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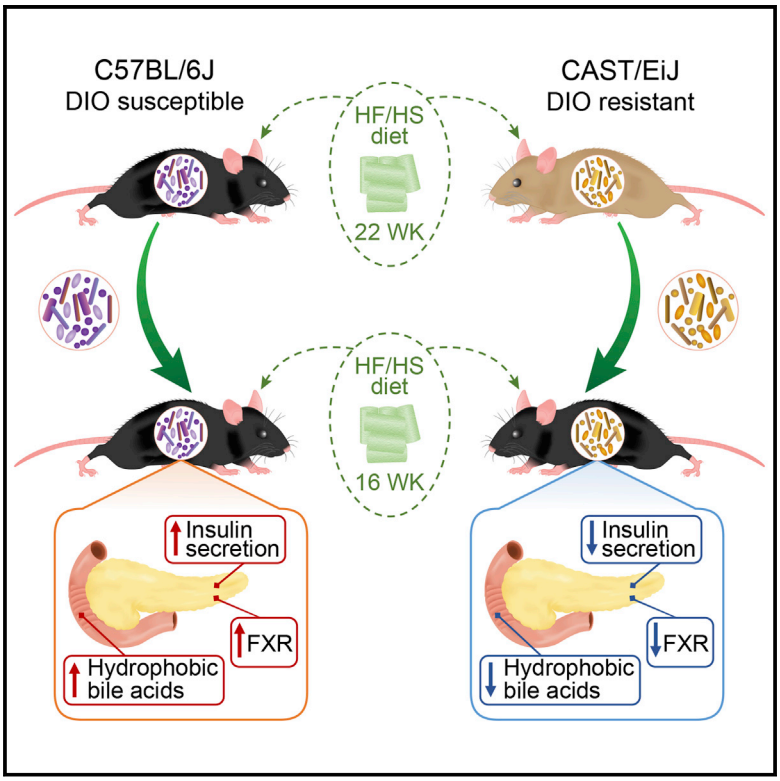
Host Genotype and Gut Microbiome Modulate Insulin Secretion and Diet-Induced Metabolic Phenotypes

Graphical Abstract



Highlights

- Host genotype affects the abundance of taxa associated with metabolic disease
- Gut microbiota affects susceptibility to diet-induced metabolic disease
- The gut microbiome modulates insulin secretion

Authors

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In Brief

Host genetics modulates the development of metabolic disease and shapes the composition of the gut microbiota. Kreznar et al. demonstrate that the gut microbiota contributes to strain-specific susceptibility to diet-induced metabolic disease and identify links between microbial metabolism and insulin secretion.

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Host Genotype and Gut Microbiome Modulate Insulin Secretion and Diet-Induced Metabolic Phenotypes

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SUMMARY

Genetic variation drives phenotypic diversity and influences the predisposition to metabolic disease. Here, we characterize the metabolic phenotypes of eight genetically distinct inbred mouse strains in response to a high-fat/high-sucrose diet. We found significant variation in diabetes-related phenotypes and gut microbiota composition among the different mouse strains in response to the dietary challenge and identified taxa associated with these traits. Follow-up microbiota transplant experiments showed that altering the composition of the gut microbiota modifies strain-specific susceptibility to diet-induced metabolic disease. Animals harboring microbial communities with enhanced capacity for processing dietary sugars and for generating hydrophobic bile acids showed increased susceptibility to metabolic disease. Notably, differences in glucose-stimulated insulin secretion between different mouse strains were partially recapitulated via gut microbiota transfer. Our results suggest that the gut microbiome contributes to the genetic and phenotypic diversity observed among mouse strains and provide a link between the gut microbiome and insulin secretion.

INTRODUCTION

The intestinal microbiota exerts a profound influence on development, physiology, and health (Clemente et al., 2012; Sommer and Bäckhed, 2013; Tremaroli and Bäckhed, 2012). Although there is substantial interpersonal variation in the composition of the gut microbiota among unrelated healthy subjects, sequencing studies have revealed distal gut community patterns associated with different pathological states, including obesity and diabetes (Ridaura et al., 2013; Qin et al., 2012; Karlsson et al., 2013). Remarkably, alterations in the intestinal microbiota composition have been shown to modulate insulin sensitivity (Vrieze et al., 2010), a key feature in metabolic disease and type 2 diabetes (T2D), and thus play a role in diabetes susceptibility.

Dietary components that are not efficiently absorbed in the proximal intestine reach the distal gut, where they are metabolized by gut microbes. Intestinal microbes impact our health in part by generating numerous metabolites from our diet. Short-chain fatty acids (SCFAs), mainly acetate, propionate, and butyrate, are produced through bacterial fermentation of dietary carbohydrates. SCFAs serve as energy and signaling molecules in the intestine and peripheral organs (den Besten et al., 2013). Specifically, SCFAs are important regulators of both energy and glucose homeostasis (den Besten et al., 2013; Koh et al., 2016). For example, butyrate improves insulin sensitivity (Gao et al., 2009; Hartstra et al., 2015) and T2D patients have reduced levels of butyrate-producing bacteria (Qin et al., 2012). Additionally, acetate modulates insulin secretion from β cells (Priyadarshini et al., 2015; Perry et al., 2016). While primarily associated with metabolic benefits, increased concentrations of butyrate and acetate have been found in the cecum of obese mice, suggesting an increased ability of the microbiome to harvest energy from the diet (Turnbaugh et al., 2006).

Gut microbes also impact host physiology by modifying bile acids (BAs) synthesized by the host (Houten et al., 2006; Kuipers et al., 2014; Ryan et al., 2014; Sayin et al., 2013). In addition to their role in emulsifying lipids, BAs function as hormones through their ability to activate nuclear hormone receptors (Parks et al., 1999) and G-coupled protein receptors (Kawamata et al., 2003). They modulate glucose homeostasis, lipid metabolism, energy expenditure, and intestinal motility (Kuipers et al., 2014). Primary BAs are synthesized from cholesterol in the liver (Russell, 2009), stored in the gallbladder, and secreted into the duodenum upon ingestion of a meal. The gut microbiota catalyzes the production of secondary BAs via deconjugation, dehydrogenation, epimerization, and dehydroxylation of primary BAs

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(Ridlon et al., 2006). BAs with different modifications vary in their ability to activate receptors and affect host physiology (Makishima et al., 1999; Kuipers et al., 2014). Subjects with T2D have altered circulating BA profiles. Treatment of T2D subjects with compounds that increase fecal excretion of BAs and modify BA composition improves their glycemic status (Handelsman, 2011).

Mouse genetics can be employed to explore the relationships between diet, host genetics, and metabolic responses (O'Connor et al., 2014; Parks et al., 2013; Ussar et al., 2015). The Collaborative Cross (CC) is a systems genetics mouse resource that consists of a panel of recombinant inbred lines and an outbred stock derived from eight genetically diverse founder strains. These include five classical inbred strains (A/J, C57BL/6J, 129S1/SvImJ, non-obese diabetic [NOD]/ShiLtJ, and NZO/HILtJ) and three wild-derived strains (CAST/EiJ, PWK/PhJ, and WSB/EiJ; Churchill et al., 2004; Roberts et al., 2007; Aylor et al., 2011).

We examined the metabolic phenotypes and gut microbiota composition of the eight CC founder strains in response to chronic consumption of two defined diets: a high-fat/high-sucrose diet (HF/HS) and a control diet. We found remarkable variation in diabetes-related phenotypes and gut microbiota composition as a function of host genotype and diet, and we identified bacterial taxa that correlate with metabolic traits, including body weight, glucose, and insulin levels. Germ-free (GF) mice were colonized with microbiota derived from two founder strains that exhibited divergent metabotypes, C57BL/6J and CAST/EiJ. The transplanted animals were maintained on the HF/HS diet and then subjected to metabolomic and metagenomic analyses. We identified functional differences attributable to the two transplanted microbial communities, including insulin secretion responses and susceptibility to diet-induced metabolic disease.

