Host Genetic Background and Gut Microbiota Contribute to Differential Metabolic Responses to High Fructose Consumption in Mice

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Running Title: Gut microbiota in metabolic response to fructose

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Abstract

Both host genotypes and gut microbiota play a role in dietary responses and the control of host phenotypes. High fructose consumption has been strongly implicated in metabolic disorders including obesity, insulin resistance, and liver disease, and individual variability in disease susceptibility in response to fructose has been observed, but the mechanisms are poorly understood. We investigated the role of gut microbiota in the host metabolic and transcriptomic responses to long-term (12-week), 8% fructose consumption in multiple mouse strains, namely, C57BL/6J (B6), DBA/2J (DBA), FVB/NJ (FVB). While DBA mice demonstrated significant increases in body weight, adiposity, and glucose intolerance, B6 and FVB mice showed significant cholesterol changes in the absence of body mass and glycemic alterations. Using 16S rRNA-sequencing analysis of gut microbiota, we found that DBA had high basal levels of Lactobacillus, Clostridiales , and Lachnospiraceae, whereas S24-7, Akkermansia, and Turicibacter are more abundant in B6 and FVB. Additionally, fructose altered several microbial taxa in B6 and DBA mice that are strongly correlated with metabolic phenotypes and host fructose-responsive genes in liver, adipose tissue, and hypothalamus. To test whether DBA microbes confer susceptibility and B6 microbes confer resistance to fructose-induced adiposity and glucose tolerance changes, we inoculated the B6 fecal content to DBA mice and vice versa. B6 microbes attenuated the fructose response in DBA mice by conferring resistance to weight/fat gain and glucose intolerance. Our findings support that strain-specific microbiota are partially responsible for fructose sensitivity among mice with different genetic backgrounds.

Introduction

The drastic increase in fructose consumption for the last few decades has been paralleled by prevalence of obesity, metabolic syndrome and subsequent cardiovascular diseases (Dhingra et al., 2007; Ludwig et al., 2001; Schulze et al., 2017; Stanhope et al., 2009; Teff et al., 2009). The use of systems nutrigenomics approaches have recently revealed the impact of fructose on the gene expression, epigenome and interactions of hundreds of genes in rodent hypothalamus-master center of metabolic control(Meng et al., 2016). In turn, studies in the liver tissue have revealed fructose-induced alterations in genes involved in several aspects of systemic metabolism (Kim et al., 2016; Koo et al., 2008; Li et al., 2016).

It is becoming to be understood that dietary intake influences trillions of gut microorganisms as early as one day post dietary intervention (David et al., 2014;Turnbaugh et al., 2006; Turnbaugh et al., 2009). and this information suggests that microbiome could mediate the effects of fructose on the host. Indeed, gut microbiome is emerging as an important modulator of metabolic capacity, underlying development of obesity and other metabolic disorders (Payne et al., 2012; Di Luccia et al., 2015). There are also indications that gut microbiome acts as a link between the genetic and phenotypic diversity among genetically diverse mouse strains in response to dietary challenges (Kreznar et al., 2017; Parks et al., 2013). Given the observed inter-individual variability in the metabolic effects of fructose (Cha et al., 2008; J. Glendinnning, L. Breinager, E. Kyrillou, K. Lacuna, R. Rocha, 2010; Montgomery et al., 2015), it is possible that gut microbiome also plays a role in mediating the genetic regulation of fructose response involved in inter-individual variability.

Dietary fructose is mostly absorbed in jejunum (middle part of small intestine) by the facilitative transporter Glut5 and by Glut2 in the presence of glucose (Burant, 1992; Barone et al., 2009). Fructose can be also absorbed in ileum and colon by Glut7 which has extremely high affinity for fructose (Cheeseman, 2008). The fructose which is not absorbed in the small intestine due to mal-absorption or excess intake undergoes colonic bacterial fermentation, resulting in the production of metabolites including short-chain fatty acids (SCFA). The fact that these fructose sub-products contribute to shaping gut environment and participating as energy source for gut microbiota, make of fructose an important modifier of microbiome functionality (Ríos-Covián et al., 2016).

