKA score†	**Intervention x MetSyn**		0.003	0.02
	MetSyn= 1			
	GSB (n=4)	FSB (n=5)		
	9.21 (6.64)	-6.46 (4.76)	0.002	
	Intervention		0.00009	0.005
	GSB (n=15)	FSB (n=16)		
	2.19 (1.69)	- 0.17 (1.77)		
KMV*†	**Intervention x MetSyn**		0.01	0.1
	MetSyn= 1			
	GSB (n=4)	FSB (n=5)		
	3.47 (1.62)	-0.85 (0.57)	0.0003	
	Intervention		0.002	0.04
	GSB (n=15)	FSB (n=16)		
	1.43 (1.94)	-0.10 (1.40)		
KIV*†	**Intervention x MetSyn**		0.01	0.09
	MetSyn= 1			
	GSB (n=4)	FSB (n=5)		
	2.61 (1.52)	-1.18 (1.25)	0.004	
	Intervention		0.003	0.05
	GSB (n=15)	FSB (n=16)		
	0.89 (4.35)	-2.81 (3.01)		
KIC*†	**Intervention x MetSyn**		0.04	0.2
	MetSyn= 1			
	GSB (n=4)	FSB (n=5)		
	3.13 (3.90)	-4.43 (3.23)	0.02	
C4/Ci4*	**Intervention**		0.006	0.10
	GSB (n=15)	FSB (n=16)		

	-0.0086 (0.037)	-0.026 (0.037)		
	Intervention x IR		0.03	0.22
	HOMA-IR= 0			
	GSB (n=6)	FSB (n=6)		
	0.0083 (0.019)	-0.050 (0.045)	0.04	
	Intervention		0.02	0.11
	GSB (n=15)	FSB (n=16)		
	10.16 (10.77)	-0.23 (9.24)		
2-HB*	**Intervention x Sex**		0.03	0.20
	Males			
	GSB (n=6)	FSB (n=6)		
	14.00 (7.48)	-2.68 (7.12)	0.0003	

Significant interaction terms were reported when the intervention effect was significant (P-value < 0.05).
* Metabolites with a VIP score ≥ 1
† Metabolites on the BCAA catabolic pathway

Supplemental Table 1. Mean metabolite concentrations (μM) before and after the fructose-sweetened beverage intervention

Metabolite	Type	Baseline *(μM)*	10 Weeks *(μM)*	P	P_{FDR}
Valine*†	Amine	240.08	267.19	0.003	0.07
KIC*†	BCKA	38.56	35.75	0.004	0.07
Isoleucine†	Amine	67.73	73.52	0.005	0.07
BCAA score†	Amine	440.47	473.49	0.009	
C20*	Acylcarnitine	0.0020	0.0034	0.02	0.12
Lysine*†	Amine	197.75	181.13	0.02	0.12
Cystine*	Amine	13.78	16.14	0.03	0.12
C5†	Acylcarnitine	0.12	0.11	0.03	0.12
1-Methylhistidine*	Amine	4.04	4.65	0.03	0.12
C4/Ci4*	Acylcarnitine	0.17	0.15	0.03	0.12
C14:1-OH*	Acylcarnitine	0.0072	0.0089	0.03	0.12
C5-DC*	Acylcarnitine	0.036	0.018	0.04	0.12
C3*†	Acylcarnitine	0.40	0.36	0.04	0.13

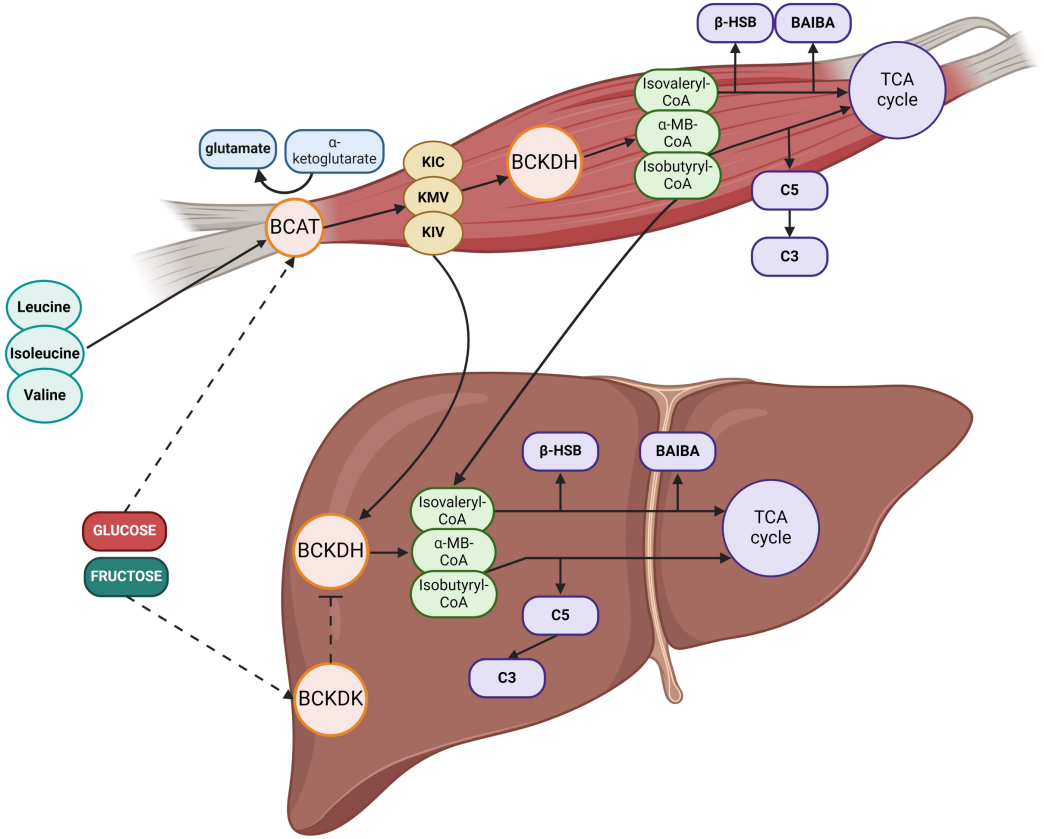
Only within-group differences in metabolites with FDR-adjusted significance level < 0.2 or within-differences in composite scores with a P-value < 0.05 are reported here.
* Metabolites with a VIP score ≥ 1
† Metabolites on the BCAA catabolic pathway

Supplemental Table 2. Mean metabolite concentrations (μM) before and after the glucose-sweetened beverage intervention

Metabolite	Type	Baseline *(μM)*	10 Weeks *(μM)*	P	P_{FDR}
1-Methylhistidine*	Amine	3.65	4.78	2.6e-06	9.3e-05
KMV*†	BCKA	16.87	19.06	9.3e-05	0.002
C8:1-DC*	Acylcarnitine	0.018	0.014	0.0004	0.005
4-Hydroxyproline*	Amine	8.37	9.40	0.002	0.01
2-HB*	HIBHB	41.10	51.27	0.003	0.02
KIV*†	BCKA	14.62	16.05	0.008	0.05
Isoleucine†	Amine	66.35	72.49	0.009	0.05
BCKA score†	BCKA	68.80	73.30	0.02	
Cystathionine*	Amine	0.19	0.26	0.02	0.08
Glutathione Reduced*	Amine	0.14	0.10	0.02	0.09
Taurine*	Amine	47.00	40.16	0.02	0.09
C18:1-DC*	Acylcarnitine	0.0055	0.0047	0.04	0.13
C10*	Acylcarnitine	0.10	0.089	0.04	0.13
C2*	Acylcarnitine	4.93	5.45	0.05	0.14

Only within-group differences in metabolites with FDR-adjusted significance level < 0.2 or within-differences in composite scores with a P-value < 0.05 are reported here.
* Metabolites with a VIP score ≥ 1
† Metabolites on the BCAA catabolic pathway

Supplemental Figure 1: Key players in the three step BCAA catabolism pathway



Transamination occurs in the muscle and adipose tissue, and decarboxylation and oxidation occur primarily in the liver but also the muscle. Proposed pathways by glucose and fructose intake are represented in dashed lines. Molecules in bold and bolded frames are measured in this study. Abbreviations: BAIBA: β-amino-isobutyric acid; BCAT: Branched-Chained Amino Transferase; BCKDH: branched-chain keto acid dehydrogenase complex; BCKDK: branched chain keto acid dehydrogenase kinase; CoA: Coenzyme A; β-HSB: β-Hydroxyisobutyrate; KIC: ketoisocaproic acid; KIV: ketoisovaleric acid; KMV: ketomethylvaleric acid; α-MB-CoA: α-methylbutyryl-CoA; TCA: tricarboxylic acid. Created with BioRender.com.

Appendix S1

MS/MS Analysis of Acylcarnitines

Acylcarnitines are analyzed by flow injection electrospray ionization tandem mass spectrometry and quantified by isotope or pseudo-isotope dilution using methods described previously [1, 2]. 50 µl of plasma is spiked with a cocktail of heavy-isotope internal standards (Cambridge Isotope Laboratories, MA, USA) and deproteinated with methanol. The methanol supernatants are dried and esterified with acidified methanol. Mass spectra for acylcarnitine esters are obtained using a precursor ion scanning method (parents of *m/*z = 99). The data are acquired using a Waters TQ (triple quadrupole) detector equipped with Acquity™ UPLC system and a data system controlled by MassLynx 4.1 operating system (Waters, Milford, MA). Ion ratios of an analyte to the respective internal standard computed from centroided spectra are converted to concentrations using calibrators constructed from authentic aliphatic acylcarnitines (Sigma, MO, USA; Larodan, Sweden) and dialyzed Fetal Bovine Serum (Sigma, MO, USA).

1 An J, Muoio DM, Shiota M, Fujimoto Y, Cline GW, Shulman GI, Koves TR, Stevens R, Millington D, Newgard CB: Hepatic expression of malonyl-CoA decarboxylase reverses muscle, liver and whole-animal insulin resistance. Nat. Med. 10:268-274, 2004.

2 Millington DS, Stevens RD. Acylcarnitines: analysis in plasma and whole blood using tandem mass spectrometry. Methods Mol Biol. 2011;708:55-72.

LC-MS/MS Analysis of Amino acids and Biogenic Amines

Amino acids and biogenic amines are analyzed by LC-MS/MS. The method is a modification of a previously published approach [1].
10 µl of plasma is spiked with a cocktail of isotope-labeled internal standards. The samples are deproteinized with methanol and the supernatants are derivatized with the AccQTag (Biosynth-Carbosynth) solution at 55° C for 10 min. Chromatographic separations are performed using a Waters Acquity UPLC system (Milford, MA) and a Waters Acquity UPLC HSS T3 column, 1.8 µm, 2.1 × 100 mm. Injection volume is 2 µL. Mobile phase A is composed of 0.1% formic acid in water. Mobile phase B is acetonitrile. The flow rate is set to 0.6 ml/min and the column temperature is 40 °C. A 6 min gradient method (t=0, %B=0; t=1.0, %B=0; t=6, %B=95) is run followed by a 1 min. wash and 1 min. equilibration. All metabolites are detected in a positive ion MRM mode based on a characteristic fragmentation reaction employing a Waters Xevo TQ-XS mass spectrometer (Milford, MA). Metabolite concentrations are computed using 26 isotope-labeled internal standards and an external calibration constructed from a serial dilution of 47 amino acids and biogenic amines.

1 Gray, N., Zia, R., King, A., Patel, V. C., Wendon, J., McPhail, M. J., Coen, M., Plumb, R. S., Wilson, I. D., & Nicholson, J. K. (2017). High-Speed Quantitative UPLC-MS Analysis of Multiple Amines in Human Plasma and Serum via Precolumn Derivatization with 6-

Aminoquinolyl-N-hydroxysuccinimidyl Carbamate: Application to Acetaminophen-Induced Liver Failure. *Anal Chem, 89*(4), 2478-2487. PMID:28194962.

LC-MS/MS Analysis of Branched-Chain Keto Acids
Branched-chain keto acids are analyzed by LC-MS/MS as previously described [1]
20 µl of plasma containing isotopically labeled internal standards KIC-d3, KIV-5C13 (Cambridge Isotope Laboratories), and KMV-d8 (Toronto Research Chemicals) is precipitated with 150 µl of 3M PCA. 200 µl of 25 M *o*-phenylenediamine (OPD) in 3M HCl is added to the supernatants and the samples are incubated at 80°C for 20 minutes. Keto acids are extracted with ethyl acetate as previously described [1]. The extracts are dried under nitrogen, reconstituted in 200 mM ammonium acetate, and analyzed on a Waters Xevo TQ-S triple quadrupole mass spectrometer coupled to a Waters Acquity UPLC system. The analytical column (Waters Acquity UPLC BEH C18 Column, 1.7 µm, 2.1 × 50 mm) is maintained at 30°C. 10 µl of the sample is injected onto the column and eluted at a flow rate of 0.4 ml/min. The gradient consists of 45% eluent A (5 mM ammonium acetate in water) and 55% eluent B (methanol) for 2 min., followed by a linear gradient to 95% B from 2 to 2.5 min., 1 min wash, and 1 min re-equilibration at initial conditions. Mass transitions of *m/z* 203 → 161 (KIC), 206 → 161 (KIC-d3), 189 → 174 (KIV), 194 → 178 (KIV-5C13), 203 → 174 (KMV), and 211 → 177 (KMV-d8) are monitored in a positive ion electrospray ionization mode.

1 White, P. J., Lapworth, A. L., An, J., Wang, L., McGarrah, R. W., Stevens, R. D., Ilkayeva, O., George, T., Muehlbauer, M. J., Bain, J. R., Trimmer, J. K., Brosnan, M. J., Rolph, T. P., & Newgard, C. B. (2016). Branched-chain amino acid restriction in Zucker-fatty rats improves muscle insulin sensitivity by enhancing efficiency of fatty acid oxidation and acyl-glycine export. Mol Metab, *5*(7), 538-551. PMC4921791, PMID:27408778.

2 Kristine C. Olson, Gang Chen, Christopher J. Lynch. (2013). Quantification of branched-chain keto acids in tissue by ultra fast liquid chromatography – mass spectrometry. Analytical Biochemistry, 439(2), 116-122. PMC3887392, PMID: 23684523

LC-MS/MS Analysis of 3-Hydroxyisobutyrate and 2-Hydroxybutyrate.3-Hydroxyisobutyric acid (3-HIB) and 2-hydroxybutyric acid (2-HB) are analyzed by LC-MS/MS. 50 µl of plasma containing isotopically labeled internal standards d6-2-hydroxyisobutyric acid and d3-hydroxybutyric acid (CDN Isotopes) is precipitated with 400 µl of methanol. The methanol supernatants are dried, reconstituted in water, and injected onto a Waters Acquity UPLC system coupled to a Waters Xevo TQ-S triple quadrupole mass spectrometer. The analytical column (Waters Acquity UPLC HSS T3 Column, 1.8 µm, 2.1 × 100 mm) is used at 30°C, 10 µl of the sample is injected onto the column, and eluted isocratically at 95% eluent A (0.1 % formic acid in water) and 5% eluent B (acetonitrile) and a flow rate of 0.4 ml/min. The total run time is 6.5 min. Mass transitions of *m/z* 103→ 73 (3-HIB), 109 → 62 (d6-2-HIB), 103→ 57 (2-HB), and 106→ 59 (d3-2-HB) are monitored in a negative ion electrospray ionization mode.

Chapter 3: Critical Review of the Metabolomic Associations of Fiber-Rich Foods

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Abstract

The effects of dietary fiber intake on the human metabolome are difficult to assess due to fiber's wide range of food sources (fruits, vegetables, whole grains, legumes, dietary supplements, etc.), structures, and properties (e.g., solubility and fermentability). We aimed to identify metabolites associated with fiber-rich foods by reviewing the data from observational and experimental studies published in the last 10 years. Using a combination of keywords related to dietary fiber (79), biomarkers (9), intake (9), and biofluid (5), we searched the PubMed, Web of Science, and CINAHL databases from 2013-2023. After screening 4,173 papers and 198 full text reviews, we created a dietary fiber – metabolite (DFM) database where a total of 662 unique metabolites from 80 articles were identified as potential fiber-related metabolites. To summarize the evidence, we assessed metabolites with positive associations to dietary fiber in 2+ studies to capture replication and developed the dietary fiber scoring system (DFSS) to assess validity. Of the 662 metabolites, five showed moderate to high specificity for sources of cereal fiber (DFSS score ≥ 3) and two showed moderate specificity for sources of legume fiber (DFSS score of 3). This panel of metabolites may be linked to intake of the fiber food sources and microbiome activity rather than candidate biomarkers of fiber intake itself. Further investigation is required to help elucidate the unique downstream metabolic effects of consuming various types of dietary fiber.

Keywords: dietary fiber; cereal fiber; metabolomics; microbiome; metabolites

1. Introduction

The Institute of Medicine defines dietary fiber as the nondigestible carbohydrates and wall lining intrinsic to plants that have been shown to have beneficial physiological effects in humans.[136] Physiological responses to different types of dietary fiber vary depending on the combination of the fiber's individual structure and properties (e.g. solubility, viscosity, fermentability). Briefly, solubility is defined as the fiber's ability to dissolve in water; viscosity occurs when a soluble fiber with a high water-holding capacity forms a gel-like substance that slows transits through the gastrointestinal (GI) tract; and fermentability refers to the fiber's rate at which it is broken down and used as a food source by the gut microbiome.[70] Whereas insoluble dietary fiber is predominantly non-viscous and poorly fermentable, soluble dietary fiber can come in different combinations of viscosity and fermentability, which leads to differential physiological health benefits. Both soluble and insoluble fibers have been linked to an increase in satiety[71-73] and weight loss,[74, 75] and a decrease in inflammation.[76] Soluble, gel-forming fibers have also been found to decrease postprandial glycemic response[77-80] and improve total and LDL cholesterol.[81-85] Fermentable fibers, which may (e.g., psyllium husk, beta-glucan) or may not (e.g., resistant starch, fructooligosaccharide) be gel-forming, have been shown to increase bacterial short-chain fatty acids (SCFA) production in the gut,[86-89] which in turn offers numerous health benefits, including supporting gut health, reducing inflammation, and enhancing metabolic function.[137] In addition to dietary fiber being highly heterogeneous in terms of structure and property,[69, 70] this key nutrient also varies in food source[86-89], with main food sources being cereals, including whole grains, legumes, fruits, and vegetables. Furthermore, disentangling dietary fiber from fiber-rich foods remains a challenge.

Metabolomics is a branch of analytical chemistry aiming to identify and annotate the metabolome, generally characterized as small molecules (molecular weight <1,500 Da)[1] that includes endogenous compounds such as amino acids, short peptides, nucleic acids, sugars, lipids, organic acids, and fatty acids, as well as exogenous chemicals such as toxins and xenobiotics.[2] Plasma metabolites are not exposed to recall bias[138] nor energy and micronutrient misreporting[139] that accompany the traditional assessment of diet

intake through semi quantitative Food Frequency Questionnaires (FFQs) and 24-hr recalls. Consequently, metabolomics is increasingly applied to food and nutrition science for identifying biomarkers of food intake as a complement to self-reported data,[12] and discovering biological processes influenced by diet in epidemiologic studies.[14]

Previous reviews of dietary fiber intake and metabolomics have focused on the effects of dietary fiber on gut microbiota composition,[140, 141] SCFA modulation,[103, 142] and disease pathophysiology.[143, 144] However, a collective assessment of observational and experimental feeding studies identifying potential metabolites linked to intake of fiber-rich foods and dietary patterns has not been reported to date. By summarizing all associations between metabolites and high intakes of dietary fiber, our objective was to identify emerging candidate metabolite biomarkers capturing intakes of total dietary fiber, dietary fiber from food groups, and dietary fiber from supplements.