RESULTS

Host Metabolic Responses to Diet Are Influenced by Genetic Background

We assessed the variability of diet-induced metabolic responses of the eight genetically diverse CC founder strains: A/J; C57BL/6J (B6); 129S1/SvImJ (129); NOD/ShiLtJ (NOD); NZO/HILtJ (NZO); CAST/EiJ (CAST); PWK/PhJ (PWK); and WSB/EiJ (WSB). All mice were obtained from The Jackson Laboratory, maintained in the same vivarium, and fed the same diet so that the only known difference among the strains is genetics. We placed 4-week-old male mice from each strain on either a control or a HF/HS diet for 22 weeks (Table S1).

The CC founder strains displayed a wide range of body weight and metabolic responses to the dietary challenge (Figures 1 and S1). Two-way ANOVA analysis of the clinical traits revealed a significant strain effect for fasting insulin ($F = 14.94$; $p < 0.0001$). We also observed significant strain-diet interactions for body weight ($F = 3.19$; $p < 0.01$) and fasting glucose ($F = 2.81$; $p < 0.01$). Significant strain and diet effects were also seen for hepatic triglyceride content ($F = 10.96$, $p < 0.0001$; $F = 11.92$, $p < 0.001$, respectively). Liver triglyceride content showed high inter-strain variation, with 129 having the most significant response to diet ($p < 0.05$; Figure 1D). NZO mice were the only strain to become overtly diabetic (glucose levels > 300 mg/dL) as a consequence of HF/HS feeding. With the exception of NZO mice, which did not survive past 18 weeks on the HF/HS diet, B6 mice were the most responsive to diet. HF/HS-fed B6 mice became obese ($p < 0.01$) and developed insulin resistance and glucose intolerance after ~8 weeks (Figures 1A and S1A–S1C). In addition to differences in diet responsiveness, the strains varied in both absolute levels of insulin and change in insulin levels over time, suggesting a significant divergence in insulin sensitivity among the strains (Figure S1B).

To assess whole-body glucose homeostasis and more directly evaluate the underlying role of the pancreatic islets in the control of plasma insulin, we measured plasma glucose and insulin during an oral glucose tolerance test (oGTT). Both plasma glucose and insulin during the oGTT varied dramatically between the strains. We computed the area under the curve (AUC) for each trait to determine the overall excursion in glucose and insulin that occurred during the oGTT (Figures 1E, 1F, and S2). We observed a wide inter-strain range of responses in plasma insulin during the oGTT ($F = 12.84$; $p < 0.0001$; Figures 1F and 2B). Changes in plasma insulin may reflect altered insulin secretion from β cells, peripheral insulin resistance, reduced insulin clearance, or any combination thereof. 129 and WSB showed diet-induced glucose intolerance but minimal changes in their insulin response during the oGTT (Figures 1E, 1F, and S2A), suggesting that their glucose intolerance may be driven by altered insulin secretion and/or enhanced insulin clearance. Remarkably, insulin secretion and glucose tolerance were completely unaffected by the HF/HS diet in CAST. Furthermore, the kinetics of the glucose and insulin responses were more rapid in CAST than in all other strains (Figure S2), suggesting that CAST mice may employ different pathways underlying glucose-stimulated insulin secretion and whole-body glucose disposal.

Diet and Host Genotype Influence Microbiota Composition

Gut microbes influence the development of metabolic disease. We characterized the cecal microbiomes of the eight CC founder strains by 16S rRNA sequencing. We compared the cecal microbiomes employing UniFrac, a phylogenetic distance metric used to measure differences in bacterial community structure (Lozupone and Knight, 2005). Principal-coordinates analysis (PCoA) of 16S rRNA unweighted UniFrac distances revealed a strong influence of strain (PERMANOVA; $p < 0.001$) and diet (PERMANOVA; $p < 0.001$) on microbial community composition (Figure S3A). Consistent with previous studies, the effect of diet on gut microbial composition varied among the strains (O'Connor et al., 2014; Parks et al., 2013; Carmody et al., 2015), where B6, CAST, and NOD mice showed the greatest microbiome response to diet (Figure S3A).

We detected eight bacterial phyla among the mice (Figure S3B). Bacteroidetes and Firmicutes dominated the gut of all strains on either diet, accounting for >90% of the sequenced reads. As reported by other studies, we observed a decrease in the Bacteroidetes:Firmicutes ratio and an increase in Proteobacteria in the HF/HS-fed mice (Ley et al., 2005; Hildebrandt et al., 2009). In fact, Proteobacteria showed the greatest fold change in abundance in response to diet: HF/HS feeding caused an

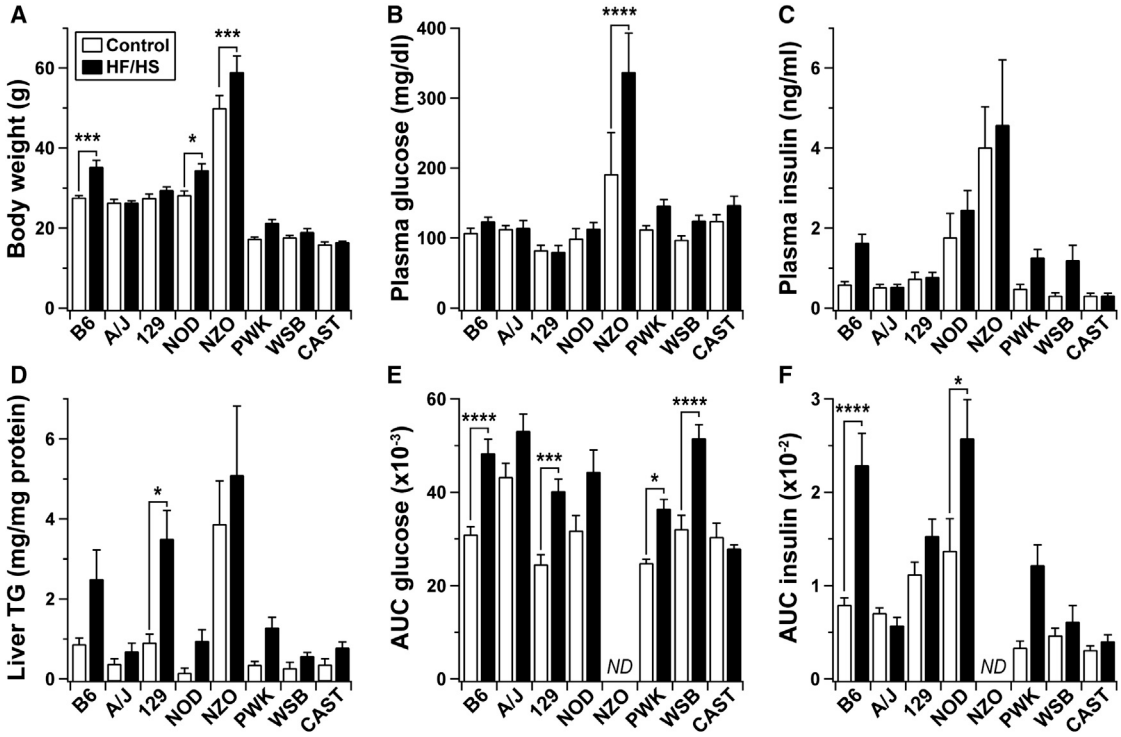

Figure 1. Segregation of Metabolic Syndrome among CC Founder Mice

Male mice were maintained on the high-fat/high-sucrose (HF/HS) or a control diet for 22 weeks beginning at 4 weeks of age.

(A–D) Body weight (A), fasting plasma glucose (B) and insulin (C), and hepatic triglyceride (D) content determined for all mice at 26 weeks of age.

