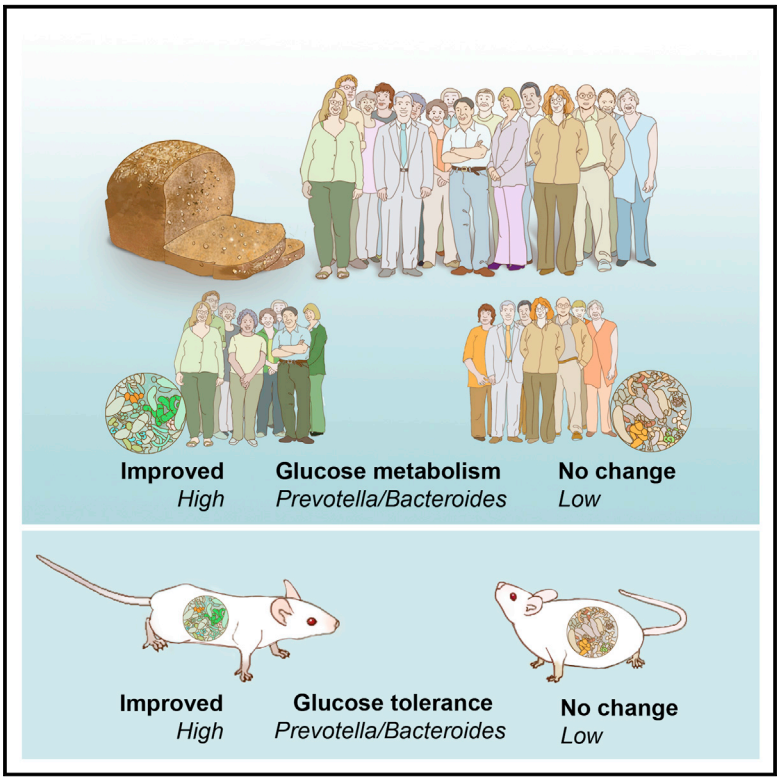
Clinical and Translational Report

Cell Metabolism

Dietary Fiber-Induced Improvement in Glucose Metabolism Is Associated with Increased Abundance of *Prevotella*

Graphical Abstract



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In Brief

Diet affects the gut microbiota composition, though large inter-individual variations exist. Kovatcheva-Datchary et al. reveal that subjects with improved glucose metabolism after barley kernel supplementation have increased *Prevotella* in their gut microbiota. *Prevotella* plays a direct role in the beneficial response, supporting the importance of personalized approaches to improve metabolism.

Highlights

- *Prevotella*/*Bacteroides* is associated with a beneficial response to barley kernels
- *Prevotella*-enriched microbial interactions are higher in barley kernel responders
- *Prevotella* protects against *Bacteroides*-induced glucose intolerance
- *Prevotella* promotes increased hepatic glycogen storage in mice

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Dietary Fiber-Induced Improvement in Glucose Metabolism Is Associated with Increased Abundance of *Prevotella*

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SUMMARY

The gut microbiota plays an important role in human health by interacting with host diet, but there is substantial inter-individual variation in the response to diet. Here we compared the gut microbiota composition of healthy subjects who exhibited improved glucose metabolism following 3-day consumption of barley kernel-based bread (BKB) with those who responded least to this dietary intervention. The *Prevotella*/*Bacteroides* ratio was higher in responders than non-responders after BKB. Metagenomic analysis showed that the gut microbiota of responders was enriched in *Prevotella copri* and had increased potential to ferment complex polysaccharides after BKB. Finally, germ-free mice transplanted with microbiota from responder human donors exhibited improved glucose metabolism and increased abundance of *Prevotella* and liver glycogen content compared with germ-free mice that received non-responder microbiota. Our findings indicate that *Prevotella* plays a role in the BKB-induced improvement in glucose metabolism observed in certain individuals, potentially by promoting increased glycogen storage.

INTRODUCTION

The gut microbiota interacts with the diet and impacts host physiology and metabolism (Tremaroli and Bäckhed, 2012). Accumulating data have demonstrated that the gut microbiota is altered in individuals with obesity (Cotillard et al., 2013; Le Chatelier et al., 2013), type 2 diabetes (Karlsson et al., 2013; Qin et al., 2012), and stroke (Wang et al., 2011), suggesting that the gut microbiota may be an important environmental factor contributing to development of metabolic diseases. A direct link between gut microbiota and metabolic disease has been established using germ-free (GF) mice, which have reduced adiposity and are protected against developing diet-induced obesity (Bäckhed et al., 2004, 2007).

Gut microbial ecology is to a large extent modulated by diet in humans (David et al., 2014): high intake of fat and protein is associated with increased levels of *Bacteroides*, whereas high fiber intake is associated with increased levels of *Prevotella* (De Filippo et al., 2010; Koeth et al., 2013; Wu et al., 2011). Dietary interventions are thus a potential tool to modulate gut microbiota and further impact host health (Dewulf et al., 2013; Kovatcheva-Datchary and Arora, 2013). We have previously shown that a barley kernel-based evening meal (rich in non-starch polysaccharides and resistant starch) improves glucose tolerance in healthy subjects with a normal body mass index (BMI) (Johansson et al., 2013; Nilsson et al., 2006, 2008) and provided evidence of a link between colonic fermentation and glucose metabolism (Johansson et al., 2013). However, there is large inter-individual variation in the response to diet (Lampe et al., 2013), and recent studies have indicated that the gut microbial composition could be used to identify those subjects who would benefit from dietary interventions (Korpela et al., 2014; Le Chatelier et al., 2013; Salonen et al., 2014).

Here we compared the gut microbiota composition of healthy subjects who exhibited improved glucose metabolism following barley kernel-based bread (BKB) with those who responded least to this dietary intervention to determine if differences in gut bacteria could explain the beneficial effect of BKB in some individuals.

RESULTS AND DISCUSSION

BKB Intervention Improves Glucose Metabolism in a Subset of Healthy Humans

We have previously shown that BKB consumption is associated with improved postprandial glucose metabolism (Nilsson et al., 2006), and in this study we selected two groups of individuals according to their response to BKB. We first measured postprandial glucose and insulin responses in 39 healthy individuals following three consecutive days of dietary intervention with BKB or white wheat flour bread (WWB) in a cross-over study (biometric measurements of these subjects are listed in Table S1). As expected, mean blood glucose and serum

