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RESEARCH ARTICLE | *Role of Gut Microbiota, Gut-Brain and Gut Liver Axes in Physiological Regulation of Inflammation, Energy Balance, and Metabolism*

Comparison of the effects of soluble corn fiber and fructooligosaccharides on metabolism, inflammation, and gut microbiome of high-fat diet-fed mice

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[1]*Université Catholique de Louvain, Université Catholique de Louvain, Louvain Drug Research Institute, Walloon Excellence in Life Sciences and Biotechnology, Metabolism and Nutrition Research Group, Brussels, Belgium;* [2]*Innovation and Commercial Development, Tate & Lyle Ingredients Americas, Hoffman Estates, Illinois;* [3]*ProDigest, Ghent, Belgium; and* [4]*Ghent University, Faculty of Bioscience Engineering, Center of Microbial Ecology and Technology, Ghent, Belgium*

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Van Hul M, Karnik K, Canene-Adams K, De Souza M, Van den Abbeele P, Marzorati M, Delzenne NM, Everard A, Cani PD. Comparison of the effects of soluble corn fiber and fructooligosaccharides on metabolism, inflammation, and gut microbiome of high-fat diet-fed mice. *Am J Physiol Endocrinol Metab* 319: E779–E791, 2020. First published August 24, 2020; doi:10.1152/ajpendo.00108.2020.—Dietary fibers are essential components of a balanced diet and have beneficial effects on metabolic functions. To gain insight into their impact on host physiology and gut microbiota, we performed a direct comparison of two specific prebiotic fibers in mice. During an 8-wk follow up, mice fed a high-fat diet (HFD) were compared with mice on a normal diet (basal condition, controls) and to mice fed the HFD but treated with one of the following prebiotics: fructooligosaccharides (FOS) or soluble corn fiber (SCF). Both prebiotic fibers led to a similar reduction of body weight and fat mass, lower inflammation and improved metabolic parameters. However, these health benefits were the result of different actions of the fibers, as SCF impacted energy excretion, whereas FOS did not. Interestingly, both fibers had very distinct gut microbial signatures with different short-chain fatty acid profiles, indicating that they do not favor the growth of the same bacterial communities. Although the prebiotic potential of different fibers may seem physiologically equivalent, our data show that the underlying mechanisms of action are different, and this by targeting different gut microbes. Altogether, our data provide evidence that beneficial health effects of specific dietary fibers must be documented to be considered a prebiotic and that studies devoted to understanding how structures relate to specific microbiota modulation and metabolic effects are warranted.

dietary fibers; gut microbiota; metabolism; obesity

INTRODUCTION

The association between fiber intake and health is now well recognized, with decades of research data supporting beneficial effects of complex dietary fibers. These include direct effects on gut functions and digestion, but also indirect effects on blood glucose control, cardiovascular functions, and host metabolism. Indeed, high dietary fiber intake have been linked to a protection against development of several noncommunicable conditions, including diabetes, cardiovascular disease, colon cancer, and obesity (60).

Traditional sources of fibers include whole grains, nuts, fruits, and vegetables. Although readily available, these ingredients are not consumed in sufficient quantities, and the average intake of the general population in the United States and the European Union remains far below recommendations (53, 62).

Fiber supplementation, therefore, represents a realistic and efficient dietary intervention. Several products are already available commercially and come in various forms and types. These are either naturally occurring fibers isolated from plants, or synthetic nondigestible soluble and insoluble carbohydrates. Given their specific structural and functional characteristics, each may act differently in the body leading to distinct health perks. Deciphering these specific mechanisms of action of these different fibers may, therefore, prove crucial to understanding their health-promoting properties.

One of the most important mechanisms of action among fibers is fermentation in the intestinal tract. Fermentation leads to the formation of short-chain fatty acids (SCFAs), of which acetate, propionate, and butyrate are the most common. These are then metabolized by the colonic epithelium, as well as other organs and contribute ~10% of the total energy intake from a Western diet (3). Fibers can also serve as prebiotics and stimulate the growth of certain beneficial bacteria (32, 36). While, more than 1,000 different known bacterial species can be found in human gut microbiota, only ~250 predominate in any given person, forming an ecosystem with the ability to exert a marked influence on the host during homeostasis and disease (13). Modulating this complex and dynamic population of microorganisms toward a more beneficial composition and metabolism is considered a crucial step in trying to improve human health. The role of fibers in directing the composition and activity of the endogenous microbiota is gaining considerable interest. Since each type of fiber is unique, with a distinct particle size, branching, solubility, viscosity, degree of polymerization and fermentability, it is implied they have distinct impacts on the host. However, comparison of different studies remains very difficult. This is largely because the human cohorts studied are very heterogeneous and interindividual comparison is complicated by a plethora of confounding factors. Indeed, individual responses to fiber consumption can vary significantly. These phenotypic variants are attributed to a combination of host genetics (33), environmental factors (22, 66), dosage of the fiber

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Table 1. *Structural and functional characteristics of FOS and SCF*

	FOS	SCF
Solubility	soluble	soluble
Viscosity	nonviscous	nonviscous
Fermentability	fermentable	fermentable
Structure component	fructose	glucose
Linkages	linear fructosyl units linked by β (2, 1) bonds with chain-terminating glucose moieties	branched glucosyl units connected by α and β bonds with varying linkages like (1, 4), (1, 6), (1, 3), and others

FOS, fructooligosaccharides; SCF, soluble corn fiber.

of interest (5, 18), and the distinct microbiota composition of the individual at baseline. Also, not many studies include more than one fiber, making direct comparison almost impossible.

In this study we assessed the effects of two types of nondigestible, soluble carbohydrates: soluble corn fiber (SCF) and fructooligosaccharides (FOS) in a mouse model of diet-induced obesity. This allows for the direct comparison of two different fibers, while the use of inbred mice in a controlled environment ensures that the number of confounding factors is kept to a minimum. The results of this comparison could be interesting to pinpoint some shared, fundamental mechanisms of action of these different fibers, while also recognizing some distinct and potentially relevant differences to be defined as prebiotic fibers.

MATERIALS AND METHODS

Animals. Nine-week-old male C57BL/6J mice (Janvier, Le Genest-Saint-Isle, France) were housed 2–3 mice per cage in specific pathogen-free conditions and in a controlled environment (room temperature of 22 ± 2°C, humidity 55 ± 10%, 12:12-h light-dark cycle) with free access to food and water. After an acclimatization period of 1 wk, mice were randomly assigned to one of four dietary conditions (n = 15 per group). The different groups were as follows: control diet (CT) (10 kcal % fat, D12450Ji; Research Diets, New Brunswick, NJ) with water, high-fat diet (HFD) (60 kcal% fat, D12492i; Research Diets) and water, high-fat diet and water supplemented with fructooligosaccharides (FOS; Orafti P95; Beneo) or a high-fat diet with water supplemented with soluble corn fiber (SCF; Promitor, Tate & Lyle) during 8 wk. Daily fiber consumption was recalculated regularly in function of the food and water intake to maintain a final consumption of 10% (fiber/food). The fiber's caloric contribution was calculated on the basis of an energy content of 1.1 kcal/g for SCF and 1.5 kcal/g for FOS, as indicated by the manufacturers). The main characteristics of both fibers are described in Table 1.