(E and F) Areas under the curve (AUC) for (E) glucose and (F) insulin during oral glucose tolerance test (oGTT) conducted at 22 weeks of age. Insulin and glucose values were determined from plasma following a 4-hr fast. No data (ND) were collected for NZO mice during oGTT.

In all panels, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$ by two-way ANOVA (diet and strain) with Bonferroni's multiple comparisons test to assess within-strain differences. Data are mean ± SEM; $n \geq 9$ mice/genotype/diet.

average 5.4-fold change ($p < 0.0001$), although the relative increase varied among strains.

Microbial Taxa Correlate with Metabolic Phenotypes

To determine whether strain-dependent variability in microbiota composition was associated with the dramatic differences in the diabetes-related clinical traits, we computed Pearson's correlations between abundance of family-level taxa and the metabolic traits among the eight CC founder mice (Figure 2A). We focused our analysis on families that were present in at least seven of the founder strains. Bacteroidaceae was among the most negatively correlated with several metabolic phenotypes, including body weight, fasting plasma insulin, and $AUC_{insulin}$ during the oGTT. The Bacteroidaceae family belongs to the Bacteroidetes phylum and is typically found at higher levels in fecal samples of lean versus obese individuals (Ley et al., 2005; Turnbaugh et al., 2009). Conversely, Clostridiaceae and Rikenellaceae showed the strongest positive correlations with plasma insulin levels. Our analysis also identified strong positive correlations between fasting plasma glucose and the Streptococcaceae and Desulfovibrionaceae families. Members of these families have previously been shown to be enriched in the fecal microbiome of patients with T2D (Qin et al., 2012; Karlsson et al., 2013).

Some of the correlations mentioned above varied significantly as a function of host diet and strain (Table S2). For example, the negative correlation observed between fasting insulin levels and Bacteroidaceae had a significant strain effect ($p < 0.0001$). We also observed a slight diet effect ($p < 0.001$), which is likely driven by the low abundance and high fasting insulin levels in the chow-fed NZO mice (Figure 2B). We also observed a significant diet effect for the relationship between Clostridiaceae and fasting insulin levels ($p < 0.05$), but there was also a strain difference that seems to be driven by NZO on chow diet ($p < 0.001$; Figure 2C).

These results suggest that diet and genetic background are major determinants of gut microbial composition and metabolic disease. However, the relative contributions of host genetic variance versus microbial-derived genetic variation across different mouse strains in the development of diet-induced metabolic phenotypes remain largely unknown.

The Gut Microbiome Is a Source of Genetic Variation that Influences Host-Associated Differences in Diet-Induced Metabotypes

To directly test the influence of gut microbes on the metabolic phenotypes observed among the founder strains, we performed cecal transplants into germ-free B6 (B6-GF) hosts, leveraging two CC founder strains that showed disparate responsiveness

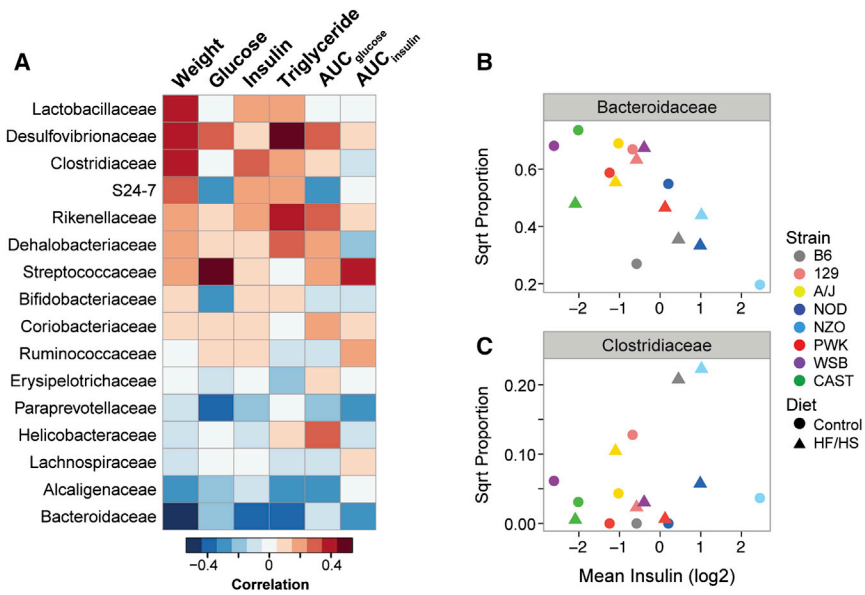

Figure 2. Gut Microbial Taxa Correlate with Metabolic Phenotypes

(A) Heatmap illustrates Pearson's pairwise correlation between microbial families and diabetes-related clinical traits measured in the eight CC founder mice (n ≥ 9 mice/genotype/diet). Microbial families are ordered by their correlation to body weight. Red, positive correlation; blue, negative. Area under the curve (AUC) values for insulin and glucose were computed from oGTT conducted at 22 weeks; other metrics were collected at 26 weeks. Correlation coefficients and p values are found in Table S2.

(B and C) Contributions of strain and diet on the correlations observed between fasting insulin and (B) the Bacteroidaceae family and (C) the Clostridiaceae family.

to the HF/HS diet. The B6 strain became obese, insulin resistant, and glucose intolerant, whereas the CAST strain remained lean and insulin-sensitive despite HF/HS feeding (Figure 1).

As mentioned above, B6 and CAST mice had significantly different intestinal microbiota (PERMANOVA; $F = 4.86$; $p < 0.001$; Figure S3A). B6 mice harbored a significantly greater abundance of microbial families with strong positive correlations with metabolic traits, such as weight and insulin (i.e., Clostridiaceae; $p < 0.05$), whereas CAST mice had a greater representation of families with significant negative correlations (i.e., Bacteroidaceae; $p < 0.01$; Figures 2A and S3C).

We transplanted cecal microbiota from either conventionally raised B6 (B6-CR) or CAST (CAST-CR) donor mice into 9-week-old B6-GF recipient mice to yield $B6_{B6}$ or $B6_{CAST}$ mice, respectively. Transplanted animals were housed by treatment group in separate vinyl gnotobiotic isolators and maintained on a HF/HS diet for 16 weeks following colonization (Figure 3A). A dietary treatment of 16 weeks allows robust development of metabolic phenotypes associated with consumption of HF/HS diet.

Recipient mice recapitulated microbial and metabolic phenotypes observed in the respective donor strains (Figures 3 and 4). $B6_{B6}$ mice gained ~25% more weight, had larger epididymal fat pad mass, and showed greater hepatic triglyceride accumulation than $B6_{CAST}$ mice (Figure 3). Additionally, oGTT revealed that, whereas the plasma glucose levels resulting from an orally administered bolus of glucose did not significantly differ between the two groups of transplanted mice (Figure 3E), the insulin responses were dramatically different (Figure 3F). The glucose challenge evoked a much larger insulin response in $B6_{B6}$ mice than in $B6_{CAST}$ mice. The low insulin response in $B6_{CAST}$ mice resembled the insulin response of the CAST-CR donors (Figures 1F and S1F). These results suggest that the effectiveness of insulin to maintain euglycemia was greater in the mice receiving the CAST microbiota than in mice receiving the B6 microbiota (Figures 3E and 3F).