The dynamic nature and ease of manipulation have made gut microbiome a suitable therapeutic target to mitigate metabolic syndrome pathogenesis (Fändriks, 2016; Jena et al.; Yadav et al., 2007). Here, we investigated gut microbiota as a potential causal link between fructose consumption and differential development of metabolic phenotypes in mice with diverse genetic background. We tested our paradigm in three mouse strains (B6, DBA, and FVB), selected based on their contrasting metabolic susceptibility to high caloric diets (Parks et al., 2013;Glendinnning et al., 2010; Montgomery et al., 2015). The results are interesting to show the importance of strain variability in in metabolic phenotypes and gut microbiota composition in response to fructose consumption. Furthermore, our results We further assessed the causal role of gut microbiota in determining strain-sensitivity to fructose via fecal transplant studies and explored the possible mechanisms by associating specific microbes with host phenotypes and fructose-responsive genes in key endocrine and metabolic tissues. Our study supporting a causal role of gut microbiota in determining inter-individual variability

in fructose response suggests that the treatment of metabolic syndrome should be based on a personalized approach, which takes into consideration the genetic and microbial background of individuals.

Materials and Methods

Animals and study design

Seven-week old male mice (20-25g) from three inbred strains, namely, C57BL/6J (B6), DBA/2J (DBA), FVB/NJ (FVB), were obtained from the Jackson Laboratory (Bar Harbor, ME, USA) and housed in a pathogen-free barrier facility with a 12-hour light/dark cycle at University of California, Los Angeles. Mice were fed a standard Chow diet (Lab Rodent Diet 5001, LabDiet, St Louis, MO) containing 3.35 kcal/mg energy (9.82 % protein, 13.427 % fat, 56.744 % carbohydrates, mono- and disaccharides negligible). After 1-week acclimation, mice from each strain were randomly divided into two groups (n=8-10 mice/ water group/strain, n = 10-12 mice/fructose group/strain). One group was provided with regular drinking water (water group) and the other group (fructose group) was given 8% (w/v) fructose (3.75 kcal/g energy; NOW Real Food, Bloomingdale, IL) in the drinking water for 12 weeks (Fig. 1). The 8% fructose water has an energy density lower than the US popular Coca Cola (11.1% sugar concentration contained 7.2 g/100 ml of fructose) (Ventura et al., 2011), and therefore is physiologically relevant. Mice were group housed by strain (n=3-4/cage) under temperature- and humidity-controlled conditions and received ad libitum access to water and Chow diet. Mice of all strains were housed within the same vivarium throughout the duration of the study. The measurement of food /drink intake and all metabolic phenotypes was performed as described in Zhang et al. companion paper. Gut microbiota composition was analyzed from cecum and feces, as detailed below. The gut microbiota differences between strains and between treatment groups were correlated with metabolic phenotypes and gene expression in individual tissues to prioritize microbial species. Lastly, fecal

transplant experiments were conducted to test the causal role of gut microbiota in determining strain-specific fructose response (Fig. 1). All experimental protocols were approved by the Institutional Animal Care and Use Committee at the University of California, Los Angeles.

Fecal sample and tissue collection

Each group/strain of mice were housed in 2-3 cages (n = 3-4 mice/cage). Fecal samples were collected weekly from each animal starting from the week of fructose treatment. Each mouse was gently restrained in a sterile empty cage, with tail lifted, to induced defecation. Feces were immediately placed into liquid nitrogen and stored at -80 °C for microbiota sequencing. At the end of 12-week fructose feeding experiment, cecal contents were collected for microbiota sequencing.

Microbiota sequencing and analysis

Fecal samples from week 1, 2, 4, and 12 and cecal samples from week 12 were used for microbiota sequencing (n=8 mice/time point/group/strain). Microbial DNA was extracted from cecal and fecal contents of each mouse using the MO BIO PowerSoil®-htp 96 Well Soil DNA Isolation Kit (MO BIO, Carlsbad, CA, USA) with an additional incubation at 65°C for 10 min prior to plate shaking. The V4 hypervariable region of the 16S ribosomal RNA gene was amplified with barcoded primers in triplicate using the 5 PRIME HotMasterMix (VWR, Radnor, PA, USA). PCR products were quantified with Quant-iTTM PicoGreen® dsDNA Assay Kit (Thermo Fisher, Waltham, MA, USA) and samples were combined in equal amounts (~250 ng per sample) and purified with the

UltraClean PCR® Clean-Up Kit (MO BIO, Carlsbad, CA, USA). Pooled amplicons were sequenced on the Illumina HiSeq 4000 platform over two lanes to generate paired-end reads.