Body weight and food and water intake were recorded weekly. Body composition (lean and fat mass) was assessed by using 7.5 MHz time domain-nuclear magnetic resonance (TD-NMR) (LF50 Minispec; Bruker, Rheinstetten, Germany). In the final week of the experiment, feces were collected for each cage by transferring the animals to clean cages for a period of 48 h. After this, feces were manually collected, dried overnight at 60°C and weighted to assess the amount of feces secreted per day. Then energy content was measured on a C1 calorimeter from IKA. Per cage containing two animals, one mean value was considered for analysis.

All mouse experiments were approved by and performed in accordance with the guidelines of the local Ethics Committee. Housing conditions were specified by the Belgian Law of May 29, 2013, regarding the protection of laboratory animals (agreement no. LA1230314).

Oral glucose tolerance test. After 7 wk of treatment, an oral glucose tolerance test (OGTT) was performed as previously described (7). Briefly, 6-h fasted mice were given an oral glucose load (2 g glucose per kg body wt), and blood glucose levels were measured at different time points: 30 min before and 15, 30, 60, 90, and 120 min after oral glucose load. Blood glucose was measured with a standard glucose meter (Accu Check, Roche, Basel, Switzerland) on blood samples collected from the tip of the tail vein.

Insulin resistance index. Plasma insulin concentration was determined using an ELISA kit (Mercodia, Uppsala, Sweden), according to the manufacturer's instructions. Insulin resistance index was determined by multiplying the area under the curve of both blood glucose (−30 to 120 min) and plasma insulin (−30 and 15 min) obtained following the oral glucose tolerance test (65). Glucose-induced insulin secretion was calculated as the difference between plasma insulin levels 30 min before and 15 min after oral glucose load.

Tissue sampling. At the end of the treatment period (*week 8*), 10 animals from each group were selected randomly and anesthetized with isoflurane (Forene, Abbott, Queenborough, Kent, UK), and blood was sampled from the portal and cava veins. After exsanguination, mice were killed by decapitation. Subcutaneous adipose tissue depots, intestines, muscles, and liver were precisely dissected, weighed, and immediately immersed in liquid nitrogen followed by storage at −80°C for further analysis.

RNA preparation and Real-time quantitative PCR analysis. Total RNA was prepared from tissues using TriPure reagent (Roche). Quantification and integrity analysis of total RNA were performed by analyzing 1 μL of each sample in an Agilent 2100 Bioanalyzer (Agilent RNA 6000 nano kit, Agilent, Santa Clara, CA). cDNA was prepared by reverse transcription of 1 μg total RNA using a reverse transcription system kit (Promega, Madison, WI). Real-time PCR was performed with the CFX96 real-time PCR system and CFX Manager 3.1 software (Bio-Rad, Hercules, CA) using Mesa Fast quantitative PCR (Eurogentec, Liège, Belgium) for detection according to the manufacturer's instructions. RPL19 was chosen as the housekeeping gene. All samples were performed in duplicate, and data were analyzed according to the $2^{-\Delta\Delta CT}$ method. The identity and purity of the amplified product were assessed by melting curve analysis at the end of amplification. The primer sequences for the targeted mouse genes are presented in Table 2.

Gut microbiota analysis. Cecal contents were collected and kept frozen at −80 °C until use. Metagenomic DNA was extracted from the cecal content using a QIAamp DNA Stool mini kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions with modifications.

The 16S rRNA gene was amplified from the cecal microbiota of the mice using the following universal eubacterial primers: 27Fmod (5′-AGRGTTTGATCMTGGCTCAG-3′) and 519Rmodbio (5′-GTNTTACNGCGGCKGCTG-3′). Purified amplicons were sequenced using a MiSeq following the manufacturer's guidelines. Sequencing was performed at MR DNA (www.mrdnalab.com; Shallowater, TX). Sequences were demultiplexed and processed using the QIIME pipeline (v1.9 using default options (Q25, minimum sequence length = 200 bp, maximum sequence length = 1,000 bp, maximum number of ambiguous bases = 6, maximum number of homopolymers = 6, maximum number of primer mismatches = 0). For the 49 samples analyzed, 162,260 OTUs have been identified (97% similarity). The minimum number of sequences per sample was 20,675, and the maximum number of sequences per sample was 79,590. The median number of sequences per sample was 40,475 and the mean number of sequences per sample was 44,066,714 ± 14,774.308 (SD). The Q25 sequence data derived from the sequencing process were analyzed with the QIIME 1.9 pipeline. Briefly, sequences were depleted of barcodes and primers. Sequences 1,000 bp were then removed; sequences with ambiguous base calls and with homopolymer runs exceeding 6 bp were also removed. Sequences were denoised, and operational taxonomic units (OTUs) were generated. Chimeras were also removed. OTUs were defined by clustering at 3% divergence (97%

Table 2. *List of primers used for quantitative PCR analysis*

Gene	Forward (5′-3′)	Reverse (5′-3′)
Rpl19	GAAGGTCAAAGGGAATGTGTTCA	CCTTGTCTGCCTTCAGCTTGT
Proglucagon	TGGCAGCACGCCCTTC	GCGCTTCTGTCTGGGA
F480	TGACAACCAGACGGCTTGTG	GCAGGCGAGGAAAAGATAGTGT
PYY	GTTTGGACCAGTGGTGAAGA	TGCCCTCTTCTTAAACCAAACA
LBP	GTCCTGGGAATCTGTCCTTG	CCGGTAACCTTGCTGTTGTT
PAI1	ACAGCCTTTGTCATCTCAGCC	CCGACACAAAGAAGGA
CD11b	GTCAGAGTCTGCCTCCGTGT	CCTGCGTGTGTTGTTCTTTG
MCP1	GCAGTTAACGCCCCACTCA	CCCAGCCTACTCATTGGGATCA
TNFa	TCGAGTGACAAGCCTGTAGCC	TTGAGATCCATGCCGTTGG
CD11c	ACGTCAGTACAAGGAGATGTTGGA	ATCCTATTGCAGAATGCTTCTTTACC
IL6	ACAAGTCGGAGGCTTAATTACACAT	TTGCCATTGCACAACTCTTTTC
IL1b	TCGCTCAGGGTCACAAGAAA	CATCAGAGGCAAGGAGGAAAAC
CD206	CCTCTGGTGAACGGAATGAT	CTTCCTTTGGTCAGCTTTGG
IL10	GCTCTTACTGACTGGCATGAG	CGCAGCTCTAGGAGCATGTG
MGL1	TGAGAAAGGCTTTAAGAA	GACCACCTGTAGTGATGTGGG
Arg1	CTCCAAGCCAAAGTCCTTAGAG	AGGAGCTGTCATTAGGGACATC

similarity). Final OTUs were taxonomically classified using BLASTn against a curated Greengenes database. Principal coordinate analysis (PCoA) was generated with QIIME using the unweighted UniFrac distance matrix between the samples (21, 52) and as previously described (25, 30).

Quantification of short-chain fatty acids. SCFAs were measured in both cecal content and fresh feces collected at the end of the experiment.

Upon weighing the samples using an analytical balance (Kern ABJ 320), samples were suspended in distilled water and incubated during 2 h at 4°C. After vortexing, SCFA were extracted from the samples with diethyl ether, upon the addition of 2-methyl hexanoic acid as an internal standard. Subsequently obtained extracts were analyzed using a GC-2014 gas chromatograph (Shimadzu, Noord-Brabant, the Netherlands), equipped with a GC SGE capillary column, 30 mm × 0.32 mm ID-BP21x 0.25 μm (Achrom, Machelen, Belgium), a flame ionization detector and a split injector. The injection volume was 1 μL and the temperature profile was set from 110 to 160°C, with a temperature increase of 6°C/min. The carrier gas was nitrogen, and the temperature of the injector and detector was both 200°C.

