Gut microbiome variation modulates the effects of dietary fiber on host metabolism

Authors:

Sofia M. Murga-Garrido[1, 2,#], Qilin Hong[3,#], Tzu-Wen L. Cross[1#], Evan R. Hutchison[1], Jessica Han [4], Sydney P. Thomas[4], Eugenio I. Vivas[1], John Denu [4], Danilo G. Ceschin[5], Zheng-Zheng Tang[3, 4*], Federico E. Rey[1*]

[1]Department of Bacteriology, University of Wisconsin-Madison, Madison, WI USA

[2]PECEM (MD/PhD), Facultad de Medicina, Universidad Nacional Autónoma de México, Coyoacán, Ciudad de México, México

[3]Department of Biostatistics and Medical Informatics, University of Wisconsin-Madison, Madison, 600 Highland Avenue, Madison, WI, USA, 53792

[4]Wisconsin Institute for Discovery, Madison, WI USA

[5]Laboratorio de Bioinformática Traslacional, Centro de Investigaciones en Medicina Traslacional Severo Amuchástegui, Instituto Universitario de Ciencias Biomédicas de Córdoba. Av. Naciones Unidas 420, CP 5000, Córdoba, Argentina

Co-first authors; order determined by relative overall contributions

* Correspondence Address:

E-mail: ferey@wisc.edu

Department of Bacteriology, University of Wisconsin-Madison

1550 Linden Dr., Madison, WI 53706

E-mail: ztang2@wisc.edu

Department of Biostatistics and Medical Informatics

600 Highland Avenue, Madison, WI, USA, 5379

ABSTRACT

Background

There is general consensus that consumption of dietary fermentable fiber improves cardiometabolic health, in part by promoting mutualistic microbes and by increasing production of beneficial metabolites in the distal gut. However, human studies have reported variations in the observed benefits among individuals consuming the same fiber. Several factors likely contribute to this variation, including host genetic and gut microbial differences. We hypothesized that gut microbial metabolism of dietary fiber represents an important and differential factor that modulates how dietary fiber impacts the host.

Results

We examined genetically identical gnotobiotic mice harboring two distinct complex gut microbial communities and exposed to four isocaloric diets, each containing different fibers: (i) cellulose, (ii) inulin, (iii) pectin, (iv) a mix of 5 fermentable fibers (assorted fiber). Gut microbiome analysis showed that each transplanted community preserved a core of common taxa across diets that differentiated it from the other community, but there were variations in richness and bacterial taxa abundance within each community among the different diet treatments. Host epigenetic, transcriptional and metabolomic analyses revealed diet-directed differences between microbiome groups, including variation in amino acids and lipid pathways that were associated with divergent health outcomes.

Conclusion

This study demonstrates that interindividual variation in the gut microbiome is causally linked to differential effects of dietary fiber on host metabolic phenotypes and suggests that a one-fits-all fiber supplementation approaches to promote health is unlikely to elicit

consistent effects across individuals Overall, the presented results underscore the importance of microbe-diet interactions on host metabolism and suggest that gut microbes modulate dietary fiber efficacy.

INTRODUCTION

Humans harbor diverse and dynamic microbial communities in their intestines that span the three domains of life [1, 2]. These microbes play key roles on host biology, including breakdown of complex dietary components, vitamin production, energy harvesting, immune system maturation, and protection against pathogens [2–4]. While many microbial functions are shared among gut communities from unrelated individuals, large interpersonal differences have also been reported [1]. Factors such as genetics, environment, and lifestyles contribute to these differences [5, 6]. Identifying the consequences of this variation as it relates to host immune responses, drug effectiveness, and metabolism is key to better understand how microbes modulate human biology and for successful implementation of precision medicine and personalized nutritional strategies.

Metabolic disease represents a major health challenge worldwide, with an estimate prevalence of 20%-25% of the world´s adult population [7, 8]. A large number of studies indicate that the gut microbiota influences the development of metabolic syndrome [9–13]. Gut microbes exacerbate metabolic disease in part by activating inflammatory pathways, and by producing compounds from diet that dysregulate host signaling and metabolism [14–17]. Microbes can also play protective roles against metabolic disease. A large body of evidence suggests that microbes and microbial metabolites derived from

dietary fiber, including short chain fatty acids (SCFAs) mediate some of the beneficial effects associated with dietary fiber consumption [18–20].

Dietary fiber are edible carbohydrate polymers resistant to host digestive enzymes with at least three monomeric units that are not broken down or absorbed in the small intestine [21]. The chemical structure of a fiber determines important physicochemical properties including its solubility and viscosity. Dietary fibers can be divided into soluble and insoluble forms [22, 23]. Insoluble forms such as cellulose have a fecal bulking effect, and resist metabolization by gut microbes, particularly in monogastrics hosts. Dietary fibers that microbes can use for energy are also referred as microbiota-accessible carbohydrates (MACs) [24]. MACs, such as inulin, pectin and resistant starches, are broken down and metabolized through complex mechanisms by different gut bacteria [25]. Multiple lines of evidence suggest that dietary MACs have important effects on the ecology of the gut ecosystem [26]. MACs can support the growth of beneficial bacteria, promote intestinal barrier function and maintain low systemic inflammation [27] and prevent high-fat induced obesity [28]. Microbial metabolism of MACs also promotes hepatic fatty acid metabolism. Microbial-derived acetate originating from degradation of dietary fiber serves as precursor for hepatic synthesis of fatty acids and related glycerophospholipid species [29].

While epidemiological studies support the notion that consumption of dietary fiber is generally beneficial for metabolic and cardiovascular health [30], results of interventions in humans vary depending on the type of dietary fibers involved. Several studies suggest that there is a degree of interpersonal variation in the benefits attained among individuals

receiving the same dietary fiber intervention [6, 31, 32], highlighting the importance of determining individual microbiota compositional and functional changes. We hypothesized that variation in the gut microbiome modulates the metabolic effects associated with the consumption of different types of dietary fibers. We colonized genetically identical germ-free (GF) mice with distinct human fecal communities and fed them isocaloric diets containing different types of fiber. We found that transplanted communities elicited divergent metabolic epigenetic and transcriptional responses to the same diet. Furthermore, responses between mice colonized with different communities were influenced by the type of fiber consumed by the animals. Lastly, we identified candidate taxa and metabolites associated with these host phenotypes.

RESULTS AND DISCUSSION

Identifying fecal microbiomes with distinct metabolic potential. We sought to identify two human gut communities that upon engraftment in mice exhibit significantly distinct metabolic capacities. We used fecal specimens from a cohort of previously analyzed samples obtained from adults in their mid-seventies [33]. We initially selected eight fecal samples that showed significant compositional differences (Fig. S1A, B) and used them to colonize eight groups of adult male GF C57BL/6 mice (n=3-4/microbiota). Mice were fed a semi-purified diet containing an assortment of fibers (i.e., assorted fiber) that included resistant starch (RS) type 2 and 4, short chain fructo-oligosaccharides (scFOS), inulin, and pectin (total fiber content 10% w/w; Table S1). Animals were placed on this diet one week prior to colonization and maintained for four additional weeks after inoculation. Unweighted UniFrac-based comparisons of bacterial 16S rRNA gene

sequences generated from the input samples and from cecal contents collected from the transplanted mice revealed that in each case the engrafted microbiomes resembled the fecal sample used to colonize the animals more closely than any of the other donor samples used in the study (Fig. S1C). Importantly, the transplanted microbiomes showed distinct organismal configurations and exhibited differences in alpha-diversity (Fig. S1D, E). As expected from this variation, transplanted communities also differed in their capacity to produce SCFAs (Fig. S2). Remarkably there was a ~4-fold range in the levels of cecal butyrate among the eight groups despite all animals consuming the same diet (Fig. S2A). Butyrate is known to vary widely among humans and has been linked with beneficial health effects on the host [34, 35]. Additionally, we used PICRUSt2 to predict the functional profiles of the 8 transplanted communities using 16S rRNA gene data [36, 37]. Principal Coordinates Analysis (PCoA) using Bray Curtis dissimilarity (Fig. S3) shows clear separation among most communities suggesting distinct functional capabilities of engrafted microbiomes. Following these analyses, we selected two markedly different communities: samples 1 and 8 (from here on referred as SubA and SubB respectively), based on differences in alpha diversity, predicted metabolic properties, and capacities to produce butyrate, to examine how variation in gut community composition modulate host responses to different types of fiber.

Effects of dietary fiber on host metabolic outcomes is influenced by gut microbial community. Six to eight-week-old male GF C57BL/6 mice were placed on the assorted fiber diet described above for one week, and subsequently colonized via oral gavage with fecal communities SubA or SubB (n=30-36 mice/community, n=66). Mice colonized with

these two communities were maintained on the same diet for two weeks to allow the engrafted communities to stabilize. After this stabilization period, each group was divided into four treatments (Fig. 1), each receiving one of four isocaloric diets (n=7-10 mice/community/diet) that differed on the type of fiber they contained: (i) cellulose (non-fermentable fiber), (ii) inulin, (iii) pectin, or (iv) assorted fiber described above (Fig. 1). We compared metabolic phenotypes of animals colonized with the two different communities consuming each of the diets described above (Fig. 2). We found significant effects of diet, gut community and their interaction on host adiposity as determined by epididymal fat pad weight (normalized by body weight) (Two-Way ANOVA $P<0.01$). Diet and community, but not their interaction also showed significant effect on liver triglycerides (TG) (Two-Way ANOVA $P<0.05$), whereas diet and its interaction with gut community showed a significant effect on plasma glucose levels (Two-Way ANOVA $P<0.05$; Table S2). Remarkably, while the pectin diet had an overall beneficial effect on metabolic phenotypes relative to non-fermentable cellulose (i.e., reduced adiposity and liver TG) for SubA colonized mice, this diet was ineffective for SubB-colonized animals, which showed the strongest benefits on the inulin fiber. We also assessed whether there were significant differences between groups through pairwise comparisons using Wilcoxon Rank Sum Test. In the cellulose diet, SubB colonized mice showed lower levels of adiposity, whereas there were no statistical differences in the levels of liver TG and fasting plasma glucose between these groups (Fig. 2). In the inulin diet, mice harboring the SubB community showed decreased adiposity, decreased liver TG and lower plasma levels of fasting glucose relative to animals colonized with the SubA community. In contrast, pectin fed mice colonized with the SubB community accumulated more fat mass relative to SubA

counterparts (Fig. 2A), whereas plasma glucose and liver TG were comparable between the two community groups. Lastly, mice colonized with the SubB community showed significantly lower levels of adiposity than those colonized with the SubA community in the assorted fiber diet, whereas plasma glucose and liver TG were comparable between the two groups (Fig. 2). Altogether, these results underscore the importance of microbe - dietary fiber interactions on host metabolism and suggest that gut microbes modulate responses to dietary fiber.

Dietary fibers cause significant restructuring of SubA and SubB communities. 16S rRNA gene sequences were generated from cecal samples collected from the mice described above. PCoA of unweighted UniFrac distances—a metric sensitive to taxonomic phylogenetic distances that does not consider abundance— of these samples show a clear clustering by community (Fig. 3A) and less by diet, supporting the notion that all four diets support colonization of similar assemblages for both communities. In all diets, each community preserved a core of common species that differentiated it from the other community (Fig. S4A). Nevertheless, there were concomitant subtle richness and pronounced abundance variations within each community across the different fiber treatments (Fig. S4). PCoA of weighted UniFrac distances between both communities in the four different diet treatments (Fig. 3B), shows that SubA and SubB post-intervention microbiomes (i.e., microbiome engrafted in each diet) cluster separately, however the inulin diet appeared to be the treatment that separated the two communities the most (Fig. S5). Furthermore, the two communities shifted consistently in response to the same fibers (Fig. 3B), suggesting that related taxa in both communities are responding similarly to a

given diet and that the different fibers have distinct effects on abundance of the taxa. PERMANOVA on weighted and unweighted UniFrac distances between engrafted microbiomes derived from the two communities for each dietary fiber intervention, showed that these are different in all four diet comparisons (P<0.05). Each diet resulted in a unique set of differences between the two microbial communities, these included phyla-level variations. Remarkably, the relative abundance of the phylum *Firmicutes* and the *Firmicutes*:*Bacteroidetes* (F/B) ratio were significantly increased in animals colonized with SubB community consuming inulin and assorted fiber relative to SubA-colonized animals in these diets respectively, whereas the level of *Firmicutes* and the F/B ratio were higher in animals colonized with SubA community consuming the cellulose diet, and there was no difference in this ratio between the two communities for mice consuming the pectin diet (Fig. S6A, B).