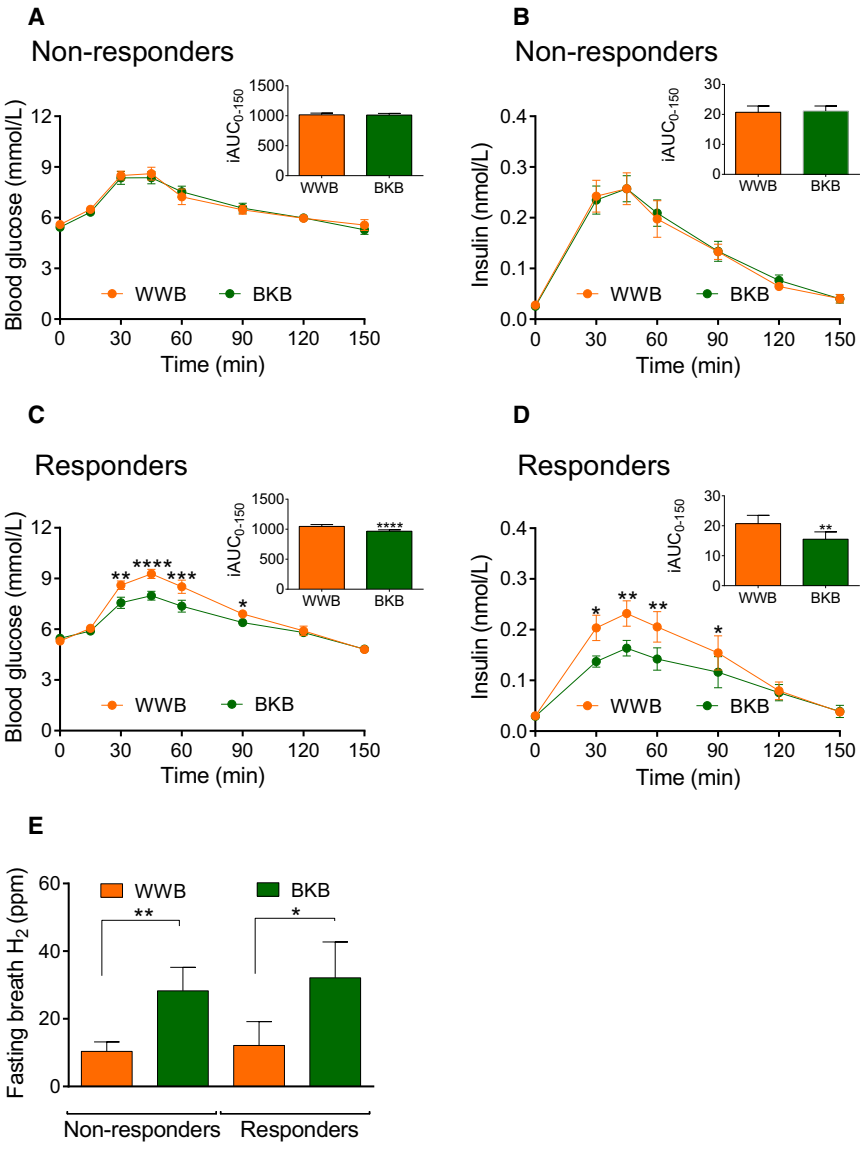

Figure 1. Glucose and Insulin Profiles in Non-responders and Responders after a Standardized Breakfast following 3-Day Consumption of WWB or BKB

(A–E) Blood glucose and serum insulin responses (A–D) and fasting breath hydrogen (H_2) (E) in non-responders (n = 10) and responders (n = 10) following a standardized breakfast after 3-day consumption of WWB or BKB. Data are mean ± SEM (*p < 0.05; **p < 0.01; ***p < 0.001; and ****p < 0.0001; Student's t test). Related to Figures S1 and S3 and Table S1.

insulin responses measured on day 4 after a standardized breakfast were improved following BKB compared with WWB in the total group (Figures S1A and S1B). In addition, breath hydrogen excretion measured at breakfast was higher following consumption of BKB compared with WWB (Figure S1C). From the total group, we selected the ten subjects who showed the least or no improvement in glucose or insulin responses (termed non-responders; Figures 1A and 1B) and the ten subjects who showed the most pronounced improvement in glucose and insulin responses (termed responders; Figures 1C and 1D) after the standardized breakfast following BKB compared with WWB. Further details about the selection criteria are included in Experimental Procedures. Fasting breath hydrogen increased in both groups following consumption of BKB (Figure 1E), indicating that the presence of indigestible carbohydrates increased the fermentative activity of the colonic microbiota in both responders and non-responders.

16S rRNA Sequencing of the Gut Microbiota Shows Increased *Prevotella* in Responders after BKB

To investigate if responders and non-responders differed in their gut microbiota composition, we analyzed fecal samples of these two groups by 454/Roche pyrosequencing of 16S rDNA genes. Principal coordinate analysis (PCoA) of the fecal microbiota sequencing data based on the individual unweighted (presence/absence information) UniFrac distances (Lozupone et al., 2006) clustered primarily by subjects (Figure S2A), suggesting that each individual's microbial community comprises distinct assemblages of microbial groups, in agreement with previous studies (Wu et al., 2011). We further assessed the microbial communities based on the unweighted and weighted UniFrac distances among responders and non-responders on WWB, BKB, and at baseline. We showed that supplementation of the diet with WWB or BKB affected the microbial community in the responders but not in the non-responders (Figures 2A and S2B). Weighted UniFrac distance between groups revealed that BKB ingestion compared to WWB ingestion led to significant changes in the relative abundance of specific members of the fecal microbiota in the responders but not in the non-responders (Figure 2A).

To further address this finding, we next analyzed the gut microbiota composition of the responders and the non-responders at baseline and after BKB and WWB consumption. Firmicutes and Bacteroidetes were the most common phyla in both responders and non-responders, and we did not observe different abundance at the phylum level between responders and non-responders at baseline or after WWB consumption (Figure 2B). However, after BKB consumption, the abundance of Bacteroidetes increased in the responders but not in the non-responders (Figure 2B). Bacteroidetes consists of two main genera, *Bacteroides* and *Prevotella*, and the increase in Bacteroidetes following BKB intervention in responders was attributed to a bloom of *Prevotella* (Figure 2C). *Prevotella* did not increase in the non-responders following BKB intervention

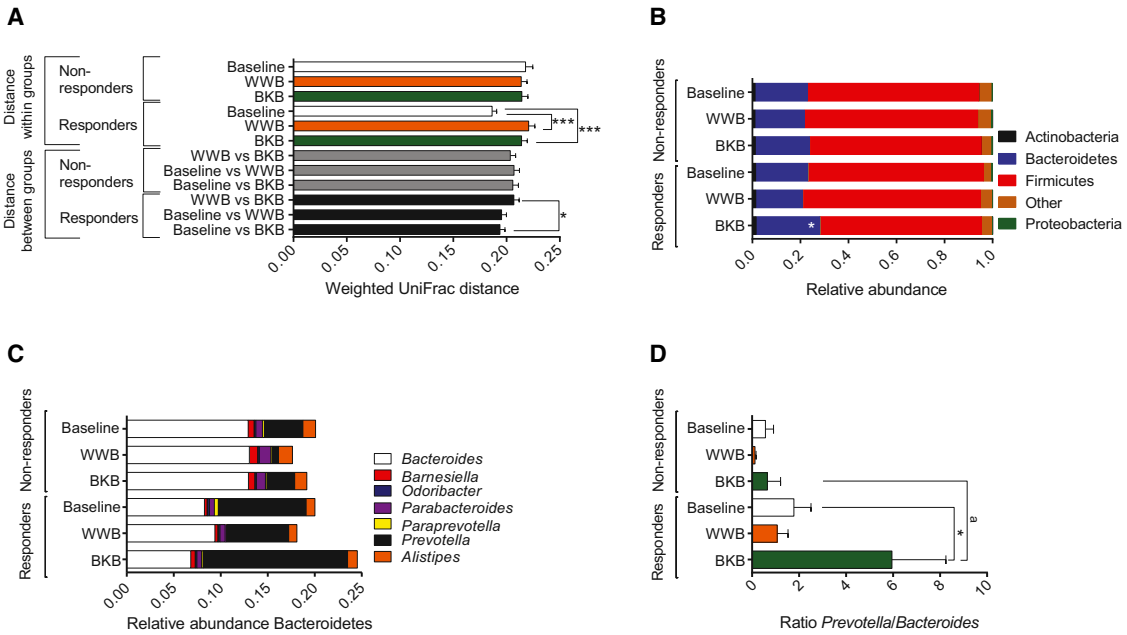

Figure 2. Altered Gut Microbiota in Non-responders and Responders following 3-Day Consumption of WWB or BKB

(A) Weighted UniFrac distances in non-responders (n = 10) and responders (n = 10) at baseline and after 3-day consumption of WWB and BKB based on bacterial V1-V2 16S rRNA pyrosequencing data. Data are mean ± SEM (*$p < 0.05$; ***$p < 0.001$; Student's t test with 1,000 Monte Carlo simulations).