Statistical analysis. Mouse data are expressed as the means ± SE. Differences between groups were assessed using nonparametric Kruskal-Wallis one-way ANOVA, followed by the Dunn's multiple-comparison test. Variance was compared using a Bartlett's test. If variances were significantly different between groups, values were normalized by Log-transformation before proceeding to the analysis. When only two groups were compared, a nonparametric Mann-Whitney *U* test was used. Regimen and treatment effects on community compositions were assessed using permutational multivariate analysis of variances after rarefaction of all communities to even sampling depths. The abundances of all phyla were computed by agglomerating the OTUs assigned to those phyla. For each such family, Mann-Whitney *U* test with Benjamini-Hochberg correction (35) were performed to detect the combinations (treatment) that were significantly different in terms of abundance.

A two-way ANOVA analysis with a Bonferroni post hoc test on repeated measurements was performed for the evolution of glycemia and insulinemia during the OGTT. For all analyses and for each group, any exclusion decision was supported by the use of the Grubbs's test for outlier detection.

RESULTS

Effects on body weight, body composition, and adipose tissue. Body weight gain and fat mass gain were both significantly greater in the high-fat diet fed mice (HFD) than in mice fed the control diet (CT). Supplementation of both FOS and SCF drastically reduced fat mass development, ultimately resulting in body weights that were comparable to that of control diet-fed mice. (Fig. 1, *A* and *B*). Lean mass was not different in any of the groups (Fig. 1*C*), suggesting that muscle development was not affected by high-fat feeding or fiber supplementation. This was confirmed by measuring the weights of four different types of muscles (Fig. 1*D*).

To confirm the results on fat mass measured by NMR, we precisely dissected and weighed four different types of adipose tissues. Subcutaneous (inguinal), epididymal, visceral (mesenteric), and brown adipose depots were all significantly smaller in mice receiving fibers (FOS and SCF), as compared with their littermates fed only high-fat diet (Fig. 1*E*).

The weights of the livers of mice treated with fibers were also reduced as compared with that of mice on HFD, suggesting reduced steatosis. The weight of the spleen was not affected in any of the groups (Fig. 1*F*), but the weight of the cecum was markedly decreased by the HFD and increased following consumption of fibers (Fig. 1*F*).

Effects on energy homeostasis. The caloric content of the diets was measured using a bomb calorimeter and energy consumption was calculated for each group by regularly weighing the amount of food eaten. In addition, the amount of water drunk was monitored, and the caloric contribution of the fiber supplementation was considered to calculate the total energy consumption. This showed that there were no differences in energy consumption between the high-fat diet-fed groups (Fig. 2*A*), indicating that potential beneficial effects associated with fiber intake could not be attributed to a lower caloric intake.

In the final week of the experiment, feces were collected during a 24-h period for each mouse, and energy content was measured by calorimetric bomb analysis (Fig. 2). There was no difference between the CT and HFD group; however, supplementation with both dietary fibers result in a significant increase of the energy content of the feces (Fig. 2*C*). In addition, the daily amount of feces excreted by mice given SCF was higher (Fig. 2*B*), resulting in a significantly increased daily energy excretion for this group (Fig. 2*D*). This suggests that SCF could partially act by reducing energy absorption, but that FOS probably has a different mode of action.

To estimate energy consumption, the body temperature was measured using a rectal probe. No differences were observed between the different groups (Fig. 2*E*).

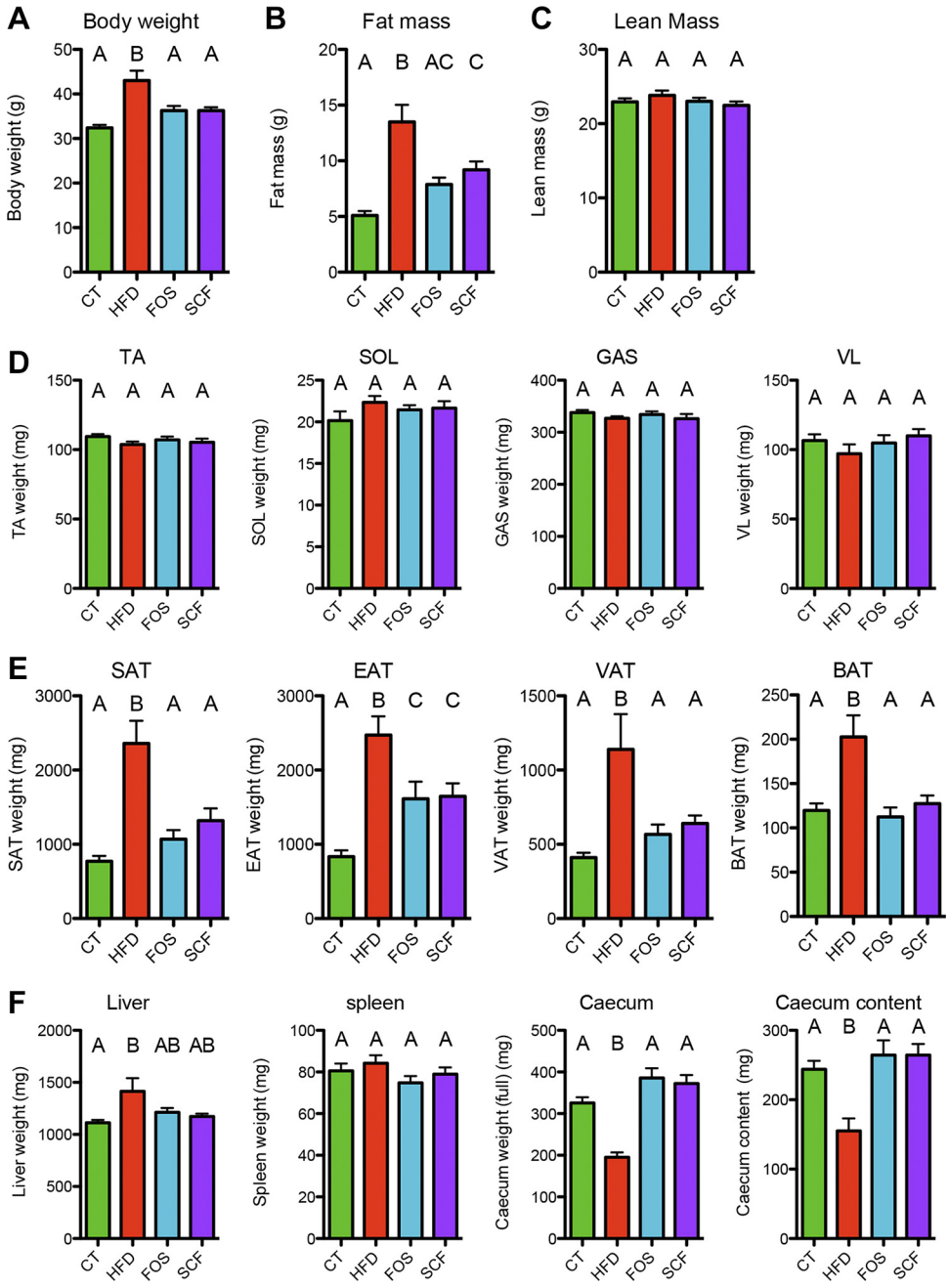

Fig. 1. Effects on body weight, body composition, organs, and adipose tissues. Body weight (g) (*A*) with corresponding fat mass (g) (*B*), and lean mass (g) (*C*) at the end of the 8-wk follow-up. Weight of different types of muscles (g) (tibialis, soleus, gastrocnemius, and vastus lateralis) (*D*) and of different adipose tissue depots (g) (subcutaneous (inguinal), epididymal, visceral (mesenteric) and brown adipose tissues) (*E*) at the end of the 8-wk period. Weights of liver (g) spleen (g), full cecum (g), and cecum content (g) at the end of the follow-up (*F*). Data are presented as the means ± SE. Data with different letters above the bars are significantly different ($P < 0.05$) according to post hoc one-way ANOVA.