Raw sequences were processed with the open source Quantitative Insights Into Microbial Ecology (QIIME) software package version 1.9.1 (Caporaso et al., 2010) to produce de-multiplexed and quality-controlled sequences. Reads were binned into OTUs at 97% similarity using UCLUST against a Greengenes reference database using the pick_closed_reference_otus.py script (Edgar, 2010; Mcdonald et al., 2011). Singletons and OTUs representing less than 0.005% total relative abundance as well as unsuccessful samples that had less than 10,000 reads were removed. The result was a total of 24,215,515 reads with an average of 126,122 per sample for 192 samples (n=8/group x 2 groups/strain x 3 strains x 4 time points) for fecal samples and 10,581,341 reads with an average of 189,262 per sample for 48 cecal samples (n=8/group x 2 groups/strain x 3 strains). Sequences were rarefied in an effort to reduce the effect of unequal sequencing depth to 60,000 and 106,815 reads per sample for fecal and cecal samples, respectively. This procedure excluded 7 fecal and two cecal samples. Five additional fecal samples were removed: two were outliers based on principle coordinate analysis (PCoA) ordination and three were contaminated based on a priori knowledge and confirmed with PCoA. A total of 180 fecal and 46 cecal samples were included in the downstream analyses.

Microbial community patterns were visualized with PCoA based on the UniFrac distance measure (Lozupone and Knight, 2005) and summarized into relative abundance for each taxonomic level in QIIME. Categorical groups (treatment, time,

strain) were confirmed to have similar multivariate homogeneity of group dispersions to allow them to be compared using the non-parametric PERMANOVA test with the adonis function (Oksanen et al., 2015). Microbial composition was analyzed at the phylum, family, and genus taxonomic levels using the Statistical Analysis of Metagenomic Profiles (STAMP) software (Parks et al., 2014). Taxa that are different between strains at baseline as well as those differ within an individual strain at 12 weeks of the study between the fructose and control groups were identified using the White's non-parametric T-test (White et al., 2009), followed by multiple testing correction using the Q value approach (Storey, 2004). Linear discriminant analysis (LDA) effect size (LEfSe) was used to identify taxa that discriminated between the microbiota of three mouse strains using standard parameters ($p < 0.05$, LDA score 2.0) (Segata et al., 2011). 2011).

Correlation analysis between gut microbes and metabolic phenotypes or tissue transcriptome

Correlation analysis was performed between the relative abundance of strain-specific or fructose-responsive microbiota with metabolic phenotypes including body weight, adiposity, and glucose intolerance ($AUC_{glucose}$). Fructose-responsive microbiota was also correlated with expression level of host genes altered by fructose in liver, omental adipose tissue, and hypothalamus, termed "fructose signature genes". Correlation was analyzed using Biweight midcorrelation (bicor), which is resistant to outliers (Zheng et al., 2014). Statistical p value was adjusted using the Benjamini Hochberg (BH) approach and adjusted $p<0.05$ was considered significant.

Functional annotation of fructose signature genes correlated with fructose-responsive microbiota

Fructose signature genes that showed significant correlation with fructose-responsive microbiota were classified for their biological functions using Molecular Signatures Database (MSigDB, http://www.broadinstitute.org/gsea/msigdb/index.jsp) (Subramanian et al., 2005), which catalogs canonical pathways or functional categories from various databases including Kyoto Encyclopedia ofGenes and Ge- nomes (KEGG; http://www.genome.jp/kegg/pathway.html), Reactome (http://www. reactome.org/), and gene sets derived from Gene Ontology (GO biological process, http://www.geneontology.org/). Over-representation of biological functions or pathways among the microbiota-correlated genes was assessed using GSEA software. Fisher's exact test was performed to calculate the enrichment p-values for each pathway or functional category within each gene signature. Results were corrected for multiple comparisons of pathways using Benjamini Hochberg correction and adjusted $p < 0.05$ was considered significant.

Fecal transplant between B6 and DBA mice

Recipient male B6 and DBA mice of 6-week age (n=16/strain) were orally gavaged with antibiotics solution of vancomycin (50mg/kg), neomycin (100 mg/kg), and metronidazole (100 mg/kg) at 9 am and 5 pm daily for 7 days according to method previously described (Reikvam et al., 2011) . Ampicillin was added in drinking water in 1 mg/ml and provided ad libitum during antibiotics oral gavage and placed on chow diet. In the end of antibiotics treatment, fecal samples were collected from the randomly