(B) Relative abundance of the major microbial phyla in non-responders and responders at baseline and after 3-day consumption of WWB and BKB (*$p < 0.05$ baseline versus BKB; Wilcoxon matched-pairs signed rank test corrected for FDR).

(C and D) Bacteroidetes phyla (C) and *Prevotella*/*Bacteroides* (D) ratio in non-responders and responders at baseline and after 3-day consumption of WWB and BKB. Data are mean ± SEM (*$p < 0.05$; Wilcoxon matched-pairs signed rank test; [a]$p = 0.056$; Mann-Whitney U test). Related to Figure S2.

(Figure 2C). Subjects with high levels of *Prevotella* usually have lower levels of *Bacteroides* (Arumugam et al., 2011; Koren et al., 2013; Wu et al., 2011), which implies that taxa from these two genera compete for the same niche in the gut. We also observed an increase in *Prevotella*/*Bacteroides* ratio following BKB intervention versus baseline in the responders and a higher *Prevotella*/*Bacteroides* ratio after BKB in responders versus non-responders (Figure 2D). The increased *Prevotella*/*Bacteroides* ratio may be a consequence of the fact that responders have a higher habitual fiber intake, as long-term intake of diets rich in fiber are associated with increased abundance of *Prevotella* (David et al., 2014; De Filippo et al., 2010; Wu et al., 2011). However, both responders and non-responders exhibited similar glucose responses to the WWB intervention (Figures 1A and 1C).

We next investigated whether the observed differences in the abundance of *Bacteroides* and *Prevotella* between responders and non-responders were accompanied by more widespread shifts involving other members of the microbiota. At baseline, the gut microbiota of the responders versus non-responders had increased relative abundance of *Dorea* ($p < 0.05$, Kruskal-Wallis test with a false discovery rate [FDR] correction) and a trend toward an increase in *Roseburia* ($p < 0.1$, Kruskal-Wallis test with an FDR correction; Figure S2C); both *Dorea* and *Roseburia* are genera of the Firmicutes phyla that are known to metabolize dietary polysaccharides (Duncan et al., 2007). However, the abundance of these genera decreased following BKB supplementation (Figure S2C), indicating that they are not the key taxa involved in the degradation of indigestible carbohydrates in responders. Methanogenic archaea may also potentially contribute to the beneficial effects of the gut microbiota as they improve the efficiency of polysaccharide fermentation in the gut by preventing accumulation of H_2 and other end products of fermentation (Pimentel et al., 2012). We therefore also assessed the abundance of methanogenic archaea in responders and non-responders before and after consumption of WWB and BKB by quantifying the methyl coenzyme-M reductase gene. We found that the abundance of methanogens was higher after BKB ingestion in the responders compared with the non-responders (Figure S2D), which could contribute to the increased efficiency of polysaccharide fermentation in these individuals (Samuel and Gordon, 2006; Zhang et al., 2009).

We hypothesized that the different dominant bacterial lineages in responders and non-responders may result in variations in how the complex carbohydrates are fermented by the microbiota into metabolites such as short-chain fatty acids (SCFAs). However, we did not observe any significant differences in the fecal SCFA profile between responders and non-responders (Figure S2E), which may be related to the inter-individual variation in the gut microbiota composition as suggested in a previous dietary intervention study (Wu et al., 2014).

Phenotype of Responders and Non-responders Remains Stable for One Year

To investigate the stability of the microbiota over time in these subjects, we repeated the cross-over study with BKB and WWB in 14 of the 20 original subjects (7 responders and 7 non-responders) 12 months after the first study. Again we observed an

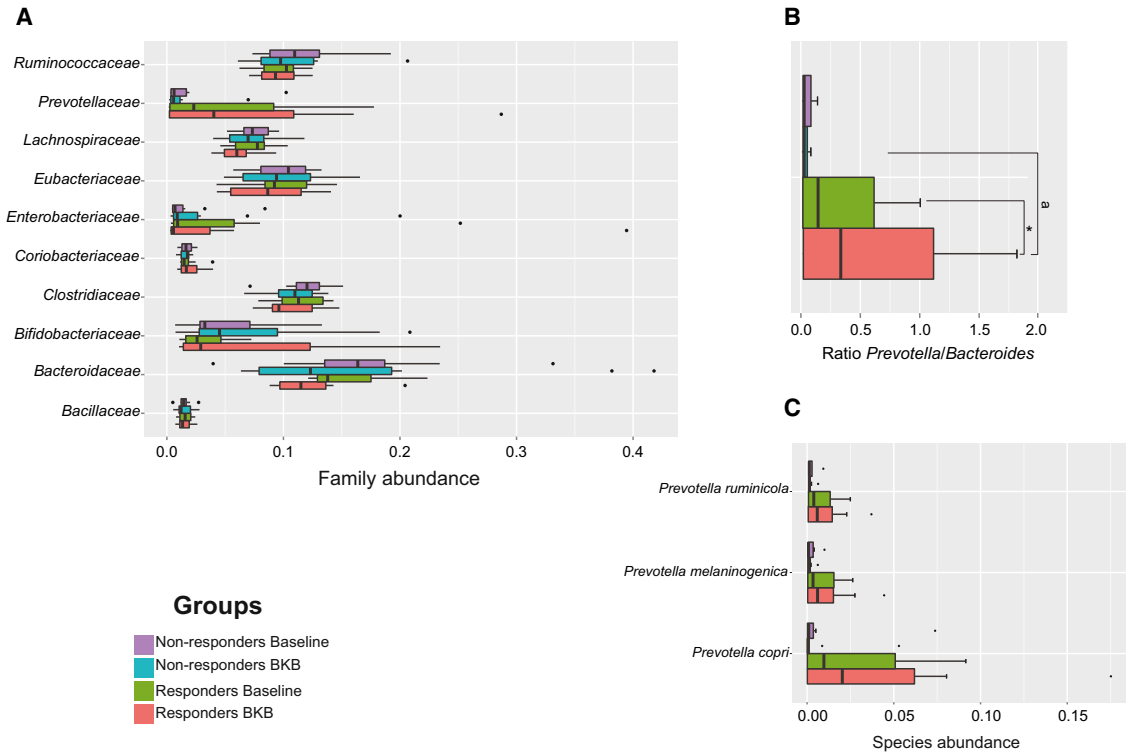

Figure 3. Taxonomic Composition of the Metagenome of Non-responders and Responders at Baseline and after BKB Consumption

(A) Microbiota composition (family level) of non-responders and responders at baseline and after BKB. The ten top abundant families are shown. Data are mean ± SD.

(B) *Prevotella*/*Bacteroides* ratio. Data are mean ± SD (*$p < 0.05$; Wilcoxon matched-pairs signed rank test; [a]$p = 0.056$; Mann-Whitney U test).

(C) *Prevotella* composition (species level) with total read abundance above 0.01. Data are mean ± SD. For statistics data, see Table S2. Related to Figure S4.

improved postprandial blood glucose response after BKB intervention in the subjects originally defined as responders but not in those originally defined as non-responders (Figures S3A and S3B). In addition, there was a trend toward an increase in the *Prevotella*/*Bacteroides* ratio (measured by 16S qPCR) at this time point after BKB in the responders versus the non-responders (Figure S3C), indicating that the microbiota remained relatively stable over time.