Effects on glucose metabolism. We have previously discovered that changing the gut microbiota by using FOS improves glucose tolerance and insulin resistance (9, 10). Therefore, an oral glucose tolerance test (OGTT) was performed after 7 wk of treatment. Mice from the HFD group clearly showed a higher increase in blood glucose levels as compared with mice from the CT group, whereas both dietary fibers (FOS and SCF) markedly and significantly improved glucose tolerance (Fig. 3, *A* and *B*). This indicated that consumption of both fibers is equivalent for improving this parameter.

As expected, fasted insulin levels were drastically increased in the HFD group, and this hyperinsulinemia was normalized after treatment with FOS and SCF (Fig. 3*C*). After an oral glucose load, the HFD group exhibited a four-fold higher insulin level compared with CT mice. This parameter was reduced by treatment with FOS and SCF but without reaching significance (Fig. 3*D*). However, the insulin resistance (IR) index was significantly lower for both fiber-treated groups (Fig. 3*E*).

Effects on adipose tissue inflammation. Since diabetes and insulin resistance were frequently associated with adipose tissue inflammation (7, 40, 57), we measured various macrophage infiltration markers in the subcutaneous (SAT) and visceral adipose tissue (VAT) using quantitative PCR analysis (Fig. 4). Lipopolysaccharide binding protein and monocyte chemoattractant protein-1 were upregulated by HFD and were reduced by both FOS and SCF in both adipose tissues. Although the number

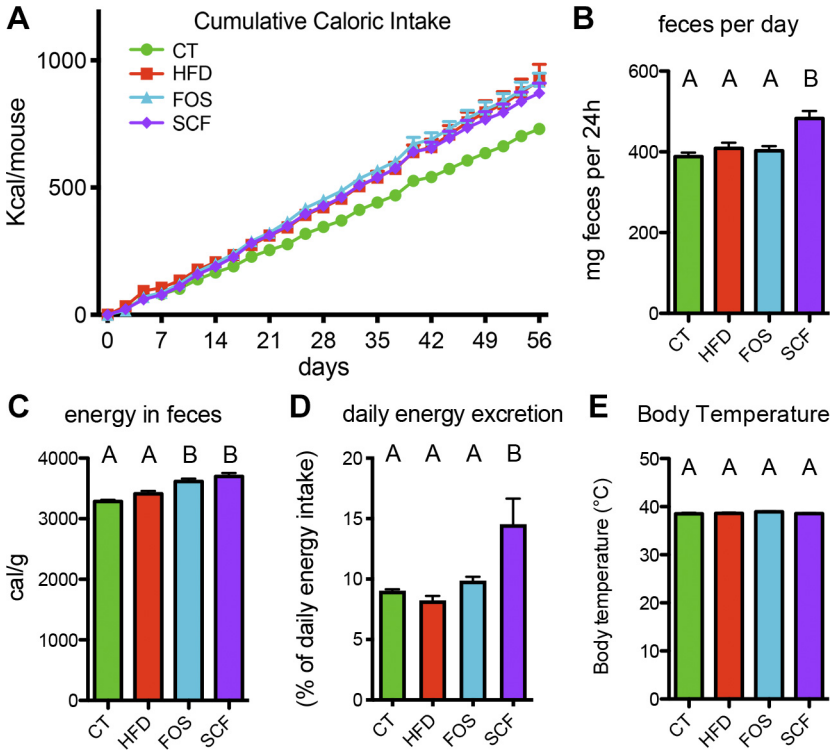

Fig. 2. Effects on energy homeostasis. Energy intake: mean cumulative caloric intake per mouse (kcal) (*A*). Energy excretion: mean amount of feces excreted per mouse in one day (mg/24 h) (*B*), mean energy content in feces (cal/g) (*C*), daily energy excretion as calculated using the previous values (cal/mouse) (*D*) and body temperature (°C) (*E*). Data are presented as the means ± SE. Data with different letters above the bars are significantly different ($P < 0.05$), according to post hoc one-way ANOVA (*B*–*E*).

of macrophages did not seem to differ significantly between groups (no effect on the global macrophage markers F4/80 and CD11b), supplementation with FOS or SCF did have an impact on M1 macrophage activation in the adipose tissue, as evidenced by a significant decrease in CD11c mRNA (SAT and VAT) and trends toward reduction of TNFa and IL6 (SAT only). M2 macrophage markers, however, remained unaffected (CD206, IL10, and MGL1), with the exception of Arg1. Plasminogen activator inhibitor-1 (PAI-1) tended to be reduced by FOS and SCF in the SAT, but not in the VAT. IL-1b was not different in any of the groups, although there was a consistent trend toward increase by the HFD.

Effects on gut microbiota. We and others have previously linked the gut microbiota with low-grade inflammation and metabolic disorders associated with HFD feeding (1, 7, 11, 46).

Here, we confirmed that HFD feeding shifted the gut microbiota composition as depicted by the PCoA scores, clearly showing a separation between the HFD and CT group (Fig. 5*A*). Interestingly, treating mice on an HFD with fiber does not restore the microbial composition to a CT-like basal signature. Instead, the different groups display very distinct clusters, indicating that they developed different bacterial compositions, and that this is specific for the fiber used. Of note, although the β-diversity (microbial composition) was clearly affected, the effects on α-diversity (species richness) were less clear: the Shannon index indicates a trend toward reduced α-diversity in the HFD group, and this was improved in the FOS-treated mice, but further decreased in the SCF-treated mice (Fig. 5*B*).

At the level of the phyla, the FOS treatment was associated with an increase in Actinobacteria and a decrease in Proteobacteria when compared with HFD-fed mice. Treatment with SCF was associated with a similar decrease in Proteobacteria, but with an increase in Firmicutes (Fig. 5*C*).

At the level of the genera, we observed some common effects between FOS and SCF (Figs. 5*D* and Fig. 6 and Supplemental Table S1, https://doi.org/10.6084/m9.figshare.12423797). For example, both were able to counteract the HFD-induced increase of *Ruminoccocus*, *Bilophila*, *Desulfovibrio*, *Oscillospira*, and *Paenibacillus*, and the decrease of *Allobaculum*, *Sutterella*, and *Dehalobacterium*. In addition, both increased *Pseudomonas* and decreased *Bacillus*.

Interestingly, we also found effects that were specific to one of the two fibers (Fig. 6, *B* and *C*). FOS treatment was able to completely restore the loss of *Prevotella* caused by the HFD, whereas this genus was not affected by SCF. In accordance with previous studies, FOS supplementation also increased *Bifidobacterium*, whereas we did not observe this for SCF-treated mice.

SCF-specific effects included an increase in abundance of *Parabacteroides*, *Coprococcus* and *Bacteroides*. In contrast to FOS, SCF was also able to counter the decrease of *Blautia* induced by the HFD and the increase of *Lactococcus* and *Odoribacter*.