selected B6 or DBA mice (one mouse from each cage; 2 cages/group/strain) and anaerobically cultured in agar plate to confirm that bacterial growth was not detected. Followed by antibiotics clearance in the recipient mice by providing autoclaved drinking water for 24 hrs, donor fecal supernatant from B6 or DBA was transplanted to the recipient mice by oral gavage (B6 fecal supernatant to DBA recipient mice, and vice versa). Fecal supernatant preparation and fecal transplant were performed as follows. First, fecal pellets were freshly collected from 4 donor B6 or DBA male mice and pooled together, and ~200 mg of feces were then placed in 5 ml of pre-reduced PBS. The fecal pellets were smashed and suspended, and allowed to settle for 2 min. Fecal supernatant (150 ul) was transferred into the recipient mouse by oral gavage. This was done twice weekly for the first two weeks and then weekly for the next two weeks (Suez et al., 2014);Gregory et al., 2015). After 1 week of fecal transplant, mice from each recipient strain were divided into two groups (water or fructose group). The fructose group mice were placed on 8 % fructose drinking water for 12 weeks while the water group received drinking water only (n= 8/group/strain, n = 4/cage). The feces were collected from individual recipient mice at 4 time points (before antibiotics treatment, and 0, 4, 8, 12 wk of fructose treatment). For the feces collection at 0 wk of fructose treatment, we collected feces from individual recipient mice at 72 hrs after oral gavage (Suppl. Fig.1). All mice were maintained in sterile cage with sterile chow diet and water throughout the experiment after antibiotics treatment. Food and drink intake were recorded twice every week, and body weight and body composition (fat mass) were measured every week. In the end of experiment, intraperitoneal glucose tolerance test (IPGTT) was also performed following an overnight fast; two grams of glucose/kg body

weight were injected intraperitoneally to each mouse and blood glucose levels from tail vein were measured at 0, 15, 30, 90, and 120 min using an Accu-check Go glucose meter (Roche diagnostic, Castle Hill, NSW, Australia).

Statistical analysis

Differences in metabolic phenotypes between control and fructose groups were analyzed using the Student's t-test. Other statistical tests for individual analyses were described in the corresponding sections above.

Results

Strain-specific metabolic responses to fructose treatment

As detailed in the Zhang et al. companion paper, B6, DBA, and FVB mice demonstrated striking differences in their metabolic responses to 8% fructose consumption for 12 weeks. There was no difference in the overall energy intake between fructose group and water group in any of the three strains. FVB drank more fructose water than the other strains by ~ 2 folds, which was accompanied by a compensatory reduction in food intake. Significant body weight gain was detected only in DBA after 5 weeks of fructose treatment ($p<0.05$). The DBA mice treated with fructose water also showed higher adiposity index ($p<0.001$) and body fat mass ($p<0.001$), along with a decrease in lean mass ($p<0.001$). In contrast, B6 and FVB mice showed no difference in body weight and fat mass between fructose and water groups. There were significant increases of plasma insulin in fructose-treated DBA and FVB mice ($p<0.05$), but no difference in plasma glucose level in any of the three strains compared with water group. The area under the curve (AUC) calculated from the intraperitoneal glucose tolerance test (IPGTT), an index of insulin resistance, was significantly elevated only in DBA mice starting from 4 weeks after fructose treatment, and this lasted throughout 12 weeks of fructose treatment, which indicates that fructose treatment decreased glucose tolerance in DBA mice. There was no significant change of AUC in the other strains. This suggests that DBA mice were more sensitive to the fructose than other two strains in terms of body composition and glucose homeostasis. In contrast, B6 showed significant increases in plasma cholesterol and FVB, interestingly, had decreases in cholesterol

levels. These results demonstrate strong inter-individual variability in fructose response in genetically different mice.

Overall effects of fructose on microbial communities

To examine the effects of fructose consumption on gut microbiota in each mouse strain, the gut microbial composition was analyzed using 16S rRNA sequencing. Principle coordinate analysis (PCoA) was performed using UniFrac distances between samples to analyze relationships among samples based on factor differences. Sample distribution in the PCoA plot showed distinctive clusters among three strains in both the cecum (pseudo-F = 5.158, p = 0.001) and the feces (pseudo-F = 12.56, p = 0.001) (Fig 2A, B). In feces, time point was also a significant overall factor (pseudo-F = 2.795, p = 0.001) with week 12 separating from the other earlier weeks along the PC3 axis in overall (Fig 2C) and individual strains of B6 and DBA (Fig. 3D-F).

Fructose treatment showed differential effects on microbiota composition based on mouse strain, time, and gut community type. There was no effect of fructose overall for either feces (pseudo-F = 1.116, p = 0.242) or cecum (pseudo-F = 1.311, p = 0.110) communities (graph not shown). However, in cecum, fructose treatment showed a distinct separation for DBA strain compared to water groups (pseudo-F = 4.357, p = 0.002), while not significant for B6 or FVB (Fig. 2G-I). In feces, differences were seen at 12 weeks of fructose vs. water treatment in B6 mice (pseudo-F = 11.702, p = 0.001) and in DBA mice (pseudo-F = 3.878, p = 0.046) while FVB did not (Fig. 2J-H). Overall, consistent with the differential effects of fructose on metabolic phenotypes described

above, DBA appears to be more sensitive to fructose than the other two strains in terms of gut microbiota shifts.