Shotgun Metagenome Sequencing of the Gut Microbiota Shows Increased *Prevotella copri* in Responders and Functional Changes in the Metagenome in Response to BKB

To validate the differences between the microbiota of non-responders and responders, we also analyzed the fecal microbiota by shotgun metagenome sequencing. We generated on average of 10.4 million (±0.89 SD) paired-end reads per subject (Table S2). Consistent with our 454-sequencing data, there was a trend toward enrichment of *Prevotellaceae* in the metagenome of responders versus non-responders, and there was a reduced abundance of *Bacteroidaceae* after BKB compared with baseline in responders but not in non-responders (Figure 3A; Table S2). The *Prevotella*/*Bacteroides* ratio was higher after BKB compared with baseline in responders and after BKB in responders versus non-responders (Figure 3B). We also showed that *Prevotella copri* was the most abundant of the *Prevotellaceae* species in the responders (Figure 3C; Table S2).

We further studied the functional changes induced in the metagenome of non-responders and responders after BKB consumption compared to baseline and quantified the abundance of genes encoding glycoside hydrolases, key enzymes in carbohydrate digestion (Dodd et al., 2011). After BKB, we observed a significant increase in abundance of genes encoding xylan 1,4-beta-xylosidase, glucan endo-1,3-beta-D-glucosidase, glucan 1,6-alpha-glucosidase, licheninase, and cellulase in the responders but not in the non-responders (Table S2). These enzymes are essential for the digestion of beta-glucans and other complex polysaccharides in the BKB. These findings thus suggest that the dietary fibers present in BKB can be more efficiently digested by the metagenome of responders than non-responders and are in agreement with a recent metagenomics analysis that linked the functional repertoire of *Prevotella* to increased capacity of the gut microbiota to ferment complex polysaccharides from the diet (Rampelli et al., 2015).

Increased Network Complexity and *Prevotella*-Enriched Microbial Interactions in the Responders after BKB

To investigate if specific microbial co-occurrences could potentially explain differences in the response to BKB, we used correlation network analysis to study how *Bacteroides* and *Prevotella*

species interact with other species in the microbiota of non-responders and responders at baseline and after BKB. The resulting networks showed that the number of nodes (species) and edges (interactions [correlations] between species) was higher in the non-responders than in the responders at baseline and after BKB (Table S3). However, the number of positive interactions between species and the complexity of the interactions (as shown by the average number of neighbors and the network density [normalization of the average number of neighbors]) increased in the responders compared with the non-responders after BKB (Table S3).

By using the network centralization parameter, which provides insight into how the network density is distributed, we observed that the interaction density was most centralized in the responders after BKB (Table S3; Figure S4). We observed a shift in centrality from *Bacteroides* in non-responders to *Prevotella* in responders; the hub nodes (nodes with the most interactions) were *Bacteroides* species in the non-responders and *Prevotella* species in the responders (Figure S4). The network analysis also revealed that *Prevotella* species were highly abundant in the responders at baseline, whereas *Bacteroides* species were dominant in the non-responders (Table S3; Figure S4). *P. copri*, which was highly abundant in the metagenome of responders (Figure 3C), was present in all individuals at baseline but was more than 2-fold higher in the responders and increased after BKB (in terms of abundance and number of interactions with other species) only in the responders (Table S3; Figure S4).

Changes in the abundance of microbial species in response to diet depend not only on their interactions with other members of the community, but also on the stability of those interactions in response to disturbances and how the population will cope afterward (Harmon et al., 2009). The network analysis showed that *Prevotella* and *Bacteroides* were competing (i.e., negatively correlated) with species from the Actinobacteria, Firmicutes, and Proteobacteria phyla in both responders and non-responders at baseline (Table S3; Figure S4). Members of the Actinobacteria and Firmicutes phyla have been suggested to include species that are primary degraders of complex carbohydrates (Flint et al., 2012; El Kaoutari et al., 2013; Leitch et al., 2007; Ze et al., 2012), suggesting that *Prevotella* and *Bacteroides* may compete with those species for the same substrate. However, after BKB in the responders, *Prevotella* species co-occurred not only with other *Prevotella* species, but also with many species from the Actinobacteria, Firmicutes, Proteobacteria, and Archaea phyla (Table S3; Figure S4), suggesting that the niche has expanded. We therefore speculate that *Prevotella* may positively interact with the other members of the community to promote increased carbohydrate fermentation in the responders. By contrast, after BKB in the non-responders, the network remained dominated by *Bacteroides* species, and the number of negative interactions between the microbes increased (Table S3), suggesting that more species compete for the same substrate in the non-responders.

Prevotella Improves Glucose Metabolism in Mice

To explore the potential of *Prevotella* species to improve glucose metabolism, we gavaged mice with *P. copri* or control daily for 7 days and compared their glucose tolerance. In mice fed a standard chow diet (low in fat and protein and high in dietary fibers), glucose tolerance was improved following treatment with live human feces-derived *P. copri* (DSM 18205) compared with heat-killed *P. copri* (experiment performed in two mouse strains; Figures 4A and 4B). By contrast, *P. copri* did not have an effect on glucose tolerance in mice fed a diet high in fat and low in dietary fiber (Figure 4C). These results support the concept that dietary fibers are important for *Prevotella* to colonize the gut and to promote the beneficial effects on glucose metabolism.

To directly investigate the effect of *Bacteroides* and *Prevotella* species on glucose metabolism, we mono- and bi-colonized GF mice with human feces-derived *Bacteroides thetaiotaomicron* (ATCC 29148) and *P. copri* (DSM 18205) for 14 days. All mice were fed a standard chow diet. While *B. thetaiotaomicron* utilizes a vast range of plant and animal glucans (Martens et al., 2008, 2011; Xu et al., 2003), it lacks essential genes for degradation of complex polysaccharides compared to other *Bacteroides* strains (Dodd et al., 2010; Martens et al., 2011). By contrast, *P. copri* possesses a number of enzymes and gene clusters essential for fermentation and utilization of complex polysaccharides (Dodd et al., 2011). Both bacteria colonized the gnotobiotic mice to similar extents, and bi-colonization resulted in lower levels of both bacteria (Figure 4D). Glucose tolerance was impaired in mice colonized with *B. thetaiotaomicron* compared with mice colonized with *P. copri* (Figure 4E). Importantly, *B. thetaiotaomicron*-induced impairment of glucose tolerance was prevented by simultaneous co-colonization with *P. copri* (Figure 4E). The levels of *B. thetaiotaomicron* did not correlate with area under the curve in the glucose tolerance test in either mono-colonized ($R^2 = 0.11$; $p = 0.22$) or bi-colonized ($R^2 = 0.10$; $p = 0.42$) mice, indicating that the lower *B. thetaiotaomicron* levels in bi-colonized mice could not account for the improvement in glucose tolerance. These data suggest that the different capacity of *P. copri* and *B. thetaiotaomicron* to perform carbohydrate fermentation in the mouse gut contributes to the differences observed in terms of glucose metabolism and highlight the importance of *P. copri* in improving glucose tolerance.