2. Material and Methods

2.1. Development of the Dietary Fiber-Metabolite (DFM) Database

2.1.1. Literature Search and Eligibility Criteria

A comprehensive search of three electronic databases, including PubMed, Web of Science, and CINAHL, was conducted to identify all potential studies that used metabolomics analyses to identify metabolites associated with consumption of high-fiber foods. We used a combination of keywords based on recent guidelines for biomarkers of food intake discovery[145] summarized in Supplementary Appendix 1. Our search criteria included clinical trials and observational studies conducted in humans published up until April 20, 2023. Of the 10,573 publications obtained, we restricted our search to publications in the last decade, from 2013-2023, due to the changes in methodologies and rapid growth in this field. Article titles were imported into Endnote (version 20.4) where duplicates were removed before transferring titles to Rayyan[146], where two reviewers (M.G. and Y.Y.) conducted independent title and abstract screening. The reviewers discussed conflicted articles, and the final list of 198 papers was re-transferred to EndNote. To ensure our restrictions represented the general adult population, we excluded studies in the elderly/children/pregnant populations. Additionally, we included common health

outcomes (overweight/obesity [o/o], issues with GI transit, abnormal cholesterol, and impaired glucose), while excluding more severe diagnoses (type 2 diabetes, cardiovascular disease, and cancer). We excluded studies that did not report the fiber amount of their food exposures and studies using symbiotic supplements, as the effects of dietary fiber cannot be isolated in either case. A further 127 articles were excluded for not meeting our inclusion criteria. A hand search of individual bibliographies of the full-text articles was conducted to add 9 more studies to the review's reference list. A total of 80 articles, 4 of which were secondary analyses of already captured interventions, were included in this review, and used to create our dietary fiber-metabolite (DFM) database (Figure 1). This database summarized all positive, negative, and insignificant associations between dietary fiber exposures and metabolites reported in the 80 articles.

2.1.2. Data Extraction

Data collected from each study included year of publication, location of study, study population and health outcomes, total sample size, study design, whether antibiotic use and background diet were accounted for, study and exposure length, exposure food item, fiber type, control type, metabolomics technique used, biofluid, and all reported metabolites that were positively, negatively, or insignificantly associated with the dietary fiber exposure.

2.1.3. Fiber Types

We collected the dietary fiber types reported in each study and categorized them as either derived from a single food or food group, isolated as a dietary supplement (SUPP), or an estimate of total dietary fiber (TDF) as part of a dietary pattern or whole diet. Dietary fiber from foods were labeled as cereal fiber (CF), fruit fiber (FF), legume fiber (LF), vegetable fiber (VF), starchy food fiber (SF), or a mixture of fruits and vegetables (FV). Supplements contained either an isolated fiber type (e.g., IF.IN, an isolated inulin supplement) or a mixed fiber supplement containing more than one fiber type (e.g. MS.CF.IN; cereal fiber and inulin supplement). When the dietary fiber exposure was part of a dietary pattern or meal, we estimated the percentage contribution of each fiber type to total dietary fiber intake.[147-151] For example, Brignardello *et al* administered meals as part of

a “healthy diet” containing 46 g of dietary fiber. Based on details of the test diets provided in their supplemental section, we deduced that the 46 g of fiber comprised 50% CF, 24% VF, 11% SF, 9% FF, and 6% LF. For articles in which dietary fiber in dietary patterns or meals was broken down as soluble and insoluble, we defaulted to the insoluble % contribution being derived from cereal sources.[152-155]

2.2. Evaluation of Candidate Biomarkers by the Dietary Fiber Scoring System (DFSS)

To identify potential dietary fiber biomarkers from this large DFM database, we developed the dietary fiber scoring system (DFSS) that accounts for the specificity and reproducibility of a metabolite’s association with a particular fiber type (Table 1). Only metabolites that were identified in 2 or more studies with positive associations with the same dietary fiber type were retained and scored to account for a first stage of replication. In studies examining foods, meals, or dietary patterns, a metabolite received +1 points for every study with a positive association with a specific fiber type and + 0.XX points for every positive association in a study with XX % amount of a specific fiber from a mixed fiber dietary pattern or meal. Using the example above, a metabolite associated with the “healthy diet” meals in the study by Brignardello *et al* would have scored 0.50 points for a positive association with CF.

In SUPP studies, because the effect of fiber was isolated, a greater weight of + 2 points was assigned for a positive association with a metabolite and +1.XX points were assigned for studies using mixed fiber supplements depending on the XX % amount of that specific fiber available. Additionally, 1 point was deducted from the metabolite score for every study reporting a negative association with a specific fiber, and 0.5 points were deducted for every study reporting insignificant associations with a specific fiber type to account for inconsistency in findings. Lastly, a metabolite score was reduced by 0.5 points for every positive association with a different fiber type (excluding TDF), suggesting some degree of misclassification. We focus on highlighting metabolites with a score of 3 or higher as the most promising potential biomarkers of our dietary fiber exposures. However, we present all the results in the DFM database for those who may be interested in metabolites with lower scores. Additionally, since each biofluid (blood, urine, and feces) is

processed differently for metabolite analysis, we scored metabolites separately by biofluid. Metabolites could be present multiple times across biofluids and fiber groups following the DFSS.

Since SCFA's are well-established physiological outcomes of fermentation and not unique enough for use to capture as biomarkers of dietary fiber intake, we collected all studies with SCFA metabolite data and analyzed this group separately. The studies that did not report adjusting or accounting for the use of antibiotics were retained in the DFM database; however, we excluded these studies when assessing dietary fiber - fecal SCFA associations due to the well-established antibiotic-induced microbiota perturbations.[156] Alkylresorcinols were included in the DFM database but excluded from our analysis as they are well-established biomarkers of rye and whole grain intake.[157]

3. Results

3.1. DFMAD Characteristics

3.1.1. Literature Search Yields

After extracting all observations for all metabolites captured in the 80 studies, we identified a total of 687 metabolites with one or a combination of positive, negative, or insignificant associations with dietary fiber sources. We then used MetaboAnalyst's ID Mapping tool[158] to join metabolites captured using different naming conventions (e.g., IUPAC vs common name). A total of 662 unique metabolites and five composite scores (branched chain fatty acids, branched chain amino acids, bile acids, alkylresorcinols, and SCFAs) categorized into 37 metabolite classes were identified (Supplemental XLS Sheet Table 1). For each metabolite found in a specific biofluid (blood, urine, or feces), we compiled studies indicating positive, negative, or insignificant associations. Each study is identified by a StudyID that can be cross-referenced with Supplemental XLS Sheet Table 2, which contains the characteristics of the 80 studies contributing to the DFM database. The type of fiber-rich food exposure is indicated in parentheses next to the StudyID. The largest metabolite classes included lipids and lipid-like molecules (25%), amino acids and derivatives (24%) and alkylresorcinols and avenanthramides (7%) (Supplemental Figure 1).

3.1.2. Study Characteristics

Characteristics of the 76 studies contributing to the DFM database are presented in Supplemental XLS Sheet Table 2. The majority of the research was carried out in the Nordic Countries (Norway, Sweden, Finland, Iceland, Denmark) (29%), followed by the United-States (25%), between 2013 and 2023 (Supplemental Figure 2). With respect to the study populations, 57 % of these studies characterized their participants as healthy, while 43% characterized participants with a wide range of health conditions (o/o, prediabetes or impaired glycemia, CVD risk, abnormal cholesterol levels, and metabolic syndrome) (Supplemental Figure 3). In total, 93% of the studies included in the DFM database were clinical trials (59% crossovers and 41% 2 or more parallel arm studies, with 96% randomized), with sample sizes ranging from 10 to 80 participants in crossover studies (median of 19) and from 15 to 210 participants in parallel studies (median of 70). The duration of the study differed by design, with crossover studies ranging from 2 days to 26 weeks, and parallel studies from 2 to 26 weeks, with most crossover studies being around 4 weeks (13 studies) and most parallel studies being around 12 weeks (8 studies) in duration. The remaining 7% of studies were observational with 3 cross-sectional (N ranged from 50 to 153 participants) and one prospective 3-year longitudinal study (N=182). One study was a cross-sectional secondary analysis of a trial (N=21).[155]

3.1.3. Distribution of dietary fiber exposure

From the 76 unique studies selected for the DFM database, a total of 95 dietary fiber exposures were captured and categorized into six different fiber types (Supplemental Figure 4). CF and SUPP were the largest fiber type categories, each contributing 31% and 40% of the dietary fiber exposures, respectively. The remainder of the categories included TDF (18%), FF (7%), LF (2%), and FV (2%). Within the SUPP category, 79% were single fiber supplements and 21% were mixed fiber supplements. Further, 44% and 32% of the supplements contained CF and inulin-type fructan, respectively. Out of the 16 studies that reported on TDF, 5 provided a breakdown on the contribution of fiber types and 4 reported the breakdown of soluble vs insoluble fiber.

3.1.4. Metabolite profiling

Methods used for metabolite profiling were reported for the 80 studies included in this review, as some of the secondary analyses used different methodologies (Supplemental Figure 5). Metabolite profiling was carried out in blood (44% studies), urine (21% studies), feces (20% studies), and in two or more of these biofluids in the remaining 15% of studies. In terms of techniques, 37% of studies used untargeted metabolomics, 18% used targeted metabolomics, and 36% quantified specific metabolites (e.g., SCFA's). The remaining 9% of studies used two or more techniques (5% used targeted and untargeted metabolomics, 3% used targeted metabolomics and quantified SCFA's/bile acids, 1% used untargeted metabolomics and quantified SCFA's). Untargeted and targeted metabolomics profiling were carried out using LC-MS in 59% of studies, NMR in 13% of studies, GC-MS in 10% of studies, two tools in 16% of studies (6% used LC-MS and GC-MS; 6% used LC-MS and NMR; and 4% used GC-MS and NMR), and FT-ICR-MS in one study.

3.2. Metabolites Selected by DFSS

Even though we included both interventions and observational studies in our review, only RCTs emerged after retaining metabolites with ≥ 2 studies with positive associations for a fiber type and biofluid and a DFSS score ≥ 3 (Table 2). This included seven crossover studies ranging from 5 days to 14 weeks,[159-165] three parallel arm studies ranging from 4 to 12 weeks,[166-168] and a secondary cross-sectional analysis of a previous RCT.[155] Several candidate biomarkers emerged for cereal and legume fiber, but none for fruit fiber or total dietary fiber. All metabolites with ≥ 2 studies that received a DFSS were summarized in Supplement Table 1.

3.2.1. Metabolites associated with Sources of Cereal Fiber

A total of five urine phenolic acids, of which pyrocatechol sulfate was also replicated in blood, were retained after limiting metabolites with ≥ 2 positive CF studies and ≥ 3 points using the DFSS (Table 2). All urine metabolites were found in two RCTs, one of which supplemented 27g of wheat aleurone (the outer layer of cells of the endosperm, high in fiber (20 g) and usually associated with the bran fraction of cereals) to 34 adults

with o/o for 4 weeks[167], and the other administered a single dose of 208 g whole grain wheat bread (16 g fiber) to 12 US adults in an acute study.[164] In both studies participants were advised to avoid changes to their habitual diets outside of the intervention foods.

Urine ferulic acid 4-O-sulfate, a phenolic compound commonly found in cell walls of plants, showed relatively strong replication, with the highest DFSS of 5. It was observed in two cross-over RCTs with one being an acute study, administering 50 g of oat bran (5.1g fiber) to 70 UK healthy participants[162], while the other administered whole meal rye bread (23 g fiber) to 20 adults with slightly elevated serum cholesterol concentrations for 4 weeks.[159] In both studies, only the diet intervention food items were administered and participants kept to their habitual diets. Ferulic acid 4-O-sulfate, along with dihydroferulic acid sulfate and dihydroferulic acid glucuronide, demonstrated consistent specificity to CF with neither negative/null associations with CF nor positive associations with other fiber types.

The metabolite pyrocatechol sulfate ranked highly with CF in both urine and blood samples (scores of 4 and 4.43 respectively). Changes in urinary pyrocatechol sulfate were observed in the three RCTs mentioned above[159, 167, 169] that administered food sources of CF. Similar changes in urinary pyrocatechol sulfate were observed in another randomized crossover trial in 50 adults with o/o at risk of metabolic syndrome, who were asked to consume ≥ 75 g/day of whole grains (23.3 g fiber) for 8 weeks; participants were instructed to substitute all cereals in their diet with cereals from a selection of study products *ad libitum*.[160] However, this urinary metabolite, pyrocatechol sulfate, lost some specificity from one parallel study reporting a null association[168] while another RCT observed a positive association with FF.[165]

Similar to urinary pyrocatechol sulfate, the study by Fava *et al*. additionally observed increases in blood pyrocatechol sulfate after a cereal fiber intervention.[167] Another study also observed increased blood pyrocatechol sulfate in a cross-sectional analysis of 21 healthy US adults on a vegan diet compared to an omnivore diet. This dietary pattern was predominantly high in insoluble dietary fiber (69% or 24 g insoluble fiber).[155] Additionally, in a randomized crossover study from Sweden, high added fiber meals were

provided daily to 25 healthy adults for 5 weeks. These meals provided 18 additional grams of fiber compared to the control diet. Participants were advised to keep their habitual diets outside the intervention products, and plant sterol-containing foods were prohibited throughout the study. Fiber types captured in this study included both CF and FF, where urine pyrocatechol sulfate scored a DFSS score of 1.74 points for CF.[163]

3.2.2. Metabolites associated with sources of Legume Fiber

The blood amino acid derivatives methylcysteine and pipecolic acid were positively associated with bean powder in a 4-week randomized dietary intervention trial of 18 colorectal cancer survivors with o/o asked to incorporate 35 g of cooked navy bean powder/ day (9 g fiber) into meals and snacks.[170, 171] Additionally, Garcia-Aloy *et al*. carried out a secondary analysis of a randomized crossover postprandial intervention study that randomized 11 healthy participants to chickpea (8.7g fiber), lentil (2.7g fiber), or control pasta (1.3 g fiber) diets, and 8 participants continued to consume white beans (17 g fiber) after a run-in period of 1 week. Both methylcysteine and pipecolic acid were found to be significantly higher after the white bean meal intervention compared to all other meals.[172]

3.2.3. SCFA's

Observations of fiber types with SCFAs were generally inconclusive. A total of 44 studies captured associations of 8 SCFAs including propionate, valerate, acetate, butyrate, isovalerate, isobutyrate, capronate, and lactate in fecal, blood, and urine biofluids with fiber (Supplemental XLS Table 1). Associations were primarily null (63-100%) for individual and total fecal SCFA metabolites. In blood, butyrate had the greatest number of studies with positive associations compared to null (61%), followed by acetate (50%). 11 out of 14 and 8 out of 11 studies that observed positive associations with butyrate and acetate, respectively, administered either cereal fiber (as food or isolated fiber) or inulin supplements as exposures.

4. Discussion

To our knowledge, this is the first review to summarize associations between metabolites and dietary fiber and aim to identify candidate biomarkers of dietary fiber

intake by food sources. Based on publications in the past decade, we identified 80 articles linking fiber to 662 metabolites (48% urine, 33% blood, 19% fecal), and created a database to summarize the current research. Top scoring metabolites included five phenolic acids associated with sources of CF (ferulic acid sulfate, dihydroferulic acid sulfate, dihydroferulic acid glucuronide, pyrocatechol sulfate and caffeic acid) and two amino acid derivatives associated with sources of LF (methylcysteine and pipecolic acid).

4.1. The need for a scoring system

Others have developed comprehensive guidelines on conducting an extensive literature search[145] and critical assessment to identify candidate biomarkers of food intake.[173] These guidelines have been applied in reviews focused on identifying candidate biomarkers of *allium* vegetables,[174] sugar-sweetened and low-calorie sweetened beverages,[175] and seaweed,[176] amongst others. The application of this methodology narrowed the search of candidate biomarkers in earlier reviews to 17-27 articles for validation of metabolites. We applied their guidelines in our search strategy and selection of databases for this review. In contrast to other studies, however, our review expands to 80 publications. Since dietary fiber is not a single food entity, rather a component of several different food sources, we developed a novel scoring system (DFSS) to identify potential candidate biomarkers of dietary fiber from food sources from the vast amount of data emerging from the 80 publications. The DFSS assesses the replication and specificity of a metabolite within dietary fiber exposure categories and weighted evidence from RCTs that administered dietary fiber supplements. This scoring system provided a greater ability to narrow down the link between certain fiber types and metabolites.

4.2. Challenges with identifying metabolite biomarkers of dietary fiber

A major challenge in this review is the inherent nature of dietary fiber, which, by definition, does not appear in blood or urine. Preliminary data supports that specific carbohydrate subunits (e.g., arabinose, xylose, mannose) may be absorbed and serve as potential biomarkers. However, the overall process is complex due to the variability introduced by the gut microbiome. Additionally, fecal metabolite analysis might overlook a significant portion of dietary fiber since most of it is removed during extraction via

centrifugation (many fibers are larger than 1500 Da).[152] Despite additional points allocated to SUPP studies, the DFSS lacked accuracy in distinguishing between dietary fiber and other components of the test foods and diets. Accordingly, our results indicate that the DFSS selected metabolites linked to the food source or metabolites related to microbiome activity rather than unique biomarkers of dietary fiber.

4.3. Metabolites Associated with Legume Intake

The studies that captured blood methylcysteine and pipecolic acid were more indicative of associations with intake of beans rather than intake of a specific legume dietary fiber. Blood methylcysteine is a well-established component of beans[177] and previous studies suggest plasma pipecolic acid originates from catabolism of dietary lysine by intestinal bacteria rather than by direct food intake.[178]

4.3. Metabolites Associated with Cereal Fiber intake

The ferulic acid compounds and pyrocatechol sulfate have well-established links with microbiome activity. Ferulic acid is a phenolic compound found in seeds and grains,[179] which has been studied for anti-inflammatory and anti-diabetic properties.[180] Ferulic acid is bound to arabinoxylan in wheat and rye, and gut microbiome metabolism can cleave the bonds, releasing ferulic acid,[181] which supports the positive association between ferulic acid and cereal fiber intake. The two dehydroferulic acid conjugates captured in this review arise from enzymatic processes during ferulic acid metabolism by gut bacteria.[182] Continued studies linking cereal fiber to health benefits in the body through the ferulic metabolic pathway are therefore warranted. The benzoxazinoid gut metabolite, pyrocatechol sulfate (also called 2-aminophenol sulfate),[183] was suggested to be a potential urine biomarker of wholegrain intake by Fava *et al*.[167] Interestingly, Roager *et al*. conducted linear regressions analysis showing pyrocatechol-glucuronide was inversely associated with c-reactive protein,[160] making this pathway worth further exploration.

4.4. Short Chained Fatty Acids

Although a significant portion of the studies reviewed reported outcomes related to SCFAs (55%), the results were largely inconclusive. No clear patterns emerged regarding the relationship between fiber intake and SCFA production, irrespective of the amount of

fiber provided, duration of exposure, or study design. The observed lack of reproducibility in SCFA findings may suggest the presence of unaccounted factors, such as variations in the gut microbiome, influencing the outcomes.

4.5. Study design, metabolomic approaches, and analytical techniques

RCTs are considered the gold standard for establishing causal relations between exposure and outcome in human nutrition, while the long-term effects of dietary intake are more likely observed in prospective cohort studies.[184] The unintentional but exclusive use of RCTs for scoring the metabolites in this review suggests robust evidence linking the metabolites to their fiber sources, however replication of these associations in observational studies is warranted for generalizability.

Untargeted approaches to metabolite identification allows for broad metabolite coverage, while targeted analysis can better quantify a known subset of metabolites.[185] Consequently, both approaches should be applied whenever possible. All phenolic compounds were identified using both targeted and untargeted methodologies, while the two amino acids associated with bean intake were identified by untargeted analysis . Evaluating the link between blood methylcysteine and blood pipecolic acid with bean intake may be worth investigating using a targeted approach to increase precision. Metabolites that were identified using two independent analytical platforms included the blood amino acids (1H-NMR and UPLC-MS/MS), urine pyrocatechol sulfate (LC-MS, GC-MS), and blood pyrocatechol sulfate (LC/MS, LC/MS/MS, GC/MS). Using two or more analytical platforms for metabolite identification has cost and volume constraints, but cross-validation increases accuracy and reliability of identification.

Overall, the lack of reproducibility between studies given the diversity of study designs and metabolomics methods points to a major challenge in isolating candidate metabolites of dietary fiber intake.

4.6. Strengths and Limitations

This review has numerous strengths and limitations. First, the development of the DFM database, which can be continuously updated, represents a valuable tool for researchers developing trials or interested in the effects of high fiber foods or diets on

human physiology. Second, we applied a novel scoring system that successfully identified metabolites most influenced by intake of high fiber food sources and strategically summarized a large amount of data. Third, our strategy to assess metabolites separately by biofluid adequately provides pertinent information on the sensitivity and specificity of metabolites in each biological medium. Limitations in this review include the risk of pertinent studies and metabolite oversight, since we limited the review to only metabolite biomarkers of dietary fiber and excluded studies that did not report fiber intake. Additionally, our scoring system could not capture distinguishing details of study design, fiber exposure amount, or metabolite-fiber association strength.