We used linear discriminant analysis effect size (LEfSe) analysis to identify bacterial taxa significantly contributing to the differences observed between engrafted microbiomes for each diet intervention [38] (Fig. 3C; Fig. S7). In the cellulose diet, SubA-colonized mice showed higher overall abundance of *Firmicutes*, *Bacteroidetes* and *Actinobacteria*, whereas in SubB-colonized animals *Verrucomicrobia* and *Proteobacteria* exhibited increased abundance. Interestingly, phylum level comparisons between the two communities yielded similar differences for the inulin and assorted fiber-fed mice. Animals colonized with the SubB microbiome showed higher levels of *Firmicutes*, *Actinobacteria*, and *Proteobacteria* whereas mice colonized with the SubA microbiome had higher relative abundance of *Bacteroidetes* in both diets. At the genus level, SubB-colonized

213 animals showed higher relative abundance of *Bifidobacterium* in these two diets, whereas
214 mice harboring the SubA microbiome showed increased levels of *Eubacterium*,
215 *Bacteroides*, *Butyricimonas*, *Lactococcus* and *Ruminococcus* (Ruminococcaceae*)*
216 relative to SubB colonized animals (P <0.05). In the pectin diet, mice colonized with the
217 SubB microbiome showed higher levels of *Proteobacteria* and higher levels of the
218 *Akkermansia, Faecalibacterium, Eubacterium and Clostridium* genera whereas SubA
219 colonized mice showed higher levels of the Lachnospiraceae family and *Dorea* genus (P
220 <0.05); some of the changes observed in pectin (e.g., *Holdemania*, *Lachnospiraceae,* and
221 *Eubacterium*) exhibit the opposite pattern seen in mice fed inulin (Fig. S7). Additionally,
222 there were several genera that show consistent differences between across diets (Fig.
223 3D, Fig. S7 and S8)**.**
224
225 Gas chromatography-based quantification analyses revealed dietary fiber-specific
226 differences in cecal levels of the SCFA acid butyrate and valerate, whereas levels of
227 acetate and propionate were comparable between communities for each diet (Fig. S9).
228 Butyrate was significantly increased in the cecum of SubB-colonized mice consuming
229 cellulose, pectin and assorted fiber relative to the SubA-colonized animals consuming the
230 same diets (Fig. S9B). Valerate was significantly higher in the gut of SubA colonized
231 animals in all diets except assorted fiber whereas cecal levels of the branched-chain fatty
232 acids (BCFA) isovalerate and isobutyrate were higher in SubA colonized mice relative to
233 SubB counterparts in the cellulose diet (P<0.05; Fig. S9E). Altogether these results
234 illustrate the divergent effects of dietary fibers on gut microbiome-derived SCFA and
235 BCFA production. These results support findings from previous studies showing

differential accumulation of these metabolites in response to dietary fiber across individuals [39, 40].

Post-intervention microbiome mediates the community effect on metabolic outcomes. We sought to evaluate the potential mediating role of post-intervention microbiome in the SubA/B community effects on metabolic outcomes (adiposity, liver TG, and plasma glucose) within each diet group. We focused this analysis on the cellulose, inulin, and pectin dietary fiber interventions, as these included a single type of fiber, as opposed to the assorted fiber which included a mixture of several fibers (RS type 2 and 4, scFOS, inulin, and pectin). We first performed association tests between post-intervention microbiomes (beta-diversity) and host metabolic phenotypes using PERMANOVA. In the inulin diet SubA and SubB communities showed differences in beta-diversity associated with changes in liver TG (P <0.05), glucose (P <0.01), and adiposity (P <0.1) phenotypes, whereas differences between the two microbiomes in pectin-fed mice were associated with variation in the adiposity phenotype (P <0.05). We then assessed the mediation effect of the gut microbiota in the relationship between communities and host metabolic phenotypes. The distance-based mediation test showed significant mediation effect of overall diversity of microbiome for the outcomes adiposity and glucose in the inulin-fed group (unweighted UniFrac and Jaccard P <0.05). To identify mediator taxa, we applied the causal mediation model on taxonomy tree (Table S3). For the adiposity outcome, we found *Clostridiales* order and *Eubacteriaceae* family as potential mediators in inulin-fed mice (P <0.1); the *Proteobacteria* phylum, the *Bacteroidales* order and the *Christensenellaceae* family in pectin-fed mice (P <0.05). For the plasma glucose

outcome, we found *Clostridiales* order in inulin-fed mice (P <0.05). Individual mediation effects for lower level taxa suggests that when fed inulin an unclassified family of the *Clostridiales* order, *Christensenellaceae*, *Clostridiaceae*, *Ruminococcaceae*, and *Lachnospiraceae* families mediate adiposity and glucose decrease. In the pectin-fed mice, the lower level taxa mediation analysis revealed that changes in *Betaproteobacteria* class, *Barnesiellaceae*, *Odoribacteraceae*, *Paraprevotellaceae* families are potential mediators of the observed metabolic phenotype. Several of these taxa, in particular members of the *Ruminococcaceae*, *Lachnospiraceae* and *Christensenellaceae* have been connected in numerous studies with metabolic outcomes [41–43]. Altogether these analyses further support the notion that dietary fiber-microbiome interactions modulate metabolic phenotypes and highlight potentially relevant taxa.

Gut microbiome-dietary fiber interactions modulate blood metabolites. We applied Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectroscopy (UPLC–MS/MS) to quantify 774 compounds in serum from the 8 groups of mice described above (6 samples /community/diet) (Table S4). Two-way ANOVA analysis, between SubB- and SubA-transplanted mice identified 1 plasma metabolite in the cellulose diet, 235 in the inulin diet, 160 in the pectin diet, and 19 in the assorted fiber diet that showed significant differences between the two communities (P <0.05, false discovery rate (FDR) adjusted-P <0.1). A large fraction of these metabolites also showed a significant diet by microbiome interaction (P <0.05; Table S4). For the purpose of generating hypotheses all metabolites showing P <0.05 were used for further analyses. Animals consuming inulin and pectin diets showed the largest number of significant changes in biochemicals between the two

communities. Inspection of the differentially abundant biochemicals within each diet revealed diet-specific changes in metabolites related to amino acids and lipid metabolic pathways, including alanine-aspartate, histidine, lysine, tyrosine, leucine-isoleucine-valine, urea cycle: arginine and proline, methionine-cysteine-taurine, fatty acids, endocannabinoid, and sphingolipid metabolic pathways (Fig. 4, Table S4). These results support the notion that consumption of dietary fiber elicits variable individual responses on blood metabolites that are influenced by differences in the gut microbiome.

Amino acids and lipid metabolic pathways associate with host phenotypes. We tested whether levels of plasma metabolites were associated with host metabolic outcomes. We used the dynamic tree cut method in weighted correlation network analysis (WGCNA, Fig. S10; Table S5) to define modules of tightly correlated plasma metabolites. The associations between these modules and host metabolic phenotypes are shown in Figure 5A. Candidate biochemicals that belong to these enriched pathways are listed in Table S4. The turquoise module is positively correlated with adiposity (r= 0.66, *P*= 8e-07), liver TG (r= 0.43, *P*= 0.003) and plasma glucose (r= 0.45, *P*= 0.002), whereas the blue module yielded negative correlations with adiposity (r= -0.47 *P*= 0.001) and liver TG (r= -0.38 *P*= 0.01). The red module showed highest association with the glucose phenotype (r= 0.46 *P*= 0.002). Pathway enrichment analysis revealed significant over-representation of metabolites related to sphingomyelins, phosphatidylcholine (PC), and hexosylceramides pathways in the turquoise module whereas the blue module is enriched for metabolites in the gama-glutamyl amino acid, branched-chain amino acid (BCAA), urea cycle, glutamate, lysine and tryptophan pathways and the red module in fatty acid metabolism (acyl glycine),

purine metabolism ((Hypo)Xanthine/Inosine containing), and acetylated peptides (Fig. 5B).

Sphingomyelins (turquoise module) are strongly associated with adiposity, TG and glucose levels. These metabolites are increased in SubB-colonized mice in the pectin diet, which exhibited increased adiposity relative to SubA-colonized mice. Previous work indicates that sphingolipids mediate cellular processes involved in apoptosis, cell differentiation, and inflammation [44]. Furthermore, plasma levels of sphingomyelins and ceramides have been associated with the development of obesity [45]. Sphingomyelins can be hydrolyzed by sphingomyelinases releasing phosphocholines and ceramides, leading to metabolic impairment [45]. Furthermore, most of the identified sphingomyelin-lipid metabolism changes are within the pectin fed group (Table S4 and S6). These results support the notion that microbiome-fiber interactions modulate host levels of sphingolipids and ceramides. This is consistent with recent work suggesting that *Bacteroides*-derived sphingolipids in the intestine provide an endogenous source of sphingolipids to the host [46].

As mentioned above, amino acids are enriched in the blue module and largely contribute to the negative association observed between this module and adiposity, liver TG, and glucose levels. These amino acids were increased in mice colonized with the SubB community in the inulin and assorted fiber diets relative to SubA-colonized animals in the same diets respectively (Table 6). Levels of histidine, leucine, isoleucine, glutamine, threonine, and arginine have been linked with health benefits including weight loss and

glucose homeostasis [47–49]. Histidine supplementation has been associated with insulin sensitivity improvement and lower body fat [50]. Remarkably, histidine levels were increased in SubB colonized mice consuming inulin diet compared to SubA colonized counterparts. BCAAs upregulate glucose transporters and activate insulin secretion [51, 52]. There is also evidence that leucine and isoleucine have a negative impact on metabolic health [53, 54], and several studies have suggested that excessive intake of amino acids could lead to inhibition of insulin signaling [55]. Additionally, the bacterial metabolite tryptophan, indolepropionate [56], which we detected at significantly higher levels in mice colonized with the SubB community consuming inulin relative to SubA counterparts has been previously associated with increased dietary fiber intake and linked to reduced risk of low-grade inflammation [57] and improved glucose homeostasis [58]. Mice fed the cellulose diet showed virtually no differences in the metabolites enriched in this module. Gamma-glutamyl amino acids were also highly enriched in the blue module. Gamma-glutamyl dipeptides are also involved in glutathione (GSH) metabolism, which plays an important role in antioxidant defense and they are produced when gamma-glutamyl transpeptidase catalyzes the transfer of the gamma-glutamyl moiety of glutathione to amino acids. Gamma-glutamyl transpeptidase is expressed in several mammalian tissues and in bacteria [59, 60]. While the role of these metabolites on metabolic health remains poorly understood a recent study showed gamma-glutamyl amino acids γ-glutamyl cysteine and γ-glutamyl valine inhibit TNF-α signaling in intestinal epithelial cells and reduce inflammation [61]. Overall, the results discussed above suggest that circulating levels of sphingolipids, amino acids and bacterial-derived metabolites, and gamma-glutamyl dipeptides are impacted by interactions between

351 microbes and dietary fibers. More research is needed to describe the extent to which
352 these pathways are differentially impacted by individual variation in gut microbiomes,
353 what microbes mediate these effects, and whether they influence differential host
354 responses to dietary fiber consumption.
355
356 *Connecting bacterial taxa, plasma metabolites and host metabolic phenotypes.* To
357 identify bacterial taxa associated with metabolites, we applied log-contrast model with
358 metabolites as the response. The association analysis linking taxa and metabolite super-
359 pathways show that the *Firmicutes* phylum is involved in most associations, followed by
360 *Bacteroidetes* (Fig. 6). Interestingly, the genus *Anaerotruncus* is negatively associated
361 with metabolites of the lipid super pathway that includes fatty acids (long chain saturated
362 and unsaturated, and branched), lysophospholipids, and monacylglycerol. This genus is
363 also positively associated with metabolites of the amino acid and the nucleotide super
364 pathways, including lysine, glycine, arginine-proline metabolism, and the purine and
365 pyrimidine. *Ruminococcus* (Ruminococcaceae) is negatively associated with metabolites
366 of the amino acid and the lipid super pathways, including BCAA, glutamate and
367 tryptophan metabolism, fatty acids, purine and gamma-glutamyl amino acid, whereas
368 *Parabacteroides* is negatively associated with metabolites in the arginine-proline and
369 dihydroxy fatty acid pathways. Interestingly, *Anaerotruncus,* and *Ruminococcus*
370 (Ruminococcaceae), along with *Parabacteroides*, showed significantly higher relative
371 abundance in mice colonized with the SubA community in the inulin diet, which exhibit
372 higher adiposity, liver TG and glucose compared to mice in the same diet colonized with
373 the SubB community (P <0.01; Fig. S8).

The *Rikenellaceae* family showed a strong positive association with tryptophan and tyrosine metabolism and a negative association with BCAA metabolism. Interestingly, *Rikenellaceae* and *Ruminococcaceae*, were also positively correlated with cecal levels of BCFAs—the end products of bacterial BCAA catabolism (Fig. S11). The relative abundance of *Rikenellaceae* was significantly increased in SubA-colonized mice consuming cellulose, inulin, assorted fiber and in SubB-colonized mice consuming pectin, which exhibited worse metabolic outcomes relative to the other community in their respective diets. This is consistent with previous reports describing increased abundance of *Rikenellaceae* in leptin-resistant obese and diabetic mice [62, 63]. Moreover, we found this family was positively associated with genes in the glycerophospholipid metabolic process, which generates lipids that can be packed in very low-density lipoproteins [64]. The *Bacteroides* genus showed a strong negative correlation with gamma-glutamyl amino acids and it was detected at significantly higher levels in SubA colonized animals except when animals were fed pectin (P <0.05). *Eubacterium* was negatively associated with plasmalogens and positively associated to purine metabolism. As discussed above this genus was also more abundant in the groups that exhibit higher adiposity, liver TG and glucose in the inulin and pectin diets (P <0.01). Altogether these results link changes in levels of bacterial taxa differentially represented in the two communities with alterations in systemic levels of metabolites and host metabolic phenotypes.