Bacteroides is known to produce propionate, whereas *Prevotella* produces succinate (Macy et al., 1978). As expected, propionate was the major metabolite in the cecum of mice mono-colonized with *B. thetaiotaomicron*, whereas mice mono-colonized with *P. copri* had high cecal levels of succinate and no propionate production (Figure S5A). To investigate whether the beneficial effects of *Prevotella* were mediated by the high production of succinate, we generated a *B. thetaiotaomicron* mutant, BT1686-89, that was unable to convert succinate into propionate. Although colonization of GF mice with this strain resulted in high cecal levels of succinate (Figure S5A), the glucose response was not improved compared with mice colonized with wild-type *B. thetaiotaomicron* (Figure S5B). Thus, the high levels of succinate observed in mice mono-colonized with *P. copri* are not sufficient to explain the beneficial effects of this bacterial species.

Impaired Glucose Tolerance Is Associated with Altered Gene Expression in Proximal Colon and Liver

To identify genes that could potentially explain the difference in glucose tolerance between mice colonized with *B. thetaiotaomicron* and *P.copri*, we performed RNA sequencing (RNA-seq) of proximal colon and liver biopsies from the

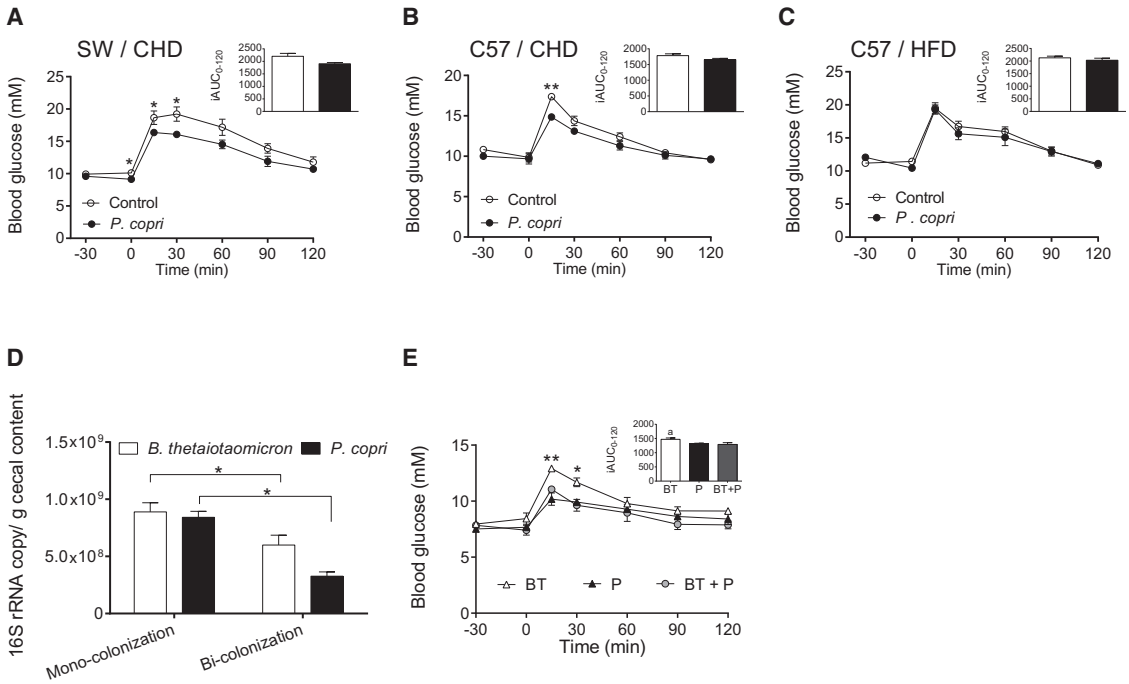

Figure 4. Beneficial Effects of *Prevotella* on Glucose Metabolism in Mice Are Diet Dependent, and *P. copri* Protects against *B. thetaiotaomicron*-Induced Glucose Intolerance

(A and B) Oral glucose tolerance test in (A) Swiss Webster and (B) C57BL/6 mice fed standard chow diet and gavaged with heat-killed *P. copri* or live *P. copri* for 7 days. Data are mean ± SEM (*$p < 0.05$, **$p < 0.01$; Student's t test).

(C) Oral glucose tolerance test in C57BL/6 mice fed high-fat diet and gavaged with *P. copri* or media for 7 days. Data are mean ± SEM.

(D) Levels of *B. thetaiotaomicron* and *P. copri* determined by 16S rRNA specific qPCR following 14-day colonization of GF mice. Data are mean ± SEM (*$p < 0.05$; Mann-Whitney U test).

(E) Oral glucose tolerance test following 14-day colonization of GF mice with *B. thetaiotaomicron*, *P. copri*, or both strains in Swiss Webster mice fed chow diet. Data are mean ± SEM (*$p < 0.05$; **$p < 0.01$; Student's t test; $^{a}p < 0.05$ BT versus BT+P; Student's t test). Related to Figure S5.

mono- and bi-colonized mice and GF mice. We generated an average of 14 million reads per sample, and more than 90% of these reads were mapped to the mouse genome (Table S4). We focused our analysis on genes that were significantly up- or downregulated by colonization with *B. thetaiotaomicron* but were not significantly altered by colonization with *P. copri* or by bi-colonization with *B. thetaiotaomicron* and *P. copri* (all compared to GF mice); we identified 601 such genes in the proximal colon and 206 in the liver (Table S4).

The most significantly affected gene in the proximal colon was *G6pc*, which encodes for glucose-6-phosphatase, a key enzyme in intestinal gluconeogenesis that induces beneficial effects on glucose and energy homeostasis (De Vadder et al., 2014); it was downregulated by colonization with *B. thetaiotaomicron* (Table 1). Two of the most significantly affected genes with an impact on glucose metabolism in the liver were *Sorbs1*, which encodes for sorbin and SH3 domain containing 1, a key protein in insulin signaling (Lesniewski et al., 2007; Menzaghi et al., 2008), and *Pygl*, which encodes for liver glycogen phosphorylase, an enzyme that catalyzes the breakdown of glycogen to glucose (Bollen et al., 1998; Izumida et al., 2013); both genes were upregulated by colonization with *B. thetaiotaomicron* (Table 1). We next investigated if the changes in *Pygl* expression were reflected by alterations in glycogen storage in liver and showed that mice mono-colonized with *B. thetaiotaomicron* had significantly lower hepatic glycogen content compared with mice mono-colonized with *P. copri* (0.60 ± 0.14 versus 2.25 ± 0.21 mg/g liver; $p < 0.0001$).

Transmission of the Beneficial Effect of BKB on Host Glucose Metabolism by Transplantation of the Human Gut Microbiota

To investigate the potential of the non-responder- and responder-associated microbiota to promote improvement in host glucose metabolism after BKB, we transferred microbiota from two non-responder and two responder human donors at baseline and after BKB into GF mice. Postprandial blood glucose and serum insulin responses after BKB and WWB and the *Prevotella*/*Bacteroides* ratio at baseline and after WWB and BKB of the selected non-responder and responder donors are shown in Figure S6. We did not observe any differences in the postprandial blood glucose response of mice colonized for 14 days with microbiota from either of the non-responder donors taken at baseline or after BKB (Figures 5A and S7A). By contrast, we observed an improved glucose response in mice colonized for 14 days with microbiota from the responder donors after BKB compared with mice colonized with microbiota from the same donors taken at baseline (Figures 5B and S7B).