5. Conclusion

We successfully reviewed and summarized the current evidence for dietary fiber-metabolite associations from studies using metabolomics analyses. Current evidence is primarily from intervention studies. The top candidate biomarkers that emerged for cereal fiber included pyrocatechol sulfate and ferulic acid derivatives and for legume fiber included methylcysteine and pipecolic acid. However, evidence was weak for candidate biomarkers of fruit, vegetable, and total fiber intake; therefore, future work is needed. The variability in the dietary fiber exposures and methods for measuring metabolites make it difficult to make strong conclusions. Findings from this review suggest further investigation is required to help elucidate the downstream metabolic effects of high fiber intake.

Tables and Figures

Figure 1. Flowchart of included studies and metabolites analyzed in this review

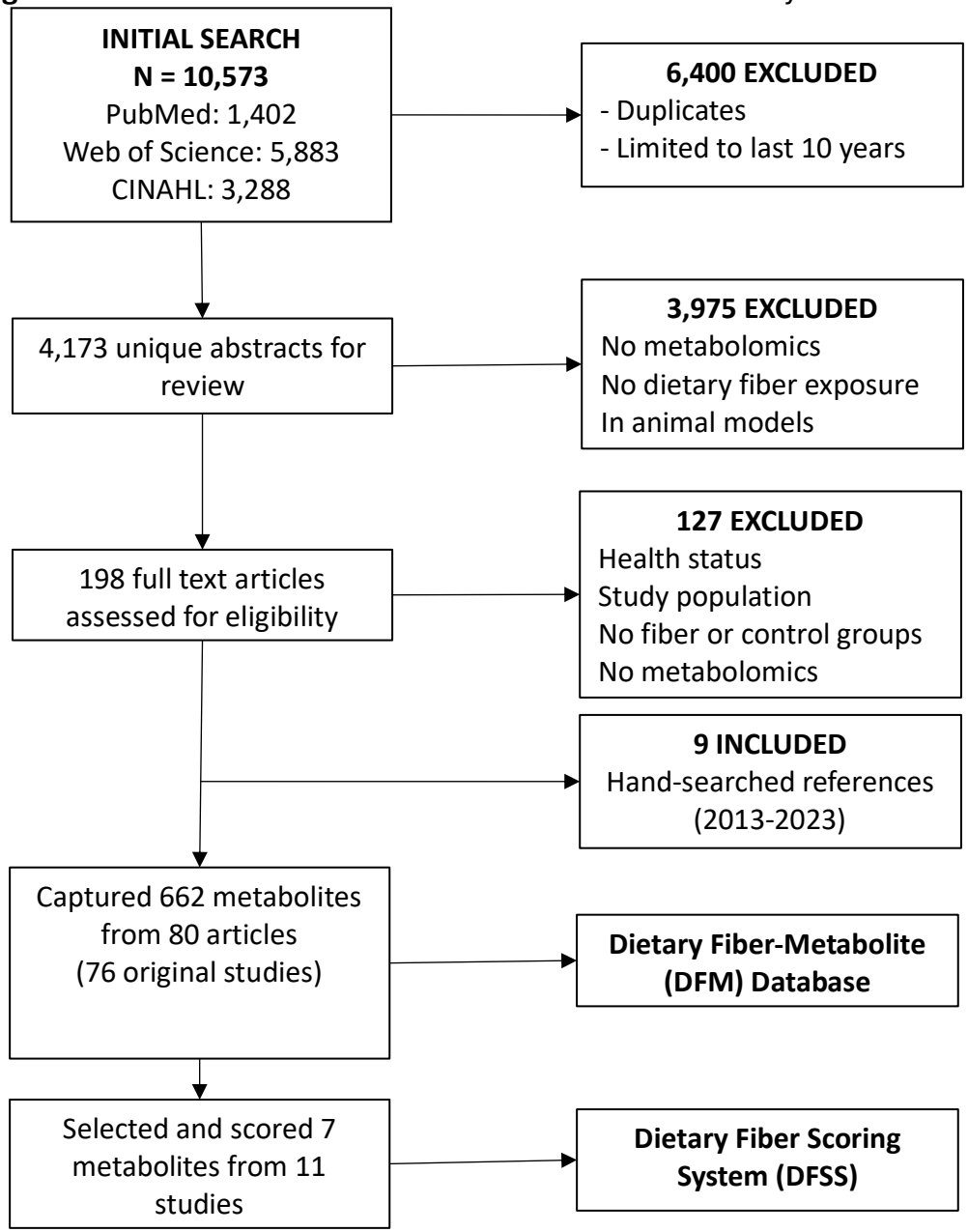


Table 1. Scoring System to capture metabolite association specificity and replicability in the literature

Reproducibility	Specificity
+ 1 for every positive specific fiber	+ 2 for a positive specific supplement study
+ 0.XX for % specific fiber present in positive TDF	+ 1.XX for % mixed supplement study
- 1 for every negative specific fiber	- 0.5 for positive association with a different fiber
- 0.5 for every null specific fiber study	
- 0.XX for fiber in mixed supplement/TDF	

XX represents the percentage point of dietary fiber present in the exposure derived by the study.

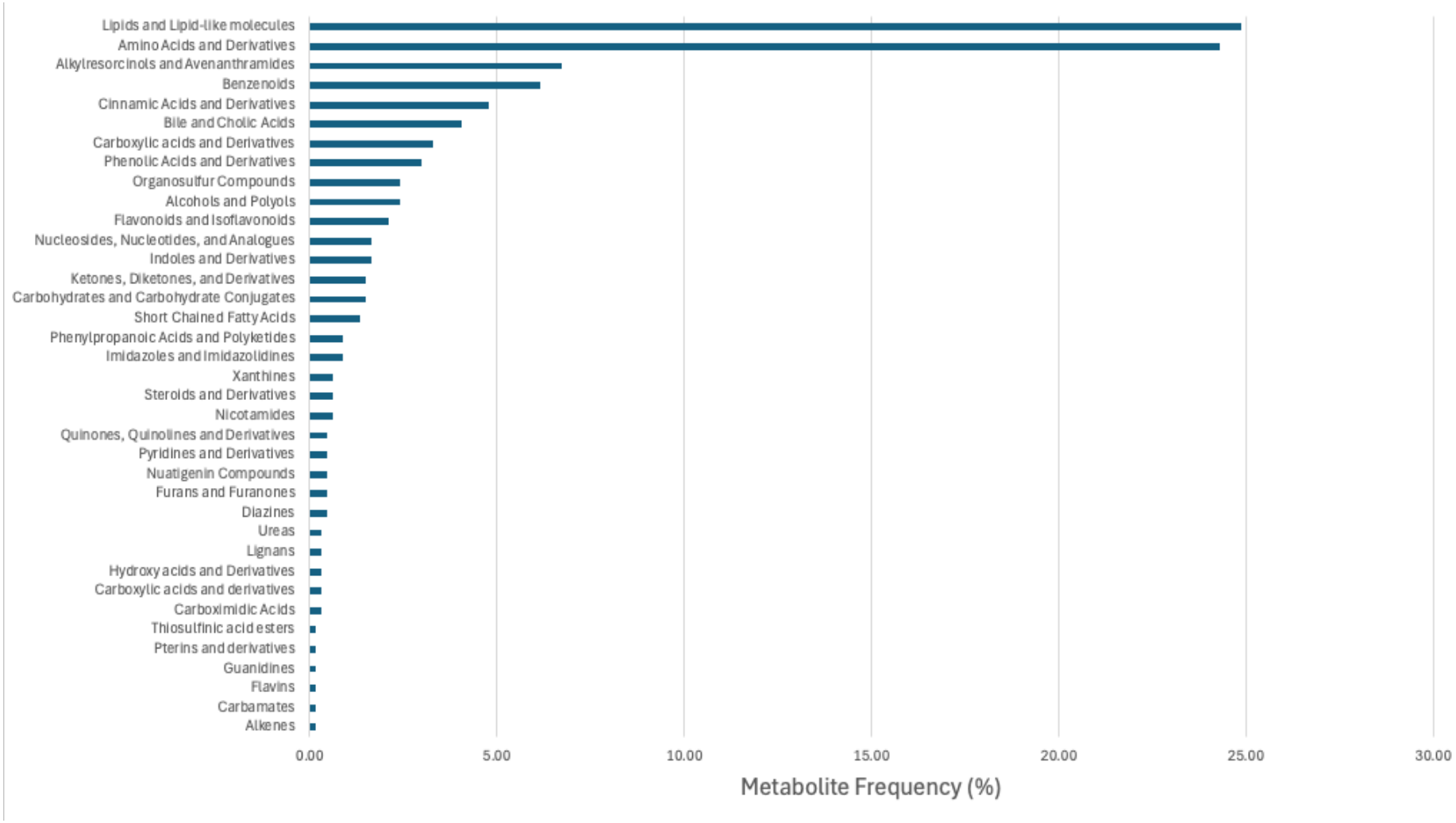
Table 2. Summary of Metabolites with a DFSS ≥ 3

Metabolite Class	Metabolite	HMDB ID	Biofluid	Positive Studies	Null Studies	Negative Studies	Other Fiber Positive Studies	Total Score
Cereal Fiber								
Phenolic Acids and Derivatives	Ferulic acid 4-O-sulfate	HMDB0029200	urine	Whole foods: PS048; PS052; PS068 Supplements: PS025				5
	Pyrocatechol sulfate	HMDB0059724	urine	Whole foods: PS022; PS068; PS052.1 Supplements: PS025	Whole foods: PS071		Whole foods: HS014 (FF)	4
	Dihydroferulic acid 4-O-sulfate	HMDB0041724	urine	Whole foods: PS052 Supplements: PS025				3
	Dihydroferulic acid glucuronide		urine	Whole foods: PS052 Supplements: PS025				3
	Caffeic acid	HMDB0001964	urine	Whole foods: PS052 Supplements: PS025				3
	Pyrocatechol sulfate	HMDB0059724	blood	Whole foods: PS082 (69%CF) Supplements:				4.43

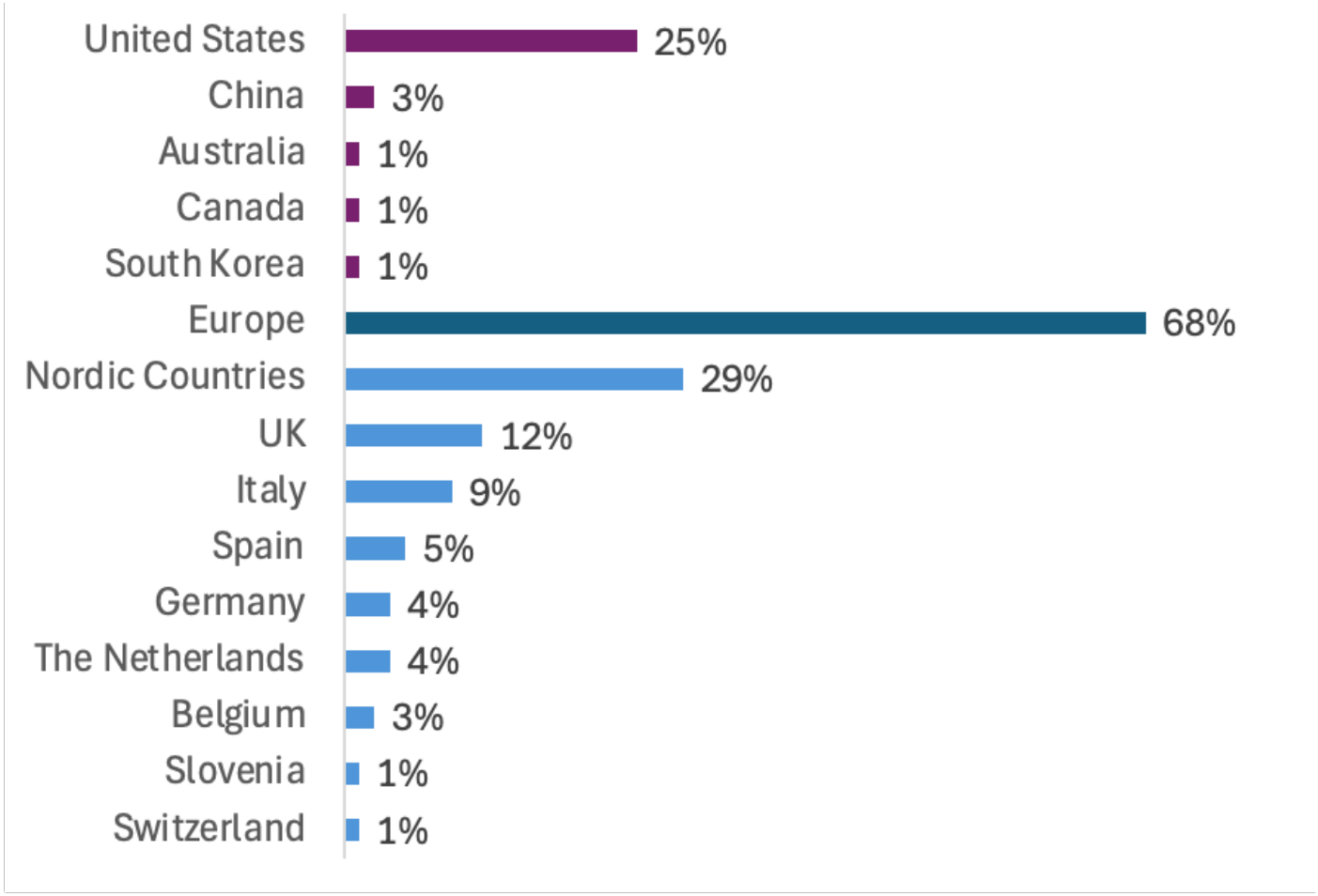
			PS025; PS091 (MS.CF.VF)	
Legume Fiber				
Amino Acids and Derivatives	Methylcysteine	blood	Whole foods: PS027 Supplements: PS005	3
	Pipecolic acid	blood	Whole foods: PS027 Supplements: PS005	3

Supplement studies without a percent specification were 100% the corresponding specific fiber type

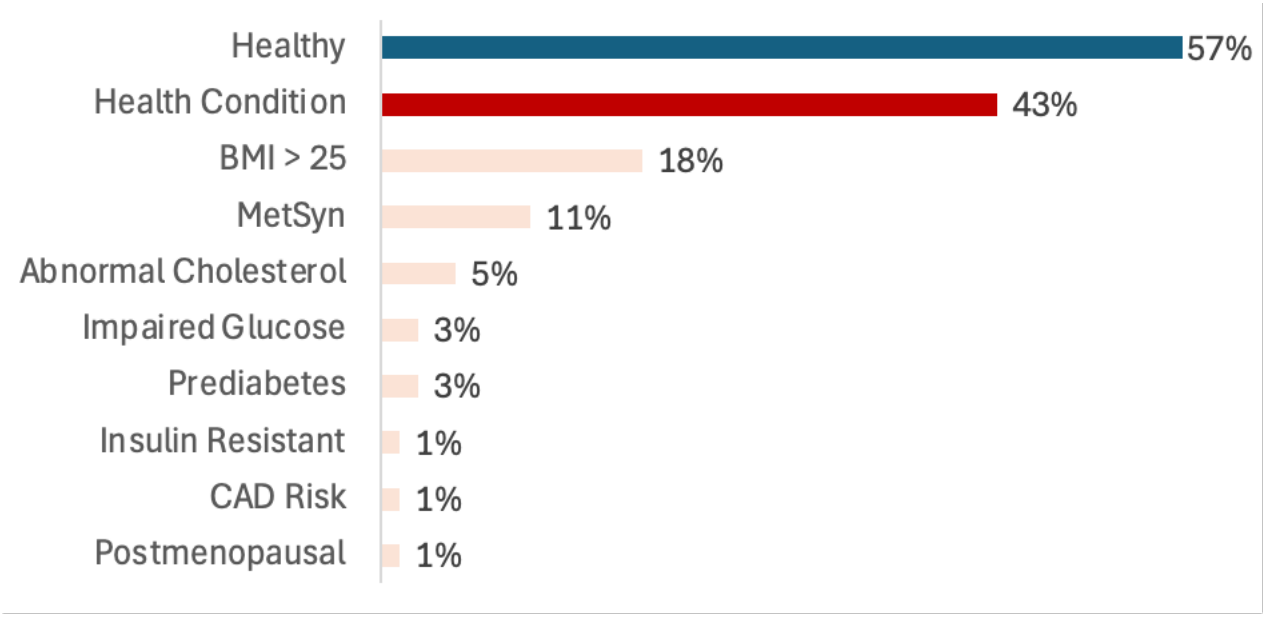
Supplemental Figure 1. Metabolites captured in the DFM database by metabolite class (N=37)



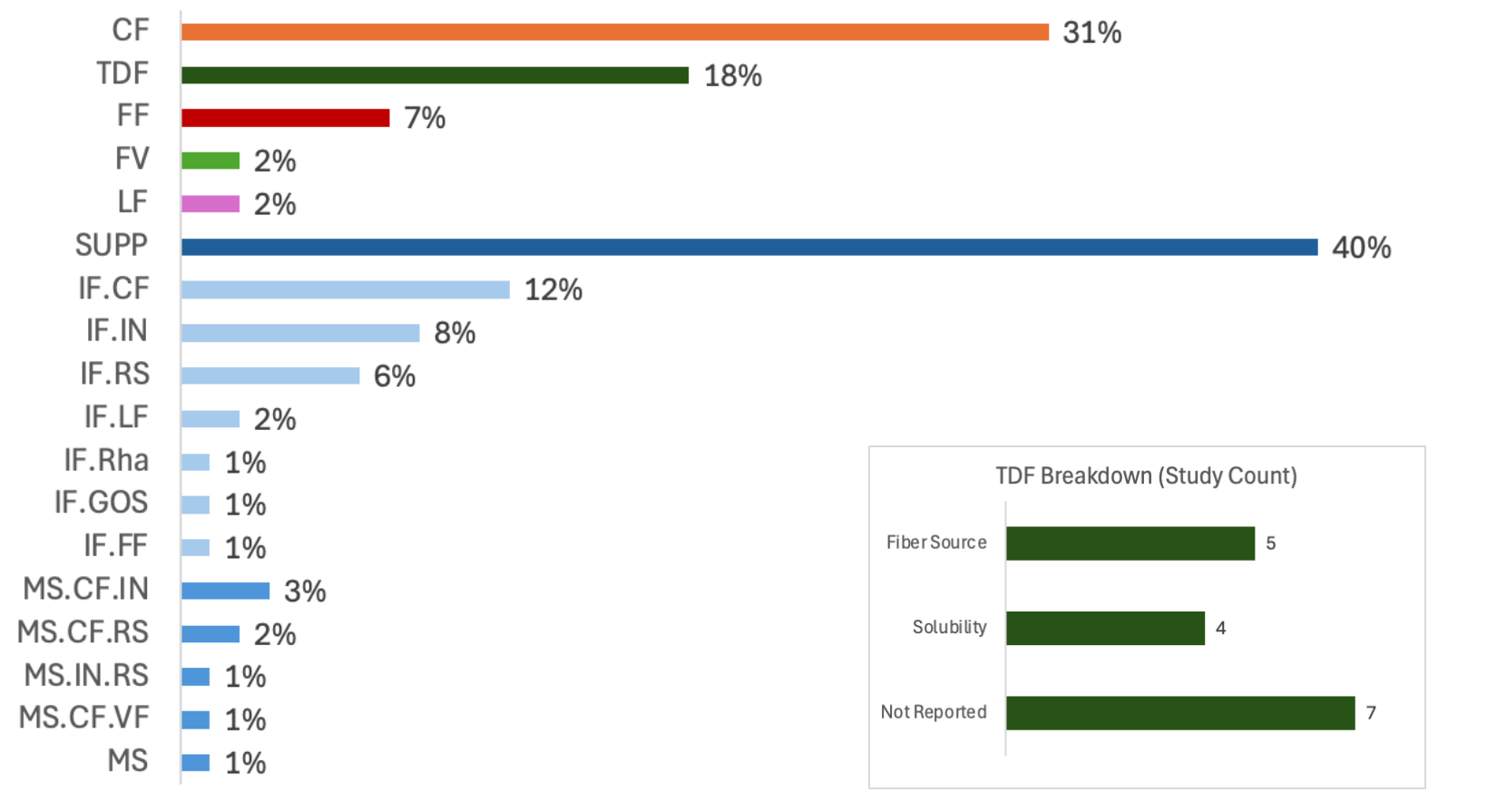
Supplemental Figure 2. Distribution of countries in the 76 primary studies contributing to DFM database