16S rRNA gene profiling of the donor cecal inoculum and transplant recipient fecal samples show that recipient mice were successfully colonized with the donor's microbiota. $B6_{B6}$ and $B6_{CAST}$ mice assumed a phylogenetically similar composition to that of their respective donors as confirmed by PCoA of unweighted UniFrac distances (Figure 4A). As seen in the founders, Bacteroidetes and Firmicutes comprised ~90% of the microbiome, although the abundance of Firmicutes was higher in $B6_{B6}$ ($p < 0.05$; Figure 4B). We identified taxonomic differences in the microbiota composition between the two recipient groups using linear discriminant analysis (LDA) effect size (LEfSe) with LDA score > 2 (Segata et al., 2011). We found 20 microbial families that were differentially enriched in the fecal microbiota of $B6_{B6}$ versus $B6_{CAST}$ mice. There were 12 microbial families that were enriched in $B6_{B6}$, of which seven belonged to the Firmicutes phyla (Figure 4C). Some of the families differentially represented in the transplanted animals overlap with taxa that are significantly correlated with metabolic phenotypes in the founder strains (Figure 2). Notably, $B6_{B6}$ mice exhibited higher levels of Clostridiaceae ($p < 0.01$), which is positively associated with insulin secretion in the founder strains (Figure 2), whereas $B6_{CAST}$ mice had higher levels of Bacteroidaceae ($p < 0.01$), which is negatively associated with body weight and insulin secretion (Figure 2). These results are concordant with the metabolic phenotypes observed in the transplanted mice and suggest that the distinct microbial gut communities influence metabolic changes evoked by HF/HS feeding, including insulin secretion.

We characterized the functional potential of transplanted communities by sequencing and analyzing their metagenomes. Metagenomic analysis of the same samples further validated that the B6- and CAST-derived microbiota were distinct from one another, with donors clustering with their respective transplant recipients (Figure 4D). We identified several thousand genes differentially represented between the B6 and CAST microbiota (Table S4). This metagenomic analysis also revealed microbial functions that were enriched in each transplanted microbial community (Table S5). The most enriched microbial pathways in $B6_{B6}$ mice included genes involved in membrane

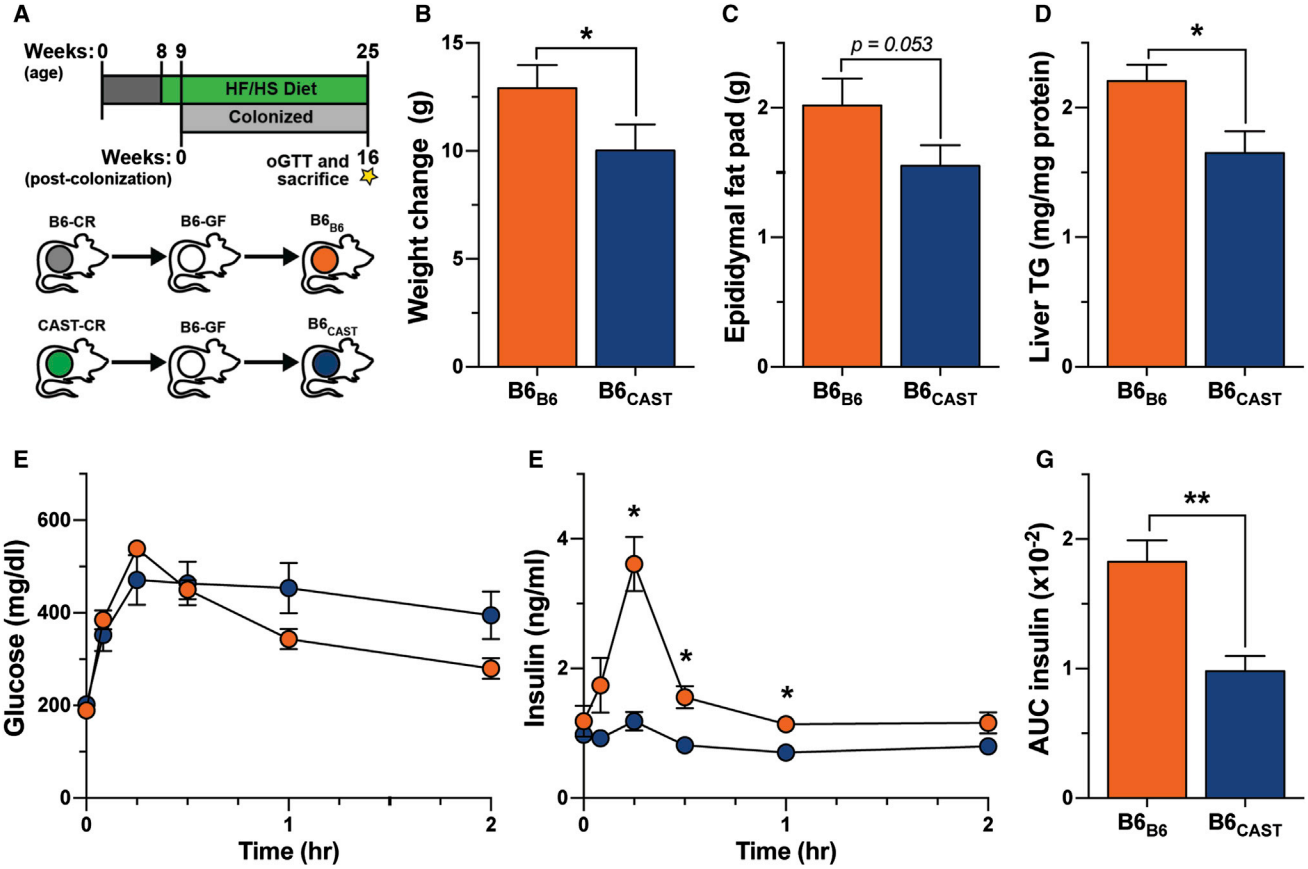

Figure 3. Divergent Effects of B6 and CAST Microbiomes on Diet-Induced Metabolic Phenotypes

(A) Transplant experimental design.

(B–D) Total weight change (B), epididymal fat pad mass (C), and quantification of hepatic triglyceride (TG) contents (D).

(E–G) Glucose and insulin values during oGTT (E and F) and AUC insulin (G) in $B6_{B6}$ and $B6_{CAST}$ mice. All measurements shown were collected 16 weeks post-colonization.

*$p < 0.05$; **$p < 0.01$ by Student's t test. Data are mean ± SEM; n = 7 for $B6_{B6}$ and n = 6 for $B6_{CAST}$ mice.

transport and carbohydrate and lipid metabolism (Figure 4E). For example, the ABC transporters and phosphotransferase system (PTS) pathways were enriched in mice colonized with the B6 microbiota ($p < 0.01$). PTS are a class of transport systems involved in the uptake and phosphorylation of a variety of carbohydrates that can be subsequently fermented to SCFAs (Deutscher et al., 2006). It has been previously reported that diet-induced obese mice have a concomitant enrichment of microbial pathways involved in PTS and elevated concentrations of SCFAs (Turnbaugh et al., 2008), reflecting an increased capacity for energy harvest. Consistent with these results, targeted gas chromatography-mass spectrometry (GC-MS) analysis of SCFAs in cecal contents disclosed that $B6_{B6}$ mice had an increased concentration of the major fermentation end products compared with $B6_{CAST}$ (Figure 4F). Conversely, $B6_{CAST}$ microbiota were enriched in genes related to the vitamin B12 (cobalamin) biosynthetic pathway (Figure S4A), synthesis of other B vitamins and enzyme co-factors, as well as lipopolysaccharide (LPS) biosynthesis (Figures 4E and S4B). A difference in LPS biosynthetic potential may reflect the composition of the $B6_{CAST}$ microbiota, which has a significantly higher relative abundance of gram-negative Bacteroidetes than the $B6_{B6}$ microbiota (Figure S3B). Our findings mirror those described previously in T2D patients relative to diabetes-free control patients (Qin et al., 2012; Karlsson et al., 2013)—both the microbiota of T2D patients and our metabolically diseased mice with B6 microbiota show enrichment in Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways involved in membrane transport, whereas diabetes-free patients and mice with the CAST microbiota exhibit enrichment in vitamin and co-factor biosynthesis.