Effects of Microbiota-fiber interactions on liver histone posttranslational modifications. The results presented above suggest that interactions between microbes and diet impact

the host metabolic milieu. Differences in abundance of metabolites have been linked with changes in host epigenetic states and gene expression. Epigenetic states of chromatin are reflected in the covalent posttranslational modifications (PTMs) on histone proteins. The N-terminal tail of histones is highly decorated with PTMs, including lysine acetylation and methylation. These PTMs are combinatorial and are thought to integrate multiple environmental signals, including microbial metabolites [65–67]. We examined histone PTM states as a function of community for each fiber treatment. We surveyed >60 acetylated, propionylated, and methylated histone PTM states in liver samples [68]. SubA and SubB communities affect histone acetylation and methylation to different extents in the four fiber groups. The most significant changes were observed when comparing the effects of pectin between the two communities (Fig. S12). Within the family of H3K9K14 peptides quantified, there is significant loss of unmodified, monoacetylation and monomethylation peptides (Fig. 7A) that is likely accounted for by an increase in H3K9me3, though the errors on this peptide species are too large to confirm this. The H3K27K36 family of peptides shows a net loss of K36 methylation but a trending gain of tri-methylation at H3K27 (Fig. S12 and Fig. 7B). Collectively, these specific changes suggest a net increase in repressive chromatin states in SubB relative to SubA with a pectin diet. Perhaps most surprising is the lack of significant increases in many acetylation sites (Fig. S12), suggesting that major differences due to butyrate production are unlikely. Altogether, these results suggest that gut microbiota-fiber interactions impact histone modifications in a complex fashion that cannot be solely isolated to the capacity to generate butyrate.

Dietary modulation of gut microbiome influences impact of gut communities on hepatic gene expression. To test directly whether the observed differences in plasma metabolites and histone PTMs were linked to changes in hepatic gene expression we performed RNA sequencing (RNA-seq) analysis of liver tissue collected from the mice described above. Consistent with the PTM results presented above, tests of differential gene expression yielded larger number of significantly regulated genes between the two communities for the inulin and pectin dietary interventions (Fig. 8), while cellulose-fed mice showed the least number of differences. Only 26 genes were differentially expressed between the two communities in cellulose-fed mice, compared to 228 genes in inulin, 123 in pectin, and 48 in the assorted fiber diets (P <0.05 and FDR adjusted-P <0.05; Fig. 8A, Table S7). Linear model fit test for over-representation of gene ontology (GO) among differentially expressed genes in biological process (BP), molecular function (MF), and cellular component (CC) categories as well as enrichment analysis on Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways revealed divergent results between dietary interventions (Table S8). Among the transcripts expressed at lower levels in SubB-relative to SubA-colonized mice in the cellulose diet there were genes associated with regulation of phosphorylation and protein modification. SubB-colonized animals consuming inulin diet showed lower levels of expression of genes associated with amino acid metabolism, fatty acid metabolic process, peroxisome components, oxidoreductase activity, and peroxisome proliferator-activated receptor (PPAR) signaling pathway relative SubA-colonized counterparts. Up-regulated genes in SubB-colonized mice were associated with ribosome biogenesis, RNA metabolic process and protein processing in endoplasmic reticulum. Mice consuming assorted fiber diet showed some similarities in

443 the enrichment of differentially expressed genes as those in the inulin diet. Genes down-
444 regulated in SubB- relative to SubA-colonized mice fed inulin and assorted fiber diets
445 show overlapping GO terms including oxidative stress, regulation of cellular ketone
446 metabolic process, lipid metabolic process, fatty acid metabolic-catabolic process and
447 lipid modification, fatty acid oxidation, long-chain fatty acid metabolic process, and
448 oxidoreductase activity. In the pectin diet, mice colonized with the SuB microbiome
449 expressed higher levels of genes involved in cofactor, vitamins, and nucleotide
450 metabolism, fatty acid metabolism, oxidoreductase activity, carbohydrate and valine,
451 leucine and isoleucine metabolism among up-regulated genes in SubB-colonized mice
452 (Fig. 8B, Table S8). These include six genes encoding cytochrome P450, some of which
453 are known to play important roles in the synthesis of steroid hormones (*Cyp2a4, Cyp17a1*)
454 and xenobiotic metabolism (*Cyp2a5*) [69, 70]. Surprisingly, SubA-colonized animals in
455 the pectin diet—which show lower levels of adiposity (Fig. 2A) relative to SuB-colonized
456 mice— exhibit higher levels of expression of genes involved in immune system process,
457 inflammatory response, phagocytic cup and vesicles cellular component, cytokine
458 receptor activity and binding, and infection pathways. Previous work has shown that
459 consumption of MACs such as inulin and pectin by mice showing alteration in their gut
460 communities results in liver inflammation and hepatocellular carcinoma [71]. Altogether
461 these results suggest that the gut microbiome is a differential factor that modulates
462 hepatic gene expression. Furthermore, data suggest that the impact of the gut
463 microbiome on liver gene expression is influenced by the type of dietary fiber consumed.

Liver gene expression is associated with host metabolic phenotypes. WGCNA was applied to the normalized read count data obtained from RNA-Seq analysis. Fourteen gene modules, clustering highly co-expressed genes, were identified (each module was assigned a different color; Fig. S13, Table S9). We performed correlation analysis between the phenotypic data and the calculated eigengene—defined as the first principal component of the expression matrix of the corresponding module— for each module identified (Fig. 9A). The most relevant module-phenotype relationship was found between the blue module and adiposity (r= 0.74, *P*= 2e-07). We narrowed down possible relevant genes contributing to this module by performing biological process GO and KEGG pathway analysis enrichment (Figure 9B and C). 614 out of the 677 transcripts in the blue module mapped into biological processes gene ontology. These showed significant enrichment in several processes including the fatty acid metabolic process, acute inflammatory response, long-chain fatty acid, and unsaturated fatty acid metabolic process (*P* <0.05 and FDR adjusted-*P* <0.05). Pathway enrichment analysis of 266 genes with KEGG annotation in this module, yielded significant enrichment in pathways that included PPAR signaling, inflammatory mediator regulation of transient receptor potential (TRP) channels, fatty acid degradation and linoleic acid metabolism (*P* <0.05 and FDR adjusted-*P*<0.05). When only considering genes in the blue module with significant positive association to each phenotype, we observed 209, 151, and 107 genes for adiposity, liver TG and glucose, respectively (*P* <0.05). The GO biological process analysis for these set of genes in the blue module showed that throughout the measured phenotypes, the fatty acid metabolic process was the gene ontology with more gene

counts, followed by long-chain fatty acid metabolic process, xenobiotic metabolic process, and epoxygenase P450 pathway. The statistically significant (P <0.05 and FDR adjusted-P<0.05) enriched KEGG pathways associated with the measured phenotypes are retinol metabolism, chemical carcinogenesis, PPAR signaling pathway, steroid hormone biosynthesis, linoleic acid metabolism, biosynthesis of unsaturated fatty acids, and fatty acid elongation. Nucleoside, ribonucleoside and purine nucleoside bisphosphate metabolic process, and fatty acid degradation are the GO terms and KEGG pathways enriched in the association with adiposity and liver TG (Fig. S14), whereas inflammatory mediator regulation of TRP channels and pyruvate metabolism pathways are enriched among the genes associated with glucose levels.

Gene expression in liver is associated with abundance of gut bacterial taxa. The association analysis linking taxa and liver gene expression modules showed that the *Firmicutes* phylum is involved in most associations, followed by *Bacteroidetes* (Fig. 10). Interestingly, *Anaerotruncus* cluster has the highest number of gene associations (334), followed by *Alistipes* (105), *Butrycimonas* (58), and *Christensenellaceae* (49) (Table S10). This association network reveals an overall positive association between *Anaerotruncus* with genes belonging to the lipid metabolic process, and a negative association with genes belonging to purine ribonucleotide and nucleoside metabolic process, cholesterol biosynthetic process, cellular lipid, fatty acid and phospholipid biosynthetic process, and response to cytokines. Interestingly, plasma metabolomics data shows an overall negative association of this genus with metabolites of the lipid super pathway (Fig. 6), suggesting that these taxa may play a role in host lipid metabolism. *Alistipes* is positively

associated with genes in the lipoprotein biosynthetic process clustered in the turquoise module, whereas *Butrycimonas* is overall negatively associated with immune system processes. Previous work has found the *Anaerotruncus* and *Alistipes* genera enriched in genetically obese (*ob/ob*) mice exhibiting severe glycolipid metabolism disorders relative to wild type and *ob/ob* mice consuming inulin with improved metabolic parameters [72, 73]. The strong correlations between intestinal microbiota, hepatic gene expression, and host metabolic phenotypes provide potential connections between dietary fiber-microbes-health that warrant further examination.

CONCLUSION

Human and mouse studies encompassing genetically diverse populations have shown that host's genetic variation impacts all facets of physiology including responses to diet [74]. However, it is now clear that these populations also contain a significant amount of genetic variation derived from their largely individual associated microbiomes. Previous work in humans showed that dietary supplementation of resistant starch increases fecal butyrate levels, but with remarkable inter-individual variation [40]. Furthermore, a recent study suggested that individual gut microbiota differences can be used to predict post-prandial glycemic responses to specific foods [75]. While these studies provide strong support to the notion that the gut microbiome is a major source of variability that influences responses to diet, dissecting the effects of microbial *vs.* host genetic variation while controlling environmental exposure is practically impossible to achieve in human studies. Modeling this variation in GF mice provides the opportunity to unravel the effects of host genetics from environmental and microbial exposures allowing discovery of causal

relationships between microbes and host phenotypes [72, 73]. Using this approach, we demonstrate that consumption of the same dietary fiber by genetically identical mice harboring different complex human-derived microbiomes can lead to different host phenotypic outcomes (Fig. 2). Remarkably, these phenotypic responses vary as a function of the type of fiber present in the diet and are associated with changes in cecal and blood metabolites, and hepatic gene expression. Altogether the results presented here suggest that a one-fits-all fiber supplementation to promote health is unlikely to elicit consistent effects across individuals. Furthermore, the upregulation of genes involved in inflammation by mice colonized with the SubA community in the pectin diet suggest that consumption of high levels of this MAC may have undesirable impacts to the host in some cases. This is line with recent work showing that chronic consumption of high-fat diet supplemented with MACs induced cholestasis and hepatocellular carcinoma while improving metabolic dysfunction in dysbiotic mice [76].

While ascertaining causal mechanisms explaining differences in host metabolic phenotypes between microbiomes for each diet is beyond the goal of the current study, our results illustrate how introduction of MACs promote divergent host phenotypes caused by the two gut communities. This result has implications not only for personalized nutrition approaches but is also relevant for animal studies in the nutrition field. The use AIN93-based purified diets [77] that contain cellulose as the sole source of fiber is common practice in nutrition studies. These diets have been powerful tools for the field, but their lack/low levels of dietary MACs might not support gut bacterial taxa that are relevant to the process being studied. While there is not sufficient evidence to prescribe

the use of a particular fiber or a specific combination of different fibers, the presented results describe some of the consequences of their inclusion or lack thereof. Furthermore, inclusion of some fermentable fibers in rodent diets will enable conditions that are more representative of human and rodent nutrition.

A major limitation of the study is that only two communities were compared. While these showed clear organismal and functional differences (Fig. S1 and S3) and elicited distinct host metabolic phenotypes, they likely capture a small fraction of the variability observed among human gut microbiomes. Expanding this study to include a wider range of communities, including samples from subjects with extreme diets, markedly different lifestyles, or different health status may expand the range of responses to dietary fiber mediated by gut microbes. Identifying gut signatures associated with desired health outcomes in response to specific fibers may reveal biomarkers of beneficial diet-microbiome interactions that guide personalized nutrition approaches. In conclusion, this study underscores the importance of the gut microbiome as a differential factor that contributes to individual variation in metabolic responses to dietary fiber and supports the notion that dietary fiber efficacy is influenced by gut microbes.

METHODS

Dietary formulation

The four diets used during this study contained 35% kcal fat, 20% kcal protein, 45% kcal carbohydrate, and different fiber type which equals 10% weight. A non-fermentable fiber, cellulose (Solka Floc), was used as control while inulin [Oliggo-Fiber Inulin instant (100010911); Cargill, Minneapolis, MN], pectin (PE1006; Gojira Fine Chemicals, LLC, Bedford Height, OH), and a formulation of assorted fibers which contained 23.4 g/kg inulin, 21.5 g/kg fructooligosaccharide (FOS), 33.3 g/kg HI-MAIZE 260 (resistant starch), 23.5 g/kg resistant wheat starch, and 23.5 pectin, as sources of fermentable fibers (Table S1). Experimental diets were manufactured and sterilized via irradiation by Envigo (10% cellulose diet; TD.170720, 10% inulin diet; TD.170721, 10% pectin diet; TD.170725, and assorted fiber diet; TD.170726).