Supplemental Figure 3. Distribution of health conditions in the 76 primary studies contributing to DFM database

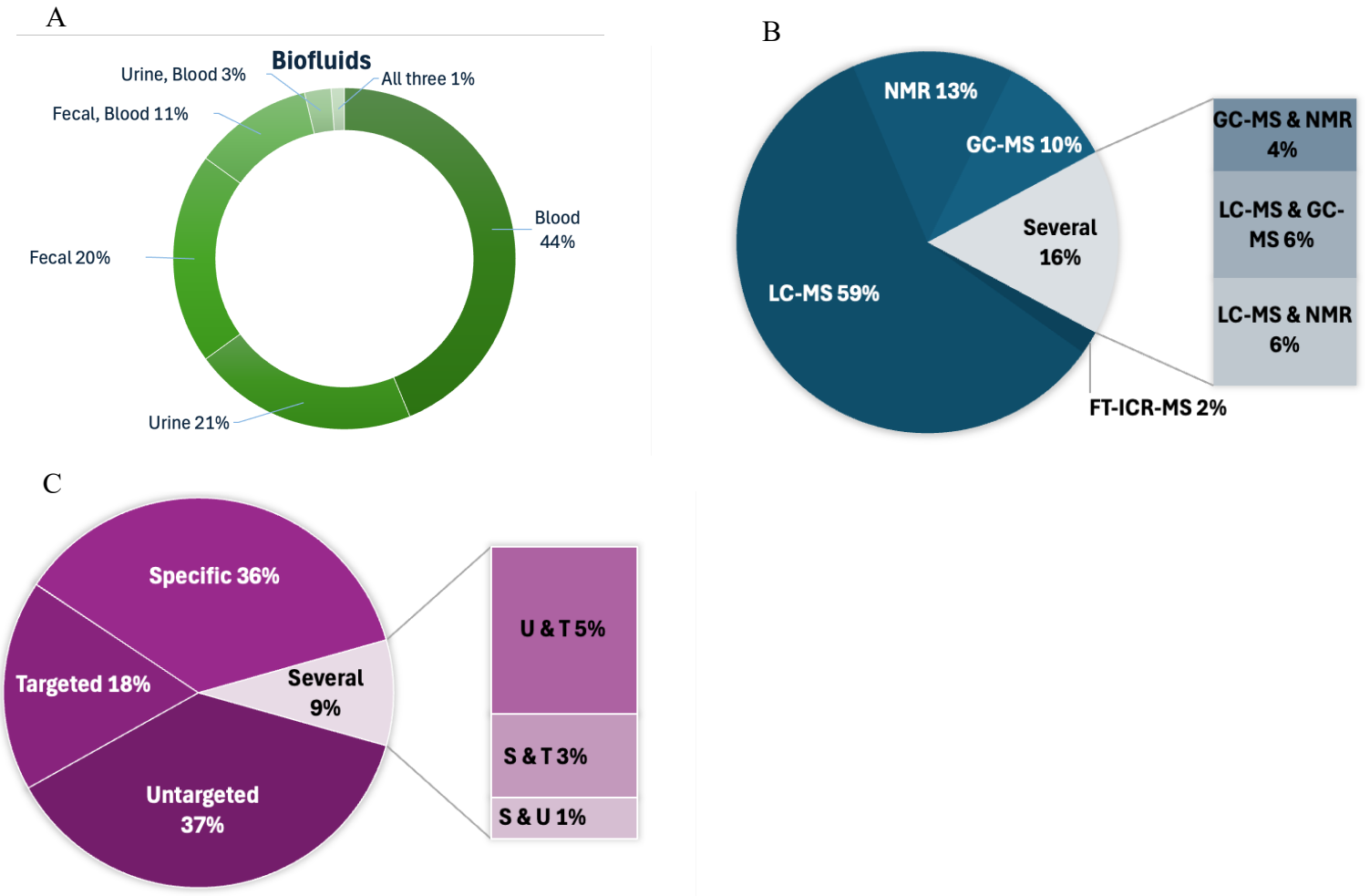


Supplemental Figure 4. Distribution of fiber sources in the 76 primary studies contributing to DFM database



Abbreviations: CF: cereal fiber; FF: fruit fiber; FV: fruit and vegetable fiber; IF: GOS: galacto-oligosaccharide; isolated fiber; IN: inulin; LF: legume fiber; MS: mixed supplement; Rha: Rhamnose; RS: resistant starch; SUPP: supplement; TDF: total dietary fiber; VF: vegetable fiber.

Supplemental Figure 5. Distribution of biofluids (A), techniques (B), and tools (C) used in the 80 primary and secondary studies contributing to the DFM database



Abbreviations: GC: gas chromatography; LC: liquid chromatography; MS: mass spectrometry; NMR: nuclear magnetic resonance; S: specific; T: targeted; U: untargeted

Supplemental Table 1. Summary of Metabolites with a DFSS > 0

Metabolite Class	Metabolite	HMDB ID	Biofluid	Positive Studies	Null Studies	Negative Studies	Other Fiber Positive Studies	Score
Cereal Fiber								
Phenolic Acids and Derivatives	Ferulic acid 4-O-sulfate	HMDB0029200	urine	Whole foods: PS048; PS052; PS068 Supplements: PS025				5
Phenolic Acids and Derivatives	Pyrocatechol sulfate	HMDB0059724	urine	Whole foods: PS022; PS068; PS052.1 Supplements: PS025	Whole foods: PS071		Whole foods: HS014 (FF)	4
Phenolic Acids and Derivatives	Dihydroferulic acid 4-O-sulfate	HMDB0041724	urine	Whole foods: PS052 Supplements: PS025				3
Phenolic Acids and Derivatives	Dihydroferulic acid glucuronide		urine	Whole foods: PS052 Supplements: PS025				3
Phenolic Acids and Derivatives	Caffeic acid	HMDB0001964	urine	Whole foods: PS052 Supplements: PS025				3
Phenolic Acids and Derivatives	Pyrocatechol sulfate	HMDB0059724	blood	Whole foods: PS082 (69%CF) Supplements: PS025; PS091 (MS.CF.VF)				4.43
Amino Acids and Derivatives	Pipecolic acid betaine	HMDB0304559	blood	Whole foods: PS071 Supplements: PS025			Whole foods: PS001 (TDF);PS054 (TDF)	**2**

Other	5-Hydroxyindole	HMDB0059805	urine	Whole foods: PS004 (30% CF) Supplements: PS024 (100% CF)		2.3
Amino Acids and Derivatives	Leucine	HMDB0000687	blood	Whole foods: HS021 Supplements: PS080 (60%CF)	Whole foods: PS075	2.1
Phenolic Acids and Derivatives	Caffeic acid sulfate	HMDB0304903	urine	Whole foods: PS052; PS109		2
Carbohydrates	2,4-Dihydroxybenzoic acid		urine	Whole foods: PS048; PS052		2
Carbohydrates	Azelaic acid	HMDB0000784	urine	Whole foods: PS068; PS052.1		2
Carbohydrates	Pimelic acid	HMDB0000857	urine	Whole foods: PS052.1; PS109		2
Carboxylic Acids	Dihydroxyphenylpropionic acid glucuronide		urine	Whole foods: PS022; PS052		2
Lipids	C23:0		blood	Whole foods: PS074; PS105		2
Lipids	Heneicosanoic acid	HMDB0002345	blood	Whole foods: PS074; PS105		2
Lipids	Nonadecanoic acid	HMDB0000772	blood	Whole foods: PS074; PS105		2
Phenolic Acids and Derivatives	Ferulic acid 4-O-glucuronide	HMDB0041733	urine	Whole foods: PS048; PS052		2
Phenolic Acids and Derivatives	Homovanillic acid	HMDB0000118	urine	Whole foods: PS048; PS052		2
Phenolic Acids and Derivatives	Sinapic acid-O-sulfate		urine	Whole foods: PS048; PS052		2

Phenolic Acids and Derivatives	Pyrogallol sulfate		urine	Whole foods: PS039 (60%CF); PS052.1				1.6
Amino Acids and Derivatives	Arginine	HMDB0000517	blood	Whole foods: PS071; PS075	Whole foods: HS021			1.5
Amino Acids and Derivatives	L-Tyrosine	HMDB0000158	blood	Whole foods: PS071; PS075	Whole foods: HS021			1.5
Other	2-Hydroxybutyric acid	HMDB0000008	blood	Whole foods: PS010 (50%CF); PS057.1				1.5
Phenolic Acids and Derivatives	N-feruloylglycine		urine	Whole foods: PS004 (30%CF); PS052; PS052.1				1.3
Carboxylic Acids	Hippuric acid	HMDB0000714	blood	Whole foods: PS082 (69%CF) Supplements: PS007	Whole foods: PS071		Whole foods: HS011 (FF); PS071 (FF)	1.19
Amino Acids and Derivatives	Isoleucine	HMDB0000172	blood	Whole foods: HS021 Supplements: PS080 (60%CF)	Whole foods: PS075	Whole foods: PS058		1.1
Amino Acids and Derivatives	Lysine	HMDB0000182	blood	Whole foods: PS071 Supplements: PS080 (60%CF)	Whole foods: PS075; HS021		Whole foods: PS027 (LF)	1.1
Amino Acids and Derivatives	Ornithine	HMDB0000214	blood	Whole foods: PS040 (80% CF); PS071	Whole foods: PS075; HS021			0.8
Fruit Fiber								
Carboxylic Acids	Hippuric acid	HMDB0000714	blood	Whole foods: HS011; PS071	Whole foods: PS071		Supplements:PS007 (CF)	1.5

Other	xi-2,3-Dihydro-2-oxo-1H-indole-3-acetic acid		urine	Whole foods: HS014; PS039 (14%FF)			1.14
Legume Fiber							
Amino Acids and Derivatives	Methylcysteine		blood	Whole foods: PS027 Supplements: PS005			3
	Pipecolic acid		blood	Whole foods: PS027 Supplements: PS005			3
Peptide	Aspartylphenylalanine		urine	Whole foods: PS004 (50%LF); PS027			1.5
Other	Lenticin	HMDB61115	urine	Whole foods: PS027; PS039 (10%LF)			1.1
Total Fiber							
Amine	Trimethylamine N-oxide		urine	Whole foods: PS004 (TDF) PS089 (TDF)			2
Amine	Trimethylamine N-oxide		blood	Whole foods: PS066 (TDF), PS001 (TDF), PS054 (TDF)	Supplements: PS044 (MS)	Supplements: PS084 (IF.RS)	2.5

Supplement studies without a percent specification were 100% the corresponding specific fiber type.

Supplemental Appendix 1

(Complex carbohydrate OR grain OR grains OR edible grain OR edible grains OR whole grain OR wholegrain OR whole grains OR wheat OR whole wheat OR oat OR maize OR brown rice OR rice OR barley OR corn OR rye OR millet OR sorghum OR cereal OR barley OR bread OR Wheat bran OR fruit fiber OR fruit fibre OR vegetable fiber OR vegetable fibre OR legume OR legumes OR legume fiber OR legume fibre OR bean OR beans OR lentil OR lentils OR glycemic index OR glycemic load OR Dietary Fiber OR Dietary Fibre OR fiber OR fibre OR high-fiber OR high-fibre OR soluble fiber OR insoluble fiber OR soluble fibre OR insoluble fibre OR roughage OR prebiotic OR fermentable OR prebiotics OR starch OR nonstarch OR non-starch OR polymer OR poly-saccharide OR polysaccharide OR oligosaccharide OR oligo-saccharide OR fructooligosaccharide OR fructo-oligosaccharide OR cellulose OR chitin OR pectin OR pectins OR psyllium OR Metamucil OR hemicellulose OR hexosan OR lignin OR polydextrose OR raffinose OR xanthan OR xylose OR galactan OR galactans OR fructan OR fructans OR inulin)
AND
(biomarker* OR marker* OR metabolite* OR metabolome* OR biomonitor* OR biosignature* OR bioavailability OR metabolic profiling OR targeted metabolomics)
AND
(intake OR diet OR dietary pattern* OR dietary habit* OR eating pattern* OR food* OR meal* OR nutrition*assessment OR nutrition* survey*)
AND
(plasma OR urine* OR serum OR blood OR hair)

Chapter 4: Metabolomic signature of cereal fiber intake favorably associated with changes in cardiometabolic risk factors among US adults

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Declarations of interest: none

Abstract

Background: Despite recommendations for a daily fiber intake of 19-38 grams, only 5% of the U.S. population achieves this goal. The U.S. dietary guidelines only emphasize that fiber should be obtained from diet rather than supplements, and does not distinguish between the different types of fiber found in food. However, recent studies have demonstrated that intake of dietary fiber from different food sources is differentially associated with cardiometabolic risk. **Objective:** This study used prospective data from the Framingham Offspring Study (N= 5,124) to identify plasma metabolite profiles predicting self-reported total, cereal, fruit, vegetable, and nut and legume fiber intake using an elastic net regression model. These metabolomic signatures were used to assess longitudinal associations between baseline dietary fiber intake and changes in cardiometabolic risk factors [waist circumference (WC, in), diastolic blood pressure (DBP, mmHg), systolic blood pressure (SBP, mmHg), fasting plasma HDL cholesterol (HDL-C, mg/dL), fasting plasma triglycerides (mg/dL), fasting serum glucose (mg/dL), and hemoglobin A1c (HbA1c, %)] over four years. **Results:** We identified metabolomic signatures that significantly predicted intakes of total fiber ($r = 0.39$; p-value < 0.001), cereal fiber ($r = 0.33$; p-value < 0.001), fruit fiber ($r = 0.32$; p-value < 0.001), vegetable fiber ($r = 0.20$; p-value < 0.001), and nut and legume fiber ($r = 0.12$; p-value < 0.001). Cereal fiber and its metabolomic signature were significantly associated with more favorable changes (β [SE]) in fasting serum glucose (-1.01 [0.31] mg/dL, $P_{adj} = 0.005$; and -0.80 [0.17] mg/dL, $P_{adj} < 0.001$), HbA1c (-0.05 [0.02] %, $P_{adj} = 0.005$; and -0.03 [0.01] %, P= 0.01), and HDL-C levels (0.98 [0.26] mg/dL, $P_{adj} < 0.001$; and 0.37 [0.15] mg/dL, $P_{adj} = 0.04$). **Conclusions**: Findings from this observational prospective cohort study suggest cereal fiber intake and its associated metabolites were more strongly related to favorable cardiometabolic risk profiles compared to the other dietary fibers.

Keywords: total dietary fiber, cereal fiber, metabolomic signatures, cardiovascular health

1. Introduction

National consumption surveys have estimated that a mere 5% of the U.S. population meets the recommended daily fiber intake, which ranges from 19-38 grams depending on age and sex.[186] The lack of dietary fiber in the American diet is directly linked to the low intake of whole-grains, fruits, vegetables, nuts, and legumes, of which 85% of the American population falls short of meeting recommended intakes.[187] The dietary guidelines only emphasize that fiber should be obtained from diet rather than supplements, and does not distinguish between the different types of fiber found in food. However, research has demonstrated that soluble and insoluble fibers have both shared and distinct effects on human metabolism,[69] with both linked to improved satiety,[71-73] weight loss,[74, 188] and inflammation.[76] However, soluble fibers form a viscous gel in the gastrointestinal tract, which is highly fermentable by gut microbiota and leads to increased production of beneficial short-chain fatty acids (SCFA)[86] and decreased postprandial glycemic index[80] as well as total and LDL cholesterol.[85] Insoluble fibers, on the other hand, are non-viscous with limited fermentability and thus have no significantly meaningful effects on modulation of the microbial composition, although have been shown to accelerate colonic transit time, increase fecal bulk, and prevent constipation.[70]

In randomized clinical trials (RCTs)[189] and prospective studies,[190] a higher dietary fiber intake has been reported to improve biomarkers of metabolic disease, including cholesterol, glucose, and insulin. Indeed, whereas greater consumption of whole grain, cereal, and legume fibers consistently shows a reduced risk in type 2 diabetes (T2D)[90, 91, 97] and improved insulin sensitivity,[98] the associated health benefit is not as consistent with fibers obtained from fruits and vegetables[69, 99]. While several cross-sectional studies have examined the association between total fiber intake and cardiometabolic risk factors[191, 192]; the impact of fiber intake by food source on changes in cardiometabolic risk factors has not yet been fully explored.

Plasma metabolites are increasingly being studied in nutritional epidemiology to examine associations between intake of a specific nutrient, food, or a general dietary pattern in relation to disease risk, such as cardiovascular disease (CVD) and T2D.[193, 194] To

date, few studies have identified metabolite signatures of dietary fiber intake. In a post-hoc analysis of data from the PREDIMED study, metabolites were found to be associated with dietary glycemic load (GL), glycemic index (GI), and overall carbohydrate quality index.[112] More recently, studies have examined links between metabolites associated with whole grain intake and liver fat,[113] total dietary fiber and T2D,[114] and healthy plant-based diets and cardiometabolic risk.[115] However, a comprehensive plasma metabolic signature of total dietary fiber and dietary fiber by food sources, and their association with changes in cardiometabolic risk factors, have not been analyzed. The study of these metabolomic signatures may help understand biological pathways of dietary fiber metabolism and potentially identify key targets for interventions.

The present analysis used a machine learning approach to identify plasma metabolites associated with self-reported total fiber intake and fiber from food sources (cereal, fruit, vegetable, nut and legume) using data from the ongoing Framingham Offspring Study (FOS). Using these fiber metabolomic signatures, we independently assessed the longitudinal associations between baseline dietary fiber intake and the adjusted 4-year mean change in cardiometabolic risk factors.

2. Material and Methods

2.1 Study Design and Subjects

The analyses were conducted in a subset of FOS, a long-standing prospective cohort study in Framingham, Massachusetts that began in 1948. All participants of these cohorts are invited for regular follow-up visits every 4-8 years. Lifestyle and health questionnaires are administered, anthropometrics are measured, and blood and urine samples are collected during these follow-ups all within a four-hour window. Data from exam 5 (1991-1995; n=3,799), exam 6 (1995-1998; n=3,532), and exam 7 (1998-2001; n=3,539) of the FOS cohort were used in the current analysis. All participants provided written informed consent before study participation.

We excluded participants who did not attend the exam 5 (n=1325); with no metabolite data (n=1,273); with diagnosed diabetes mellitus (n=178 and 1 missing) or CVD (n= 16) at baseline (exam 5); with non-fasting samples (n=15 and 7 missing); with invalid

food frequency questionnaire (FFQ) at baseline, which was defined as ≥ 12 blanks and/or calorie intake < 600 kcal or ≥ 4,000 kcal for women and ≥ 4,200 kcal for men (n=196); and with missing covariate data at baseline (n=50). Participants were excluded if their change in outcome was more than ± 4 SDs of the mean change for that outcome. Final sample sizes varied by outcome (waist circumference n = 1,861; diastolic blood pressure n = 1,684; systolic blood pressure n = 1,683; HDL-C n = 1,625; triglycerides n = 1,624; serum glucose n = 1,598; and HbA1c n = 1,140) (Figure 1). The institutional review boards at Boston University Medical Campus and Tufts University Health Sciences approved all study protocols and procedures for the current study.

2.2. Assessment of Plasma Metabolites

Methods for the metabolomic profiling on plasma samples collected at the in-person exam 5 after participants were asked to fast overnight were previously described elsewhere.[33] In brief, metabolite profiling was performed using a 4000 QTRAP Triple Quadrupole Mass Spectrometer (Applied Biosystems/Sciex), coupled to a 1200 Series Pump (Agilent Technologies) and an HTS PAL Autosampler (Leap Technologies). MultiQuant software (version 1.1; Applied Biosystems/Sciex) was used for automated peak integration, and peaks were manually reviewed for quality of integration.[195]

Subjects with missing values for >75% of metabolites within a batch were removed from the data. For batches having ≤ 75% missing data, the missing values were replaced with half of the batch minimum. We then winsorized metabolite levels to ± 4SD followed by standardizing metabolites with mean at 0 and SD at 1. Finally, for unmeasured metabolites between batches, we utilized the k-Nearest Neighbors imputation method (k=10) to obtain a final dataset of 217 metabolites measured in 1,863 participants with no missing metabolite data. Supplemental Table 1 depicts the number of individuals with imputations for each metabolite due to variation in the number of metabolites measured between batches.