Differences in the baseline microbial composition among three mouse strains and the correlation with host metabolic phenotypes

Differences in baseline microbial composition can drive the response to the dietary changes or differential phenotypic responses (Dao et al., 2016; Kootte et al., 2017). We considered the gut microbiota in water group as the baseline microbiota. At the phylum level, DBA feces has significantly higher Fermicutes/Bacteroidetes ratio (1.78±0.17, $p<0.05$) than B6 (0.35±0.24) and FVB (0.90±0.09). Veruccomicrobia was more enriched in B6 and FVB compared with DBA, and higher level of Tenericutes was observed in FVB feces (Fig.S2). Linear Discriminant Analysis (LDA) for the baseline microbiota of three strains revealed 51 and 44 microbial taxa to be differentially abundant between strains in cecum and feces, respectively (Fig.S3A, B), among which 21 cecal and 14 fecal microbial genera, respectively, were differentially enriched in each strain (Fig. S3C, D).

We reasoned that if any baseline microbe determines the differential fructose response among strains, its abundance likely shows contrasting patterns between DBA and the other two strains, since DBA was particularly sensitive to fructose. Compared to B6 and FVB, DBA mice were more enriched for Lactobacillus, unknown bacteria of order Clostridiales, unknown bacteria of family Lachnospiraceae in feces. On the other hand, DBA samples were least enriched for unknown bacteria of family S24-7, Akkermansia, and Turicibacter in feces (Fig. 3).

To further understand the potential role of these microbial taxa in metabolic regulation, we correlated their baseline level with the gained adiposity using Biweight midcorrelation (bicor). All of those fecal microbiota but Turicibacter were positively or negatively correlated with adiposity (Fig. 4).

Effect of fructose on gut microbiota abundance

Next, to explore differential microbial response to the fructose in the three strains, we analyzed the differential abundance of gut microbiota after 12 weeks of fructose treatment. In cecum, family Erysipelotrichaceae as well as its two genera (Clostridum and an unknown genus), and *Anaerostipes* were all significantly decreased by fructose in DBA, 2 families, and 3 genera were significantly increased in FVB ($p<0.05$). No significant change was observed in B6. In feces, B6 microbiota were altered by fructose treatment the most (nine families and three genera). Among these, 5 families are of phylum Firmicutes, and all were significantly decreased. Verrucomicrobiaceae and its species *Akkermansia*, which is known as anti-obesity bacteria, were increased. S24-7 of phylum Bacteroidetes encompasses ~ 50% of microbiota community and was significantly increased by fructose treatment. In DBA, fructose altered the abundance of Rikenellaceae and Pseudomonadaceae, and their genera. These two families were also found to be fructose-responsive in B6, however, the changes were more dramatic in DBA mice: 2.7 fold vs 1.5 fold decrease in Rikenellaceae in DBA versus B6, respectively, and 30 fold vs 3 fold increase in Pseudomonadaceae in DBA versus B6, respectively (Table 1, Fig. 5).

Correlation of fructose-responsive microbiota with host metabolic phenotypes

To see if the fructose responsive microbiota is associated with metabolic phenotypes, we correlated their abundance with metabolic phenotypes. Most of the fructose-responsive microbiota in DBA was correlated with their metabolic phenotypes. In cecum, an unknown genus of Erysipelotrichaceae was correlated with adiposity and $AUC_{glucose}$ in IPGTT. In feces, families Rikenellaceae and Pseudomonadaceae, and their genera in fructose group were correlated with one or more of the phenotypes including body weight, adiposity, and $AUC_{glucose}$ with no significant correlation in water group. It is notable that Pseudomonadaceae and its genera were positively correlated with metabolic phenotypes, while Rikenellaceae and its genera were negatively correlated. (Fig. 6, Fig. S4). These two families were also identified in B6 mice but no correlation was detected, suggesting even same microbiota works differently in mouse strains with different genetic background. No correlation was observed with fructose-responsive bacteria in B6 and FVB.

Correlation of fructose-responsive microbiota with differentially expressed genes in host peripheral tissues