We also used 16S qPCR to measure the levels of *Prevotella* and *Bacteroides* in the cecum of mice colonized with non-responder

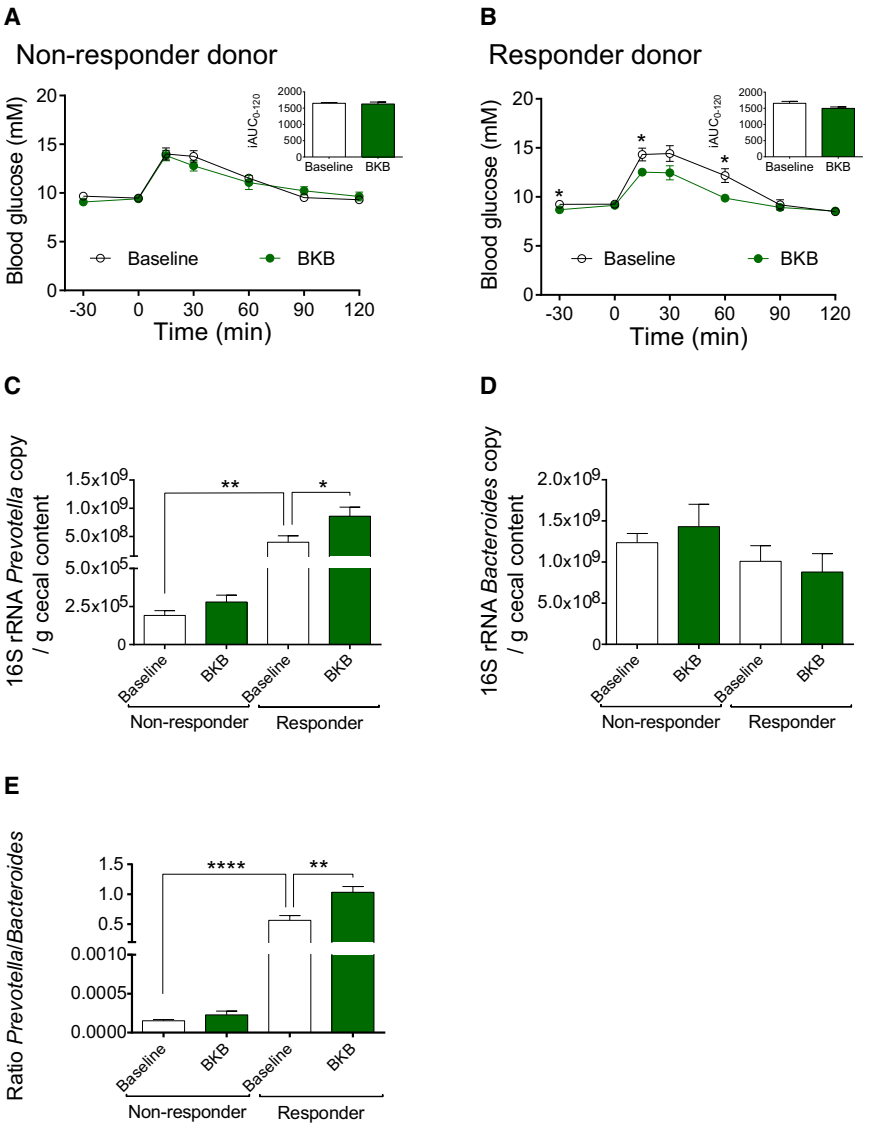

Figure 5. Transfer of a Responder's Gut Microbiota Taken after BKB to GF Mice Improves Glucose Tolerance

(A and B) Oral glucose tolerance test in recipient Swiss Webster male mice colonized with (A) non-responder donor 1 or (B) responder donor 1 microbiota taken at baseline and after BKB. Data are mean ± SEM (*$p < 0.05$; Student's t test).

(C and D) Levels of (C) *Prevotella* and (D) *Bacteroides* in the cecum of recipient mice after 14 days of colonization, determined by 16S rRNA specific qPCR. Data are mean ± SEM (*$p < 0.05$; **$p < 0.01$; Mann-Whitney U test).

(E) *Prevotella* to *Bacteroides* ratio. Data are mean ± SEM (**$p < 0.01$; ****$p < 0.0001$; Mann-Whitney U test). Related to Figures S6 and S7.

and responder microbiota at baseline and after BKB. The levels of *Prevotella* were higher in mice colonized with responder versus non-responder donor microbiota taken at baseline and were further increased in mice colonized with responder microbiota taken after BKB (Figures 5C and S7C). Levels of *Bacteroides* were slightly but not significantly higher in the cecum of the mice colonized with non-responder versus responder donor microbiota and were similar in mice colonized with microbiota taken at baseline or after BKB (Figures 5D and S7D). In addition, the *Prevotella*/*Bacteroides* ratio was significantly higher in mice colonized with responder versus non-responder donor microbiota taken at baseline and increased further in mice that received responder microbiota taken after BKB (Figures 5E and S7E). These observations further support our hypothesis that BKB-induced changes in the microbiota are sufficient to promote beneficial responses in the host and, specifically, that increased abundance of *Prevotella* after BKB improves glucose tolerance.

We also measured relative expression of genes that were differentially regulated in mice colonized with *B. thetaiotaomicron* and *P.copri* and that could potentially play a role in glucose metabolism (Table 1) in tissues from mice that received microbiota from responder and non-responder donors at baseline and after BKB. We identified significantly increased expression of *G6Pc* in proximal colon biopsies from the mice colonized with responder versus non-responder microbiota taken after BKB (Figure 6A). We also observed a significant decrease in *Sorbs1* in liver from mice colonized with responder and non-responder microbiota taken after BKB compared with the respective microbiota taken at baseline (Figure 6B). However, *Pygl* decreased significantly in liver from mice colonized with responder but not non-responder microbiota taken after BKB versus microbiota taken at baseline (Figure 6C).

We next investigated if the changes in *Pygl* expression were reflected by alterations in glycogen storage in liver from mice colonized with responder versus non-responder microbiota. We showed that hepatic glycogen content was significantly higher in mice colonized with responder microbiota taken after BKB than in mice colonized with responder microbiota taken at baseline or with non-responder microbiota taken after BKB or at baseline (Figure 6D). These findings suggest that *Prevotella* may contribute to the BKB-induced improvement in glucose metabolism observed in some (but not all) individuals by promoting increased glycogen storage.

Perspective

In summary, we have shown that alterations in the gut microbiota contribute to the beneficial metabolic benefits induced by short-term dietary supplementation with indigestible carbohydrates in a subgroup of healthy humans. Our study also highlights the potential importance of individual members of the gut microbiota (both in terms of abundance and their interactions with other microbial species) to host metabolism and responses to changes in diet. Our findings support the importance of personalized

Table 1. Top Ten Significantly Regulated Genes in Biopsies from Proximal Colon and Liver of Mice Mono-Colonized with *B. thetaiotaomicron*