B6 and CAST Microbiota Produce Divergent Bile Acid Profiles

Gut microbes impact host physiology in part by modulating the composition of the BA pool. We determined fecal BA profiles of the transplanted mice and HF/HS-fed B6-CR and CAST-CR mice by ultra performance liquid chromatography-mass spectrometry (UPLC/MS)-based quantification of primary and the most abundant secondary BAs. The BA composition of $B6_{B6}$ mice closely resembled that of B6-CR donor mice, whereas

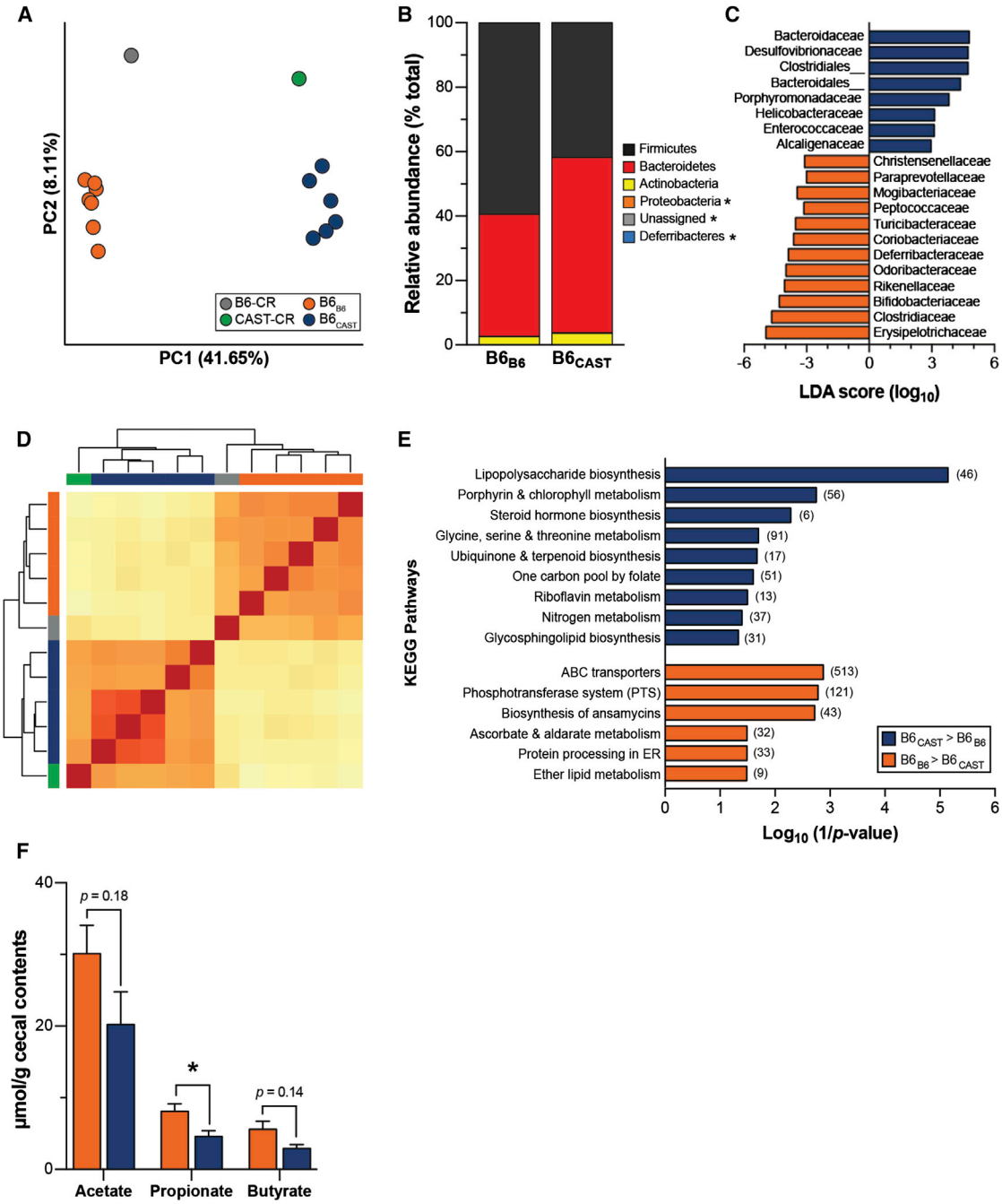

Figure 4. Gut Microbiota Composition and Function of Transplant Recipients

(A) Principal-coordinate analysis (PCoA) of unweighted UniFrac distances for the fecal microbiota of transplant donors and recipients at sacrifice. Each circle represents an individual mouse. Percent variation explained by each PC is shown in parentheses.

(B) Relative abundance of major microbial phyla ordered by increasing mean abundance; * denotes mean phyla abundance <1%.

(C) Microbial families differentially enriched in either $B6_{CAST}$ (blue) or $B6_{B6}$ (orange) as determined by linear discriminant analysis (LDA) with effect size (LEfSe).

(D) Clustering of mice based on relative abundance of KEGG metabolic pathways using Euclidian distance measurement with complete linkage hierarchical clustering; B6-CR (gray), CAST-CR (green), $B6_{B6}$ (orange), and $B6_{CAST}$ (blue).

(E) KEGG categories enriched in either CAST (blue) or B6 (orange) transplanted microbiomes.

(F) Targeted GC-MS analysis of cecal short-chain fatty acids; *$p < 0.05$ by Student's t test.

Data are mean ± SEM; n = 6–7 mice/recipient group and n = 2–3 mice/donor group. For metagenomics analysis, n = 5 mice/recipient group.

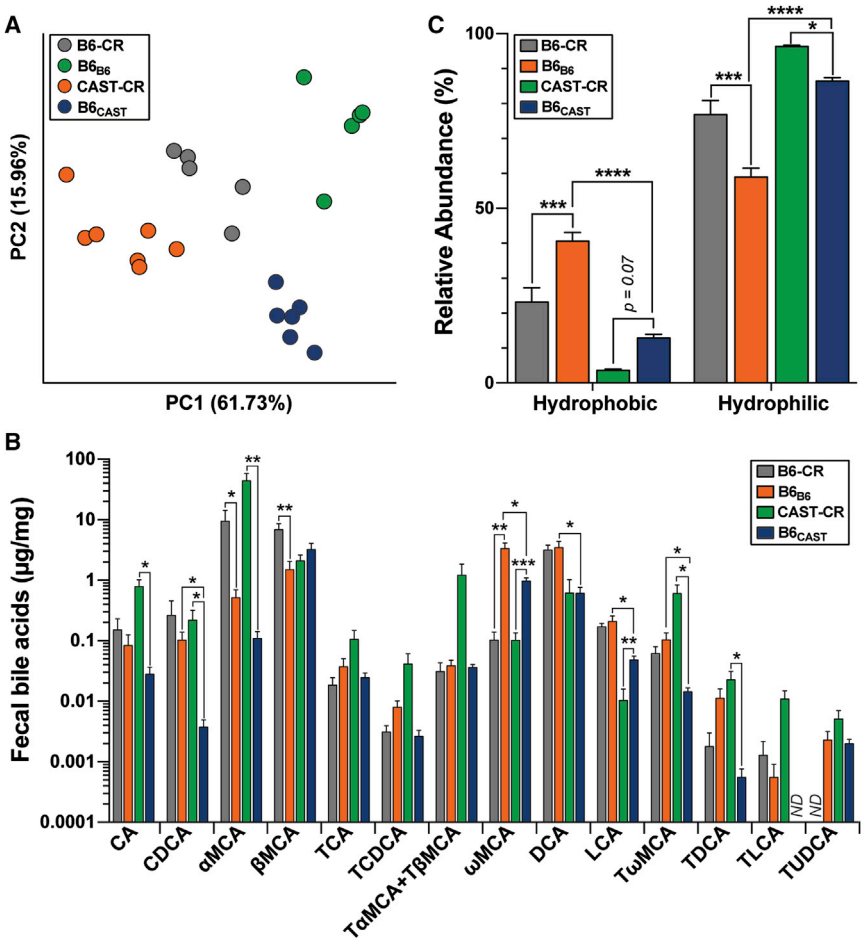

Figure 5. B6 and CAST Microbiota Produce Different Bile Acid Profiles

(A) Principal-component analysis of the square root proportion of 14 major bile acid species (ng/mg). Each dot represents the bile acid profile of an individual mouse. Percent variation explained by each PC is shown in parentheses.