Next, we questioned whether the fructose-responsive gut microbiota may regulate host gene expression, which may bridge the development of metabolic phenotypes. To address this, we investigated the correlation between abundance of fructose-responsive microbiota and host genes altered by fructose in liver, adipose tissue, and hypothalamus, termed “fructose signature genes” (Table 2). We found distinct patterns

of correlation between gut microbiota and host gene expression at $p < 0.05$ between mouse strains: the fructose-responsive microbiota in B6 was associated with only hypothalamic fructose signature genes, while the microbiota in DBA cecum or feces were associated with the signature genes from only liver or adipose tissue, respectively. No correlation was found in FVB microbiota. In B6, all fructose-responsive bacteria except Turicibacteraceae were correlated with hypothalamic signature genes. By classifying correlated genes for their biological functions, these hypothalamic genes are involved in pathways including neuron development, response to alcohol, steroid hormones, regulation of heart contraction, transmembrane transport of small molecules, metabolism of protein and mRNA, protein targeting to membrane, etc. Unknown genus of family Mogibacteriaceae has the most correlated genes by 130 genes. *Dehalobacterium* was decreased by fructose consumption (Table 1), and positively correlated with the genes encode neurotransmitter transporter Slc6a3, notch signaling component, Nrarp, autophagy-related marker Atg3. Akkermansia was increased in response to fructose (Table 1) and correlated with several neurotransmitter encoding genes; Akkermansia was positively correlated with Oxt which acts like insulin, while it was negatively correlated with Th which encodes tyrosine hydroxylase, main enzyme in the synthesis of catecholamines (dopamine). In DBA cecum, both *Anaerostipes* and *Clostridium* were associated with liver genes including Cyp8b1, which is responsible for canonical bile acid synthesis and related with lipid accumulation in response to fructose (Yamada et al., 2016). All fructose-responsive bacteria in DBA feces were correlated with numerous host signature genes of adipose tissue, with Rikenellaceae correlating with ~40% of adipose signature genes. The overlapping taxa, Rikenellaceae and

Pesudomonadaceae, between B6 and DBA were correlated with signature genes in mouse strain or tissue specific manner. Contrary to the hypothalamic genes in B6 mice, the correlated genes in DBA adipose tissue are involved in pathways such as response to lipid, cytokines, lipid metabolism, immune system, vesicle mediated transport, cell communication, which more relevant to metabolic syndrome. Adipose genes such as Abhd3, Msr1, Ccr1, Creb, Fas were correlated with both Rikenellaceae and Pseudomonadaceae and their genera, but the correlation direction was opposite (Table 2).

Fecal transplant to test the functional relevance of gut microbiota to strain differences in fructose response

Since B6 and DBA mice showed disparate metabolic responses to fructose, we tested whether B6 confers resistance and DBA microbiota confers vulnerability using fecal transplant experiments. B6 feces were transferred to antibiotics-treated DBA mice. We observed the increased basal levels of body weight gain and fat mass in DBA mice receiving B6 feces (Fig. 7B) compared to the DBA mice (Fig. 7A), however, B6 mice receiving DBA feces had consistent basal level with B6 mice. Remarkably, DBA mice receiving B6 feces became resistant to body weight gain, fat mass and glucose intolerance in spite of the fructose treatment, recapitulating the resistance phenotype of B6 mice. In contrast, B6 mice receiving DBA feces stayed lean and glucose tolerance, suggesting that DBA microbiota failed to induce fructose sensitivity in B6 (Fig. 7).

Discussion

In this study, we examined the role of gut microbiota in the differential metabolic responses to high fructose consumption among genetically diverse mouse strains. Fructose consumption had little effect on adiposity and glucose homeostasis in B6 and FVB mice, but led to dramatic weight gain, high adiposity, and glucose intolerance in DBA. We also observed significant differences in baseline gut microbiota composition among the strains as well as differential responses in microbiota to fructose consumption, with many differential baseline or fructose-responsive microbes associated metabolic phenotypes and/or metabolic pathways in host tissues. Our fecal transplant experiments support that B6 mice carry microbiota that confers resistance to fructose-induced metabolic dysfunctions Taken together, these results support a causal role of gut microbiota in determining differential sensitivity to fructose consumption among genetically diverse mouse strains.

High dose of fructose spill over and reach colonic microbiota, and undergoes colonic fermentation. The resulting metabolites become energy source of bacteria and shapes gut environment (Ríos-Covián et al., 2016). Here we explore the role of gut microbiota in modulating fructose response in hosts with different genetic background. As initial colonizing microbial species are important for the developing immune system and the establishment of favorable environment for bacterial growth (Jost et al., 2012), differences in the baseline microbiota can help explain variations in the metabolic processes of individuals in response to diet (Kootte et al., 2017; Lozupone et al., 2012). At the phylum level, the fructose-sensitive DBA mice showed higher