Gnotobiotic husbandry

All experiments involving gnotobiotic mice were performed under protocols approved by the University of Wisconsin-Madison Animal Care and Use Committee. All germ free (GF) C57BL/6 mice were maintained in a controlled environment in plastic flexible film gnotobiotic isolators under a strict 12 h light/dark cycle and received sterilized water and standard chow (LabDiet 5021; LabDiet, St. Louis, MO) *ad libitum*. Sterility of GF animals was periodically assessed.

Colonization of germ-free mice with human fecal samples and dietary fiber interventions

Richness and diversity metrics (i.e., alpha and beta) obtained from previous publication [33] plus additional Faith's phylogenetic diversity calculated using QIIME 2 [78] were used

to describe 8 Wisconsin Longitudinal Study (WLS) samples selected for this study [79]. Fecal suspensions were prepared under anaerobic conditions in Hungate tubes. A ~0.5 cm piece of frozen Fecal Aliquot Straw Technique (FAST) straw material were resuspendend in 5 ml mega media as previously described [16, 80]. Adult 6-8 week-old male C57BL/6 GF mice were inoculated by oral gavage with ~200 µl of fecal inocula, after being fed with an assorted fiber diet (see below) for a week in sealed positive pressure individually ventilated cages (IVCs; Allentown). Mice were maintained on the same irradiated assorted fiber diet for 2 more weeks. Upon euthanasia, cecal contents were collected for SCFAs and 16S rRNA gene sequencing analysis from each of the 8 gnotobiotic groups. The two gut microbial communities exhbitings the highest (SubB) vs. lowest (SubA) butyrate producing activity were then selected for subsequent experiments.

Fecal samples from SubA and SubB were prepared and inoculated into sixty-six 6-8-week-old GF male mice consuming assorted fiber diet as described above. All gnotobiotic mice continued with the same irradiated assorted fiber diet for another week. Bedding between cages and wires with food were mixed between groups colonized with the same community to minimize cage effects within groups. At 11 weeks old, gnotobiotic mice were switched to one of the fiber type of diets until sample collection 4 weeks after dietary intervention of age when they were euthanized after 4h of fasting.

Measurements of Short-Chain Fatty Acids

SCFA Analysis of Human Fecal Samples. The analysis was performed as previously described [81]. Frozen, weighed samples (~ 50 - 100 mg) were added to chilled 20 ml

625 headspace vials (Restek, Bellefonte, PA) containing 2.0 g NaHSO4, distilled water (300
626 µl – sample weight), and 1.0 ml of 60 µM 2-butanol (internal standard; added just prior to
627 the sample). Vials were crimp sealed immediately after sample addition and vortexed
628 periodically to disperse and mix the contents. Headspace GC analyses were performed
629 using a Shimadzu (Columbia, MD) HS-20 headspace sampler connected to a Shimadzu
630 GC-2010 Plus GC equipped with a SH-Stabilwax column (30 m, 0.25 mm ID, 0.10 µm df)
631 linked to a FID. Samples were equilibrated with shaking to 80°C for 20 min, pressurized
632 to 80 kPa for 3 min prior to column injection (2 ml injection loop, load time 0.2 min, sample
633 and transfer line temperature 150°C, 1:15 split ratio, N2 column flow 1.2 ml/min), with a
634 column temperature program starting at 40°C/2 min, increased to 200°C (20°C/min), held
635 2 min, decreased to 120°C (20°C/min), decreased to 40°C (40°C/min), and stabilized 1
636 min prior to the subsequent injection. The GC cycle time was approximately 23 minutes.
637 Standard mixtures were prepared and analyzed by the same method, and peak areas
638 determined using Shimadzu Lab Solution software (version 5.92), with adjustment for
639 fecal sample size.
640
641 SCFA Analysis of Mice Cecal Samples. Mice cecal SCFAs were measured as previously
642 described [19]. A mixture of 10 µl of internal standards (200 mM for mice and 20 mM for
643 human each; acetic acid-D4, Sigma-Aldrich no. 233315; propionic acid-D6, Sigma-Aldrich
644 no. 490644; and butyric acid-D7, CDN isotopes no. D-171) was subsequently added,
645 followed by 20 µl of 33% HCl and 1 ml diethyl ether. The vials were sealed, vortexed
646 vigorously for 3 min and then centrifuged (4,000g, 10 min). The upper organic layer was
647 transferred to another vial and a second diethyl ether extraction was performed. After

combining the 2 ether extracts, a 60µl-aliquot was removed, combined with 2 µ l N-tert-butyldimethylsilyl- N-methyltrifluoroacetamide (Sigma-Aldrich no. 394882) in a GC auto-sampler vial with a 200 µ l glass insert, and incubated for 2 h at room temperature. Derivatized samples (1 µl) were injected onto an Agilent 7890B/5977A GC/MSD instrument with an Agilent DB1-ms 0.25 mm × 60 m column with a 0.25 µ m bonded phase. A discontinuous oven program was used starting at 40 °C for 2.25 min, then ramping at 20 °C min−1 to 200 °C, then ramping at 100 °C min−1 to 300 °C and holding for 7 min. The total run time was 18.25 min. Linear column flow was maintained at 1.26 ml min−1. The inlet temperature was set to 250 °C with an injection split ratio of 15:1. Acquisition B.07.02.1938. The m/z values of monitored ions in mice cecal measurements were as follows: 117 (acetic acid), 120 (acetic acid-D4), 131 (propionic acid), 136 (propionic acid-D6), 145 (butyric acid) and 152 (butyric acid-D7). Concentrations were normalized to milligrams of cecal contents.

Measurements of Branched-Chain Fatty Acids. BCFA were measured using the headspace GC analysis method described above.

Tissue collection and analysis

Blood was collected via cardiac puncture of anesthetized mice following a 4h fast. Serum was obtained by centrifugation and stored at -80°C. Cecal contents, liver, and gonadal fat pads were collected at the time of euthanasia, snap frozen in liquid nitrogen, and stored at -80°C until analysis. Glucose was using ultrahigh performance liquid chromatography-tandem mass spectroscopy (UPLC–MS/MS) by Metabolon**.**

671 **Liver triglyceride measurement and analysis**

672 Liver triglycerides (TG) were quantified as previously described [19, 82]. Briefly, between
673 30 and 40 mg of frozen liver tissue was homogenized in 30ml of 2:1 chloroform:methanol
674 and disrupted using a bead beater (BioSpec Products, Barlesville, OK; maximum setting
675 for 6 min at room temperature). Samples were incubated overnight at 4°C with gentle
676 agitation and 1ml of 4mM MgCl was added for phase separation. The organic solvent
677 (500 ul) was left to evaporate overnight and the dried lipids were reconstituted in 200 ul
678 butanol:triton-x114 mix (3:2 vol:vol). TG content was determined by colorimetric assay
679 from Sigma (Sigma, F6428), according to the manufacturer's instructions and expressed
680 in nmol per g of wet tissue for final concentration.

681

682 **Statistical analysis of mouse phenotypes**

683 To assess differences on metabolic phenotypes measured between microbiota
684 communities within each dietary intervention and between the same microbiota across
685 different diets we performed a nonparametric test and use permutation approach
686 to obtain the P-value for two-group comparison through Wilcoxon rank sum test.
687 Homogeneity of variance was tested using Levene´s test (P>0.05) previous to performing
688 a two-way ANOVA to investigate the effect of dietary intervention, transplanted microbiota
689 community, and their interaction on each phenotype (P <0.05).

690

691 **16S rRNA gene sequencing**

692 Genomic DNA was extracted from cecal contents using a bead-beating protocol [72].
693 Briefly, ~50 mg of fecal pellet sample were re-suspended in a solution containing 500 µl

694 of extraction buffer [200 mM Tris:HCl (pH 8.0), 200 mM NaCL, 20 mM EDTA], 210 µl of
695 20% SDS, 500 µl phenol:chloroform:isoamyl alcohol (pH 7.9, 25:24:1) (Invitrogen 15593-
696 049) and 500 µl of 0.1-mm diameter zirconia/silica beads. Samples were mechanically
697 disrupted using a bead beater (BioSpec Products, Barlesville, OK; maximum setting for
698 3 min at room temperature), followed by centrifugation, recovery of the aqueous phase
699 with 60 µl 3M NaAcetate, and precipitation with isopropanol. QIAquick 96-well PCR
700 Purification Kit was used to remove contaminants. Isolated DNA was eluted in 10 mM
701 Tris (pH 8.0) buffer and was stored at -20°C until further use.
702 Amplification of 16S rRNA genes (V4) was done from DNA by PCR using unique 8-bp
703 barcodes on the forward and reverse primers and fused with Illumina sequencing
704 adapters [83]. Each sample was amplified in duplicate in a reaction volume of 12.5µl using
705 KAPA HiFi HotStart DNA polymerase (KAPA Biosystems, Wilmington, MA, cat. #
706 KK2602), 10µM of each primer and ~12.5ng of genomic DNA. PCR was carried out under
707 the following conditions: initial denaturation for 3 min at 95°C, followed by 25 cycles of
708 denaturation for 30 s at 95°C, annealing for 30 s at 55°C and elongation for 30 s at 72°C,
709 and a final elongation step for 5 min at 72°C. PCR products were purified with the
710 QIAquick 96-well PCR Purification Kit and then quantified using Qubit dsDNA BR Assay
711 kit (Invitrogen, Oregon, USA). Samples were equimolar pooled and sequenced on the
712 Illumina MiSeq 2x250bp platform.
713
714 Sequences were processed using QIIME 2 pipeline [84]. Demultiplexed 250 bases
715 paired-end sequences were imported using Casava 1.8 format and denoised using
716 DADA2 [85, 86] to obtain amplicon sequence variant (ASV) table. Singletons (ASV

present < 2 times) and ASVs that are present in less than 10% of the samples were discarded. Greengenes [87] reference sequences (clustered at 99% similarity) was used to train a naïve Bayes taxonomy classifier to further annotate ASVs taxonomically. ASV were then collapsed based on genus or lowest-level (i.e. family, order, class, phylum) taxonomy possible. An even sampling depth of 5,795 and 33,714 sequences per sample was used for assessing alpha- and beta-diversity measures in the screening and study phase, respectively. Shannon diversity Index and Faith's phylogenetic diversity (PD) was used to measure alpha diversity. Beta-diversity was calculated using Principal Coordinates Analysis (PCoA), Jaccard, and weighted and unweighted UniFrac metrics [88]. Weighted UniFrac distances between microbiota communities were tested by pairwise PERMANOVA using Qiime2 beta-group-significance command with the -p-pairwise parameter [89]. Also, Linear Discriminant Analysis (LDA) effect size (LEfSe Galaxy Version 1.0) was performed to each microbiota pair (SubA and SubB) for each dietary fiber intervention to elucidate significantly different abundances of bacterial taxa. The parameters used for these analyses were set with default P-value ($\alpha = 0.05$) and LDA score of 2.0 [38].

PICRUSt2 was used to predict functional content or microbiome 16S rRNA genes [36, 37] using QIIME2 generated data. An even sampling depth of 3,919,286 gene counts was used to rarefy all samples to further analyze diversity using Bray Curtis analysis.

Untargeted metabolomics of serum samples

Untargeted mass spectrometry data was collected at Metabolon Inc from 100 µl serum samples of 6 randomly selected mice in each treatment. The 48 samples were prepared using the automated MicroLab STAR system (Hamilton Company). Recovery standards were added and protein, dissociate small molecules bound to protein or trapped in the precipitated protein matrix were removed. To recover chemically diverse metabolites, proteins were precipitated with methanol under vigorous shaking for 2 min (Glen Mills GenoGrinder 2000) followed by centrifugation. The resulting extract was divided into five fractions: two for analysis by two separate reverse-phase UPLC–MS/MS methods with positive ion mode electrospray ionization (ESI), one for analysis by reverse-phase UPLC–MS/MS with negative ion mode ESI, one for analysis by HILIC/UPLC–MS/MS with negative ion mode ESI, and one sample was reserved for backup. Samples were placed briefly on a TurboVap (Zymark) to remove the organic solvent. The sample extracts were stored overnight under nitrogen before preparation for analysis.

Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectroscopy (UPLC–MS/MS). All methods utilized a Waters ACQUITY ultra-performance liquid chromatography system and a Thermo Scientific Q-Exactive high-resolution/ accurate mass spectrometer interfaced with a heated ESI source and an Orbitrap mass analyzer operated at 35,000 mass resolution. The sample extract was dried and then reconstituted in solvents compatible to each of the four methods. Each reconstitution solvent contained a series of standards at fixed concentrations to ensure injection and chromatographic consistency. One aliquot was analyzed using acidic positive ion conditions, chromatographically optimized for more hydrophilic compounds. In this method, the

762 extract was gradient eluted from a C18 column (Waters UPLC BEH C18-2.1 × 100 mm,
763 1.7 µm) using water and methanol, containing 0.05% perfluoropentanoic acid and 0.1%
764 formic acid. Another aliquot was also analyzed using acidic positive ion conditions;
765 however, it was chromatographically optimized for more hydrophobic compounds. In this
766 method, the extract was gradient eluted from the same aforementioned C18 column using
767 methanol, acetonitrile, water, 0.05% perfluoropentanoic acid and 0.01% formic acid and
768 was operated at an overall higher organic content. Another aliquot was analysed using
769 basic negative ion optimized conditions using a separate dedicated C18 column. The
770 basic extracts were gradient eluted from the column using methanol and water, amended
771 with 6.5 mM ammonium bicarbonate at pH 8. The fourth aliquot was analyzed via negative
772 ionization following elution from a HILIC column (Waters UPLC BEH Amide 2.1 × 150
773 mm, 1.7 µm) using a gradient consisting of water and acetonitrile with 10 mM ammonium
774 formate, pH 10.8. Raw data was extracted, peak-identified and QC processed using
775 Metabolon's hardware and software. Compounds were identified by comparison to library
776 entries of purified standards or recurrent unknown entities based on retention time/index,
777 mass to charge ratio (m/z) and chromatographic data, and peaks were quantified using
778 area-under-the curve.
779
780 Statistical analysis. The dataset comprises a total of 774 biochemicals. Metabolic profiles
781 were quantified in terms of relative abundance and median scaled to 1. Following log
782 transformation and imputation of missing values, if any, with the minimum observed value
783 for each compound, two-way ANOVA contrast were used to identify biochemicals that
784 differed significantly between experimental groups. An FDR adjusted-*P* value (i.e. q-

785 value) is calculated to take into account the multiple comparisons that normally occur in
786 metabolomic-based studies and all metabolites with q-value < 0.05 were included.
787
788 **RNA-seq analysis of liver**
789 Mouse liver tissue samples were submitted to the University of Wisconsin Biotechnology
790 Center (UWBC) Gene Expression Center for total RNA extraction. In a 96-well format,
791 tissue samples were lysed using QIAzol Lysis Reagent (Qiagen, Hilden, Germany) and
792 the TissueLyser. Following phase separation by centrifugation, the aqueous phase was
793 recovered, ethanol was added and the solution was added to an RNeasy 96 Universal
794 Tissue plate. Plate was processed following the RNeasy 96 Universal Tissue protocol. An
795 on-column DNase treatment step was included. RNA was eluted in nuclease-free water.
796 Each sample was quantified and analyzed on a NanoDrop One Spectrophotometer
797 (Thermo Fisher Scientific, Waltham, MA, USA) and Agilent 2100 Bioanalzyer (Santa
798 Clara, CA, USA) for purity and integrity, respectively.
799 Total RNA samples that met the Illumina sample input guidelines were prepared
800 according the TruSeq® Stranded mRNA Sample Preparation Guide (Rev. E) using the
801 Illumina® TruSeq® Stranded mRNA Sample Preparation kit (Illumina Inc., San Diego,
802 California, USA). For each library preparation, mRNA was purified from 1000ng total RNA
803 using poly-T oligo-attached magnetic beads. Subsequently, each poly-A enriched sample
804 was fragmented using divalent cations under elevated temperature. The mRNA
805 fragments were converted to double-stranded cDNA (ds cDNA) using SuperScript II
806 (Invitrogen, Carlsbad, California, USA), RNaseH and DNA Pol I, primed by random
807 primers. The ds cDNA was purified with AMPure XP beads (Agencourt, Beckman

Coulter). The cDNA products were incubated with Klenow DNA Polymerase to add an ‘A’ base (Adenine) to the 3’ end of the blunt DNA fragments. DNA fragments were ligated to unique dual index (UDI) adapters (IDT for Illumina- TruSeq RNA UD Index- catalog 20022371, IDT for Illumina - Nextera DNA Unique Dual Indexes, Set A and custom synthesized UDIs), which have a single ‘T’ base (Thymine) overhang at their 3’end. The adapter-ligated DNA products were purified with AMPure XP beads. Adapter ligated DNA was amplified in a Linker Mediated PCR reaction (LM-PCR) for 10 cycles using Phusion TM DNA Polymerase and Illumina's PE genomic DNA primer set followed by purification with AMPure XP beads. Finally, the quality and quantity of the finished libraries were assessed using an Agilent DNA1000 chip (Agilent Technologies, Inc., Santa Clara, CA, USA) and Qubit® dsDNA HS Assay Kit (Invitrogen, Carlsbad, California, USA), respectively. Libraries were standardized to 2nM. Paired-end 2x150bp sequencing was performed, using standard SBS chemistry on an Illumina NovaSeq6000 sequencer. Images were analyzed using the standard Illumina Pipeline, version 1.8.2.

High quality reads were obtained after removal of adaptor sequences and high content of unknown bases. Filtered reads were mapped to mouse genome reference GRCm38.p6 from Ensemble (release 97) database using Spliced Transcripts Alignment to Reference tool (STAR, v2.7.2a). Further quantification of mapped transcript reads to the reference was performed using featureCounts tool (v1.6.4). Readcount data was analyzed for differential gene expression using edgeR Bioconductor package (version 3.28.0) performing the generalized linear model quasi-likelihood F-test from empirical Bayes methods to estimate gene-specific biological variation [90]. Quasi-likelihood method accounts for uncertainty in dispersion estimation, therefore, gives stricter error rate control

which makes it ideal for differential expression analyses of our RNA-seq data [90]. Filtering of low expressed genes was performed by keeping worthwhile counts in a minimum number of samples computed through the built-in function “filterByExpr”. Normalization for RNA composition effect was performed in order to compare relative changes in expression levels between conditions using trimmed mean of M-values (TMM) between each pair of samples [91]. Biological and technical variability estimation was performed by analyzing sample replicates. Negative binomial generalized linear models were fitted and tagwise dispersion estimates were obtained using Cox-Reid profile-adjusted likelihood method in order to determine differential expression. Differentially expressed genes (DEGs) were obtained after filtering by logarithmic fold change 1 and -1, P-value 0.05, and false discovery rate (FDR adjusted-*P*) 0.05, yielding 55 573 genes in total. Gene Ontology (GO) functional enrichment analysis for Biological Process (BP), Cellular Component (CC), Molecular Function (MF) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were obtained as part of the downstream procedure to interpret the differential expression of genes. After the linear model fit test for over-representation was applied, GO and KEGG analysis data was filtered by P-value (<0.05) after correction, by a minimum of total number of genes that are annotated with each GO term (=>10), and by minimum number of genes with the GO term that are significantly regulated in the differential expression comparison (=>5).

Mass-spectrometry analysis of post-translational modification (PTM) of histones from liver

Tissue fractionation and histone extraction and label-free chemical derivatization from liver. Tissue fractionation and histone acid extraction was performed using previously published protocols [67, 68]. Briefly, 50-100 mg of frozen mice liver tissue (n=32, 4 samples per treatment) was dounce-homogenized on ice in a hypotonic lysis buffer containing histone deacetylase and protease inhibitors, followed by the centrifugation to pellet nuclei. Histones were acid extracted, and a Bradford assay was performed to quantify protein yield. 5ug of dried histone extract was then subjected to hybrid chemical derivatization [92] using heavy acetic anhydride. This procedure was followed by trypsinization for 4 hours and derivatization of newly generated peptide N-termini with phenylisocyanate (PIC), Finally, labeled histones were desalted using C18 stage tips.

Nano-liquid chromatography and electrospray ionization tandem MS. For each sample, derivatized histone peptides were injected onto a Dionex Ultimate3000 nanoflow HPLC with a Waters nanoAcquity UPLC C18 column (100 m x 150 mm, 3m) coupled to a Thermo Fisher Q-Exactive mass spectrometer at 700 nL/ min. Mobile phase consisted of water + 0.1% formic acid (A) and acetonitrile + 0.1% formic acid (B). Histone peptides were resolved with a 2-step linear gradient of 2% to 25% mobile phase B over 60 minutes followed by 25% to 40% mobile phase B over 15 minutes. Data was acquired using data-independent acquisition (DIA) mode. The mass spectrometer was operated with a MS1 scan at resolution = 35,000, automatic gain control target = 1 x 106, and scan range = 390-910 m/z, followed by a DIA scan with a loop count of 10. DIA settings were as follows: window size = 10 m/z, resolution = 17,500, automatic gain control target = 1 x 106, DIA

maximum fill time = AUTO, and normalized collision energy = 30. For each cycle, one full MS1 scan was followed by 10 MS2 scans using an isolation window size of 10 m/z.

Histone PTM quantification. EpiProfile 2.0 was used for quantification of histone PTMs [93]. The R script provided by Denu lab (https://github.com/DenuLab/HistoneAnalysisWorkflow) was used to perform data cleaning, normalization, statistical analysis, and visualization.

Data processing for microbiome, metabolome, and transcriptome

Normalization/transformation/filtering. For the taxa that were unclassified at the genus level, their identities at higher levels were used. We combined all ASVs belonging to the same genus and filtered out the genera that appear in fewer than 20% of total samples, leaving 5 phyla, 11 classes, 11 orders, 25 families and 45 genera. For the metabolome data, we normalized biochemicals by using inverse normal transformation and transformed variables that did not follow a normal distribution (Shapiro-Wilk test $P < 0.05$) were removed, resulting in 712 biochemicals. The RNA-seq data were normalized with voom methodology [94]. The voom method estimates the mean-variance relationship and computes appropriate observation-level weights to transform count data to log2-counts per million. We used median absolute deviation to measure the variability of each gene because two genes without notable variance between samples will be highly correlated. As a heuristic cut-off, the top 5000 most variant genes had been used in the downstream analysis.

Weighted Correlation Network Analysis (WGCNA) for metabolome and transcriptome. In order to group the biochemicals that were highly correlated, we built the co-expression network using WGCNA [95]. The WGCNA is an efficient and robust method in grouping metabolomic and transcriptomic data [96, 97] and allowed us to summarize each module by its module eigenvalue. A one-sided Fisher test was used to determine if a pathway was enriched within the turquoise and blue modules in metabolomic data. P-values were then adjusted using Benjamini-Hochberg method and a cut-off of $P < 0.05$ and FDR adjusted-$P < 0.05$ were chosen to determine if a pathway was significantly enriched. We used Pearson's correlation between expression profile of each gene and module eigenvalue to identify module membership. Using the module eigenvalue, the module-traits relationships were estimated by calculating the Pearson's correlations between the module eigenvalue and the traits of interest. We considered 0.90 as a correlation cut-off to choose soft-thresholding power and set the minimal module size as 20. For metabolome, the metabolites were clustered into 8 modules plus 43 unclustered metabolites. The transformed values of the unclustered metabolites were combined with standardized module eigenvalues in the following analysis. For the transcriptome data, 14 modules (defined as clusters of highly interconnected genes) were identified by using DynamicTree Cut algorithm. WGCNA led to 14 different modules by using DynamicTree Cut algorithm. Over-representation of genes in the blue module was characterized based on gene ontology biological process and KEGG pathways using clusterProfiler [98].

Microbiome association with host omics

We applied the sparse linear log-contrast model [99] to pinpoint important genera that are associated with individual metabolite/gene/peptide. In this model, the host omics variable is the response and the genus-level microbial taxa are compositional covariates. The sparse linear log-contrast model respects the compositional nature of the microbiome data and avoids choosing an arbitrary reference taxon, in which the unit-sum constraint on the compositional vector is translated into the zero-sum constraint on the association coefficients across taxa in log-ratio scale. In our analysis, we used 10-fold cross validation to choose the tuning parameter. To obtain stable selection results, we generated 100 bootstrap samples and used the same cross validation procedure to select the genera. We also followed the stability selection approach [100] to assess the stability of the selected genera, where 100 subsamples of half sample size were taken to compute the selection probabilities. In the association network (Fig. 6, 10), we kept genera with stability selection probability large than 0.85 and filter out the lowly associated genera which the absolute value of coefficient is less than 0.1.

Causal mediation analysis

We performed mediation analyses to investigate how the post-intervention gut microbiome/metabolites/gene expressions may mediate the effect of fecal colonization on various phenotypes (adiposity, liver TG, glucose) under a given diet. We employed the causal mediation model with batch effects as confounder, SubA/B microbial communities as exposure and different phenotypes as outcome. All the mediation effect hypotheses were tested by using the resampling method. Below we described two approaches for testing microbiome mediation effects on the global community level and on the

subcompositions defined on the taxonomy tree. For the metabolites and genes, we applied mediation analysis to the corresponding WGCNA modules.

Beta-diversity mediation analysis for microbiome. For beta-diversity distance matrices, we performed the distance-based mediation test by using the MedTest package in R language [101]. The Jaccard and unweighted UniFrac distance matrices were calculated based on the rarefied genus-level abundance matrix (rarefied to the minimum sequence depth) to reduce potential sequence depth-dependent bias.

Tree-based mediation analysis microbiome. We used maximum round error 0.5 to replace 0 [102] in full-composition abundance matrix, then calculated the sub-composition relative abundance matrix for each high-rank internal node. We removed extremely rare taxa (only detected in 10% of the observations or less). We selected the most abundant taxa in the relative abundance matrix as the reference taxa and took additive logarithm transformation on the compositional data so that the transformed data could be considered as multivariate variables. Finally, we applied the causal mediation model on each high-rank internal node in the taxonomy tree.