(B and C) Abundance of fecal bile acids (B) and relative abundance of hydrophobic and hydrophilic BA species (C) determined by UPLC-MS/MS from fecal samples collected at 12 weeks post-colonization. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ by one-way ANOVA with Bonferroni's multiple comparisons test.

Data are mean ± SEM; n = 6–7 for transplant recipients and n = 5 for CR mice.

$B6_{CAST}$ exhibited a BA profile that was intermediate between CAST-CR and B6-CR mice (Figure 5A). Microbiota composition was also a significant predictor of BA composition. Bray-Curtis dissimilarity-based principal-component analysis (PCA) revealed clustering of the BA profiles by microbiota composition.

Although the $B6_{CAST}$ microbiota composition resembled that of CAST-CR (Figure 4A), there were significant differences in BA profiles between these groups, suggesting that variation in circulating BAs is under the control of both host genetics and gut microbiota. For example, the primary BAs cholic acid (CA), chenodeoxycolic acid (CDCA), and α-muricholic acid (α-MCA) were significantly higher in CAST-CR mice compared to $B6_{CAST}$ mice ($p < 0.01$, $p < 0.05$, and $p < 0.01$, respectively; Figure 5B). Moreover, taurine-conjugated muricholic acids (MCAs) were significantly higher in CAST-CR mice compared with $B6_{CAST}$ mice. In contrast, these differences in taurine conjugation were not present between B6-CR and $B6_{B6}$ mice. Taurine conjugation of MCAs is a host process (Ridlon et al., 2006), further highlighting the interaction of host genetics and microbiome in modulating host BA profiles.

B6-CR and $B6_{B6}$ mice had a significantly greater representation of hydrophobic BA species (e.g., deoxycholic acid and lithocholic acid; Figures 5B and 5C), which are elevated in humans and mice with insulin resistance (Ryan et al., 2014; Prawitt et al., 2011). Microbial metabolism of bile acids generally leads to a more hydrophobic bile acid pool, which facilitates fecal elimination of bile acids. Bile salt hydrolases (BSH) are involved in the hydrolysis of conjugated BAs, a necessary step for the production of secondary BAs. Consistent with the results presented above, there were a higher number of distinct BSH genes in the B6 microbiota relative to CAST microbiota (13 annotated BSH genes highly abundant in the B6 microbiota relative to CAST versus two annotated BSH genes highly abundant in the CAST microbiota relative to B6; Table S4). Furthermore, the two groups of recipient mice had vastly different fecal BA profiles. Chenodeoxycholic acid (CDCA) ($p < 0.05$), deoxycholic acid (DCA) ($p < 0.01$), lithocholic acid (LCA) ($p < 0.01$), ω-muricholic acid (ωMCA) ($p < 0.05$), and tauro-ω-muricholic acid (TωMCA) ($p < 0.05$) were all significantly higher in $B6_{B6}$ than in $B6_{CAST}$ (Figure 5B). DCA was the most abundant BA species in $B6_{B6}$ mice and was also ~5-fold more abundant in B6-CR versus CAST-CR mice. DCA contributes to microbial dysbiosis, a hallmark of metabolic disease, and is positively associated with higher levels of Firmicutes (Islam et al., 2011). Tauroursodeoxycholic acid (TUDCA) was >2-fold higher in CAST-CR mice compared to the transplanted animals but was not detected in B6-CR mice. Interestingly, administration of TUDCA has been shown to decrease hepatic steatosis and improve insulin resistance in genetically obese mice (Kars et al., 2010; Ozcan et al., 2006), suggesting a potential protective role. These results reveal differences in BA profiles linked to both host genotype and gut microbial composition. They also suggest that the differential responses to prolonged HF/HS diet consumption between B6 and CAST mice could be mediated at least in part by differences in microbial BA metabolism.

Gut Microbiota Influences Insulin Secretion

The most dramatic phenotype difference we observed between $B6_{B6}$ and $B6_{CAST}$ mice was in insulin secretion, where $B6_{CAST}$

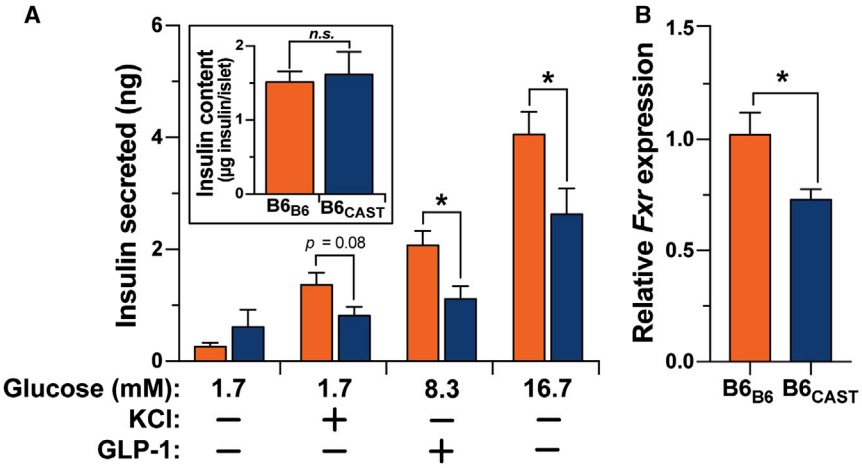

Figure 6. CAST and B6 Microbiomes Differentially Regulate Insulin Secretion and *Fxr* Expression in Pancreatic Islets

(A) Total islet insulin content and glucose-stimulated insulin secretion in response to low glucose (3.3 mM), low glucose plus KCl (40 mM), high glucose (16.7 mM), and high glucose plus GLP-1 (100 mM) from islets isolated from $B6_{B6}$ and $B6_{CAST}$ mice. The number of islets and the insulin content per islet were not different between the groups.

(B) Relative expression of *Fxr* mRNA from isolated islets. Figure S6 shows microbiota composition for donor and transplanted communities.

*$p < 0.05$ by Student's t test. Data are mean ± SEM; n = 5.

mice had a blunted insulin response during the oGTT (Figure 3E). This attenuated response in $B6_{CAST}$ mice may also reflect low insulin secretion from β cells and/or increased insulin clearance. To determine whether the differential insulin response during the oGTT in the $B6_{B6}$ versus $B6_{CAST}$ mice resulted from altered insulin secretion, we performed ex vivo insulin secretion assays on isolated islets. Islets were harvested from B6-GF mice 1 month after successful colonization with either CAST-CR or B6-CR cecum-derived microbiota (Figure S5).

The isolated islets partially recapitulated the reduced insulin secretion observed in the CAST-colonized mice in vivo (Figure 3E). The comparison between the B6-GF mice receiving B6 versus CAST microbiota allowed us to estimate the contribution of the microbiota to the strain difference in insulin secretion (Figure 6A). Accordingly, the reduction in insulin secretion caused by CAST microbiota colonization in B6 mice was ~33%.

Circulating acetate is capable of modulating insulin secretion from pancreatic islets. Specifically, recent studies have shown that acetate directly enhances glucose-stimulated insulin secretion through activation of free fatty acid receptors on β cells (Priyadarshini et al., 2015) and the parasympathetic nervous system (Perry et al., 2016). Therefore, we measured concentrations of SCFAs in plasma and cecum but found no differences in levels of acetate between $B6_{B6}$ and $B6_{CAST}$ mice (Figures S6A and S6B), suggesting that the divergent effects of the B6 and CAST microbiota on insulin secretion are unlikely to stem from differences in acetate.