Effects on short-chain fatty acids. In the cecum, a nonsignificant reduction of total short-chain fatty acids (SCFA) amounts was observed in all HFD-fed mice when compared with CT diet-fed mice (Fig. 7*A*). This was mostly due to an HFD-induced reduction of propionate amounts. This decrease was countered upon dosing SCF that significantly increased propionate amounts, as compared with the HFD group, with a similar, albeit milder, trend being observed for FOS. Further, also acetate tended to decrease upon dosing

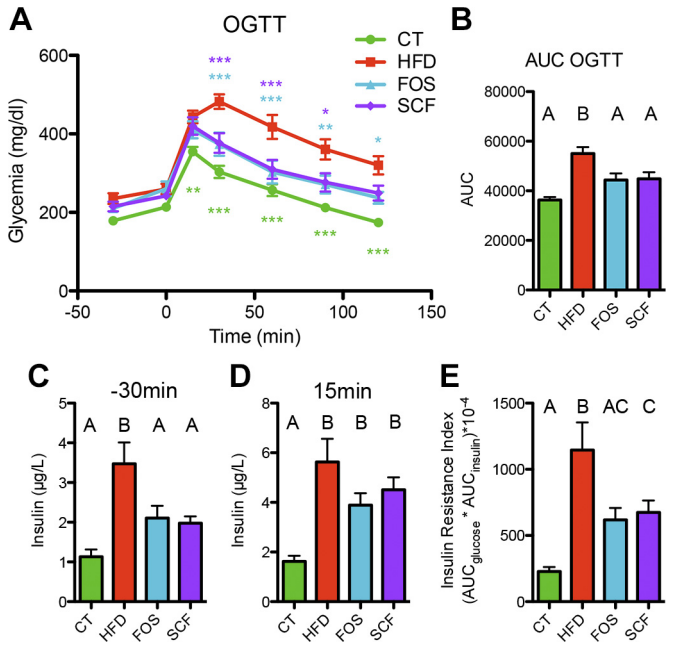

Fig. 3. Effects on glucose metabolism. Plasma glucose (mg/dL) profile (*A*) and the mean area under the curve (AUC) (*B*) (using the oral glucose tolerance test (OGTT) measured between 0 and 120 min after glucose loading ($mg \cdot dL^{-1} \cdot min^{-1}$). Plasma insulin levels at 30 min before (*C*) and 15 min after glucose loading (µg/L) (*D*). Insulin resistance index determined by multiplying the AUC of blood glucose by the AUC of insulin between 30 min before and 15 min after glucose loading (*E*). Data are presented as means ± SE. $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$, significant difference vs. HFD, as determined by a two-way ANOVA. *A*: data with different letters above the bars are significantly different ($P < 0.05$), according to post hoc one-way ANOVA (*B*–*E*).

the HFD. Neither FOS nor SCF treatment could counter this reduction.

In feces, propionate and butyrate concentrations were significantly decreased in the HFD-group, as compared with the control group, whereas both SCF and FOS treatment significantly increased propionate and butyrate (Fig. 7*B*). Additionally, FOS significantly increased acetate concentrations.

DISCUSSION

The gut microbiota plays an important role in health (6, 16, 48). More and more studies are being conducted to understand the complex interplay between gut microbes and their host. These studies have implicated many mediators ranging from bacterial components and metabolites to hormones, neurotransmitters, and bioactive lipids (13). Concurrently, efforts were made to identify which bacterial populations are associated with health or disease risk. The ultimate goal is to be able to understand how an individual's microbiota can be modulated to maximize health.

Dietary interventions are one of the most obvious ways to achieve changes of the microbiota (17). In this context, fibers, in particular, have a crucial role to play. These carbohydrates are undigestible by the human gut but can be used by certain microbes in the colon. They have the capacity to significantly impact the gut microbiota and have been linked with beneficial health effects. Unfortunately, there are only a few human dietary intervention studies that assess well-defined diets in well-characterized study populations to objectively monitor wide-ranging responses (61, 71). This type of trials is needed to make correlation-causation connections. Indeed, confounding factors, such as metabolic, physiological and genetic differences among individuals result in substantial interindividual variation in the response to diet and fiber intake, making it almost impossible to characterize phenotypes under controlled conditions.

Animal studies allow population-based confounding factors to be avoided and can be performed in controlled experimental setups. Therefore, they are very useful when it comes to assessing causality of the complex host-microbiota interactions and deciphering the ground rules of gut microbiota functioning.

In this study, we set out to compare two different fibers that are both classified as soluble, fermentable, nonviscous fibers to which prebiotic effects have been attributed. Both FOS and SCF are known for their influence on colonic microbiota content (26, 27, 42, 51, 69) and have been associated with improved intestinal health (2, 4, 20, 43).

When supplemented to a high-fat diet, both components reduced body weight gain and inhibited the development of fat mass in mice. This was independent of the amount of energy consumed. In addition, the weight of the liver, an indicator of steatosis, was lower in the fiber-treated mice. Glucose homeostasis was also improved to a similar degree for both fiber-supplemented groups, as evidenced by lower glucose plasma levels after an oral glucose load and reduced insulin resistance indexes. Also, adipose tissue inflammation was ameliorated. These combined observations lead to the conclusion that both FOS and SCF have a positive impact on host metabolism and health.

The most obvious underlying mechanism of the two tested fibers is gut bacterial fermentation to SCFA, which are known to interact with the host metabolism on several levels (13). In the cecum, we only found an increase of propionate for SCF and, to a lesser extent, for FOS. However, the lack of effects could be masked by the high degree of absorption of SCFA in this part of the intestines. Additionally, we also measured the SCFA in the feces and found that treatment with either FOS or SCF could completely alleviate the HFD-induced reduction of propionate and butyrate. In addition, we observed a marked effect of FOS on acetate.

Of course, other potential mechanisms may also explain the improved phenotype. For example, it is interesting to note that only mice treated with SCF had a significantly increased daily energy excretion. This suggests that SCF could partially act by reducing energy absorption, possibly as a result of this fiber's stool-bulking effect. Since we did not find similar results for FOS, it is probable that this mode of action is not relevant for this type of fiber, confirming our statement that it is essential to individually assess the specific health-promoting properties of specific fibers. Another potential mechanism that cannot be excluded is the effect on gastrointestinal motility. Indeed, we found that mRNA expression of proglucagon, the precursor of glucagon-like peptide, and peptide YY to be increased in the ileum and colon of FOS-treated mice (Supplemental Fig. S1, https://doi.org/10.6084/m9.figshare.12423920). Both hormones have been shown to delay gut transit, suggesting that SCF-treated mice had shorter transit times.