Metabolite modules/gene modules mediation analysis. In order to identify the microbial community status effect on different phenotypes which were transmitted through the metabolite modules/gene modules, we applied causal mediation model by considering the corresponding WGCNA modules as multiple independent mediators.

List of abbreviations:

ANOVA: Analysis of variance

Af: assorted Fiber

ASV: amplicon sequence variant

BCAA: branched-chain amino acid

BCFA: branched-chain fatty acids

BP: biological process

C: cellulose

CC: cellular component

DEGs: differentially expressed genes

ds cDNA: double-stranded cDNA

F/B: *Firmicutes*:*Bacteroidetes* ratio

FAST: Fecal Aliquot Straw Technique

FC: fold-change

FDR: false discovery rate

GF: germ-free

GO: gene ontology

GSH: glutathione

I: inulin

KEGG: Kyoto Encyclopedia of Genes and Genomes

LEfSe: Linear discriminant analysis Effect Size

MACs: microbiota-accessible carbohydrates

MF: molecular function

ob/ob: genetically obese

P: pectin

PC: phosphatidylcholine

PCoA: Principal Coordinates Analysis

PD: phylogenetic diversity

PERMANOVA: Permutational multivariate analysis of variance

PPAR: peroxisome proliferator-activated receptor

PTMs: posttranslational modifications

RNA-seq: RNA sequencing

RS: resistant starch

SCFAs: short chain fatty acids

scFOS: short chain fructo-oligosaccharides

SubA: Subject A

SubB: Subject B

TG: liver triglycerides

TMM: trimmed mean of M-values

TNFα: tumor necrosis factor alpha

TRP: Transient receptor potential

UPLC–MS/MS: Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectroscopy

uwUF: unweighted UniFrac

WGCNA: weighted correlation network analysis

WLS: Wisconsin Longitudinal Study

wUF: weighted UniFrac

DECLARATIONS

Ethics approval and consent to participate

The use of Wisconsin Longitudinal Study fecal samples was approved by the Institutional Review Board at the University of Wisconsin-Madison.

Consent for publication

Not applicable

Availability of data and material

The data reported in this paper are accessible in the NCBI Short Read Archive (SRA) under accession ID PRJNA665643 and European Nucleotide Archive (ENA) accession number PRJEB40242. Original R scripts and data used for statistical analysis are available in GitHub (https://github.com/KiRinHong/fiberNmicrobiome).

Competing interests

The authors declare that they have no competing interests

Funding

This work was supported in part by grants NIH DK108259 (to F.E.R), GM140464 (to Z.T) and by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award number 2018-08000 (to F.E.R.) and Hatch grant WIS03073. This work was also supported in part by a Transatlantic Networks of Excellence Award from the Leducq Foundation. T.-W.L.C is supported by the National Institutes of Health, under Ruth L. Kirschstein National Research Service Award T32 HL 007936 from the National Heart Lung and Blood Institute to the University of Wisconsin-Madison Cardiovascular Research Center. S.M.M.G. was supported by “El Consejo Nacional de Ciencia y Tecnologia – CONACYT” (The National Council for Science and Technology) with register number 895733 and by “Programa 2018 para Actividades Especiales

de Cooperación Interinstitucional con Fines de Internacionalización para alumnos y egresados de nivel licenciatura de la UNAM" (PAECI Scholarship) offered by the "Dirección General de Cooperación e Internacionalización (DGECI)" of the "Universidad Nacional Autónoma de México (UNAM)". Funding for this research was also provided by Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) PICT-2017-4497.D.G.C is staff researcher of CONICET.

Authors' contributions

TWLC and FER conceived the study. TWLC, SMMG, ERH and EIV performed mouse studies and collected phenotypic and transcriptomic data. JH, SPT and JD conducted and analyzed epigenetic studies. QH, SMMG, DGC and ZZT conducted statistical analyses. SMMG, QH, ZZT and FER wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors thank the University of Wisconsin Biotechnology Center DNA Sequencing Facility for providing sequencing and support services, and the University of Wisconsin Center for High Throughput Computing (CHTC) in the Department of Computer Sciences for providing computational resources, support, and assistance. Part of the bioinformatics analysis was performed in the Cluster Navira, CIDIE - CONICET - UCC (Plataforma Nacional de Bioinformática).

References

1. Li J, Jia H, Cai X, Zhong H, Feng Q, Sunagawa S, et al. An integrated catalog of reference genes in the human gut microbiome. Nature Biotechnology. 2014;32:834–41. doi:10.1038/nbt.2942.

2. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography. Nature. 2012;486:222–7.

3. Kau AL, Ahern PP, Griffin NW, Goodman AL, Gordon JI. Human nutrition, the gut microbiome and the immune system. Nature. 2011;474:327–36.

4. Thursby E, Juge N. Introduction to the human gut microbiota. Biochemical Journal. 2017;474:1823–36.

5. Deschasaux M, Bouter KE, Prodan A, Levin E, Groen AK, Herrema H, et al. Depicting the composition of gut microbiota in a population with varied ethnic origins but shared geography. Nature Medicine. 2018;24:1526–31. doi:10.1038/s41591-018-0160-1.

6. Falony G, Joossens M, Vieira-Silva S, Wang J, Darzi Y, Faust K, et al. Population-level analysis of gut microbiome variation. Science. 2016;352:560–4.

7. Afshin A, Forouzanfar MH, Reitsma MB, Sur P, Estep K, Lee A, et al. Health effects of overweight and obesity in 195 countries over 25 years. New England Journal of Medicine. 2017;377:13–27.

8. Wei B, Liu Y, Lin X, Fang Y, Cui J, Wan J. Dietary fiber intake and risk of metabolic syndrome: A meta-analysis of observational studies. Clinical Nutrition. 2018;37:1935–42. doi:10.1016/j.clnu.2017.10.019.

9. Bäckhed F, Manchester JK, Semenkovich CF, Gordon JI. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. Proceedings of the National Academy of Sciences of the United States of America. 2007;104:979–84.

10. Cho I, Yamanishi S, Cox L, Methé BA, Zavadil J, Li K, et al. Antibiotics in early life alter the murine colonic microbiome and adiposity. Nature. 2012;488:621–6.

11. Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science. 2013;341.

12. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006;444:1027–31.

13. Vrieze A, van Nood E, Holleman F, Salojärvi J, Kootte RS, Bartelsman JFWM, et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. Gastroenterology. 2012;143:913-916.e7. doi:10.1053/j.gastro.2012.06.031.

14. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, et al. Intestinal microbiota metabolism of l-carnitine, a nutrient in red meat, promotes atherosclerosis. Nature Medicine. 2013;19:576–85.

15. Koh A, Molinaro A, Ståhlman M, Khan MT, Schmidt C, Mannerås-Holm L, et al. Microbially Produced Imidazole Propionate Impairs Insulin Signaling through mTORC1. Cell. 2018;175:947-961.e17.

16. Romano KA, Vivas EI, Amador-noguez D, Rey FE. Intestinal Microbiota Composition Modulates Choline Bioavailability. mBio. 2015;6:1–8.

17. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature. 2011;472:57–65.

18. de Vadder F, Kovatcheva-Datchary P, Goncalves D, Vinera J, Zitoun C, Duchampt A, et al. Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. Cell. 2014;156:84–96.

19. Kasahara K, Krautkramer KA, Org E, Romano KA, Kerby RL, Vivas EI, et al. Interactions between Roseburia intestinalis and diet modulate atherogenesis in a murine model. Nature Microbiology. 2018;3:1461–71. doi:10.1038/s41564-018-0272-x.

20. Koh A, de Vadder F, Kovatcheva-Datchary P, Bäckhed F. From dietary fiber to host physiology: Short-chain fatty acids as key bacterial metabolites. Cell. 2016;165:1332–45.

21. Makki K, Deehan EC, Walter J, Bäckhed F. The Impact of Dietary Fiber on Gut Microbiota in Host Health and Disease. Cell Host and Microbe. 2018;23:705–15.

22. Deehan EC, Duar RM, Armet AM, Perez-Muñoz ME, Jin M, Walter J. Modulation of the Gastrointestinal Microbiome with Nondigestible Fermentable Carbohydrates To Improve Human Health. Microbiology Spectrum. 2017;5:1–24.

23. Wang H, Hong T, Li N, Zang B, Wu X. Soluble dietary fiber improves energy homeostasis in obese mice by remodeling the gut microbiota. Biochemical and Biophysical Research Communications. 2018;498:146–51.

24. Sonnenburg ED, Sonnenburg JL. Starving our microbial self: The deleterious consequences of a diet deficient in microbiota-accessible carbohydrates. Cell Metabolism. 2014;20:779–86. doi:10.1016/j.cmet.2014.07.003.

25. Martens EC, Kelly AG, Tauzin AS, Brumer H. The devil lies in the details: How variations in polysaccharide fine-structure impact the physiology and evolution of gut microbes. Journal of Molecular Biology. 2014;426:3851–65. doi:10.1016/j.jmb.2014.06.022.

26. Gentile CL, Weir TL. The gut microbiota at the intersection of diet and human health. Science. 2018;362:776–80.

27. Cani PD, Possemiers S, van de Wiele T, Guiot Y, Everard A, Rottier O, et al. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut. 2009;58:1091–103.

28. Chassaing B, Miles-Brown J, Pellizzon M, Ulman E, Ricci M, Zhang L, et al. Lack of soluble fiber drives diet-induced adiposity in mice. American Journal of Physiology - Gastrointestinal and Liver Physiology. 2015;309:G528–41.

29. Kindt A, Liebisch G, Clavel T, Haller D, Hörmannsperger G, Yoon H, et al. The gut microbiota promotes hepatic fatty acid desaturation and elongation in mice. Nature Communications. 2018;9. doi:10.1038/s41467-018-05767-4.

30. Veronese N, Solmi M, Caruso MG, Giannelli G, Osella AR, Evangelou E, et al. Dietary fiber and health outcomes: An umbrella review of systematic reviews and meta-analyses. American Journal of Clinical Nutrition. 2018;107:436–44.

31. Jovanovski E, Mazhar N, Komishon A, Khayyat R, Li D, Blanco Mejia S, et al. Can dietary viscous fiber affect body weight independently of an energy-restrictive diet? A systematic review and meta-analysis of randomized controlled trials. American Journal of Clinical Nutrition. 2020;111:471–85.

32. Masson LF, McNeill G, Avenell A. Genetic variation and the lipid response to dietary intervention: A systematic review. American Journal of Clinical Nutrition. 2003;77:1098–111.

33. Dill-McFarland KA, Tang ZZ, Kemis JH, Kerby RL, Chen G, Palloni A, et al. Close social relationships correlate with human gut microbiota composition. Scientific Reports. 2019;9:1–10. doi:10.1038/s41598-018-37298-9.

34. Kasubuchi M, Hasegawa S, Hiramatsu T, Ichimura A, Kimura I. Dietary gut microbial metabolites, short-chain fatty acids, and host metabolic regulation. Nutrients. 2015;7:2839–49.

35. McOrist AL, Miller RB, Bird AR, Keogh JB, Noakes M, Topping DL, et al. Fecal Butyrate Levels Vary Widely among Individuals but Are Usually Increased by a Diet High in Resistant Starch. The Journal of Nutrition. 2011;141:883–9.

36. Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, et al. PICRUSt2 for prediction of metagenome functions. Nature Biotechnology. 2020;38:685–8. doi:10.1038/s41587-020-0548-6.

37. Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nature Biotechnology. 2013;31:814–21.

38. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. Genome Biology. 2011;12:1–18.

39. Cantu-Jungles TM, Hamaker BR. New View on Dietary Fiber Selection for Predictable Shifts in Gut Microbiota. mBio. 2020;11:1–9.

40. Venkataraman A, Sieber JR, Schmidt AW, Waldron C, Theis KR, Schmidt TM. Variable responses of human microbiomes to dietary supplementation with resistant starch. Microbiome. 2016;4:1–9. doi:10.1186/s40168-016-0178-x.

41. Aguilar T, Nava GM, Olvera-Ramírez AM, Ronquillo D, Camacho M, Zavala GA, et al. Gut Bacterial Families Are Associated with Body Composition and Metabolic Risk Markers in School-Aged Children in Rural Mexico. Childhood Obesity. 2020;16:358–66.

42. Udayappan S, Manneras-Holm L, Chaplin-Scott A, Belzer C, Herrema H, Dallinga-Thie GM, et al. Oral treatment with Eubacterium hallii improves insulin sensitivity in db/db mice. NPJ Biofilms Microbiomes. 2016;2:1–10. doi:10.1038/npjbiofilms.2016.9.

43. Waters JL, Ley RE. The human gut bacteria Christensenellaceae are widespread, heritable, and associated with health. BMC Biology. 2019;17:1–11.

44. Heaver SL, Johnson EL, Ley RE. Sphingolipids in host–microbial interactions. Current Opinion in Microbiology. 2018;43:92–9. doi:10.1016/j.mib.2017.12.011.