Recent in vitro studies have also identified BAs as important regulators of islet function (Düfer et al., 2012; Renga et al., 2010). We investigated the plasma BA profiles in the $B6_{B6}$ and $B6_{CAST}$ mice used for insulin secretion studies (Figures S6C and S6D). $B6_{CAST}$ BA profiles were composed of a significantly higher percentage of hydrophilic BAs (Figure S6C). Consistent with a previous report (Sayin et al., 2013), BA profiles were dominated by taurine-conjugated species, with TωMCA and TβMCA being the two most abundant in both groups of animals (Figure S6D). In $B6_{B6}$ mice, the hydrophobic secondary BAs DCA and LCA were significantly higher than in $B6_{CAST}$ mice (Figure S6D).

BAs regulate insulin secretion through the activation of specific receptors in islets. For instance, BAs can directly increase insulin secretion and production through activation of farnesoid X receptor (*Fxr*) in β cells (Düfer et al., 2012; Renga et al., 2010). Expression of *Fxr* is increased in an agonist-dependent manner (Lee et al., 2006). Remarkably, we found that expression of *Fxr* was significantly higher in $B6_{B6}$ islets compared with $B6_{CAST}$ islets (Figure 6B). These results suggest that the gut microbiota modulate BA-dependent signaling in pancreatic islets.

DISCUSSION

The collective genetic variance of the eight CC strains is roughly equivalent to that of the entire human population, with the three wild-derived strains (WSB, CAST, and PWK) accounting for ~75% of the genetic diversity within the cohort (Roberts et al., 2007). Remarkably, these three wild-derived strains captured the full scope of dietary responsiveness observed across the panel (Figures 1 and S1). HF/HS feeding had no effect on any of the phenotypes measured in CAST mice, whereas it resulted in weight gain, glucose intolerance, and insulin resistance in B6 mice. Additionally, the diet caused a simultaneous increase in weight and glucose in NZO mice. We also identified significant differences in the gut microbiota composition among strains and between diets. All animals were obtained from the same facility and subject to the same environmental conditions throughout the study, and genetic differences among the mice are the only known variable. Together, these results support a role for host genetics to regulate the composition of the microbiota. However, it is important to note that, although large population studies have identified highly heritable taxa, the genetic architecture underlying these taxa is highly complex with relatively small effect sizes that are difficult to replicate (Benson, 2016).

From the CC founder panel, we identified B6 and CAST as the two strains with the most divergent phenotypes. Previous studies have exploited the differential response to diet-induced metabolic disease between B6 and CAST to identify genetic loci associated with metabolic disease (Mehrabian et al., 1998,

2000). In these studies, the gut microbiome may have contributed to the metabolic differences between strains.

In order to dissect the contribution of the microbiome of B6 and CAST to their contrasting metabolic profiles, we resorted to fecal transplantation experiments. B6-GF mice colonized with the CAST microbiota were less affected by chronic HF/HS feeding relative to B6-GF mice colonized with the B6 microbiota. The mice receiving the CAST microbiota secreted far less insulin in response to a glucose challenge but were still able to maintain normal blood glucose levels.

We consistently identified microbial taxa in both the CC founders and transplant recipients associated with metabolic traits. Clostridiaceae showed the strongest positive correlation with plasma insulin levels and weight gain (Figure 2A). Clostridiaceae also had a strong positive correlation with AUC insulin, a proxy for pancreas function. OTUs within the Clostridiaceae family have previously been both positively and negatively associated with metabolic traits (Ussar et al., 2015; Karlsson et al., 2013), and a recent study showed a positive correlation between an increase in BMI and an increase of SCFA-producing Closdiria species in Danish infants (Bergström et al., 2014). In contrast to the elevated Clostridiaceae in mice with a B6 microbiota, Bacteroidaceae was significantly higher in CAST-CR and $B6_{CAST}$ mice (Figures S3C and 4C). Bacteroidaceae was negatively correlated with body weight, circulating insulin, and $AUC_{insulin}$ (Figure 2A). A previous report found that daily oral administration of *Bacteroides uniformis*, a member of the Bacteroidaceae family, ameliorated metabolic dysfunction resulting from a high-fat diet (Gauffin Cano et al., 2012). This species also evoked a reduction in hepatic triglyceride levels, consistent with our observations that $B6_{CAST}$ mice have lower hepatic lipid levels compared to $B6_{B6}$ mice. Fecal abundance of members of the Bacteroidaceae family, including *Bacteroides vulgatus*, has also been reported to be lower in humans with T2D (Wu et al., 2010). Despite the high abundance of Bacteroidaceae in $B6_{CAST}$ mice, we did not observe complete protection from diet-induced metabolic disease that we observed in CAST-CR mice, suggesting that host factors, or taxa that failed to colonize transplanted mice (e.g., Verrucomicrobiaceae), contribute to the metabotype differences.

Vitamin B12 is exclusively produced by microbes (Martens et al., 2002), and several members of the Bacteroidaceae family transport, metabolize, and produce vitamin B12 analogs (Goodman et al., 2009; Degnan et al., 2014; Wu et al., 2015). Metagenomic analysis of the microbial communities from mice with the CAST microbiota revealed microbial functional enrichment for pathways involved in the biosynthesis of vitamin B12 (Figure S4A), which is necessary for DNA synthesis, neurological function, hematopoiesis, epigenetic modifications, and propionate metabolism (Kibirige and Mwebaze, 2013). Importantly, deficiencies in vitamin B12 are commonly observed in individuals with T2D and gestational diabetes (Kibirige and Mwebaze, 2013; Krishnaveni et al., 2009), and B12 therapy improves insulin resistance and endothelial function in patients with metabolic syndrome by mechanisms that are not fully elucidated (Setola et al., 2004).

Our metagenomic analysis also revealed that genes involved in LPS production are enriched in the CAST-transplanted microbiome (Figures 4E and S4B). This finding was surprising given that increased levels of LPS have been causally linked to the development of metabolic disease, yet $B6_{CAST}$ mice are partially protected from the effects of HF/HS feeding relative to $B6_{B6}$ animals (Figure 3). Taxonomic evaluation of the metagenomic data indicated that the Bacteroidetes phylum is the major contributor to the increased abundance of genes from this pathway (Table S4). This is relevant because unrelated bacteria generate structurally distinct LPS molecules with varying capacity to elicit an innate immune response (Whitfield and Trent, 2014). Notably, a recent study showed that LPS derived from *E. coli* generates a strong inflammatory signal, whereas LPS derived from members of the Bacteroidetes phylum inhibited the host immune response (Vatanen et al., 2016). The differential ability of LPS sub-types to modify host physiology may explain why LPS has been shown to both stimulate (Nguyen et al., 2014) and attenuate (Amyot et al., 2012) insulin secretion. Studies aimed at testing the roles of LPS derived from phylogenetically diverse taxa on metabolic disease and insulin secretion are warranted to further clarify how structural differences in this molecule affect host metabolism.

In addition to LPS, gut microbes produce SCFAs, which are important energy and signaling molecules implicated in metabolic disease. For instance, butyrate has been shown to improve whole-body insulin sensitivity (Gao et al., 2009) and patients with T2D have reduced levels of butyrate-producing bacteria (Qin et al., 2010). SCFAs are also elevated in individuals with diet-induced obesity, which is consistent with the elevated cecal SCFA levels in $B6_{B6}$ mice (Turnbaugh et al., 2008). Interestingly, SCFAs are also known regulators of insulin sensitivity and secretion. Acetate can modulate insulin secretion from β cells either directly through FFAR2 or via parasympathetic activation (Priyadarshini et al., 2015; Perry et al., 2016). However, we did not observe differences in concentrations of plasma or cecal acetate in the transplanted animals (Figures 4F, S6A, and S6B). Therefore, it is unlikely that the differences in insulin secretion could be attributed to SCFAs and consequentially implies there are multiple pathways through which the gut microbiota can modulate insulin secretion from β cells.