2.3. Dietary Assessment of Fiber Intake

Intake of dietary fiber was assessed using the previously validated Harvard semiquantitative FFQ designed to capture a person's usual diet over the previous year.[196]

The FFQ includes a list of foods and nine categories of intake frequency ranging from < 1 standard serving per month to ≥ 6 standard servings per day. Dietary fiber of each food item was calculated by multiplying their frequency of consumption by the dietary fiber content of the specified portion. Nutrient composition was based on the USDA food composition database and supplemented with other published sources.[196] Groups of fiber-containing food items (cereal, fruit, vegetable, and nut and legumes) were gathered and their dietary fiber amounts were summed to obtain total fiber, cereal fiber, fruit fiber, vegetable fiber, and nut and legume fiber variables (g/d) (Supplement Table 2).

2.4. Cardiometabolic Outcomes

The primary outcomes of the present analyses were the changes in metabolic syndrome risk factors, namely waist circumference (WC, in), diastolic blood pressure (DBP, mmHg), systolic blood pressure (SBP, mmHg), fasting plasma HDL cholesterol (HDL-C, mg/dL), fasting plasma triglycerides (mg/dL), and fasting circulating serum glucose (mg/dL); ,as well as hemoglobin A1c (HbA1c, %). We captured the outcome change between exam 5 and exam 7 and standardized them by dividing the raw change by the number of years between exam dates and converting the data to reflect mean 4-year changes.

2.5. Covariates

Several potential confounders in the associations between dietary fiber, metabolites, and cardiometabolic risk factors were considered as covariates in our analyses. These included age at baseline (years), sex (female/male), education (no high school degree/high school degree/some college/college graduated), smoking status (yes/no/change of status), physical activity index (PAI), alcohol intake (g/d), menopausal status (yes/no/change of status), and standardized 4-year change in waist circumference (inch). These measures were obtained from standard questionnaires and measurements administered at each exam.[197] The PAI is a score based on the sum of sedentary, light, moderate, and vigorous metabolic equivalent tasks (MET, hrs/wk) completed in a week.[198]

In addition, we included a measure of overall diet quality, which was calculated as the fiber-adjusted 2015 Dietary Guidelines Adherence Index (DGAI) score previously

described elsewhere.[199, 200] For the HDL-C and triglyceride outcomes, we additionally adjusted for the use of lipid-lowering medication (yes/no/yes at baseline and no at follow-up/no at baseline and yes at follow-up). For the SBP and DBP outcomes, we accounted for the use of anti-hypertensive medications by adding 15 mm Hg to the blood pressure values of these participants.[201] We used baseline covariate values captured at exam 5 for continuous variables, and we accounted for change in status of the categorical variables smoking status, menopausal status, and use of medication (yes/no/changed status) from exams 5 to 7. Finally, we used both baseline and an adjusted 4-year change for waist circumference as both an outcome and a covariate.

2.6. Statistical Analysis

For the development of the metabolite signatures, linear regression models were first used to examine the cross-sectional association between individual metabolites (n=217) and energy-adjusted fiber sources (total, cereal, fruit, vegetable, nut and legume), adjusting for baseline age, sex, education, daily energy intake, alcohol intake, smoking status, PAI, waist circumference, and fiber source adjusted DGAI score. Intake of total fiber and fiber sources were energy adjusted and the DGAI scores was fiber adjusted to help isolate the effect of dietary fiber from total energy intake and overall dietary pattern, using the residual method.[202] Metabolites with a significant associations ≤ 0.05 were fed to an elastic net regression model to develop metabolomic signatures.

A metabolomic signature for each fiber source was developed using a randomly selected training set (2/3rd of cohort, n=1,117) and testing set (1/3rd of cohort, N=746). For each dietary fiber source, an elastic net regression (α=0.5) with 10-fold cross-validation analysis was first applied to the training set to select a minimum λ shrinkage parameter and a set of metabolites predictive of dietary fiber intake. The elastic net regression methodology assigns a weight to each metabolite through a stepwise regression that combines Lasso and Ridge regression models to reduce model overfitting. When the elastic net model uses an α value of 0.5, it either includes or excludes groups of correlated features, similar to Lasso regression. This approach has proven effective in selecting sets of metabolites that predict continuous outcomes in prior analyses.[203] We conducted

separate training models for total, cereal, fruit, vegetable, and nut and legume fiber intake, and selected the most common model among 100 repetitions. The metabolomic signature was calculated as the weighted sum of the selected metabolites with weights equal to coefficients (coeff's) from the elastic net regression. To avoid overfitting, the metabolic signature of the training set was applied to the testing set. Pearson correlation coefficients (R, ranging from 0-1) were then used to determine how well the signature predicts the fiber source intake, and a p-value of < 0.05 was used to test significance.

Multiple linear regression models were used to examine the association between both the energy adjusted fiber intakes and the corresponding metabolomic signatures with change in cardiometabolic risk factors. Model 1 adjusted for age, sex, and daily caloric intake. Model 2 further adjusted for lifestyle factors, including education, smoking status, number of cigarettes smoked daily, PAI, daily alcohol intake, menopausal status, and lipid medication use for the HDL-C and triglyceride outcomes only. Model 3 additionally adjusted for both baseline and change in waist circumference to consider if the change in abdominal adiposity was mediating the observed associations. Finally, model 4 adjusted for other attributes of a healthy diet by including the fiber adjusted DGAI score. We modeled the 4-year change in outcomes after a 5-g increase in dietary fiber intake for the diet exposures and a per-SD increase in corresponding fiber metabolomic signature. A Bonferroni corrected p-value was used to account for multiple testing of four dietary exposures (fiber sources). All analyses were conducted using R (version 2023.03.0+386),[127] where the elastic net regressions were performed using the *glmnet* package. All reported p-values are two-sided and global $\alpha < 0.05$ indicates statistical significance.

3. Results

3.1 Baseline Characteristics

Exam 5 characteristics are presented for all participants and by tertiles of energy-adjusted total fiber intake in Table 1. At baseline, participants had a mean ± SD age of 54 ± 10, 45% were male, and had a mean BMI of 27.2 ± 4.7 kg/m^2. Study participants had median energy-adjusted total fiber intake of 16.2 ± 0.1 g/d, cereal fiber intake of 5.7± 0.1 g/d, fruit fiber intake of 3.5 ± 0.1 g/d, vegetable fiber intake of 6.3 ± 0.1 g/d, and nut and

legume fiber intake of 1.5 ± 0.03 g/d. The trend analysis suggested increased age, % female, % smoker status, % college graduate, DGAI score, cereal/fruit/vegetable/nuts and legume fiber intake, and decreased triglycerides, glucose, waist circumference, and alcohol intake across increasing tertiles of energy adjusted total dietary fiber intake at baseline.

3.2 Metabolomic Signatures

Of 217 metabolites captured at exam 5, 91 unique metabolites were nominally associated (p-value < 0.05) with intakes of dietary fiber and fiber sources. Most metabolites were lipids (60%) or amino acids (17%), followed by nucleotides (7%) and carboxylic acids (4%). The remaining 10 metabolites included 2 hydroxy acids, 2 ketones, 2 alcohols, indoxyl sulfate, hippurate, inositol, and indole propionate. Metabolite lists varied by fiber source, where 32 metabolites were nominally associated with total dietary fiber intake ($p < 0.05$); 36 metabolites were associated with cereal fiber intake; 59 metabolites were associated with fruit fiber intake; 26 metabolites were associated with vegetable fiber intake; and 26 metabolites were associated with nut and legume fiber intake.

Twenty-seven metabolites (14 positive, 13 negative) were selected 10 times by the elastic net regressions with 10-fold cross-validation to construct a metabolomic signature that significantly predicted total fiber intake ($R = 0.39$; p-value < 0.001). Similarly, 28 metabolites (14 positive, 14 negative) were selected for the cereal fiber metabolomic signature ($R = 0.33$; p-value < 0.001); 29 metabolites (12 positive, 17 negative) were selected for the fruit fiber metabolomic signature ($R = 0.32$; p-value < 0.001); 22 metabolites (10 positive, 12 negative) were selected for the vegetable fiber metabolomic signature ($R = 0.20$; p-value < 0.001); and 8 metabolites (2 positive, 6 negative) were selected for the nut and legume fiber metabolomic signature ($R = 0.12$; p-value < 0.001) (Table 2). Metabolite names, class, and weights for each signature are presented in Supplemental Table 3.

Across the different metabolomic signatures, phosphatidylcholine C38:6 PC had the strongest positive coefficient value for total fiber (coeff = 1.30), fruit fiber (coeff = 0.37), and vegetable fiber (coeff = 0.35). Indole propionate and hippurate also had strong positive

coefficients in the metabolomic signatures of dietary fiber (coeff = 0.90 and 0.52, respectively), cereal fiber (coeff = 0.32 and 0.26, respectively), and fruit fiber (coeff = 0.32 and 0.25, respectively). Metabolites with strong negative coefficient values included urate in the total fiber (coeff = - 0.92) and fruit fiber (coeff = -0.34) signatures and several of the sphingomyelins (C18:0 SM, C14:0 SM, C22:0 SM) in the total and cereal fiber metabolomic signature (coeffs range from -0.25 to -0.83). The cereal fiber metabolomic signature was made up of 12 lipids, 6 amino acids, 3 nucleotides, tri- and di-carboxylic acids, α- and ß-hydroxybutyrate, indole propionate, and a-ketoglutarate (Figure 2). The fruit fiber metabolomic signature was made up of 14 lipids, 2 alcohols, 4 amino acids, 2 nucleotides, hippurate, α-ketoglutarate, kynurenine, indoxylsulfate, indole propionate, oxalate, and sorbitol (Figure 3).The total fiber metabolomic signature was made up of 15 lipids, 3 carboxylic acids, 2 alcohols, 2 amino acids, 2 nucleotides, hippurate, indole propionate, and α-ketoglutarate (Figure 4).

Correlations between metabolomic signatures are presented in Supplemental Table 4, where the strongest correlations were observed between total, cereal, and fruit fiber metabolomic signatures (R = 0.65 – 0.88). Additionally, correlations between exam 5 metabolomic signatures for total, cereal, and fruit fiber and energy adjusted intakes of dietary fibers at exams 6 and 7 remained similar to correlations with intakes at exam 5 (Supplemental Table 5). Since nut and legume fiber intake at baseline was low (1.5 g/d, Table 1) and poorly correlated with its metabolomic signature (R= 0.12, Table 2), we excluded both dietary and metabolite exposures from further outcome associations studies.

3.3 Associations with Cardiometabolic Outcomes

Tables 3-4 and Supplementary Table 6 present the 4-year changes in cardiometabolic risk factors per 5-g increase in energy adjusted total dietary fiber, cereal fiber, and fruit fiber intakes and 1-SD difference in their corresponding fiber metabolomic signature. Neither vegetable fiber nor its corresponding metabolomic signature were significantly associated with changes in cardiometabolic risk factors (Supplemental Table 7). The associations between dietary fibers and outcomes remained statistically significant

or nominally associated after further adjustment for diet quality using the fiber adjusted DGAI-2015 scores (Supplement Table 8); as such model 2 and 3 data were presented as final models.

Higher cereal fiber intake and its metabolomic signature were significantly associated (β [SE]) with more favorable changes in fasting glucose (-1.01 [0.31] mg/dL, P_{adj} = 0.005; and -0.80 [0.17] mg/dL, P_{adj} < 0.001), HbA1c (-0.05 [0.02] %, P_{adj} = 0.01; and -0.03 [0.01] %, P_{adj}= 0.01), and HDL-C levels (0.98 [0.26] mg/dL, P_{adj} < 0.001; and 0.37 [0.15] mg/dL, P_{adj}= 0.04). These associations were slightly attenuated, but remained significant, after adjusting for change in abdominal adiposity, proxied by change in waist circumference (Tables 3-4). Additionally, higher cereal fiber intake was nominally associated with more favorable changes in SBP (-0.82 [0.42] in, P = 0.047) and DBP (-0.59 [0.26] in, P = 0.02) (Supplemental Table 6).

Higher total fiber intake was significantly associated with more favorable changes in HDL-C (0.37 [0.13] mg/dL, P_{adj} = 0.01) while its metabolomic signature only showed a nominal association (0.36 [0.15] mg/dL, P = 0.01). The total fiber metabolomic signature, but not total fiber intake, was significantly associated with a lower mean changes in fasting glucose (-0.53 [0.18] mg/dL, P_{adj} = 0.01) and HbA1c (-0.02 [0.01] mg/dL, P_{adj} = 0.04). Higher total fiber intake and HDL-C association remained significant after adjusting for waist circumference (0.30 [0.15] mg/dL, P_{adj} = 0.04), and the total fiber metabolomic signature showed nominal association with HDL-C after adjusting for waist circumference (0.30 [0.15] mg/dL, P = 0.04). The total and cereal fiber signatures were significantly associated with more favorable changes in triglycerides (-2.39 [0.91], P_{adj} = 0.03; and -2.76 [0.90], P_{adj} = 0.008). The association remained signficant for the cereal fiber signature -2.46 [0.90], P_{adj} = 0.02) and was became nominal for the total dietary fiber signature (-2.05 [0.92], P = 0.03) after adjusting for waist circumference.

For the fruit fiber exposures, only the fruit fiber signature was nominally associated with more favorable changes in fasting glucose (-0.45 [0.20], P = 0.02) before adjusting for waist circumference and triglycerides (-2.01 [1.01], P = 0.046) after adjusting for waist circumference.

We carried out sensitivity analyses in the associations between the cereal fiber metabolomics signature and the lipid and glycemic outcomes to help account for the confounding of associations between other fiber sources and signatures. We further adjusted Model 3 by either energy-adjusted total fiber intake, energy adjusted cereal fiber intake, or the fruit fiber metabolomic signature. The associations remained consistent after all adjustments for both serum glucose (β between -0.48 and – 0.72 [0.17-0.22] mg/dL, $P < 0.01$) and HbA1c (-0.02 [0.01] mg/dL, $P < 0.05$). Additionally, the associations between the cereal fiber metabolomic signature and triglycerides remained significant after adjusting for energy adjusted total fiber intake (-2.89 [0.92], $P = 0.01$) (Supplemental Table 9).

4. Discussion

Using data from a middle-aged to older cohort, we successfully demonstrated that intake of dietary fiber from different food sources is differentially associated with cardiometabolic risk, which may be in part driven by differences in metabolite profiles.

Several large prospective cohort studies have found that higher cereal fiber intake, but not fruit or vegetable fiber, was consistently linked to lower inflammation levels and reduced CVD incidence and T2D; however, the cereal fiber-specific mechanisms remained elusive.[204, 205] In line with the literature, cereal fiber intake and its metabolomic signature seemed to be the most effective for improving glycemic and lipid control, with the observed benefit being independent of changes in abdominal adiposity. Additionally, the protective association between cereal fiber intake and change in waist circumference in the present analysis is consistent with the previously observed association of greater whole grain intake and smaller increase in waist circumference over time in this cohort.[206] Our findings align with a recent RCT in women with overweight or obesity, which found greater reductions in waist circumference, SBP, fasting glucose, and triglycerides after whole grains consumption compared to fruit and vegetable consumption or the combination of both diets.[207]

Although the effect sizes in this study were relatively small, their protective trends are in line with the well-established link of dietary fiber consumption and disease

prevention.[208, 209] Our findings serve to explain the discrepancy in the results from different fiber sources, which seem to be driven in part by metabolite profiles rather than changes in body composition. Using a machine learning approach, we identified a total of 91 unique metabolites that are associated with dietary fiber intake by food source (total, cereal, fruit, vegetable, and nut and legume) using cross-sectional data from the FOS. All our fiber metabolomic signatures, except nut and legume fiber, displayed significant correlations with their corresponding self-reported fiber intake ($R > 0.15$), suggesting that further investigation of the individual metabolites constituting the signatures is warranted. We observed that indole propionate and hippurate were positively weighed metabolites in the metabolomic signatures predicting total dietary fiber (coeff = 0.90 and 0.52, respectively), cereal fiber (coeff = 0.32 and 0.26, respectively) and fruit fiber (coeff = 0.32 and 0.25, respectively). Interestingly, a recent review of the metabolites associated with fiber sources identified blood hippurate positively associated to total dietary fiber,[155] cereal fiber,[210] and fruit fiber.[211, 212] [Guirette 2024, in preparation] Circulating levels of indole propionic acid were also previously associated with higher intake of total fiber, mainly from whole grains, and lower risk of T2D.[213, 214] Both metabolites are produced when gut bacteria break down high fiber and high polyphenol food sources,[215, 216] and results from this study further elucidate dietary fiber's role in metabolic health potentially mediated by the gut microbiota derived metabolites. In terms of negatively weighted metabolites, our results confirm previously observed associations between higher dietary fiber intake and lower risk of hyperuricemia, leading to improved insulin sensitivity.[217, 218] These studies found that the strongest associations were in total dietary fiber and cereal fiber only, however given the highly weighted coefficient in the fruit fiber metabolomic signature observed in the present analysis (coefficient = -0.35), the relationship between fruit fiber intake and urate may be worth exploring. Although there are no previous studies on the link between dietary fiber and C38:6 PC, several studies have associated it and other phosphatidylcholines with higher intake of animal products.[219, 220] Given the cross-sectional nature of the metabolite-fiber associations studied in these analyses, the positively weighted phosphatidylcholines in these fiber metabolomic signatures may be a result of reverse causality.

The elastic net regression developed metabolomic signatures that robustly predicted total, cereal, and fruit fiber intake; and only the cereal fiber metabolomic signature was significantly and nominally associated with beneficial changes in cardiometabolic risk factors, particularly changes in lipid (HDL-C, and triglycerides) and glycemic (fasting glucose and HbA1c) profiles after adjusting for abdominal adiposity. Our sensitivity analyses further suggest the metabolomic effect of cereal fiber on glycemic profiles is independent of cereal fiber intake and the metabolomic signatures of the other fibers, which further isolates the effect of cereal fiber's metabolite profile. Hence, the links between disease prevention and the metabolites that were unique to the cereal fiber metabolomic signature are worth further exploration. One of the 28 metabolites we identified as negatively correlated with cereal fiber was ß-hydroxybutyrate (coeff = -0.19), which in high levels has recently been suggested as a possible predictor of adverse cardiovascular events and disease progression.[221] Furthermore, thiamine (coeff = 0.17), a nutrient found in enriched grains and whole grains, has been linked to benefits in endothelial function in diabetes.[222] Continued exploration of these metabolic pathways may help detect individuals at high risk of cardiometabolic dysfunction and potentially identify new treatments for disease prevention or progression.

Our study has several strengths, which include a large sample size, detailed collection of covariates adjusting for potential residual confounding, and the longitudinal nature of our outcome-association analyses. However, the observational nature of this study and the cross-sectional associations between fiber and metabolite data to develop the metabolomic signatures do not allow for inferring causality, and we cannot rule out potential residual confounding by measured and unmeasured factors.

The discrepancy between higher statistical significance between our cardiometabolic outcomes and the metabolomic signatures compared to the self-reported fiber intake may be attributed to several factors. Although we used metabolites that were associated with self-reported fiber intake in multivariable adjusted regression models to feed our elastic net regression, the coefficients obtained by the elastic net regression are derived in an unadjusted model and residual confounding might be

introduced. Consequently, the weights and associations may be reflective of factors associated with fiber consumption. Although some of the discrepancy may be due to residual confounding, it is possible that we are also revealing inter-individual differences in response to dietary fiber by capturing downstream metabolites of the physiological effects of dietary fiber.