45. Boini KM, Xia M, Koka S, Gehr TWB, Li PL. Sphingolipids in obesity and related complications. Frontiers in Bioscience - Landmark. 2017;22:96–116.

46. Johnson EL, Heaver SL, Waters JL, Kim BI, Bretin A, Goodman AL, et al. Sphingolipids produced by gut bacteria enter host metabolic pathways impacting ceramide levels. Nature Communications. 2020;11:1–11. doi:10.1038/s41467-020-16274-w.

47. Holeček M. Branched-chain amino acids in health and disease: metabolism, alterations in blood plasma, and as supplements. Nutrition & Metabolism. 2018;15:1–12.

48. Layman DK, Walker DA. Potential Importance of Leucine in Treatment of Obesity and the Metabolic Syndrome. The Journal of Nutrition. 2006;136:319S–323S.

49. Ma Q, Zhou X, Sun Y, Hu L, Zhu J, Shao C, et al. Threonine, but Not Lysine and Methionine, Reduces Fat Accumulation by Regulating Lipid Metabolism in Obese Mice. Journal of Agricultural and Food Chemistry. 2020;68:4876–83.

50. Dinicolantonio JJ, McCarty MF, Okeefe JH. Role of dietary histidine in the prevention of obesity and metabolic syndrome. Open Heart. 2018;5:1–4.

51. Floyd JC, Fajans SS, Conn JW, Knopf RF, Rull J. Stimulation of insulin secretion by amino acids. The Journal of clinical investigation. 1966;45:1487–502.

52. Nishitani S, Takehana K, Fujitani S, Sonaka I. Branched-chain amino acids improve glucose metabolism in rats with liver cirrhosis. American Journal of Physiology - Gastrointestinal and Liver Physiology. 2005;288 6 51-6:1292–300.

53. Connelly MA, Wolak-Dinsmore J, Dullaart RPF. Branched Chain Amino Acids Are Associated with Insulin Resistance Independent of Leptin and Adiponectin in Subjects with Varying Degrees of Glucose Tolerance. Metabolic Syndrome and Related Disorders. 2017;15:183–6.

54. Lynch CJ, Adams SH. BCAA in metabolic signalling and insulin resistance. Nat Rev Endocrinol. 2014;10:723–36.

55. Tremblay F, Lavigne C, Jacques H, Marette A. Role of Dietary Proteins and Amino Acids in the Pathogenesis of Insulin Resistance. Annual Review of Nutrition. 2007;27:293–310.

56. Elsden SR, Hilton MG, Waller JM. The end products of the metabolism of aromatic amino acids by clostridia. Archives of Microbiology. 1976;107:283–8.

57. Brial F, le Lay A, Dumas ME, Gauguier D. Implication of gut microbiota metabolites in cardiovascular and metabolic diseases. Cellular and Molecular Life Sciences. 2018;75:3977–90. doi:10.1007/s00018-018-2901-1.

58. de Mello VD, Paananen J, Lindström J, Lankinen MA, Shi L, Kuusisto J, et al. Indolepropionic acid and novel lipid metabolites are associated with a lower risk of type 2 diabetes in the Finnish Diabetes Prevention Study. Scientific Reports. 2017;7 March:1–12.

59. Jump RLP, Polinkovsky A, Hurless K, Sitzlar B, Eckart K, Tomas M, et al. Metabolomics analysis identifies intestinal microbiota-derived biomarkers of colonization resistance in clindamycin-treated mice. PLoS ONE. 2014;9:1–18.

60. Olson CA, Vuong HE, Yano JM, Liang QY, Nusbaum DJ, Hsiao EY. The Gut Microbiota Mediates the Anti-Seizure Effects of the Ketogenic Diet. Cell. 2018;173:1728-1741.e13. doi:10.1016/j.cell.2018.04.027.

61. Zhang H, Kovacs-Nolan J, Kodera T, Eto Y, Mine Y. γ-Glutamyl cysteine and γ-glutamyl valine inhibit TNF-α signaling in intestinal epithelial cells and reduce inflammation in a mouse model of colitis via allosteric activation of the calcium-sensing receptor. Biochimica et Biophysica Acta - Molecular Basis of Disease. 2015;1852:792–804. doi:10.1016/j.bbadis.2014.12.023.

62. Geurts L, Lazarevic V, Derrien M, Everard A, van Roye M, Knauf C, et al. Altered gut microbiota and endocannabinoid system tone in obese and diabetic leptin-resistant mice: Impact on apelin regulation in adipose tissue. Frontiers in Microbiology. 2011;2 JULY:1–17.

63. Kim KA, Gu W, Lee IA, Joh EH, Kim DH. High Fat Diet-Induced Gut Microbiota Exacerbates Inflammation and Obesity in Mice via the TLR4 Signaling Pathway. PLoS ONE. 2012;7.

64. Rauschert S, Uhl O, Koletzko B, Kirchberg F, Mori TA, Huang RC, et al. Lipidomics reveals associations of phospholipids with obesity and insulin resistance in young adults. Journal of Clinical Endocrinology and Metabolism. 2016;101:871–9.

65. Fan J, Krautkramer KA, Feldman JL, Denu JM. Metabolic regulation of histone post-translational modifications. ACS Chemical Biology. 2015;10:95–108.

66. Krautkramer KA, Rey FE, Denu JM. Chemical signaling between gut microbiota and host chromatin: What is your gut really saying? Journal of Biological Chemistry. 2017;292:8582–93.

67. Krautkramer KA, Kreznar JH, Romano KA, Vivas EI, Barrett-Wilt GA, Rabaglia ME, et al. Diet-Microbiota Interactions Mediate Global Epigenetic Programming in Multiple Host Tissues. Molecular Cell. 2016;64:982–92. doi:10.1016/j.molcel.2016.10.025.

68. Krautkramer KA, Reiter L, Denu JM, Dowell JA. Quantification of SAHA-Dependent Changes in Histone Modifications Using Data-Independent Acquisition Mass Spectrometry. Journal of Proteome Research. 2015;14:3252–62.

69. Gilep AA, Sushko TA, Usanov SA. At the crossroads of steroid hormone biosynthesis: The role, substrate specificity and evolutionary development of CYP17. Biochimica et Biophysica Acta - Proteins and Proteomics. 2011;1814:200–9. doi:10.1016/j.bbapap.2010.06.021.

70. Lindberg R, Burkhart B, Ichikawa T, Negishi M. The structure and characterization of Type I P-450(15α) gene as major steroid 15α-hydroxylase and its comparison with Type II P-450(15α) gene. Journal of Biological Chemistry. 1989;264:6465–71.

71. Singh V, Yeoh BS, Chassaing B, Xiao X, Saha P, Aguilera Olvera R, et al. Dysregulated Microbial Fermentation of Soluble Fiber Induces Cholestatic Liver Cancer. Cell. 2018;175:679-694.e22. doi:10.1016/j.cell.2018.09.004.

72. Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JI. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice

SI13. Science translational medicine. 2009;1:6ra14.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2894525/.
73. Smits SA, Marcobal A, Higginbottom S, Sonnenburg JL, Kashyap PC. Individualized Responses of Gut Microbiota to Dietary Intervention Modeled in Humanized Mice. mSystems. 2016;1:3–8.
74. Meddens SFW, de Vlaming R, Bowers P, Burik CAP, Linnér RK, Lee C, et al. Genomic analysis of diet composition finds novel loci and associations with health and lifestyle. Molecular Psychiatry. 2020.
75. Zeevi D, Korem T, Zmora N, Israeli D, Rothschild D, Weinberger A, et al. Personalized Nutrition by Prediction of Glycemic Responses. Cell. 2015;163:1079–94.
76. Singh A, Zapata RC, Pezeshki A, Reidelberger RD, Chelikani PK. Inulin fiber dose-dependently modulates energy balance, glucose tolerance, gut microbiota, hormones and diet preference in high-fat-fed male rats. Journal of Nutritional Biochemistry. 2018;59:142–52. doi:10.1016/j.jnutbio.2018.05.017.
77. Reeves PG, Nielsen FH, Fahey GC. AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. Journal of Nutrition. 1993;123:1939–51.
78. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet C, Alghalith GA, et al. QIIME 2: Reproducible, interactive, scalable, and extensible microbiome data science. PeerJ Preprints. 2018; October:1–21. doi:10.7287/peerj.preprints.27295v1.
79. Herd P, Carr D, Roan C. Cohort profile: Wisconsin longitudinal study (WLS). International Journal of Epidemiology. 2014;43:34–41.
80. Romano KA, Dill-McFarland KA, Kasahara K, Kerby RL, Vivas EI, Amador-Noguez D, et al. Fecal Aliquot Straw Technique (FAST) allows for easy and reproducible subsampling: assessing interpersonal variation in trimethylamine-N-oxide (TMAO) accumulation. Microbiome. 2018;6:91.

81. Cruwys JA, Dinsdale RM, Hawkes FR, Hawkes DL. Development of a static headspace gas chromatographic procedure for the routine analysis of volatile fatty acids in wastewaters. Journal of Chromatography A. 2002;945:195–209.
82. Rector RS, Thyfault JP, Morris RT, Laye MJ, Borengasser SJ, Booth FW, et al. Daily exercise increases hepatic fatty acid oxidation and prevents steatosis in Otsuka Long-Evans Tokushima Fatty rats. American Journal of Physiology - Gastrointestinal and Liver Physiology. 2008;294:619–26.
83. Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the miseq illumina sequencing platform. Applied and Environmental Microbiology. 2013;79:5112–20.
84. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology. 2019;37:852–7.
85. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods. 2016;13:581–3.
86. Callahan BJ, McMurdie PJ, Holmes SP. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME Journal. 2017;11:2639–43. doi:10.1038/ismej.2017.119.
87. McDonald D, Price MN, Goodrich J, Nawrocki EP, Desantis TZ, Probst A, et al. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. ISME Journal. 2012;6:610–8. doi:10.1038/ismej.2011.139.
88. Lozupone C, Knight R. UniFrac: A new phylogenetic method for comparing microbial communities. Applied and Environmental Microbiology. 2005;71:8228–35.

89. Anderson MJ. Permutational Multivariate Analysis of Variance (PERMANOVA). Wiley StatsRef: Statistics Reference Online. 2017;:1–15.

90. Robinson M, McCarthy… D. edgeR: differential expression analysis of digital gene expression data. BioconductorFhcrcOrg. 2010; September 2008:1–76. http://bioconductor.fhcrc.org/packages/2.6/bioc/vignettes/edgeR/inst/doc/edgeR.pdf.

91. Robinson MD, Oshlack A. A scaling normalization method for differential expression analysis of RNA-seq data. Genome Biology. 2010;11.

92. Maile TM, Izrael-Tomasevic A, Cheung T, Guler GD, Tindell C, Masselot A, et al. Mass spectrometric quantification of histone post-translational modifications by a hybrid chemical labeling method. Molecular and Cellular Proteomics. 2015;14:1148–58.

93. Thomas SP, Haws SA, Borth LE, Denu JM. A practical guide for analysis of histone post-translational modifications by mass spectrometry: Best practices and pitfalls. Methods. 2019. doi:https://doi.org/10.1016/j.ymeth.2019.12.001.

94. Law CW, Chen Y, Shi W, Smyth GK. Voom: Precision weights unlock linear model analysis tools for RNA-seq read counts. Genome Biology. 2014;15:1–17.

95. Langfelder P, Horvath S. WGCNA: An R package for weighted correlation network analysis. BMC Bioinformatics. 2008;9.

96. McHardy IH, Goudarzi M, Tong M, Ruegger PM, Schwager E, Weger JR, et al. Integrative analysis of the microbiome and metabolome of the human intestinal mucosal surface reveals exquisite inter-relationships. Microbiome 2013,. 2013;1:1–19.

97. Tang ZZ, Chen G, Hong Q, Huang S, Smith HM, Shah RD, et al. Multi-omic analysis of the microbiome and metabolome in healthy subjects reveals microbiome-dependent relationships between diet and metabolites. Frontiers in Genetics. 2019;10 MAY.

98. Yu G, Wang LG, Han Y, He QY. ClusterProfiler: An R package for comparing biological themes among gene clusters. OMICS A Journal of Integrative Biology. 2012;16:284–7.

99. Lin W, Shi P, Feng R, Li H. Variable selection in regression with compositional covariates. Biometrika. 2014;101:785–97.

100. Meinshausen N, Bühlmann P. Stability selection. Journal of the Royal Statistical Society Series B: Statistical Methodology. 2010;72:417–73.

101. Zhang J, Wei Z, Chen J. A distance-based approach for testing the mediation effect of the human microbiome. Bioinformatics. 2018;34:1875–83.

102. Aitchison J. The Statistical Analysis of Compositional Data. Journal of the Royal Statistical Society. 1982;44:139–77.

Figure Legends

Fig. 1. Timeline for colonization of germ-free (GF) mice and diets used in the study. Six to eight-week-old C57 Bl6 GF male mice were placed on irradiated diet containing a mix of five fibers (Assorted Fiber diet; Af); a week later mice were colonized with one of two different human fecal samples (SubA or SubB). Cage bedding and wires with food were mixed between cages of mice colonized with the same community to minimize cage effects within groups. Two weeks after colonization gnotobiotic mice received one of four isocaloric diets that vary on the type of fiber (10% wt/wt): Cellulose (C), Inulin (I), Pectin (P) and Assorted fiber (Af). Mice were maintained in these diets for four weeks.