Gut microbes are responsible for the production of the highly hydrophobic secondary BAs DCA and LCA through the dehydroxylation of the primary BAs, CA and CDCA, in the colon. Removal of glycine/taurine BA conjugates via BSH enzymes is a prerequisite for 7α/β-dehydroxylation of primary BAs into secondary BAs (Batta et al., 1990). Interestingly, there were 13 predicted BSH genes that were more abundant in the B6 metagenome but only two in the CAST metagenome. One possible interpretation of this result is that there may be more bacterial species present in the B6 microbiome that are able to deconjugate BA. Consistent with this, $B6_{B6}$ mice had significantly higher levels of secondary BA as well as hydrophobic BA species than $B6_{CAST}$ mice (Figures 5B, 5C, S6C, and S6D), both of which are elevated in humans and mice with insulin resistance (Ryan et al., 2014; Prawitt et al., 2011). Furthermore, DCA has been positively associated with higher levels of Firmicutes (Islam et al., 2011). This is consistent with our findings as B6-CR founders and $B6_{B6}$ had a significantly greater relative abundance of Firmicutes and fecal DCA than CAST-CR and $B6_{CAST}$ (Figures S3B and 5B). Conversely, $B6_{CAST}$ had a higher abundance of hydrophilic BAs and the majority of the BA pool was comprised of the mouse primary BA, βMCA (Figures 5B and 5C).

The BA receptor FXR is expressed in pancreatic β cells, and its activation via BAs enhances insulin secretion (Kumar et al., 2012; Renga et al., 2010). Hydrophobic BAs, such as CDCA, DCA, LCA, and their taurine conjugates, are known ligands of FXR. The hydrophobic tauro-chenodeoxycholic acid (TCDCA) increases insulin production and secretion through an FXR-dependent regulation of K_{ATP} channels (Düfer et al., 2012). Moreover, β cell FXR activation in diabetic-leptin-receptor-deficient (db/db) mice and NOD mice increases insulin secretion and delays the development of diabetes (Renga et al., 2010; Zhang et al., 2006). We detected higher levels of LCA and DCA in the feces and plasma of $B6_{B6}$ mice relative to $B6_{CAST}$ mice (Figures 5B, S6C, and S6D), along with increased expression of *Fxr* in pancreatic islets from $B6_{B6}$ mice (Figure 6B). Altogether, this suggests that the gut microbiota and BA composition could modulate pancreatic function and insulin secretion.

We have highlighted four examples of microbial-derived products, vitamin B12, SCFAs, LPS, and BAs, as plausible mediators of the microbiome effect on insulin secretion. However, there are thousands of other metabolites that were not characterized in our study and could also play an important role in regulating host metabolism. Future experiments using gnotobiotic mice colonized with defined communities that have different metabolic capabilities will provide mechanistic insights into the communication between gut microbes and the host.

EXPERIMENTAL PROCEDURES

Mouse Husbandry

Animal care and study protocols were approved by the University of Wisconsin-Madison Animal Care and Use Committee.

CC Mouse Husbandry

Mice were housed on a 12-hr light:dark cycle. CC founder strains were obtained from The Jackson Laboratory and were bred at University of Wisconsin, Madison. Mice were group housed by strain (two mice/cage) and diet under temperature- and humidity-controlled conditions and received ad libitum access to water and food. After 4 weeks of age, mice were maintained on either a control (TD.08810; Envigo Teklad; 16.8% kcal fat; 60.9% carbohydrate; 22.3% protein) or a high-fat, high-sucrose diet (TD.08811; Envigo Teklad; 44.6% kcal fat; 40.6% carbohydrate; 14.8% protein; Table S1). Strains were housed within the same vivarium throughout the duration of the study.

Gnotobiotic Mouse Husbandry

C57BL/6J germ-free mice were bred and housed in the Microbial Sciences Building vivarium at University of Wisconsin-Madison to generate mice used in this study. B6-CR and B6-GF mice were housed in separate plastic flexible vinyl gnotobiotic isolators under temperature- and humidity-controlled conditions (12-hr light:dark). Fresh cecal contents were collected from 15-week-old conventional B6-CR and CAST-CR mice maintained on the HF/HS diet (n = 2–3 mice per donor cecal microbiota samples). Cecal contents from B6 and CAST donor mice were resuspended in rich medium (1:100 w/vol) inside an anaerobic chamber. Suspensions were transferred into anaerobic sealed tubes and moved into gnotobiotic isolators. Nine-week-old B6-GF male mice were inoculated via a single oral gavage with ~0.2 mL of cecal inocula (Turnbaugh et al., 2009). Each group of mice was housed in a controlled environment in separate plastic flexible vinyl gnotobiotic isolators under standard conditions. Recipient mice received sterilized water and HF/HS diet (TD.0.8811) ad libitum beginning 1 week before colonization.

Metabolic Phenotypes

Mice were fasted for 4 hr before blood collection. Fasting levels of glucose, insulin, and triglycerides were quantified at regular intervals, along with body weight. Glucose tolerance tests were performed by administering an oral dose of glucose (2 g/kg body weight) after a 4-hr fast. Blood was collected at 0, 5, 15, 30, 60, and 120 min to assess glucose and insulin levels. Hepatic triglycerides were extracted following the Bligh and Dyer method.

Microbiome Sequencing and Analysis

DNA was isolated from cecal contents and feces by extraction using a bead-beating protocol (Turnbaugh et al., 2009). The V4 region of the 16S rRNA gene was amplified using barcoded primers, and sequencing was performed using the Illumina MiSeq platform. 16S rRNA sequences were analyzed using QIIME (Quantitative Insights Into Microbial Ecology) software package (Caporaso et al., 2010). LEfSe analysis was performed using standard parameters ($p < 0.05$ and LDA score 2.0; Segata et al., 2011). For the metagenomic analysis, DNA fragments ~350–400 bp were sequenced using Illumina Rapid HiSeq 2000. Details about metagenomic sequence analysis, 16S rRNA sequencing, and analyses are provided in Supplemental Experimental Procedures.

Statistical Analysis

The data are expressed as mean ± SEM and analyzed using GraphPad Prism 6.0 (GraphPad Software). Multiple groups were analyzed by one-way or two-way ANOVA followed by Bonferroni's multiple comparisons test. Significant differences between two groups were evaluated by two-tailed unpaired Student's t test or Mann-Whitney U test for samples that were not normally distributed. Pearson's correlations between microbiota and phenotypes and association testing were performed in R. The level of significance was set at $p < 0.05$; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.

See Supplemental Information for detailed description of methods related to (1) plasma and liver measurements, (2) high-performance liquid chromatography-mass spectrometry (HPLC-MS) and GC-MS assays of plasma and cecal contents, (3) microbiome analyses, (4) islet isolation and GSIS, and (5) qRT-PCR assays.

ACCESSION NUMBERS

The accession number for the data reported in this paper is ENA: PRJEB15120.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, six figures, and five tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2017.01.062.

AUTHOR CONTRIBUTIONS

F.E.R., A.D.A., M.P.K., and J.H.K. conceived the project; J.H.K., E.I.V., M.E.R., D.S.S., K.L.S., B.S.Y., A.T.B., and W.Z. performed experiments and interpreted results; J.H.K., M.P.K., and L.L.T. prepared figures; J.H.K., M.P.K., A.D.A., and F.E.R. wrote the manuscript; and W.Z., L.L.T., M.E.R., and B.H. provided critical feedback. All authors read and agreed on the final version of the manuscript.

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