Tissue	Gene Name	Log_2 Fold change	p value	p-adj	Description
Proximal colon	*G6pc*	−2.75	1E-10	5.4E-08	glucose-6-phosphatase, catalytic
	Apoa4	−3.51	4E-08	8.1E-06	apolipoprotein A-IV
	Sprr2a3	1.15	5E-08	1E-05	small proline-rich protein 2A3
	Saa2	−1.09	8E-08	1.6E-05	serum amyloid A 2
	Pepd	−0.57	8E-08	1.6E-05	peptidase D
	Enpep	−1.01	2E-07	2.9E-05	glutamyl aminopeptidase
	Gsdmcl2	2.41	3E-07	5E-05	gasdermin C-like 2
	Nfkbia	−0.39	4E-07	6.1E-05	nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor
	Slc38a2	0.36	1E-06	0.00015	solute carrier family 38, member 2
	Mfsd8	−0.45	1E-06	0.00015	major facilitator superfamily domain containing 8
	Edn1	−0.82	2E-06	0.00025	Endothelin
Liver	*Sorbs1*	0.60	5E-06	0.00033	sorbin and SH3 domain containing 1
	Bdh2	0.97	1E-05	0.00062	3-hydroxybutyrate dehydrogenase, type 2
	Chka	−0.81	1E-05	0.00068	choline kinase alpha
	Camk2b	1.57	1E-05	0.00073	calcium/calmodulin-dependent protein kinase II, beta
	Cd9	−0.69	2E-05	0.00102	CD9 antigen
	Lrig2	−0.60	3E-05	0.00138	leucine-rich repeats and immunoglobulin-like domains 2
	Hist1h1e	−0.85	5E-05	0.00188	histone cluster 1, H1e
	Ergic1	0.41	6E-05	0.00223	endoplasmic reticulum-golgi intermediate compartment (ERGIC) 1
	Pygl	0.41	7E-05	0.00244	liver glycogen phosphorylase
	Atp6v0e2	1.10	8E-05	0.00268	ATPase, H^+ transporting, lysosomal V0 subunit E2

Related to Table S4.

approaches to improve host metabolism and emphasize the possibility of using a synbiotic approach involving both diet and microbiota to treat metabolic disorders. It is important to note that our findings need to be validated in a larger cohort. A further step will be to perform a prospective follow-up study that uses gut microbiota composition as a strategy to identify the individuals who would benefit from a dietary intervention.

EXPERIMENTAL PROCEDURES

Test Subjects and Study Design

In total, 39 subjects (6 men, 33 women) participated in the study (Table S1). The inclusion criteria were: age 50–70 years, BMI normal to slightly overweight (BMI 18–28 kg/m^2), fasting plasma glucose $\leq$ 6.1 mmol/l, non-smoker, overall healthy, and no known metabolic disorders or food allergies. Anti-hypertensive medications and prescription-free painkillers without any anti-inflammatory action were accepted. None of the participants in the study had a partner or spouse who participated in the study. The study was approved by the Regional Ethical Review Board in Lund, Sweden (Reference 2010/457).

We used a randomized cross-over design to test the effect of BKB versus WWB on glucose metabolism. The composition of the BKB and WWB and the experimental procedure have been described earlier (Nilsson et al., 2015). In brief, each subject participated in two 3-day interventions where they consumed either BKB or WWB in a random order separated by a minimum of 2 weeks. After the evening meal on day 3 of the intervention period, the subjects fasted until the standardized breakfast, which was served the next morning at the experimental department (the test day). Fasting blood samples were collected and breath hydrogen was registered before breakfast was served at 8:00. Finger-prick capillary blood samples were taken for determination of blood glucose (HemoCue B-glucose). Venous blood samples were collected to determine serum insulin. Serum was separated by centrifugation and immediately stored in a freezer (−40°C) until analysis. Breath hydrogen in expired air was measured using a Gastro+ (Bedfont EC60 Gastrolyzer). Results on glucose and insulin responses and breath hydrogen excretion in 20 of these subjects have been reported previously (Nilsson et al., 2015).

Fecal samples were collected before the dietary intervention (defined as baseline) and from the first defecation that occurred on day 4 after BKB or WWB (the test days). The sample was immediately frozen and handed over to the experimental department within 24 hr for continued storage at −80°C until analysis.

Selection of Responders and Non-responders

From the total group, we selected a group of ten responders (two men, eight women) who showed all of the following responses after the standardized breakfast following BKB compared with WWB: (1) incremental blood glucose area (IAUC, 0–90 min) decreased by at least 25%, (2) total AUC (0–90 min) decreased, and (3) insulin IAUC (0–90 min) decreased by at least 15%. From the remaining 29 subjects, we selected a group of ten non-responders (all women), defined as those who showed the least improvements in glucose and/or insulin responses after the standardized breakfast following BKB compared with WWB. Of these, neither the glucose nor insulin responses were affected by BKB in four subjects, the glucose response but not the insulin response was slightly affected in three subjects, and the insulin response but not the glucose response was slightly affected in three subjects.

Genomic DNA Purification, Amplification, Pyrosequencing, and Data Analysis

After isolation of genomic DNA from feces using repeated bead beating (Salonen et al., 2010), the V1-V2 variable region of the 16 rRNA gene was amplified using the 27F and 338R primers as previously reported (De Vadder et al., 2014). The pooled products were sequenced using the 454 GS FLX

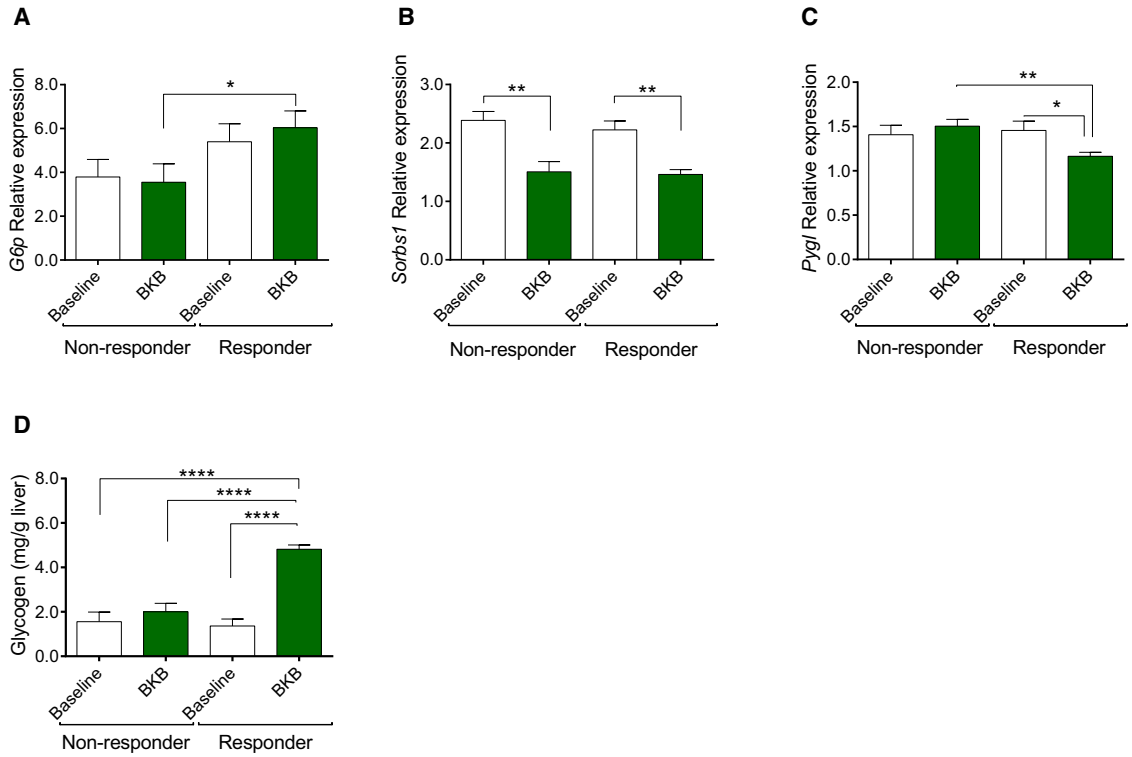

Figure 6. Transfer of a Responder's Gut Microbiota Taken after BKB to GF Mice Promotes Hepatic Glucose Storage as Glycogen

(A–C) Relative expression of (A) *G6pc* in proximal colon, (B) *Sorbs1* in liver, and (C) *Pygl* in liver of recipient mice colonized with non-responder or responder microbiota taken at baseline and after BKB. Data are mean ± SEM (*$p < 0.05$;**$p < 0.01$; Mann-Whitney U test).