Firmicutes/Bacteroidetes ratio compared to B6 and FVB mice (Suppl. Fig. 2), agreeing with the known association of Firmicutes with obesity and the increased adiposity in DBA (Ley et al., 2005). In contrast, the resistant strain FVB has higher level of fecal Tenerictues. The only class in Tenericutes is Mollicutes, which uses sugars (glucose, fructose) as source of carbon and energy via glycolysis (Halbedel et al., 2006). The favorable bloom of Tenericutes in FVB mice may be related with their high consumption of fructose and reduced food consumption while maintaining unaltered total caloric intake. At genus level, Akkermancia and Turicibacter are the top microbes which were almost depleted in DBA mice but are abundant in B6 and FVB mice, whereas *Lactobacillus* was a top microbe with higher baseline levels in DBA mice, all of which were correlated with adiposity (Fig. 3,4). Akkermansia muciniphila has long been associated with obesity resistance and improving metabolic parameters in humans and mice (Dao et al., 2016; Derrien et al., 2004; Everard et al., 2013). Turicibacter is related with the assimilation or sequestration of cholesterol, and low abundance has pathophysiological importance in the development of cardiometabolic diseases (Dimova et al., 2017). The lack of both Akkermansia and Turicibacter in DBA agree with the heightened potential for metabolic syndrome in response to fructose consumption. On the other hand, Lachnospiraceae blooms in high fat diet induced obesity and S24-7, the major constituent group of Bacteroidetes, decreases (Evans et al., 2014). S24–7 has protective effects against diabetes whereas members of Lachnospiraceae promote pathogenesis (Krych et al., 2015). The observed higher abundance in Lachnospiraceae and lower S24-7 in DBA again agree with the vulnerability of this strain to fructose-induced metabolic syndrome. Therefore, all of these bacterial species that differ

significantly at baseline between strains have the potential to regulate the differential response to fructose consumption. In addition to differences in baseline microbes that can explain inter-individual variability, bacteria altered by fructose may also play a role in the variability in fructose response. It has been suggested that fructose conditions gut microbiota and leads to an acquisition of a westernized microbiome with altered metabolic capacity, resulting in development of obesity or metabolic disorders (Payne et al., 2012; Di Luccia et al., 2015). In our study, we found various microbes with significantly altered abundances in DBA and B6 but not FVB, which may relate to their differential sensitivity to fructose. In DBA cecum, Erypsipelotricaceae and its genera were decreased in abundant by fructose consumption (Table 1, Fig.5). Erysipelotricaceae and its genus (Anaerostipes) is known as a butyrate-producing bacteria (Levine et al., 2013). Butyrate promotes the intestinal barrier development, and the decreased butyrate production can increase intestinal permeability (Peng et al., 2009). In both B6 and DBA feces, we also identified the differential abundance of Rikenellaceae (decrease) and Pseudomonadaceae (increase) by fructose consumption (Table 1). Increased abundance of Pseudomonadaceae and Pseudomonas also identified in obese individual with higher insulin resistance (Moreno-Indias et al., 2016).

We detected more fructose-responsive microbiota in B6 mice than in DBA and FVB mice (Table 1), and, surprisingly, most of these taxa were associated with the hypothalamic signature genes (Table 2). Among signature genes correlated with Dehalobacterium, Nrarp (positively correlated) encodes an intracellular component of the Notch signaling pathway, which regulates differentiation of mouse hypothalamic arcuate neurons important for feeding and energy balance. Dysregulation of this

homeostatic mediator underlies diseases ranging from growth failure to obesity. (Lamar et al., 2001; Aujla et al., 2013). Akkermansia was increased by fructose consumption, and was also associated with 44 hypothalamic genes, among which Th gene (negatively correlated) encodes tyrosine hydroxylase (TH). Hypothalamic arcuate nucleus TH neurons play orexigenic role in energy homeostasis. Activation of TH neurons increases feeding, whereas silencing reduces body weight (Zhang and Van Den Pol, 2016). Meng et al.(2016) reported fructose as a powerful inducer of genomic and epigenomic variability with the capacity to reorganize gene networks critical for central metabolic regulation and neuronal processes in the brain. Our study suggests fructose effect on hypothalamic gene expression in B6 mice may be partly via fructose-responsive gut microbiota.

In DBA, fructose responsive microbiota was more associated with genes in the adipose tissue involved in lipid metabolism and inflammation. Abhd3 is required for the anti-lipolytic action of insulin and a crucial factor of insulin resistance in adipose tissue (Xia et al., 2018), Ccr1 is chemokine receptor, and is elevated on inflammatory cells in omental and subcutaneous adipose tissues of obese patients (Yao et al., 2014). Creb promotes expression of transcriptional factors of adipogenesis in preadipocytes to form adipocytes, and adipocytes Creb promotes insulin resistance in obesity (Qi et al., 2010; Zhang et al., 2004). Fas in adipocytes contributes to adipose tissue inflammation, hepatic steatosis, and insulin resistance induced by obesity (Wueest et al., 2010). Therefore, these adipose-tissue genes correlating with fructose-responsive bacteria in DBA are relevant to the increased adiposity and compromised insulin sensitivity seen in DBA mice. Although the above analyses suggest a relationship between gut microbiota

and differential metabolic sensitivity to fructose, these are correlative in nature and cannot support causality. Our fecal transplant study helps establish that B6 mice carry gut microbes that confer resistance to fructose-induced metabolic syndrome. Future efforts testing the pathogenic or protective role of other individual bacteria revealed, such as Akkermansia, Lactobacillus, Turicibacrer, or Pseudomonas, are warranted.