Another target of the tested fibers is the microbiota itself. Changes in microbial composition due to fiber supplementation may result in altered microbial functions, which can have an impact on specific metabolites such as bile acids, branched-

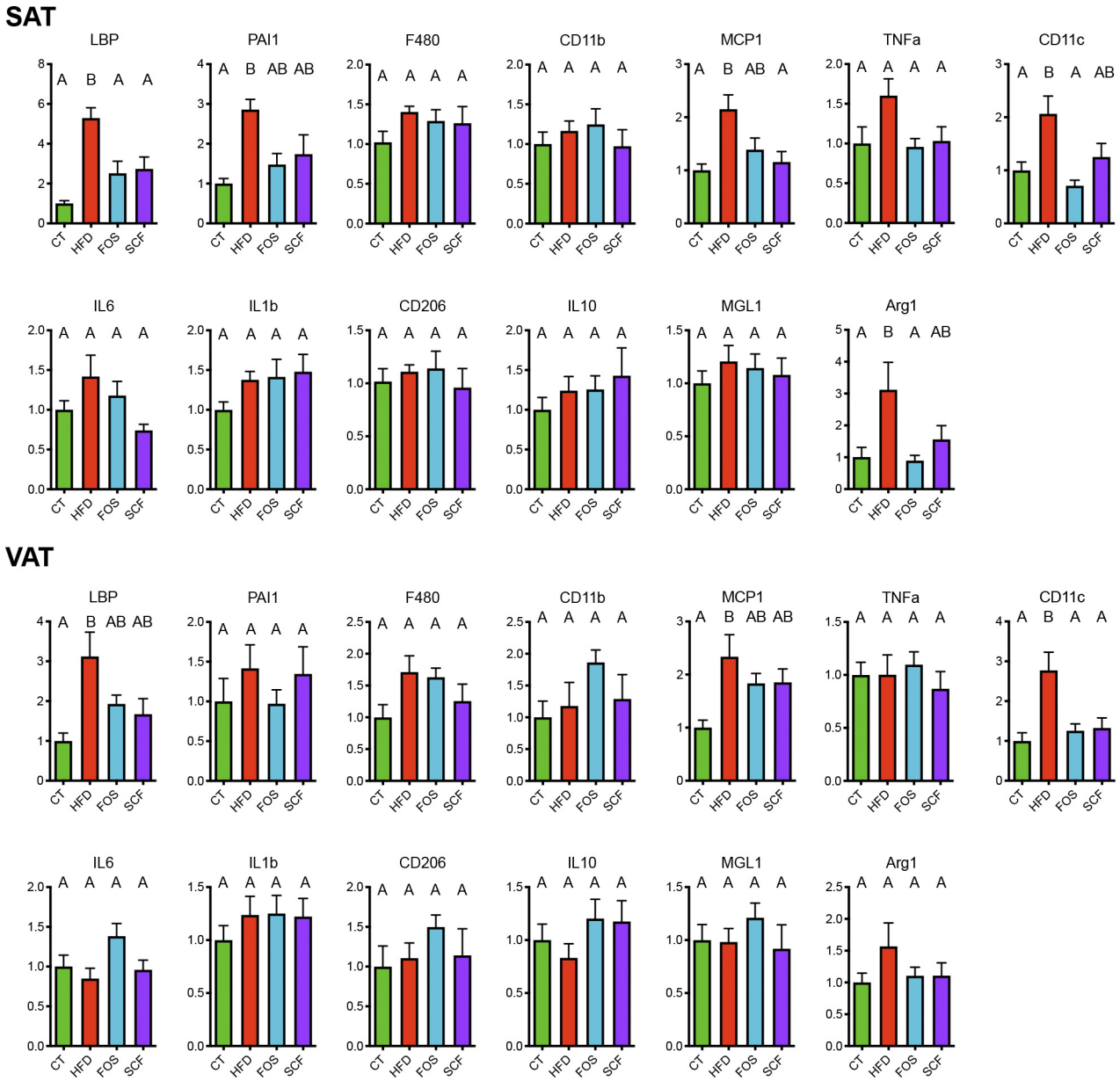

Fig. 4. Effects on adipose tissue inflammation. Relative expression of genes related to inflammation and immune system in the subcutaneous (SAT) and visceral adipose tissue (VAT). Arg1, arginase 1; IL-1b, interleukin 1β; LBP, lipopolysaccharide binding protein; MCP1, monocyte chemoattractant protein-1; MGL1, macrophage galactose-type lectin 1; PAI1, plasminogen activator inhibitor-1. Data are presented as means ± SE. Data with different letters above the bars are significantly different ($P < 0.05$), according to post hoc one-way ANOVA.

chain amino acids, indole propionic acid, and endocannabinoids (13). These metabolites can, in turn, affect systemic energy expenditure by influencing energy consumption, thermogenesis, and adipose tissue browning.

In this study, we confirm that the gut microbiota is strongly affected by both FOS and SCF fibers. The microbial composition after treatment with both soluble fibers is not a mere restoration to the control situation of normal diet-fed mice, but each fiber is characterized by a distinct microbial signature. There are changes that are common to both components, but there are also modifications that are fiber-specific. Common effects of both fibers include increases of *Allobaculum*, *Pseudomonas*, *Sutterella* and *Dehalobacterium*, and decreases of *Bacillus*, *Ruminoccocus*, *Bilophila*, *Desulfovibrio*, *Oscillospira* and *Paenibacillus*. Several of these genera are thought to be involved in beneficial physiological effects. For example, *Allobaculum* is a known producer of butyrate, a SCFA that is rapidly taken up by enterocytes, where it serves as an energy source (23). *Allobaculum* has been shown to be increased by prebiotics (27, 63) and has been associated with improved intestinal integrity, increased Reg3γ levels in the colon (27), and with resistance to NAFLD development (46). Moreover, metformin and berberine, two clinical drugs used for the treatment of diabetes, are associated with increases in *Allobaculum* abundance (73).