(D) Glycogen in the liver of recipient mice colonized with non-responder or responder microbiota taken at baseline and after BKB. Data are mean ± SEM (****$p < 0.0001$; ANOVA with Tukey post-hoc test).

System and Titanium Chemistry (Roche) at National Genomics Infrastructure (Stockholm).

Raw data were quality filtered to remove sequences that were shorter than 200 nucleotides, were longer than 1,000 nucleotides, or contained primer mismatches, ambiguous bases, uncorrectable barcodes, or homopolymer runs in excess of six bases. Quality-filtered reads were trimmed from their 454 adapters and barcode sequences and were analyzed with the software package Quantitative Insights into Microbial Ecology (QIIME) (version 1.5.0). In total, 755,963 reads passed the quality filter (mean 12,599 sequences/sample). The sequencing data were de-noised with denoise_wrapper.py in QIIME.

Sequences were assigned to operational taxonomic units (OTUs) using UCLUST with a 97% threshold of pairwise identity. The most abundant sequence was picked as representative for each OTU and was taxonomically assigned using the Ribosomal Database Project Classifier. Representative OTUs were aligned using Pynast and used to build a phylogenetic tree with FastTree, which was used to estimate β-diversity of samples (weighted and unweighted UniFrac).

Shotgun Metagenome Analysis

The metagenome DNA samples were sequenced in paired-end mode (2*151 bp) with NextSeq500 at the core facility of Gothenburg University. Each single read sequence was annotated individually for both functional and taxonomical profiles using the Metagenome rapid annotation (MG-RAST) server (Meyer et al., 2008). More detailed information of the analysis is included in the Supplemental Information.

Network Co-occurrence Analysis

For the network analysis, all possible Spearman's correlations between abundance of *Bacteroides* and *Prevotella* species with the other species in the microbiota were calculated in responders and non-responders at baseline and after BKB in each group separately (using data from the metagenomic analysis). To reduce network complexity, we only used species that had a minimum of five sequences in at $\geq$ 4 individuals (out of 10 in each group). All the statistical analyses were performed in R (R Core Team, 2015), and p values were corrected using the FDR method (Benjamini and Hochberg, 1995). An acceptable co-occurrence interaction was considered to have an absolute correlation coefficient of > 0.6 and an adjusted p value of < 0.05. Networks were visualized using Cytoscape 3.0.2 (Kofia et al., 2015) with a Prefuse Force Directed Layout. NetworkAnalyzer tool in Cytoscape was used to analyze the networks individually.

16S rRNA Quantitative PCR

16S rRNA quantitative PCR was performed with a CFX96 Real-Time System (Bio-Rad). All reactions were performed in duplicate in one run and in duplicate PCR runs. qPCR was performed as reported previously: for *Bacteroides* (Walker et al., 2011), for the methyl coenzyme-M reductase gene (Denman et al., 2007), for *Prevotella* (Matsuki et al., 2002), and for *B. thetaiotaomicron* (Samuel and Gordon, 2006). More detailed information of the analysis is included in the Supplemental Information.

Mouse Experiments with *P. copri*

We performed three experiments to test the effect of *P. copri* in mice. In the first experiment, adult Swiss Webster and C57BL/6 male mice (Taconic) on a chow diet were gavaged daily for 7 consecutive days with live or heat-killed control *P. copri* (five mice per group). In the second experiment, C57BL/6 male mice were fed a high-fat diet (40.6% kcal from fat; TD.09683 Harlan Teklad) for 4 weeks and gavaged for 7 consecutive days with live *P. copri* or media control. In the third experiment, 10- to 12-week-old GF Swiss Webster male mice on a chow diet were inoculated with single gavage of 10^8 CFU *P. copri* strain DSM18205 and/or *B. thetaiotaomicron* strain ATCC 29148 or the isogenic

B. thetaiotaomicron mutant strain (BT1686-89). Mono- and bi-colonized mice were housed in an iso-cage system for 14 days. An oral glucose tolerance test (OGTT) was performed at the end of all of these experiments. All mice experiments were performed using protocols approved by the University of Gothenburg Animal Studies Committee.

Transfer of Human Feces into Mice

Fecal samples from selected responders and non-responders at baseline and after BKB consumption (see Figure S6) were added to PBS buffer supplemented with reducing solution (Na_2S and cysteine dissolved in $NaHCO_3$ buffer) and transferred to 10- to 12-week-old GF Swiss Webster male and female mice (5–7 per group) via oral gavage. The mice were colonized for 14 days. An OGTT was performed at the end of the experiment. Liver, cecum, and colon were harvested, immediately snap-frozen in liquid nitrogen, and stored at −80°C until further processing.

RNA Extraction and RNA Sequencing

Mouse tissues from liver and proximal colon were homogenized in RLT buffer using a TissueLyzer (QIAGEN). RNA was isolated using the Qiacube (QIAGEN) following the manufacture protocol. RNA quantity and quality were examined with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies).

The RNA samples were sequenced at the genomics core facility of Gothenburg University in a paired-end mode with Nextseq500 (2*75). The qualities of the sequences (reads) were evaluated using MAXINFO algorithm in Trimmomatic software (Bolger et al., 2014). More detailed information is included in the Supplemental Information.

RT qPCR

cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). qRT-PCR reactions were set up in a 10 μl volume containing 1× SYBR Green Master Mix buffer (Thermo Scientific) and 900 nM gene-specific primers (or 300 nM L32 primers). Reactions were run on a CFX96 Real-Time System (Bio-Rad). Gene expression data were normalized to the ribosomal protein L32 using the $\Delta\Delta C_T$ method. The primer sequences are listed in the Supplemental Information.

Glycogen Measurement

Glycogen determination was carried out on grinded frozen liver homogenates as described previously (Pfleiderer, 1974).

Calculations and Statistical Methods

Values are presented as means ± SEM. For graph plotting and statistical analysis, we used GraphPad Prism (version 6, GraphPad Software) unless otherwise indicated. Statistical comparison of two groups was performed by Student's t test, Wilcoxon matched-pairs signed rank test, and Mann-Whitney U test; comparisons of three or more groups were performed by one-way analysis of variance (ANOVA) and corrected for multiple comparison with Tukey post-tests.

ACCESSION NUMBERS

The accession number for the 454 pyrosequencing sequences reported in this paper is NCBI: SRP062889. The accession number for the metagenome sequences reported in this paper is MG-RAST: 13068 (http://metagenomics.anl.gov/linkin.cgi?project=13068). The accession number for the RNA-seq sequences reported in this paper is NCBI: SRP064375.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, seven figures, and four tables and can be found with this article online at http://dx.doi.org/10.1016/j.cmet.2015.10.001.

AUTHOR CONTRIBUTIONS

P.K.D., A.N., I.B., and F.B. conceived and designed the project. P.K.D., A.N., and A.H. collected samples. P.K.D., A.N., R.A., Y.S.L., F.D.V., T.A., A.H., and E.M. performed experiments. P.K.D, A.N, R.A., Y.S.L, F.D.V, T.A., I.B., and F.B. analyzed and interpreted the data. P.K.D, A.N., I.B., and F.B. wrote the paper. All authors commented on the manuscript.

CONFLICTS OF INTEREST

A.N., I.B., and F.B. are founders and shareholders of ProPrev AB.

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