Fig. 2. Gut microbiome impact on host metabolic phenotypes in different dietary fibers. Phenotypes were measured six weeks after colonization and four weeks of specific dietary fiber exposure (~15-weeks old) . **A.** Epididymal fat-pad weight expressed a percentage of body weight (n=7-10/microbiome/diet). **B.** Liver triglycerides levels (n=7-

10/microbiome/diet). **C.** Serum glucose levels (arbitrary units) were measured by UPLC/MS/MS (untargeted metabolomics platform; n=6/microbiome/diet). Wilcoxon rank sum test was conducted to examine whether two samples are likely to derive from the same population. *P <0.05, **P <0.01, ***P <0.001**.** SubA, magenta; SubB, yellow.

Fig. 3. Dietary fibers cause significant restructuring of transplanted human-derived microbial communities. 16S rRNA gene sequence analysis of cecal communities of gnotobiotic mice colonized with SubA and SubB microbiomes exposed to diets containing Cellulose (orange), Inulin (purple), Pectin (pink) or Assorted fiber (green) for four weeks**.** **A-B.** Principal Coordinates Analysis (PCoA) of unweighted and weighted UniFrac distances, respectively. SubA community is represented by squares and SubB by circles. **C.** Cladograms generated using LEfSe analysis; comparison results are presented for the two communities in each diet, colors distinguish taxa differences between SubA (magenta) and SubB (yellow) communities. **D.** Genus/family level relative abundances of taxa. Taxa that showed significant differences in relative abundance through the interventions are marked with an asterisk (Kruskal-Wallis test and LDA P <0.05).

Fig. 4. Gut microbiome variation directs changes in plasma levels of amino acid and lipid metabolites. Heatmap indicating differences in plasma levels of amino acid and lipid metabolites as determined by Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectroscopy (UPLC–MS/MS). Biochemicals exhibiting a difference of at least 20% difference between SubB *vs.* SubA are indicated for each diet; P <0.05 (Two-

way ANOVA). List of metabolites, *P* values and fold-changes are listed in Supplemental Table 4.

Fig. 5. Changes in plasma levels of amino acids and lipids associated with metabolic phenotypes. A. Correlation matrix between metabolites consensus modules and host phenotypes measured in mice described in Figure 1. Modules were determined based on patterns of co-abundance of metabolites using WGCNA. Each of the modules was labelled with a unique color as an identifier. Each module was tested for correlation with host metabolic phenotypes. Within each cell, upper values are correlation coefficients between module and the phenotypes; lower values are the corresponding FDR adjusted-*P* values. **B.** Pathways enriched in the blue and turquoise modules as determined by Fisher´s test.

Fig. 6. Association network between gut microbiota and blood metabolites. Association strength is denoted by width of the lines; red lines show positive association while blue ones show negative. Phylum of taxa is indicated by colored boxes and metabolite super-pathway (listed in Supplemental table 5) by colored circles. *Anaerotruncus* (*Firmicutes*) is the genus with the largest number of negative associations with the lipid super-pathway, while the family *Chistensenellaceae* (*Firmicutes*) has the largest number of positive associations with metabolites in the same super-pathway. *Enterobacteriaceae* family (*Proteobacteria*) is the taxa with more positive association with the amino acid super-pathway.

Fig. 7. Gut community-mediated epigenetic changes in liver are sensitive to dietary fiber. Abundance of histone PTMs on H3 lysines (K9, K14,K27, and K36). **A**. H3K9K14 peptide. **B.** H3K27K36 peptide (n=4/community/diet). *P <0.05, **P <0.01; ac, acetylated; unmod, unmodified; meth1,2,3, mono- di- and try- methylated respectively; pr, propionylated.

Fig. 8. Effects of gut microbiome variation on hepatic gene expression across different fibers. A. MA-plots showing differentially expressed genes in the liver of mice colonized with SubB *vs.* SubA microbiome consuming: (i) Cellulose diet; (ii) Inulin diet; (iii) Pectin diet (iv) Assorted fiber diet (n=5 animals/microbiome/diet). Differentially Express Genes (DEGs) $P < 0.05$, FDR adjusted-P <0.05. **B.** Heatmap showing differentially expressed KEGG pathways between SubB and SubA. The ratio of significantly regulated KEGG terms and genes annotated with each KEGG term (minimum of 10) comparing SubB *vs*. SubA in each dietary intervention are shown in the heatmap. Up-regulated pathways are shown in red and down-regulated pathways are shown in blue. Inulin and pectin comparisons show the largest number of differentially expressed genes.

Fig. 9. Liver gene expression is associated with host metabolic phenotypes. A. Correlation matrix between gene expression consensus modules and host phenotypes measured in mice described in Fig. 1**.** Modules were determined based on patterns of co-abundance of transcripts using WGCNA. Each of the modules was labelled with a unique color as an identifier. Each module was tested for correlation with host metabolic

phenotypes. Within each cell, upper values are correlation coefficients between module and the phenotypes; lower values are the corresponding FDR adjusted-*P* values. **B** Gene Ontology (biological process term) and KEGG pathways over-represented in the blue module (gene count >5; P and FDR adjusted-*P* <0.05).

Fig. 10. Taxa associated with liver transcriptomic modules. Association strength is denoted by width of the lines, red lines show positive association while blue ones show negative. Phylum level classification of the taxa is marked by colored boxes and transcriptomic WGCNA module by colored circles. *Anaerotruncus* (*Firmicutes*) and *Alistipes* (*Bacteroidetes*) are the taxa with the most associations with the clustered genes.

Supplemental Material

Fig. S1. Screening phase. 16S rRNA gene sequence analysis **A.** Principal Coordinates Analysis (PCoA) of unweighted UniFrac (uwUF) distances from eight human fecal samples collected for Wisconsin Longevity Study. **B.** Gut bacterial relative abundance summarized at the phylum level. **C.** UwUF distances between donor fecal samples and each engrafted cecal community. Averages of distances between corresponding human donor-mouse engrafted community are indicated as DONOR. Average of uwUF distances between non-matched donor-mouse community are indicated as OTHER. **D.** PCoA of uwUF distances of the eight human fecal samples engrafted in the mouse cecum**.** Circles with the same colors indicate biological replicates colonized with the

same community. **E.** Alpha diversity as determined by Faith's phylogenic diversity of each of the eight engrafted communities. ***P <0.001

Fig. S2. Variation in cecal short-chain fatty acids among transplanted communities. Cecal levels of **(A)** butyrate; **(B)** acetate; and **(C)** propionate (μMoles/g wet weight) for each the eight transplanted groups of mice described in Fig. S1.

Fig. S3. Variation in predicted metabolic capacity among engrafted gut communities. Principal Coordinates Analysis (PCoA) of Bray Curtis dissimilarity using the PICRUSt2 predicted metabolic functions of the eight transplanted human microbiota samples used in this study. Circles with the same colors indicate biological replicates colonized with the same community.

Fig. S4. **Characterization of transplanted communities in mice.** 16S rRNA gene sequence analysis of engrafted cecal communities. Germ-free mice were colonized with SubA or SubB microbiomes exposed to one of four diets containing aa different type of fiber; (i) Cellulose; (ii) Inulin; (iii) Pectin; or (iv) Assorted fiber for four weeks **A.** Heatmap showing presence/absence of bacterial taxa in the gut of transplanted animals across the four different diets. Red indicates presence and black absence. Each column represents an individual mouse. **B.** Alpha diversity (Shannon Index) of SubA and SubB communities after dietary intervention**.** *P <0.05, **P <0.01, ***P <0.001 ****P <0.0001.

Fig. S5. **Differences in gut microbiota between SubA- and SubB-colonized animals across the different diets used.** Weighted UniFrac distances between fecal microbiomes of SubA and SubB colonized mice. The UniFrac matrix was permuted 999 times; n=7-10 animals/microbiome/diet.

Fig. S6. Individual effect of dietary fibers on *Firmicutes* to *Bacteroidetes* ratio. Comparison of *Firmicutes* and *Bacteroidetes* between engrafted SubA and SubB communities across different diets. **A.** Relative abundance of *Bacteroidetes* (white) and *Firmicutes* (black) in SubA (magenta) and SubB (yellow) colonized mice for each dietary fiber intervention. **B.** *Firmicutes*:*Bacteroidetes* ratio in SubA (magenta) and SubB (yellow) for the four dietary interventions. **P* <0.05, ***P* <0.01, ****P* <0.001, *****P* <0.0001.

Fig. S7. Linear discriminant analysis Effect Size (LEfSe) summary. List of taxa differentially abundant between gut community SubA (magenta) and SubB (yellow) through the four fiber dietary interventions. LDA score (log 10) is indicated at the bottom of each graph.

Fig. S8. Relative abundance of gut bacterial taxa for SubA and SubB across communities. Box plots indicating relative abundance of taxa of interest relevant to the diversity, association, and mediation analyses. This figure shows relative abundance of taxa that has at least one significant difference between SubA and SubB within a dietary intervention. SubA is represented with the color magenta and SubB with the color yellow. **P* <0.05, ***P* <0.01, ****P* <0.001, *****P* <0.0001.

Fig. S9. Short Chain Fatty Acids (SCFA). Cecal levels of **(A)** acetate; **(B)** butyrate, **(C)** propionate and total SCFA (uMoles/g wet wt) **E.** valerate and Branched-chain Fatty Acids (BCFA) Isobutyrate and Isovalerate of SubA (magenta) and SubB (yellow) by diet. Wilcoxon test comparison *P <0.05, **P <0.01, ***P <0.001, ****P <0.0001

Fig. S10. Dendrogram of plasma metabolites from transplanted mice. Clustering dendrograms of 712 plasma metabolites with dissimilarity based on topological overlap, together with assigned module colors. There are 9 modules that cluster different numbers of metabolites.

Fig. S11. Branched-Chain Fatty Acids and taxa correlation. The heatmap shows all correlations (P <0.05) using Spearman method between BCFA and taxa in each dietary intervention.

Fig. S12. Effect of gut community on liver histone post-translational modifications (PTMs). Heatmap show relative difference in abundance for each histone PTM quantified in liver for mice colonized with the two communities (Log2 fold-change of SubB/SubA) in each diet. *P<0.1, **P<0.01, ***P<0.001 (n= 3-4/condition). ac, acetylated; unmod, unmodified; meth1,2,3, mono- di- and try- methylated respectively; pr, propionylated.

Fig. S13. Dendrogram of liver transcripts from transplanted mice. Clustering dendrograms of genes with dissimilarity based on topological overlap, together with

assigned module colors. There are 14 modules that cluster different numbers of transcripts.

Fig. S14. Gene Ontology and KEGG pathway enrichment of transcripts in the blue module associated with metabolic phenotypes. A. Biological Process GO and KEGG enrichment of blue module associated with adiposity, **B.** Association with liver triglycerides. C. Association with glucose. Gene counts and FDR adjusted-*P* values are indicated for each enrichment box.

Supplemental Tables

Table S1. Diets used in the study.

Table S2. Table S2. Statistical analysis of metabolic Phenotypes in transplanted mice.

Table S3. Mediation analysis results. For each dietary fiber intervention associated phenotype a mediator type was evaluated, numbers following the mediator report effect estimates and numbers within the parentheses are *P*-values.

Table S4. **List of plasma metabolites measured in plasma of transplanted mice.** Fold-change(FC) between SubB and SubA is indicated as follow: darker green indicates FC<1 with P≤0.05, lighter green indicates FC<1 with 0.05<P<0.1, darker read indicates

FC≥1 with P≤0.05,and lighter red indicates FC≥1 with 0.05<P<0.1. Two-Way ANOVA main effects: Community, diet, and their interaction are indicated in blue shaded cells when significant (P≤0.05) ANOVA effect; light blue shaded cells indicate 0.05<P<0.10. P and FDR adjusted-*P* values for two-way ANOVA are indicated in columns P through W and mean values in columns Y through AH.

Table S5. **List of metabolites cluster in modules by WGCNA.**

Table S6. **WGCNA assignment of metabolites into modules.** Metabolites (biochemicals) contained in the blue and turquoise modules by subject and super pathway. The fourth to seventh column shows the fold change of SubB compared to SubA within each fiber dietary intervention, the last two columns shows metabolite significance (MS) which reports the association of each metabolite with each phenotype and the corresponding P-value (p.MS).

Table S7. Liver gene expression analysis in transplanted mice. List of differentially expressed genes in SubB compared with SubA per dietary fiber intervention. (LogFC >1 and <-1, P and FDR adjusted-*P* <0.05)

Table S8. Analysis of differentially expressed liver genes. Number of differentially regulated genes contained per pathway, sub and super hierarchy in the KEGG and Gene Ontology fold change comparison for SubB versus SubA microbiome community with their respective *P*-value.

1574

1575 **Table S9**. **List of genes cluster in modules by WGCNA.**

1576

1577 **Table 10. List of genes associated with taxa.**