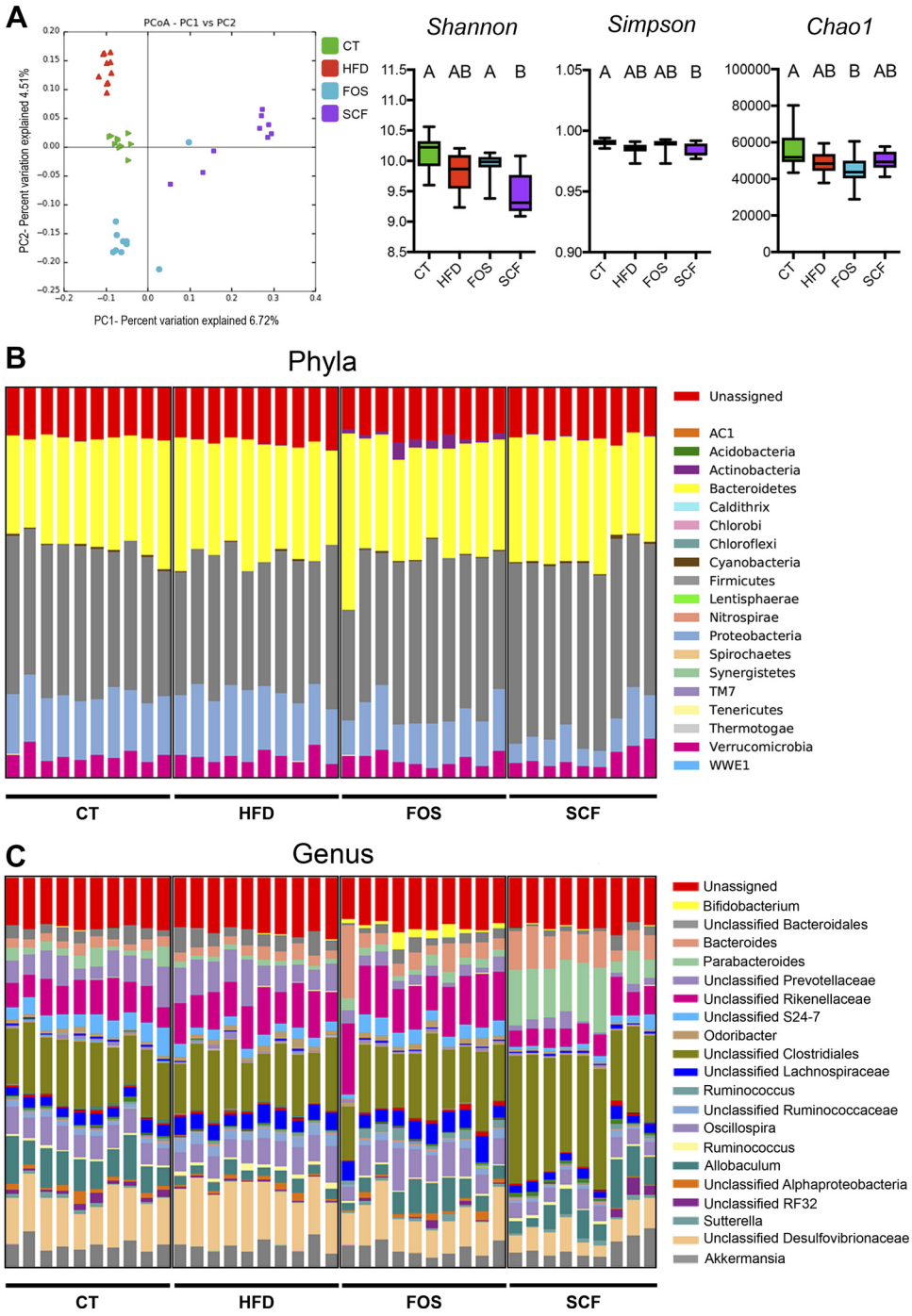

Fig. 5. Effects on gut microbiota. Gut bacterial community analysis by 16S rRNA gene high-throughput sequencing. *A*: principal coordinate analysis based on the weighted UniFrac analysis (PCoA) on operational taxonomic units (OTUs). Each symbol representing a single sample is colored according to the group. Data with different letters above the bars are significantly different ($P < 0.05$), according to post hoc one-way ANOVA. *B*: richness of the cecal microbiota based on 16S rRNA gene sequences analysis presented by Shannon, Simpson, and Chao1 indexes. Data are presented as box plots. *C*: relative abundances (percentage of 16S rRNA gene sequences) of the different bacterial phyla and genera in each sample among the different groups.

Lower abundances of *Dehalobacterium* were previously associated with a high body mass index (BMI) (28, 58), and *Dehalobacteria* appeared to be protective against atherosclerosis in apolipoprotein E knockout ($ApoE^{-/-}$) mice (14). *Ruminococcaceae* and *Desulfovibrio* have previously been associated with a high-fat diet and obesity (27, 31, 41, 72) and decreased with FOS in genetically obese mice (26).

Several genera belonging to the *Desulfovibrionaceae* family are considered opportunistic pathogens. They produce endotoxins and have been linked to inflammatory diseases (50, 68). They also have the capacity to reduce sulfate to H_2S (67), thereby damaging the intestinal barrier (38).

Two genera were modified only by FOS supplementation. We observed a restoration of the *Prevotella* abundance to a similar level as that of control mice, as well as a major increase in *Bifidobacterium*. Humans consuming more carbohydrates and fiber have been suggested to predominantly have a *Prevotella*-driven enterotype (19, 44, 49, 70), which has been negatively correlated with body weight, BMI, fat mass, and leptin concentrations (29). *Prevotella* are known to produce acetate and

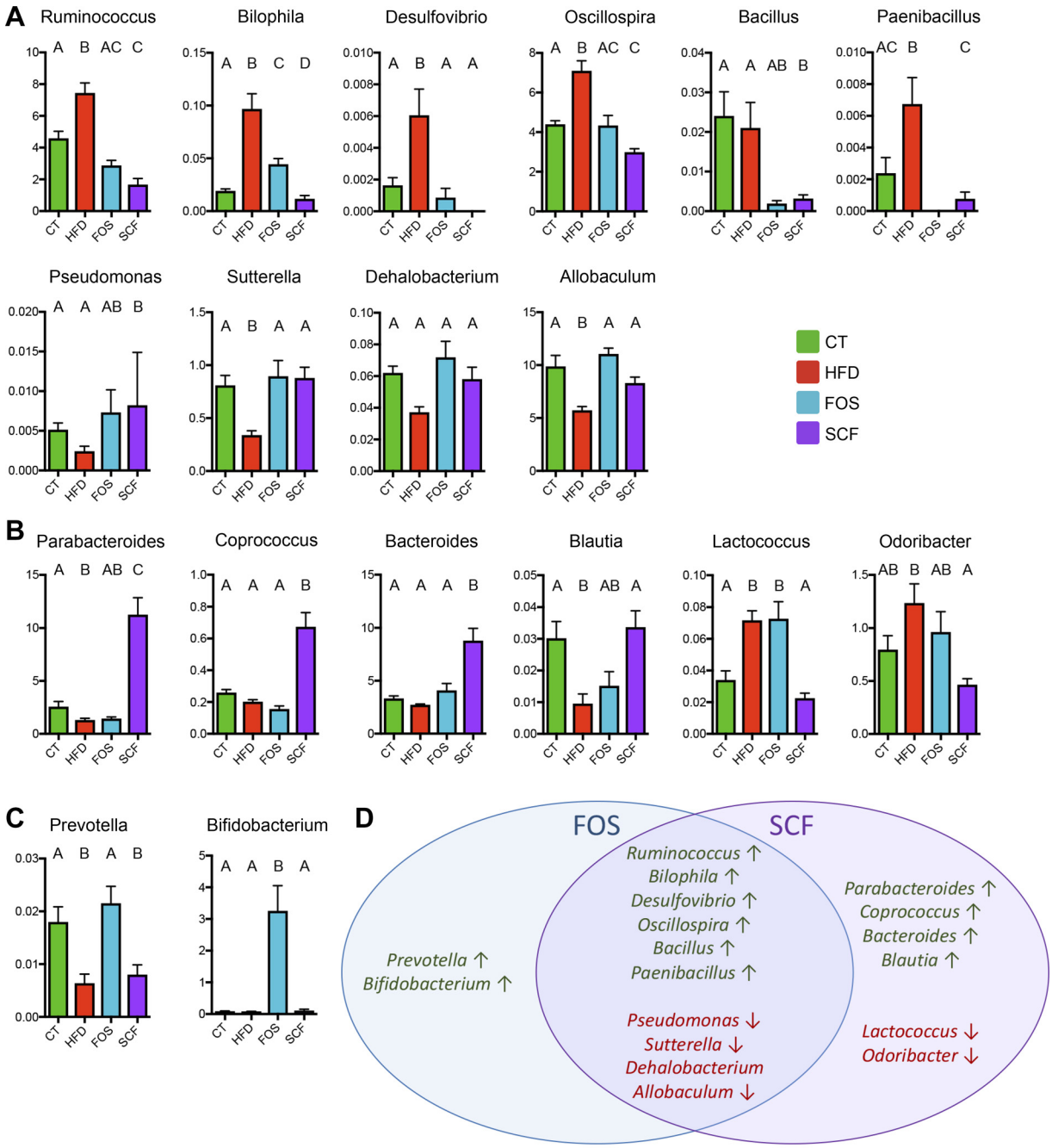

Fig. 6. Effects on bacterial genera. Relative abundance of the bacterial genera significantly altered by both fructooligosaccharides (FOS) and soluble corn fiber (SCF) (*A*), by FOS only (*B*), or by SCF only (*C*). Data are presented as the means ± SE. Data with different letters above the bars are significantly different ($P < 0.05$), according to post hoc one-way ANOVA.

succinate, which can be converted by certain bacterial species into propionate. Both acetate and propionate are increased in the feces of FOS-treated mice. *Bifidobacterium* are acetate-producing members of the *Actinobacteria* phylum, and some strains have been attributed health-promoting properties (34).