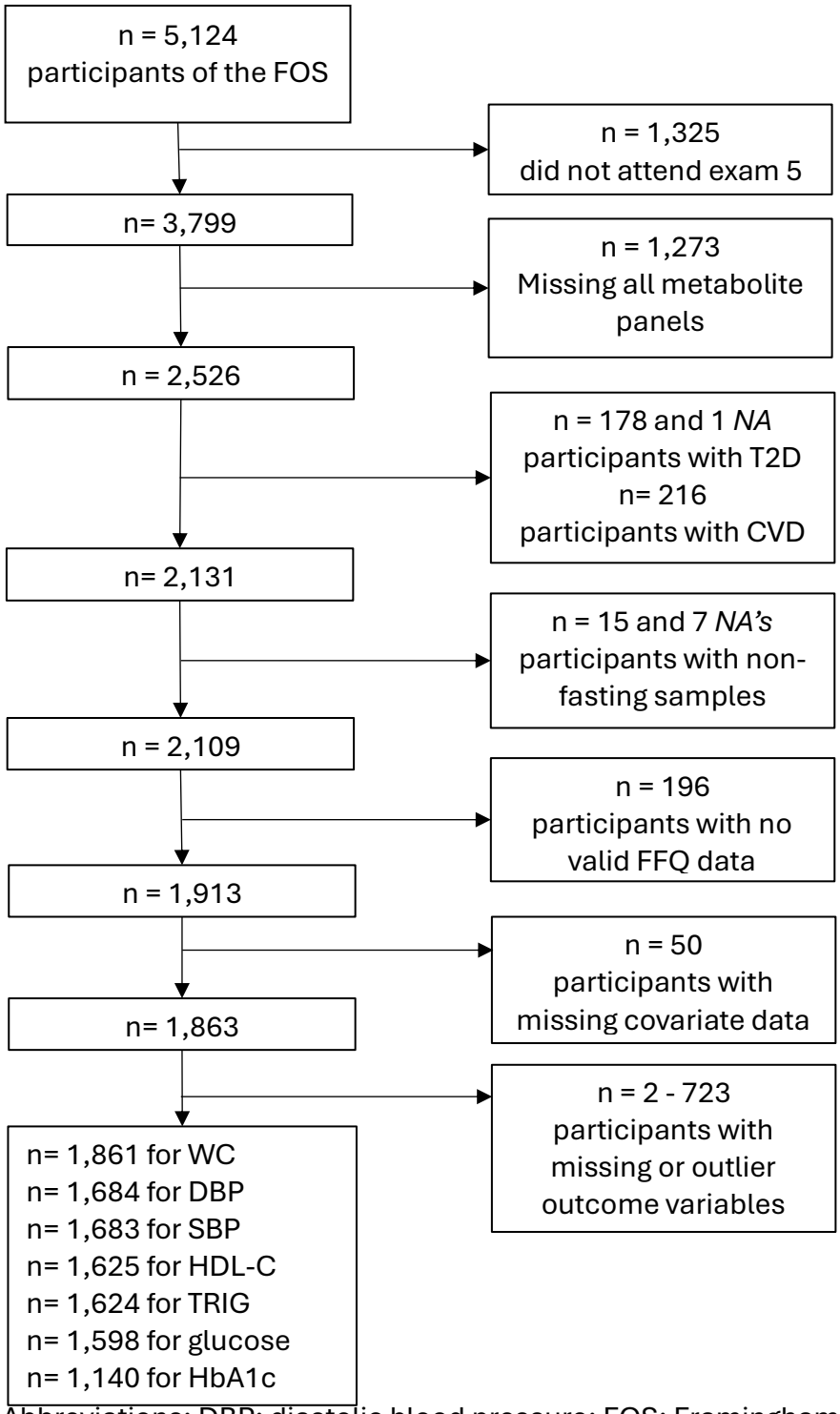
Despite the large sample size, the cohort was primarily middle-aged white participants; therefore, the findings are not generalizable to populations of different age-groups and races. Dietary fiber intake was captured by FFQs, which are known to be subject to a certain level of recall bias. Finally, the use of a targeted metabolite panel limits our discovery of fiber-associated metabolites. Future validation studies in other populations, untargeted metabolomic analyses, and cohorts with a larger number of metabolites measured are warranted.

5. Conclusion

We identified metabolomic signatures predicting total, cereal, fruit, and vegetable fiber intake. Findings from this observational prospective cohort study suggest cereal fiber intake and its associated metabolites were more strongly related to beneficial cardiometabolic risk profiles compared to the other dietary fibers. This hypothesis-generating study may help inform trials to investigate specific fiber types and their impact on cardiometabolic health, potentially leading to early detection of high-risk individuals, targeted dietary recommendations, and interventions for improving metabolic health outcomes.

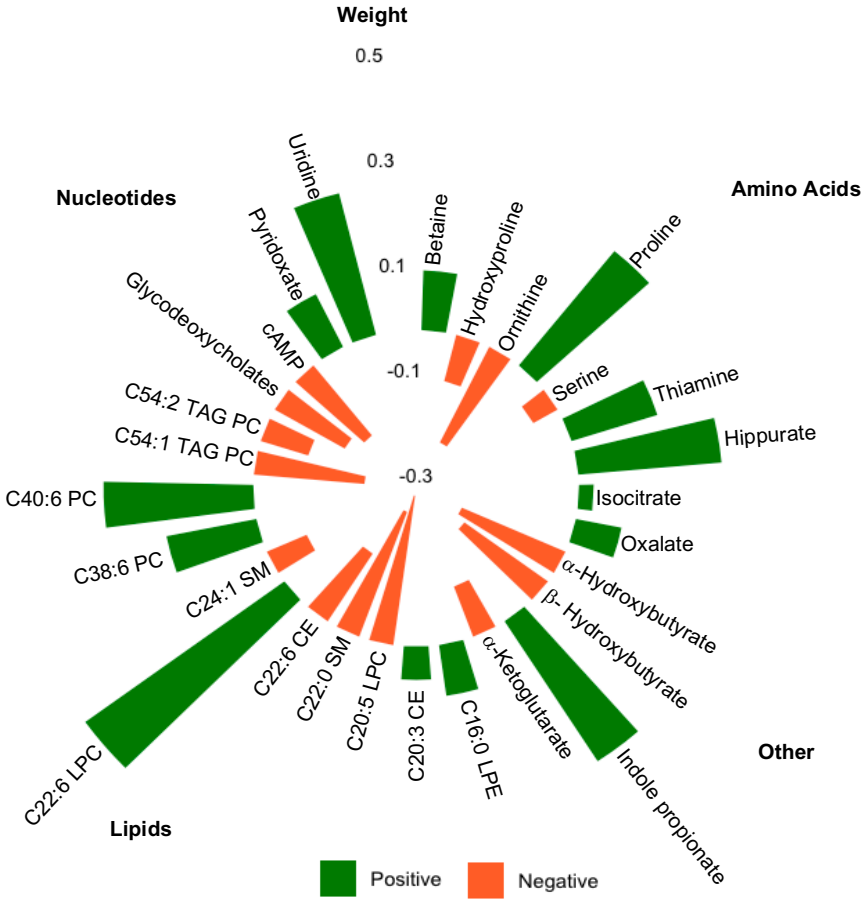
Tables and Figures

Figure 1 Flowchart of included participants from the Framingham Cohort Study



Abbreviations: DBP: diastolic blood pressure; FOS: Framingham Offspring Study; HDL-C: high density lipoprotein cholesterol; SBP: systolic blood pressure; WC: waist circumference; TRIG: triglycerides; HbA1c: Hemoglobin A1C

Figure 2. Circos plot of metabolite weights selected 10 times in the 10 cross-validations of elastic net regression for cereal fiber intake



Abbreviations: cAMP: cyclic adenosine monophosphate

Figure 3. Circos plot of metabolite weights selected 10 times in the 10 cross-validations of elastic net regression for fruit fiber intake

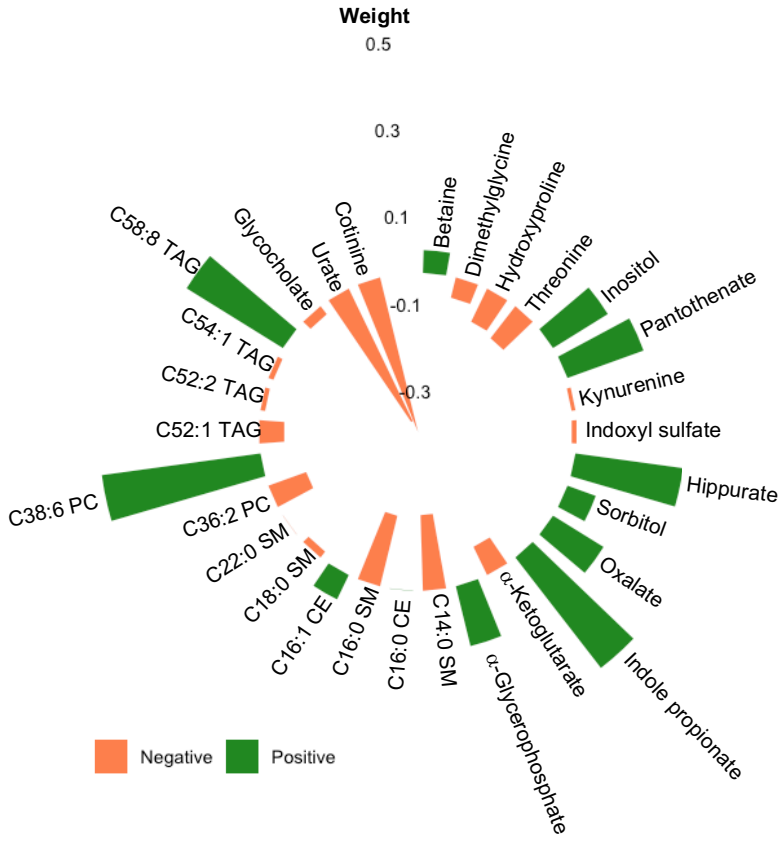


Figure 4. Circos plot of metabolite weights selected 10 times in the 10 cross-validations of elastic net regression for total fiber intake

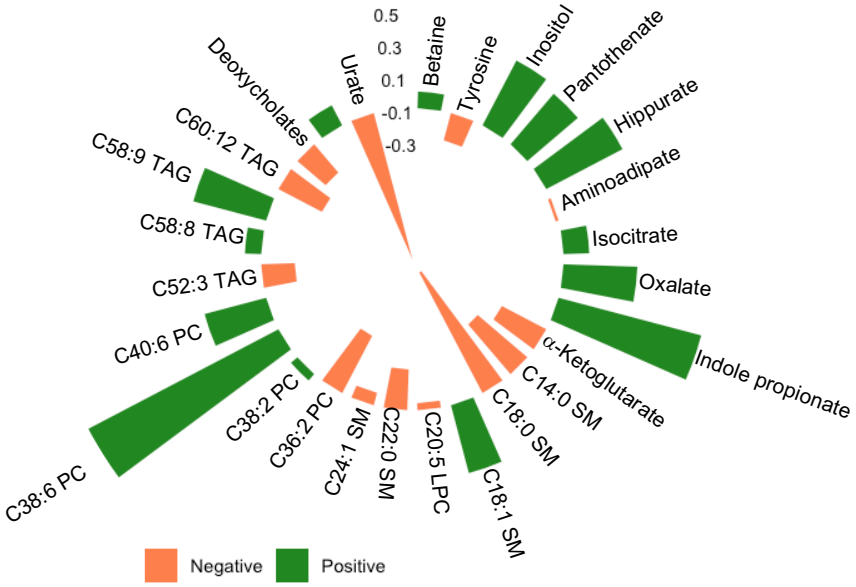


Table 1. Participant Baseline Characteristics by tertiles of energy adjusted median total fiber intake in 1863 participants from the Framingham Offspring Study[1]

	Total	Tertiles of Total Fiber Intake (g/d)			P-trend[4]
		1	2	3	
Energy Adjusted Median Total Fiber Intake	16.2 (0.1)	11.9 (0.1)	16.2 (0.1)	21.7 (0.2)	<0.001
Characteristics[2]					
n	1863	621	621	621	
Age, y	54 (10)	51 (0.4)	55 (0.4)	56 (0.4)	<0.001
Male Sex, %	45	57	41	37	<0.001
BMI	27.2 (4.7)	27.3 (4.9)	27.2 (4.7)	26.9 (4.6)	0.1
HDL cholesterol, mg/dL	50.2 (13.4)	50.4 (13.3)	50.2 (13.7)	49.7 (14.0)	0.5
Triglycerides, mg/dL	143.5 (103.5)	144.3 (114.8)	145.5 (106.5)	139.8 (92.4)	0.09
SBP, mm Hg	127.2 (18.4)	126.0 (18.5)	126.9 (17.5)	128.5 (20.2)	0.2
DBP, mm Hg	76.9 (11.8)	76.6 (12.3)	76.9 (11.1)	76.9 (12.5)	0.6
Glucose, mg/dL	95.1 (9.3)	95.4 (9.3)	95.1 (9.7)	94.8 (9.5)	0.01
Waist circumference, inch	36.3 (4.8)	36.4 (5.1)	36.3 (4.9)	36.1 (4.6)	0.03
HbA1c, %	5.27 (0.69)	5.25 (0.65)	5.28 (0.67)	5.29 (0.79)	0.6
PAI score	35 (6)	35 (7)	34 (5)	35 (6)	0.5
Current smoker, %	17	31	14	8	<0.001
Hypertension, %	15	14	15	18	0.9
Lipid Medication, %	6	4	5	7	0.07
Menopausal, % among females	34	22	36	45	0.2
College graduate, %	36	30	37	41	<0.001
Dietary Intakes[3]					
Total energy, kcal/d	1877 (603)	1950 (669)	1715 (530)	1959 (599)	0.2
Total alcohol, g/d	11.3 (15.3)	14.9 (20.3)	10.2 (13.0)	8.1 (10.9)	<0.001
Cereal fiber, g/d	5.7 (2.8)	4.5 (1.7)	5.1 (1.9)	7.7 (3.4)	<0.001
Fruit fiber, g/d	3.5 (2.8)	1.7 (1.3)	3.0 (1.6)	5.7 (3.5)	<0.001
Vegetable fiber, g/d	6.3 (2.8)	4.9 (1.6)	5.7 (1.9)	8.3 (3.6)	<0.001
Nut and Legume fiber, g/d	1.5 (1.3)	1.2 (0.9)	1.3 (0.9)	2.1 (1.8)	<0.001
2015 DGAI score	59.8 (10.6)	51.3 (9.0)	59.6 (7.8)	68.9 (7.7)	<0.001

[1]Values are means (SD) or percentages

[2]Adjusted for age and sex

[3]Adjusted for age, sex, and energy intake

[4] P-trends derived from models treating tertiles as a continuous variable, adjusting for age, sex, and energy intake accordingly

Abbreviations: BMI: body mass index; DBP: diastolic blood pressure; DGAI: Dietary Guidelines Adherence Index; HDL: high density lipoprotein; SBP: systolic blood pressure; PAI: physical activity index

Table 2. Correlations between metabolomic signatures predicting intake of total fiber, cereal fiber, fruit fiber, vegetable fiber, and nut and legume fiber and their self-reported intakes

Fiber Metabolic Signature	R[1]	p-value	95% CI	Metabolites #[2]	Top + Met (coefficient)	Top - Met (coefficient)
Total	0.38	< 0.0001	0.32-0.42	27	C38-6-PC (1.30)	Urate (-0.92)
Cereal	0.33	< 0.0001	0.27-0.38	28	C22-6-LPC (0.45)	C20-5-LPC (-0.29)
Fruit	0.32	< 0.0001	0.27-0.37	29	C38-6-PC (0.37)	Cotinine (-0.36)
Vegetable	0.20	< 0.0001	0.14-0.25	22	C38-6-PC (0.35)	PEP (-0.27)
Nut and Legume	0.12	0.0001	0.05-0.17	8	Citrulline (0.01)	C18-0-SM (-0.09)

[1] Pearson correlation between fiber metabolomic signature and FFQ-derived fiber intake
[2] Number of metabolites selected by the elastic net regression model (full list in Supplemental Table 3)
Abbreviations: Met: metabolite, PEP: phosphoenolpyruvate

Table 3. Associations of glycemic risk factors with both energy-adjusted dietary fiber intake and its corresponding fiber metabolomic signature

	Model 2[1]			Model 3[2]		
	β (SE)	P[3]	P-adj[4]	β (SE)	P	P-adj
Fasting glucose (mg/dL)			**n = 1593**			
Total Fiber Intake[5]	-0.15 (0.14)	0.31	1.00	-0.16 (0.15)	0.29	1.00
Total Fiber Signature[6]	-0.57 (0.17)	0.001	0.004	-0.53 (0.18)	0.003	0.01
Cereal Fiber Intake	-0.81 (0.30)	0.01	0.03	-1.01 (0.31)	0.001	0.005
Cereal Fiber Signature	-0.75 (0.17)	0.00001	0.00003	-0.80 (0.17)	0.00000	0.00002
Fruit Fiber Intake	-0.03 (0.30)	0.93	1.00	-0.05 (0.31)	0.86	1.00
Fruit Fiber Signature	-0.48 (0.17)	0.005	0.02	-0.45 (0.20)	0.02	0.08
HbA1c (%)			**n = 1140**			
Total Fiber Intake	-0.004 (0.007)	0.58	1.00	-0.01 (0.01)	0.48	1.00
Total Fiber Signature	-0.03 (0.01)	0.004	0.02	-0.02 (0.01)	0.01	0.04
Cereal Fiber Intake	-0.04 (0.02)	0.01	0.06	-0.05 (0.02)	0.001	0.005
Cereal Fiber Signature	-0.02 (0.01)	0.004	0.02	-0.03 (0.01)	0.002	0.01
Fruit Fiber Intake	0.01 (0.02)	0.44	1.00	0.01 (0.02)	0.55	1.00
Fruit Fiber Signature	-0.02 (0.01)	0.02	0.09	-0.02 (0.01)	0.07	0.27

[1]Adjusting for baseline and lifestyle factors
[2] Additionally adjusted for baseline and 4-year mean change in waist circumference
[3] Unadjusted p-value
[4] Bonferroni adjusted p-value for four fiber exposures
[5] Per 5-gram increase in fiber intake
[6] Per 1-SD unit increase in the metabolomic signature
Abbreviations: β: Beta coefficient; SE: standard error

Table 4. Associations of lipid risk factors and both energy adjusted dietary fiber intake and corresponding fiber metabolomic signature

	Model 2[1]			Model 3[2]		
	β (SE)	P[3]	P-adj[4]	β (SE)	P	P-adj
HDL-C (mg/dL)			**n = 1625**			
Total Fiber Intake[5]	0.30 (0.12)	0.014	0.05	0.37 (0.13)	0.003	0.013
Total Fiber Signature[6]	0.35 (0.14)	0.02	0.06	0.36 (0.15)	0.01	0.06
Cereal Fiber Intake	0.72 (0.25)	0.004	0.02	0.98 (0.26)	0.0002	0.0008
Cereal Fiber Signature	0.29 (0.14)	0.04	0.16	0.37 (0.15)	0.01	0.04
Fruit Fiber Intake	0.11 (0.26)	0.67	1.00	0.22 (0.26)	0.41	1.00
Fruit Fiber Signature	0.16 (0.15)	0.27	1.00	0.31 (0.16)	0.05	0.22
Triglycerides (%)			**n = 1624**			
Total Fiber Intake	0.86 (0.75)	0.25	0.99	0.99 (0.78)	0.20	0.81
Total Fiber Signature	-2.36 (0.89)	0.008	0.03	-2.39 (0.91)	0.009	0.03
Cereal Fiber Intake	-2.18 (1.55)	0.16	0.63	-2.19 (1.63)	0.18	0.71
Cereal Fiber Signature	-2.63 (0.87)	0.002	0.010	-2.76 (0.90)	0.002	0.008
Fruit Fiber Intake	1.32 (1.57)	0.40	1.00	1.41 (1.62)	0.39	1.00
Fruit Fiber Signature	-1.88 (0.89)	0.04	0.14	-2.31 (1.01)	0.02	0.09

[1]Adjusting for baseline and lifestyle factors
[2] Additionally adjusted for baseline and 4-year mean change in waist circumference
[3] Unadjusted p-value
[4] Bonferroni adjusted p-value
[5] Per 5-gram increase in fiber intake
[6] Per 1-SD unit increase in the metabolomic signature
Abbreviations: β: Beta coefficient; SE: standard error