We found four bacterial genera that were increased specifically by the SCF treatment, as compared with the HFD group: *Blautia* were restored to control levels, whereas *Parabacteroides*, *Coprococcus*, and *Bacteroides* were increased to levels several folds higher than that of the control group. *Blautia* has been suggested to be important for gut health, as reductions in *Blautia* are associated with increased incidence of colorectal cancer (15, 55). Reductions in *Blautia* were also found in diabetic adults and children (45, 54) and patients suffering from liver cirrhosis (39). On the other hand, high levels of *Blautia* were observed in obese humans and rats (37, 47, 56, 58), as well as in human patients with inflammatory bowel disease (59). Whether, *Blautia* is deleterious in these cases, or perhaps increased in an attempt to restore homeostasis, remains unknown. Interestingly, berberine markedly enriched the SCFA-producing *Blautia* in the gut of rats and,

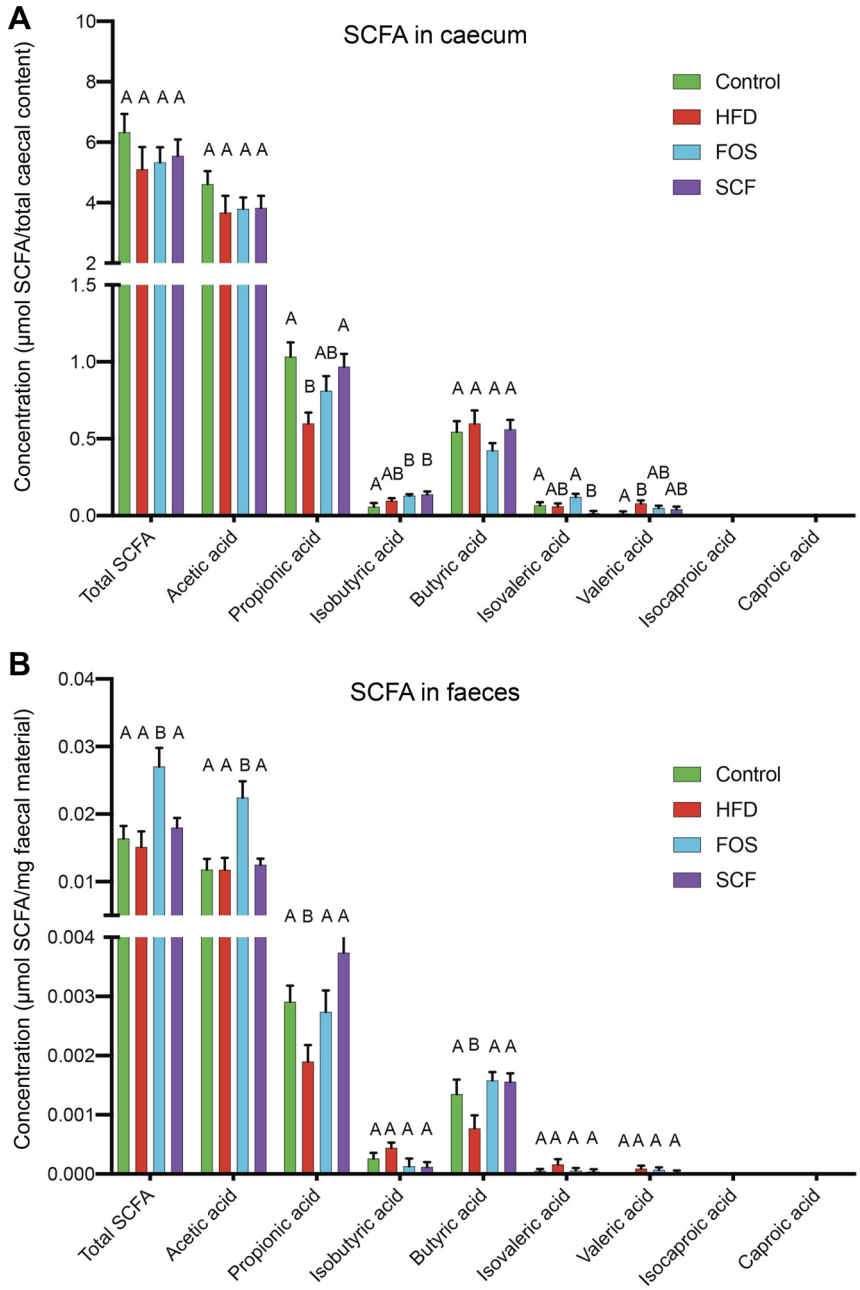

Fig. 7. Short-chain fatty acids (SCFA). Total pool of SCFA and iso-SCFA in the cecum (µmol) (*A*) and fecal concentrations of aforementioned metabolites (µmol/mg) (*B*). Data are expressed as means ± SE. Data with different letters above the bars are significantly different ($P < 0.05$), according to post hoc one-way ANOVA.

accordingly, the intestinal SCFAs were increased as well. Coprococci are considered to be butyrate producers and might, thus, contribute to the improvement of glucose tolerance. We have previously observed that FOS was associated with higher *Akkermansia* levels (24, 26, 27); however, this effect has not been reproduced in this study. This suggests that an increase of this genus is not always necessary to obtain beneficial effects using prebiotic fibers (8). As discussed in previous papers (8, 12, 64), we advocate for caution when attributing specific effects to changes of a single taxonomic species. Especially in the context of dietary fibers or polyphenols, much broader functional modifications may be at play.

Consumption of dietary fibers is sometimes associated with gastrointestinal effects (e.g., bloating, borborygmi, flatus, and diarrhea), particularly at high-consumption levels. These effects are due to the production of gases by fermentation, as well as osmotic effects in the large intestine. In this study, we did not observe any adverse effects or discomfort in the mice at the end of the follow-up, indicating that maintaining a final fiber consumption of 10% (fiber/food) was well tolerated.

In conclusion, we show that two different fibers with similar overall properties (both nondigestible and soluble), and with similar metabolic outcomes (reduced fat mass, improved glucose homeostasis, reduced inflammation), may have very distinct mechanism of actions and result in quite different gut microbial profiles. This study was conducted in inbred mice, however; therefore, we should remain very careful about the possible extrapolation of these results to humans. Even so, we believe

these kind of experiments remain essential for gaining the necessary understanding of the complex processes involved in host metabolism, in general.

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AUTHOR CONTRIBUTONS

M.V.H., K.K., K.C., A.E., and P.D.C conceived and designed research; M.V.H., A.E., and P.V. performed experiments; M.V.H., P.V., M.M., A.E., and P.D.C. analyzed data; M.V.H., K.K., K.C., P.V., M.M., A.E., and P.D.C. interpreted results of experiments; M.V.H. and A.E. prepared figures; M.V.H., A.E., and P.D.C. drafted manuscript; M.V.H., K.K., K.C., M.D., P.V., M.M., N.M.D., A.E., and P.D.C. edited and revised manuscript; M.V.H., K.K., K.C., M.D., P.V., M.M.,N.M.D., A.E., and P.D.C. approved final version of manuscript.

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