Supplemental Table 1. Metabolites by the number of imputations (n) using the K-NN method

n =1	n = 209-310	n = 543	n = 743	n = 1034	n = 1242
alanine	X__OH_anthranilic_acid	C46_0_TAG	triiodothyronine	aspartate	X___adenosylhomocysteine
asparagine	glycerol	C50_1_TAG		argininosuccinate	
arginine	C14_0_LPC	C54_1_TAG		carnosine	
glutamic_acid	C16_1_LPC	C56_2_TAG			
glutamine	C16_0_LPC	C58_7_TAG			
glycine	C18_2_LPC	C58_6_TAG			
histidine	C18_1_LPC				
isoleucine	C18_0_LPC				
leucine	C20_5_LPC				
lysine	C20_4_LPC				
methionine	C20_3_LPC				
phenylalanine	C22_6_LPC				
proline	C16_0_LPE				
serine	C18_2_LPE				
threonine	C18_1_LPE				
tryptophan	C18_0_LPE				
tyrosine	C20_4_LPE				
valine	C22_6_LPE				
creatine	C32_2_PC				
creatinine	C32_1_PC				
xanthosine	C32_0_PC				
kynurenic_acid	C34_4_PC				
anthranilic_acid	C34_3_PC				
allantoin	C34_2_PC				
aminoisobutyric_acid	C34_1_PC				

betaine	C36_4_PC_A				
choline	C36_4_PC_B				
dimethylglycine	C36_3_PC				
trimethylamine_n_oxide	C36_2_PC				
citrulline	C36_1_PC				
ornithine	C38_6_PC				
carnitine	C38_5_PC				
cis_trans_hydroxyproline	C38_4_PC				
GABA	C38_3_PC				
NMMA	C38_2_PC				
alpha_glycerophosphocholine	C40_6_PC				
n_carbamoyl_B_alanine	C14_0_SM				
niacinamide	C16_1_SM				
taurine	C16_0_SM				
thiamine	C18_1_SM				
thyroxine	C18_0_SM				
serotonin	C22_1_SM				
X__HIAA	C22_0_SM				
ADMA	C24_1_SM				
SDMA	C24_0_SM				
cotinine	C34_2_DAG				
adenosine	C34_1_DAG				
	C36_2_DAG				
	C36_1_DAG				
	C14_0_CE				
	C16_1_CE				
	C16_0_CE				

C18_3_CE				
C18_2_CE				
C18_1_CE				
C18_0_CE				
C20_5_CE				
C20_4_CE				
C20_3_CE				
C22_6_CE				
C44_1_TAG				
C46_2_TAG				
C46_1_TAG				
C48_4_TAG				
C48_3_TAG				
C48_2_TAG				
C48_1_TAG				
C48_0_TAG				
C50_5_TAG				
C50_4_TAG				
C50_3_TAG				
C50_2_TAG				
C52_6_TAG				
C52_5_TAG				
C52_4_TAG				
C52_3_TAG				
C52_2_TAG				
C52_1_TAG				
C54_9_TAG				
C54_8_TAG				

C54_7_TAG				
C54_6_TAG				
C54_5_TAG				
C54_4_TAG				
C54_3_TAG				
C54_2_TAG				
C56_10_TAG				
C56_9_TAG				
C56_8_TAG				
C56_7_TAG				
C56_6_TAG				
C56_5_TAG				
C56_4_TAG				
C56_3_TAG				
C58_12_TAG				
C58_11_TAG				
C58_10_TAG				
C58_9_TAG				
C58_8_TAG				
C60_12_TAG				
cmh_aminoadipate				
cmh_phosphoglycerate				
cmh_a_glycerophosphate				
cmh_aconitate				
cmh_adipate				
cmh_amp				
cmh_adp				
cmh_citrate				

cmh_isocitrate				
cmh_f1p_f6p_g1p_g6p				
cmh_fruc_gluc_galac				
cmh_fum_mal_val				
cmh_glucuronate				
cmh_hippurate				
cmh_kynurenine				
cmh_lactate				
cmh_lactose				
cmh_malate				
cmh_pantothenate				
cmh_quinolinate				
cmh_sorbitol				
cmh_sucrose				
cmh_urate				
cmh_uridine				
cmh_glycocholate				
cmh_glyco_deoxychol				
cmh_gentisate				
cmh_phosphocreat				
cmh_methyladi_pimelate				
cmh_a_hydroxybutyrate				
cmh_b_hydroxybutyrate				
cmh_hydroxyglutarate				
cmh_indoxylsulfate				
cmh_pyridoxate				
cmh_oxalate				
cmh_xanthurenate				

cmh_deoxycholates				
cmh_salicylurate				
cmh_hydroxyp_acetate				
cmh_t_deoxycholates				
cmh_xanthine				
cmh_pep				
cmh_inosine				
cmh_indole_pro				
cmh_inositol				
cmh_udp_g_g				
cmh_ribo_ribu_p				
cmh_cystathionine				
cmh_hypoxanthine				
cmh_orotate				
cmh_a_ketoglutarate				
cmh_gmp				
cmh_propionate				
cmh_gdp				
cmh_udp				
cmh_camp				
cmh_suberate				
cmh_taurocholate				
cmh_pyruvate				

Supplemental Table 2. Fiber containing Food Items captured by the FFQ grouped into categories of fiber source

Cereal Fiber	Fruit Fiber	Vegetable Fiber	Nut and Legume Fiber
COLD CEREAL	RAISINS	TOMATOES	TOFU OR SOYBEANS
COOKED OATMEAL	PRUNES	TOMATO JUICE	PEAS/LIMA BEANS
OTHER HOT CEREAL	BANANAS	TOMATO SAUCE	BEANS/LENTILS
WHITE BREAD	CANTALOUPE	STRING BEANS	PEANUT BUTTER
DARK BREAD	WATERMELON	BROCCOLI	NUTS
ENG. MUFF./BAGELS	APPLES/PEARS, FRESH	CABBAGE/COLE SLAW	
MUFFINS/BISCUITS	APPLE JUICE/CIDER	CAULIFLOWER	
BROWN RICE	ORANGES	BRUSSELS SPROUTS	
WHITE RICE	ORANGE JUICE	CARROTS, RAW	
PASTA	GRAPEFRUIT	CARROTS, COOKED	
OTHER GRAINS	GRAPEFRUIT JUICE	CORN	
PANCAKES/WAFFLES	OTHER FRUIT JUICE	MIXED VEGETABLES	
CRACKERS	STRAWBERRIES	WINTER SQUASH	
PIZZA	BLUEBERRIES	SUMMER SQUASH	
COOKIES, HOMEMADE	PEACHES	YAMS/SWEET POTATOES	
COOKIES, READYMADE		SPINACH, COOKED	
BROWNIES		SPANACH, RAW	
DOUGHNUTS		KALE/MUSTARD/CHARD	
CAKE, HOMEMADE		ICEBERG/HEAD LETTUCE	
CAKE, READYMADE		ROMAINE/LEAF LETTUCE	
SWEETROLL, HOMEMADE		CELERY	
SWEETROLL, READYMADE		BEETS	
PIE, HOMEMADE		ALFALFA SPROUTS	
PIE, READYMADE		GARLIC	
POPCORN		FRENCH FRIES	
BRAN		POTATOES	
WHEAT GERM		CHIPS	
		CHOWDER/CREAM SOUP	

Supplemental Table 3. Metabolites selected by elastic net regression and their corresponding weights to predict total, cereal, fruit, vegetable, and nut and legume fiber intake

Positively weighted Metabolites			Negatively weighted metabolites		
Name	**Class**	**Coefficient**	**Name**	**Class**	**Coefficient**
Total Dietary Fiber					
C38:6 PC	Phosphatidylcholine	1.30	Urate	Purine	-0.92
Indole propionate	Indole	0.90	C18:0 SM	Sphingomyelin	-0.83
Hippurate	Benzenoid	0.52	C14:0 SM	Sphingomyelin	-0.40
C58:9 TAG	Triacylglycerol	0.46	C36:2 PC	Phosphatidylcholine	-0.39
Oxalate	Dicarboxylic Acid	0.45	alpha Ketoglutarate	Keto Acid	-0.30
Inositol	Cyclohexanols	0.44	C60:12 TAG	Triacylglycerol	-0.30
C18:1 SM	Sphingomyelin	0.43	C22:0 SM	Sphingomyelin	-0.25
Pantothenate	Secondary Alcohol	0.40	Deoxycholates	Bile acid	-0.23
C40:6 PC	Phosphatidylcholine	0.39	C52:3 TAG	Triacylglycerol	-0.20
Isocitrate	Tricarboxylic Acid	0.16	Tyrosine	Amino acid	-0.17
Pyridoxate	Pyridine	0.14	C24:1 SM	Sphingomyelin	-0.08
Betaine	Amino acid	0.10	C20:5 LPC	Lysophosphatidylcholine	-0.04
C58:8 TAG	Triacylglycerol	0.10	Aminoadipate	Amino Acid	-0.02
C38:2 PC	Phosphatidylcholine	0.05			
Cereal Fiber					
C22:6 LPC	Lysophosphatidylcholine	0.45	C20:5 LPC	Lysophosphatidylcholine	-0.29
Indole propionate	Indole	0.32	C22:0 SM	Sphingomyelin	-0.25
Proline	Amino Acid	0.28	alpha Hydroxybutyrate	Hydroxy Acid	-0.21
Uridine	Pyrimidine	0.28	C54:1 TAG	Triacylglycerol	-0.20
C40:6 PC	Phosphatidylcholine	0.28	Ornithine	Amino acid	-0.20
Hippurate	Benzenoid	0.26	beta Hydroxybutyrate	Hydroxy Acid	-0.19
C38:6 PC	Phosphatidylcholine	0.17	cAMP	Purine	-0.17

Thiamine	Amino Acid	0.17	C22:6 CE	Cholesterol ester	-0.15
Betaine	Amino Acid	0.11	Deoxycholates	Bile acid	-0.15
Pyridoxate	Pyridine	0.11	alpha Ketoglutarate	Keto Acid	-0.10
C16:0 LPE	Lysophosphatidylethanolamine	0.10	C54:2 TAG	Triacylglycerol	-0.09
Oxalate	Dicarboxylic Acid	0.09	Hydroxyproline	Amino acid	-0.09
C20:3 CE	Cholesterol ester	0.06	C24:1 SM	Sphingomyelin	-0.08
Isocitrate	Tricarboxylic Acid	0.03	Serine	Amino acid	-0.05
Fruit Fiber					
C38:6 PC	Phosphatidylcholine	0.37	Cotinine	Pyridine	-0.36
Indole propionate	Indole	0.32	Urate	Purine	-0.34
C58:8 TAG	Triacylglycerol	0.26	C14:0 SM	Sphingomyelin	-0.17
Hippurate	Benzenoid	0.25	C16:0 SM	Sphingomyelin	-0.17
Pantothenate	Secondary Alcohol	0.18	Threonine	Amino acid	-0.09
Inositol	Cyclohexanols	0.15	C36:2 PC	Phosphatidylcholine	-0.09
alpha Glycerophosphate	Glycerophosphocholine	0.14	Hydroxyproline	Amino acid	-0.08
Oxalate	Dicarboxylic Acid	0.14	alpha Ketoglutarate	Keto Acid	-0.07
Sorbitol	Sugar Alcohol	0.07	C52:1 TAG	Triacylglycerol	-0.06
C16:1 CE	Cholesterol ester	0.06	Dimethylglycine	Amino Acid	-0.05
Betaine	Amino acid	0.05	Glycocholate	Bile acid	-0.02
C16:0 CE	Cholesterol ester	0.001	C18:0 SM	Sphingomyelin	-0.02
			C54:1 TAG	Triacylglycerol	-0.01
			Indoxylsulfate	Arylsulfates	-0.01
			C52:2 TAG	Triacylglycerol	-0.01
			Kynurenine	Alkyl-phenylketone	-0.01
			C22:0 SM	Sphingomyelin	-0.001
Vegetable Fiber					
C38:6 PC	Phosphatidylcholine	0.35	PEP	Phosphate Ester	-0.27

C22:1 SM	Sphingomyelin	0.25	C38:3 PC	Phosphatidylcholine	-0.23
Inositol	Cyclohexanols	0.15	C18:1 LPC	Lysophosphatidylcholine	-0.22
C58:10 TAG	Triacylglycerol	0.13	C14:0 SM	Sphingomyelin	-0.17
C40:6 PC	Phosphatidylcholine	0.12	Ornithine	Amino acid	-0.16
Hydroxyproline	Amino Acid	0.11	C56:8 TAG	Triacylglycerol	-0.12
NMMA	Amino Acid	0.09	C16:0 SM	Sphingomyelin	-0.12
C58:7 TAG	Triacylglycerol	0.07	Choline	Amino acid	-0.12
alpha Hydroxybutyrate	Hydroxy Acid	0.07	C18:2 LPE	Lysophosphatidylethanolamine	-0.11
Propionate	Carboxylic Acid	0.05	C22:0 SM	Sphingomyelin	-0.11
			C58:9 TAG	Triacylglycerol	-0.01
			C56:7 TAG	Triacylglycerol	-0.01
Nut and Legume Fiber					
Citrulline	Amino Acid	0.055	C18:0 SM	Sphingomyelin	-0.090
Arginine	Amino Acid	0.040	alpha Glycerophosphate	Glycerophosphocholine	-0.080
			Aminoadipate	Amino Acid	-0.065
			C44:1 TAG	Triacylglycerol	-0.048
			C14:0 SM	Sphingomyelin	-0.042
			Urate		-0.006

Supplemental Table 4. Pearson correlation coefficients between fiber metabolomic signatures

Fiber Intake[1]		17.8 (0.13)	5.7 (0.1)	3.5 (0.1)	6.3 (0.1)	1.5 (0.03)
	Fiber Signatures	**Total Fiber**	**Cereal Fiber**	**Fruit Fiber**	**Vegetable Fiber**	**Nut & Legume Fiber**
17.8 (0.13)	**Total Fiber**	1	0.7	0.88	0.52	0.17
5.7 (0.1)	**Cereal Fiber**	0.7	1	0.65	0.25	0.12
3.5 (0.1)	**Fruit Fiber**	0.88	0.65	1	0.45	0.06
6.3 (0.1)	**Vegetable Fiber**	0.52	0.25	0.45	1	0
1.5 (0.03)	**Nut & Legume Fiber**	0.17	0.12	0.06	0	1

[1] Presented as mean (SE)

Supplemental Table 5. Pearson correlation coefficients between fiber metabolomic signatures and their corresponding energy adjusted fiber intakes across exams 5, 6 and 7

	Exam 5			Exam 6			Exam 7		
Metabolic Signature	**R**	**p-value**	**95% CI**	**R**	**p-value**	**95% CI**	**R**	**p-value**	**95% CI**
Total Fiber	0.38	< 0.0001	0.32-0.42	0.31	< 2.2e-16	0.27 - 0.34	0.28	< 2.2e-16	0.23-0.33
Cereal Fiber	0.33	< 0.0001	0.27-0.38	0.21	< 2.2e-16	0.17 - 0.24	0.21	< 2.2e-16	0.16-0.26
Fruit Fiber	0.32	< 0.0001	0.27-0.37	0.32	< 2.2e-16	0.29-0.35	0.28	< 2.2e-16	0.23-0.32
Vegetable Fiber	0.20	< 0.0001	0.14-0.25	0.10	2.01E-08	0.07-0.14	0.10	0.0002	0.046-0.15
Nut & Legume Fiber	0.12	0.0001	0.05-0.17	0.04	0.02	0.006-0.080	0.07	0.007	0.019-0.12

Supplementary Table 6. Associations of blood pressure and waist circumference outcomes and both energy adjusted dietary fiber intake and corresponding fiber metabolomic signature

	Model 2[1]			Model 3[2]			Model 4[3]		
	β (SE)	P[4]	Adj P[5]	β (SE)	P	Adj P	β (SE)	P	Adj P
Waist circumference (inch)				**n = 1693**					
Total Fiber Intake[6]	-0.06 (0.04)	0.12	0.46				-0.06 (0.04)	0.16	0.64
Total Fiber Signature[7]	-0.05 (0.05)	0.24	0.98				-0.05 (0.05)	0.31	1.00
Cereal Fiber Intake	-0.16 (0.08)	0.06	0.23				-0.16 (0.08)	**0.06**	0.22
Cereal Fiber Signature	-0.06 (0.05)	0.16	0.65				-0.06 (0.05)	0.21	0.83
Fruit Fiber Intake	-0.10 (0.08)	0.24	0.94				-0.10 (0.08)	0.22	0.87
Fruit Fiber Signature	-0.02 (0.05)	0.68	1.00				-0.01 (0.05)	0.80	1.00
SBP (mm Hg)				**n = 1682**					
Total Fiber Intake	-0.15 (0.20)	0.44	1.00	-0.13 (0.20)	0.52	1.00	-0.14 (0.21)	0.49	1.00
Total Fiber Signature	-0.07 (0.23)	0.76	1.00	-0.04 (0.24)	0.88	1.00	-0.04 (0.24)	0.86	1.00
Cereal Fiber Intake	-0.82 (0.42)	**0.05**	0.19	-0.74 (0.42)	0.08	0.32	-0.74 (0.42)	0.08	0.32
Cereal Fiber Signature	-0.24 (0.23)	0.29	1.00	-0.18 (0.23)	0.44	1.00	-0.21 (0.24)	0.39	1.00
Fruit Fiber Intake	-0.20 (0.41)	0.64	1.00	-0.15 (0.42)	0.73	1.00	-0.15 (0.42)	0.73	1.00
Fruit Fiber Signature	0.01 (0.26)	0.96	1.00	0.02 (0.26)	0.94	1.00	0.02 (0.27)	0.94	1.00
DBP (mm Hg)				**n = 1684**					
Total Fiber Intake	-0.03 (0.12)	0.81	1.00	-0.01 (0.12)	0.96	1.00	-0.01 (0.13)	0.94	1.00
Total Fiber Signature	-0.10 (0.14)	0.47	1.00	-0.08 (0.15)	0.60	1.00	-0.08 (0.15)	0.57	1.00
Cereal Fiber Intake	-0.59 (0.26)	0.02	0.08	-0.48 (0.26)	0.07	0.26	-0.47 (0.26)	0.067	0.27
Cereal Fiber Signature	-0.20 (0.14)	0.15	0.59	-0.14 (0.14)	0.34	1.00	-0.16 (0.15)	0.27	1.00
Fruit Fiber Intake	0.22 (0.25)	0.39	1.00	0.23 (0.26)	0.36	1.00	0.23 (0.26)	0.37	1.00
Fruit Fiber Signature	-0.07 (0.16)	0.68	1.00	-0.05 (0.16)	0.78	1.00	-0.03 (0.17)	0.84	1.00

[1]Adjusting for baseline and lifestyle factors

[2] Additionally adjusted for baseline and 4-year mean change in waist circumference

[3] Additionally adjusted for fiber adjusted DGAI score
[4] Unadjusted p-value
[5] Bonferroni adjusted p-value
[6] Per 5-gram increase in fiber intake
[7] Per 1-SD unit increase in the metabolomic signature
Abbreviations: β: Beta coefficient; DBP: diastolic blood pressure; SBP: systolic blood pressure; SE: standard error; WC: waist circumference

Supplemental Table 7. Associations of cardiometabolic risk factors and both energy adjusted vegetable fiber intake and corresponding fiber metabolomic signature

	Model 2[1]			Model 3[2]			Model 4[3]		
	β (SE)	P	Adj P[4]	β (SE)	P	Adj P	β (SE)	P	Adj P
Energy Adjusted Vegetable Fiber Intake									
Fasting glucose (mg/dL)	0.19 (0.30)	0.52	1.00	0.21 (0.29)	0.46	1.00	0.21 (0.29)	0.46	1.00
HbA1c (%)	0.02 (0.02)	0.17	0.67	0.02 (0.01)	0.15	0.59	0.02 (0.02)	0.14	0.58
Waist circumference (inch)	-0.003 (0.078)	0.97	1.00				-0.001 (0.078)	0.99	1.00
SBP (mm Hg)	0.44 (0.39)	0.26	1.00	0.41 (0.40)	0.31	1.00	0.41 (0.40)	0.31	1.00
DBP (mm Hg)	0.18 (0.24)	0.45	1.00	0.13 (0.25)	0.59	1.00	0.13 (0.25)	0.59	1.00
HDL-C (mg/dL)	0.38 (0.25)	0.13	0.54	0.40 (0.25)	0.10	0.41	0.40 (0.25)	0.10	0.41
Triglycerides (mg/dL)	2.45 (1.55)	0.11	0.46	2.29 (1.53)	0.14	0.54	2.27 (1.53)	0.14	0.56
Vegetable Fiber Metabolomic Signature									
Fasting glucose (mg/dL)	-0.26 (0.17)	0.13	0.51	-0.18 (0.16)	0.27	1.00	-0.20 (0.17)	0.24	0.95
HbA1c (%)	0.001 (0.009)	0.93	1.00	0.002 (0.009)	0.78	1.00	0.003 (0.009)	0.72	1.00
Waist circumference (inch)	-0.01 (0.04)	0.83	1.00				0.001 (0.045)	0.98	1.00
SBP (mm Hg)	0.22 (0.22)	0.32	1.00	0.23 (0.23)	0.32	1.00	0.25 (0.23)	0.27	1.00
DBP (mm Hg)	-0.07 (0.14)	0.60	1.00	-0.07 (0.14)	0.63	1.00	-0.07 (0.14)	0.64	1.00
HDL-C (mg/dL)	0.20 (0.14)	0.16	0.63	0.19 (0.14)	0.18	0.72	0.18 (0.14)	0.21	0.82
Triglycerides (mg/dL)	-0.58 (0.88)	0.51	1.00	-0.54 (0.87)	0.54	1.00	-0.68 (0.88)	0.44	1.00

[1]Adjusting for baseline and lifestyle factors

[2] Additionally adjusted for baseline and 4-year mean change in waist circumference

[3] Additionally adjusted for vegetable fiber adjusted DGAI score

[4] Bonferroni corrected p-value accounting for four related exposures.

Abbreviations: β: Beta coefficient; DBP: diastolic blood pressure; HDL-C: high density lipoprotein cholesterol; SBP: systolic blood pressure; SE: standard error; WC: waist circumference

Supplemental Table 8. DGAI Adjusted Associations of cardiometabolic risk factors and energy adjusted total, cereal, and fruit fiber intake and corresponding fiber metabolomic signature (Model 4)

	Model 4					
	β (SE)	P[1]	Adj P[2]	β (SE)	P	Adj P
Fasting glucose (mg/dL)				**HbA1c (%)**		
Total Fiber Intake[3]	-0.11 (0.15)	0.48	1.00	-0.003 (0.008)	0.71	1.00
Total Fiber Signature[4]	-0.31 (0.18)	0.08	0.33	-0.02 (0.01)	0.04	0.17
Cereal Fiber Intake	-0.80 (0.30)	0.008	0.030	-0.04 (0.02)	0.006	0.02
Cereal Fiber Signature	-0.63 (0.17)	0.0002	0.0009	-0.03 (0.01)	0.01	0.02
Fruit Fiber Intake	0.15 (0.30)	0.62	1.00	0.02 (0.02)	0.34	1.00
Fruit Fiber Signature	-0.25 (0.19)	0.20	0.79	-0.01 (0.01)	0.16	0.66
HDL-C (mg/dL)				**Triglycerides (mg/dL)**		
Total Fiber Intake	0.33 (0.13)	0.01	0.03	1.08 (0.79)	0.17	0.69
Total Fiber Signature	0.31 (0.15)	0.04	0.17	-2.31 (0.93)	0.01	0.05
Cereal Fiber Intake	0.84 (0.26)	0.0011	0.005	-1.63 (1.61)	0.31	1.00
Cereal Fiber Signature	0.32 (0.15)	0.03	0.13	-2.87 (0.91)	0.002	0.006
Fruit Fiber Intake	0.15 (0.26)	0.57	1.00	1.83 (1.60)	0.25	1.00
Fruit Fiber Signature	0.25 (0.17)	0.13	0.53	-2.28 (1.03)	0.03	0.10
SBP (mm Hg)				**DBP (mm Hg)**		
Total Fiber Intake	-0.14 (0.21)	0.49	1.00	-0.01 (0.13)	0.94	1.00
Total Fiber Signature	-0.04 (0.24)	0.86	1.00	-0.08 (0.15)	0.57	1.00
Cereal Fiber Intake	-0.74 (0.42)	0.08	0.32	-0.47 (0.26)	0.067	0.27
Cereal Fiber Signature	-0.21 (0.24)	0.39	1.00	-0.16 (0.15)	0.27	1.00
Fruit Fiber Intake	-0.15 (0.42)	0.73	1.00	0.23 (0.26)	0.37	1.00
Fruit Fiber Signature	0.02 (0.27)	0.94	1.00	-0.03 (0.17)	0.84	1.00
Waist circumference (inch)						
Total Fiber Intake	-0.06 (0.04)	0.16	0.64			
Total Fiber Signature	-0.05 (0.05)	0.31	1.00			

Cereal Fiber Intake	-0.16 (0.08)	0.06	0.22
Cereal Fiber Signature	-0.06 (0.05)	0.21	0.83
Fruit Fiber Intake	-0.10 (0.08)	0.22	0.87
Fruit Fiber Signature	-0.01 (0.05)	0.80	1.00

[1] Unadjusted p-value
[2] Bonferroni adjusted p-value
[3] Per 5-gram increase in fiber intake
[4] Per 1-SD unit increase in the metabolomic signature
Abbreviations: β: Beta coefficient; DBP: diastolic blood pressure; SBP: systolic blood pressure; SE: standard error; WC: waist circumference

Supplemental Table 9. Sensitivity analyses for associations between the cereal fiber metabolomic signatures and cardiometabolic risk factors

Model	Beta (SE)	P-value
HDL-C (mg/dL)		
M3	0.30 (0.14)	**0.04**
M3 + cereal fiber intake	0.22 (0.15)	0.14
M3 + total fiber intake	0.24 (0.15)	0.11
M3 + fruit fiber signature	0.27 (0.18)	0.14
Triglycerides (mg/dL)		
M3	-2.45 (0.90)	0.02
M3 + cereal fiber intake	-2.36 (0.92)	0.01
M3 + total fiber intake	-2.89 (0.92)	**0.01**
M3 + fruit fiber signature	-2.26 (1.15)	0.25
Serum glucose (mg/dL)		
M3	-0.56 (0.17)	**0.001**
M3 + cereal fiber intake	-0.48 (0.17)	**0.01**
M3 + total fiber intake	-0.57 (0.17)	**0.001**
M3 + fruit fiber signature	-0.72 (0.22)	**0.001**
HBA1C (%)		
M3	-0.02 (0.01)	**0.01**
M3 + cereal fiber intake	-0.02 (0.01)	**0.048**
M3 + total fiber intake	-0.02 (0.01)	**0.01**
M3 + fruit fiber signature	-0.02 (0.01)	**0.03**

[1]Bolded values are < 0.05
Abbreviations: DBP: diastolic blood pressure; CFI: cereal fiber intake; HDL: high density lipoprotein; SE: standard error; SBP: systolic blood pressure

Chapter 5: Summary and Discussion

Based on the results of these three aims, we successfully provide evidence that carbohydrates differing by their structure (glucose vs fructose) and dietary sources (fiber from cereal, fruit, vegetables, and nuts and legumes) demonstrate differential effects on cardiometabolic pathways linked to metabolic risk factors that can be identified through metabolomics. This research sheds light on the primary research question by elucidating how different types of carbohydrates and dietary fiber sources may impact metabolic processes and the components of MetSyn.

Aim 1: Sugar Metabolism

In Aim 1, we found that in individuals with overweight/obesity, glucose versus fructose sweetened beverages differentially affect the metabolism of branched-chain amino acids (BCAAs), particularly within the isoleucine catabolic pathway. Changes in sixteen metabolites (five of which are branched-chain amino acid catabolic pathway metabolites) and the branched chain keto acid (BCKA) composite score showed nominal differences in response to the two interventions. The results demonstrate an increase in the immediate BCAA-derived metabolites, the BCKAs, in response to glucose compared to fructose feeding. Conversely, we observed an increase in valine and a decrease in the valine-derived metabolite C3 acylcarnitine elicited by fructose feeding. The differences in these responses may relate to tissue-specific impact of the two sugars on BCAA metabolism, with glucose exerting a dominant effect in muscle, while fructose acts mainly in the liver. Consistent with this idea, the increase in BCKA levels in response to glucose supplementation was more prominent in those with MetSyn. Although our analysis remains exploratory, this is the first report of comprehensive metabolomic changes in response to high doses glucose versus fructose consumption, which furthers scientific knowledge about the interactions of sugar and BCAA catabolism. The changes in metabolites related to BCAA catabolism, insulin resistance, and fatty acid synthesis demonstrate differences in glucose vs fructose effects on metabolism.

Further exploration of the links between BCAAs and sugar intake are not only important to elucidate molecular mechanisms, but also reveal potential health

implications. Consumption of sugar sweetened beverages (SSBs) remains high among the general population, representing the largest single source of sugar in the U.S. diet.[223] High intake of SSB's, which are primarily composed of glucose and fructose, is a well-established risk factor for MetSyn.[224] Furthermore, SBBs' harmful effects have even been demonstrated to be independent of adiposity.[224] Our results may indicate that one of SSBs' pathway to metabolic dysregulation may be through its influence on circulating BCAAs. Large, prospective epidemiological studies could examine the relationship between BCAA metabolism and SSB consumption further to elucidate metabolic dysregulation in the general population.

Aims 2 and 3: Dietary Fiber Metabolism

Aim 2 provided a comprehensive review of the current evidence linking dietary fiber from various sources to specific metabolites in plasma, urine, and feces. The DFM database categorized the 662 metabolites into 30 metabolite classes, where the largest metabolite classes included lipids and lipid-like molecules (25%), amino acids and derivatives (24%) and alkylresorcinols and avenanthramides (7%). The scoring system (DFSS) enabled the identification of candidate biomarkers for dietary fiber intake, highlighting metabolites associated with cereal and legume fiber. The top candidate biomarkers for cereal fiber included urine ferulic acid derivatives (scores 3-5) and pyrocatechol sulfate (scored 4.43 in blood and 4 in urine). Ferulic acid is a phenolic compound found in seeds and grains.[179] In wheat and rye, ferulic acid is bound to arabinoxylan, the major dietary fiber in these two cereals. Gut microbiome metabolism can cleave the bonds and release ferulic acid,[181] which may contribute to the strong association between ferulic acid and cereal fiber intake. The benzoxazinoid gut metabolite, pyrocatechol sulfate (also called 2-aminophenol sulfate),[183] was suggested to be a potential biomarker of wholegrain in urine intake by Fava *et al* (2022).[167] Additionally, Roager *et al* (2019) conducted linear regressions showing pyrocatechol-glucuronide was inversely associated with c-reactive protein,[160] making this pathway worth further exploration.

The variability in dietary fiber exposures and metabolite measurement methods make it difficult to make strong conclusions and underscores the need for more standardized research in this area. Nevertheless, this aim successfully reviewed and summarized dietary fiber-metabolite associations from studies using metabolomics analyses and greatly informed our analyses for Aim 3. The long-term plans for Aim 2 would be to continuously update the DFM database and turn it into a publicly available, valuable tool for researchers developing trials or interested in the effects of high-fiber foods or diets on human physiology.

Aim 3 demonstrated that dietary fiber intake from different food sources is differentially associated with cardiometabolic risk factors. In terms of individual metabolites, indole propionate was identified in our review (Aim 2) and had a strong positive weights in the metabolomic signatures of total dietary fiber (coeff = 0.90) and modest weights in the cereal (coeff = 0.32) and fruit (coeff = 0.32) fiber signatures. This metabolite is produced when gut bacteria break down high-fiber and high polyphenol food sources.[215, 216] and has been linked to lower risk of T2D[213] and increased kidney function.[214] Urate, a metabolite produced in purine metabolism, displayed a strong inverse weight in the total dietary fiber signature (coeff = - 0.92) and a modest weight in the association fruit fiber signature (coeff = -0.34). This inverse observation with urate and total fiber aligns with previously observed associations between higher dietary fiber intake and lower risk of hyperuricemia, leading to improved insulin sensitivity.[217, 218]

Using a machine learning approach, we identified metabolomic signatures that predict intakes of total, cereal, fruit, vegetable, and legume fiber, and associated these signatures with metabolic health outcomes. Notably, cereal fiber and its metabolomic signature were significantly associated with more favorable changes in fasting glucose, HbA1c, and HDL-C levels, suggesting that cereal fiber intake may have a particularly beneficial impact on cardiometabolic health. These associations remained signficant after adjusting for abdominal adiposity, proxied by waist circumference, which may indicate changes are being driven by metabolite profiles rather than body composition. Future

validation studies in other populations using untargeted metabolomic analyses are warranted.

The findings from this scoping review and longitudinal analysis suggest that cereal fiber intake and its associated metabolites may be related to more favorable cardiometabolic risk profiles. Findings from this hypothesis-generating study may help inform trials to investigate specific fiber types and their impact on metabolites and cardiometabolic health, potentially leading to targeted dietary recommendations and interventions for improving metabolic health outcomes.

Concluding Remarks

This research significantly contributes to the broader field of nutrition metabolomics by providing insights into the emerging roles of sugar in BCAA catabolism and the impact of dietary fiber on metabolic health, potentially mediated by gut microbiota-derived metabolites. The findings suggest that glucose and fructose have distinct effects on BCAA metabolism, with glucose having a more pronounced effect on BCKA levels in individuals with metabolic syndrome. Additionally, dietary fiber intake, particularly from cereal sources, is associated with favorable changes in cardiometabolic risk factors, emphasizing the health benefits of a high-fiber diet, especially one high in whole grains. Understanding how different types of carbohydrates and fiber influence metabolic pathways can help identify specific dietary components that exacerbate or mitigate MetSyn risk factors. This knowledge can inform dietary guidelines and interventions aimed at preventing or managing MetSyn, ultimately reducing the burden of chronic diseases on public health.

Despite these advancements, several unanswered questions remain. The differential effects of glucose and fructose on BCAA metabolism and the associations between dietary fiber intake and cardiometabolic risk factors need validation in larger, more diverse populations. Further elucidation of the specific metabolic pathways involved in sugar and dietary fiber metabolism is essential, including a deeper investigation into the tissue-specific impacts of glucose and fructose on BCAA metabolism. Large, prospective epidemiological studies could examine the relationship between BCAA metabolism and

SSB consumption. Given the established link between high SSB intake and metabolic syndrome, understanding how SSBs influence circulating BCAA levels could reveal pathways to metabolic dysregulation in the general population. Furthermore, intervention trials investigating the impact of different fiber types on metabolic health could lead to targeted dietary recommendations and interventions for improving health outcomes.

Developing standardized methods for measuring dietary fiber intake and its metabolic effects is crucial for advancing this field. The variability in dietary fiber exposures and metabolite measurement methods currently limits the ability to make strong conclusions. Standardization would enable more accurate and comparable results across studies. Additionally, investigating the role of gut microbiota in mediating the effects of dietary fiber on metabolism and health outcomes is a promising area for future research. The identification of gut microbiota-derived metabolites, such as the ferulic acid derivatives and indole propionate, highlights the importance of understanding the interaction between diet, gut microbiota, and metabolic health.

In summary, this research enhances our understanding of how different types of carbohydrates and sources of dietary fiber impact metabolic health. It highlights the need for further studies to validate these findings, develop standardized methods, and explore the emerging role of gut microbiota in mediating these effects.

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Appendices

This thesis is a series of secondary analyses of previously collected data. No primary data collection took place. The following section summarizes the methods of the intervention (aim 1) that are not fully described in the thesis chapter 2.

Methods Aim 1: Inpatient, Outpatient (IPOP) Intervention

Recruitment: This aim was conducted using the data collected on participants in the IPOP study, which was a double blind, parallel arm intervention among thirty-one male and female subjects with overweight or obesity matched by sexual phenotype, BMI, and fasting triglycerides and insulin, which took place at the University of California (UC) Davis' Clinical and Translational Science Center's Clinical Research Center (CCRC) in 2009.[120] Participants were recruited through newspaper advertisements and were assessed for eligibility with two interviews (one over the telephone and one in person), a review of their medical history, and a complete blood count and serum biochemistry panel. Main criteria for inclusion were an age range of 40-72 years, a BMI of 25-35 kg/m^2, and a self-reported stable body weight (BW) during the prior 6 months. Exclusion criteria included abnormal triglyceride concentrations (< 400 mg/dl), hypertension (>140/90 mmHg), history of surgery for weight loss, smoking, vigorous exercise (> 3.5 hours/week), evidence of diabetes/renal disease/hepatic disease, use of thyroid/lipid-lowering/glucose-lowering/antihypertensive/antidepressant/weight-loss medication, and habitual consumption of more than one sugar-sweetened beverage or two alcoholic beverages per day. Women were post-menopausal. Out of thirty-nine subjects enrolled in the study, seven subjects dropped out due to inability or unwillingness to comply with protocol. A total of fifteen subjects were assigned to the glucose-sweetened beverage (GSB) arm and seventeen subjects to the fructose-sweetened beverage (FSB) arm. There were no significant anthropometric or metabolic differences between the two arms at baseline.[120]

Intervention Timeline and Dietary Exposure: The IPOP study ran for a total duration of 12 weeks. The first two weeks were an inpatient baseline period at the CCRC, where participants consumed an energy-balanced diet (55% of total energy provided from

complex CHO; 15% protein; 30% fat). This was followed by 8 weeks of outpatient intervention and two weeks of inpatient intervention periods. Participants were asked to come in for a 2-day inpatient intervention period in Week 3 of the outpatient intervention phase. During the inpatient periods, a 4-day rotating menu of three daily meals with 25% of energy provided at breakfast (0900 hours), 35% at lunch (1300 hours), and 40% at dinner (1800 hours) were distributed. During the outpatient period (Weeks 3-10), participants were instructed to consume their usual diet with three daily servings of either glucose- or fructose-sweetened beverages flavored with an unsweetened drink mix (Kool-Aid; Kraft). During the 2-day inpatient period of Week 3 and the second prolonged in-patient period (Weeks 11-12), participants consumed an energy-balanced diet with the addition of their assigned GSB or FSB (30% of total energy provided from complex CHO; 25% from intervention SB; 15% protein; 30% fat). Energy requirements were calculated using the Mifflin equation with a 1.5 activity factor; on days of 26-hour isotope infusion and 24-hour serial blood collections, the activity factor used was 1.3. Anthropometric and clinical measures were taken over the course of the intervention.

During the Outpatient period (Weeks 3-10), subjects were instructed to consume their usual diet with three daily servings of either GSB or FSB flavored with an unsweetened drink mix (Kool-Aid; Kraft). The SSB's contained riboflavin, which was measured fluorometrically in urine samples at the time of beverage pickup (twice weekly) to measure compliance. A registered dietitian carried out 24-hour food intake recall interviews via telephone using the USDA 5-step multiple pass method[225] on six random outpatient days (Week 4 and Week 9) to measure adherence to calculated energy requirements. Both groups of subjects were comparably compliant for the intervention SSB intake and reported significantly higher energy consumption than their total calculated energy requirements; although there were no significant differences in total energy intake and fat, sugar, or alcohol intake as a percentage of energy intake by sex nor intervention arm.

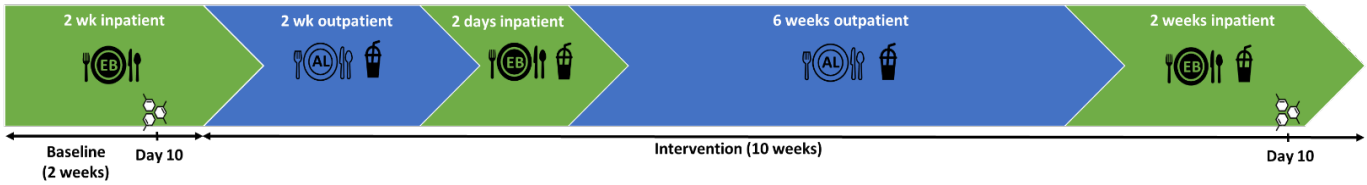

Figure 1. 12-week, IPOP study timeline of procedures and diet schedules where inpatient days are in green and outpatient days are in blue. EB: Energy balanced meals (55% complex CHO, 30% FAT, 15% PRO; AL: ad libitum usual diet. Icon of beverage present when study GSB or FSB accounting for 25% of energy requirements was provided. Metabolites were captured at day 10 of the first and last inpatient period.

Metabolomics Data: Plasma metabolite data were measured from fasting and postprandial samples using the 24-hr blood collection conducted on Week 1 and Week 12. Specifically, at both the Week 1 and Week 12 visits, the dinner meal was served at 18:00 hour. The fasting and post prandial samples were gathered from pooled plasma drawn at 8:00, 8:30, 9:00 hours and 22:00, 23:00, 23:30 hours, respectively. These two blood collections were performed after the subjects had consumed energy-balanced, weight-maintaining diets in the CCRC for 10 days. A summary of the study timeline and diet schedule is illustrated in Figure 1.