In summary, our multi-strain, multi-omics (gut microbiome, transcriptome, and phenome) integrative study of inter-individual variability in fructose-induced metabolic syndrome help establish the causal role of gut microbiota in modulating host metabolic and tissue-specific transcriptomic responses to fructose, a significant metabolic risk in modern societies. Our study also helps prioritize key microbial species that may serve as preventative and therapeutic targets for metabolic syndrome.

Figure legends

Fig. 1. Overall study design. Three mouse strains, C57BL/6J (B6), DBA/2J (DBA), FVB/NJ (FVB), were used to study the differential metabolic responses to high fructose consumption. Followed by 8 % fructose consumption for 12 weeks, mice were examined for metabolic phenotypes, microbiome from strain-specific microbiota and fructose-responsive microbiota, and transcriptome from host individual tissues (liver, adipose tissue, and hypothalamus). The gut microbiota differences between strains and between treatment groups were correlated with metabolic phenotypes and gene expression in individual tissues to prioritize microbial species. The causal role of gut microbiota was validated by fecal transplant between B6 mice (fructose resistant) and DBA mice (fructose sensitive).

Fig. 2. Clustering of cecal and fecal microbiota of three mouse strains with fructose consumption. Principal coordinates analysis (PCoA) of UniFrac distances based on 16S rRNA analysis is done from cecal and fecal microbiota. Mice were treated by 8 % fructose drinking water for 12 weeks. Feces were collected at 1, 2, 4, and 12 weeks of fructose consumption. Cecum was collected in the end of experiment. Each circle represents the microbiota profiles of individual mouse, and is colored according to different strains (purple, yellow, and green for B6, DBA, and FVB mouse, respectively), different treatment (pink and cyan for fructose and water treatment), and treatment time (red, blue, orange, and green for 1, 2, 4, 12 week treatment, respectively). (A) cecal microbiota of three strains (n = 16 for each strain), (B) fecal

microbiota of three strains (n = 64 for each strain), and fecal microbiota of all strains (C, n = 64), B6 (D, n = 16), DBA (E, n = 16), FVB (F, n = 16) mice with the different treatment time. Cecal microbiota of DBA (G, n = 16), B6 (H, n = 16), FVB (I, n = 16) mice with the different treatment. Fecal microbiota of B6 (D, n = 64), DBA (E, n = 64), FVB (F, n = 64) mice with the different treatment and treatment time. Percent variance is shown in parentheses.

Fig. 3. Differences in the baseline microbial composition among three strains Relative abundance of strain-specific baseline microbiota was compared between B6, DBA, and FVB mouse strains in the feces. The baseline microbiota levels were analyzed using the microbiota from water group mice. (A) Lactobacillus, (B) Clostridiales, (C) Lachnospiraceae, (D) S24-7, (E) Akkermansia, (F) Turicibacrer. Each bar represents each mouse sample (n = 30-32 for each mouse strain).

Fig. 4. Correlation of relative abundance of baseline microbiota with the gained adiposity in three mouse strains. Relative abundance of baseline microbiota was correlated with the gained adiposity by fructose consumption for 12 weeks (n = 7-8/ mouse strain). r = Biweight midcorrelation, p = adjusted p value corrected using Benjamini Hochberg procedure.

Fig. 5. Differential abundance of gut microbiota by fructose consumption at the genus level. Differential abundance between mean proportions of microbiota in water

(cyan) and fructose (pink) groups was identified using extended error bar plot. (A) DBA, (B) FVB in cecum, (C) B6, (D) DBA in feces. Corrected q values are shown at right.

Fig 6. Correlation between significant DBA microbiota in genus level and metabolic phenotypes. The relative abundance of the differentially abundant microbiota by fructose treatment (in Table 1) was correlated with metabolic phenotypes. A-C; cecal microbiota (n = 7-8 each group), Data were collected at 12 weeks of fructose consumption, D-I: fecal microbiota. Data were collected at 1, 2, 4, 12 weeks of fructose consumption. Water (W) group and fructose (F) group were analyzed separately (n = 7-8/ group/time point). r = Biweight midcorrelation, p = adjusted p value corrected using Benjamini Hochberg procedure.

Fig. 7. Effects of B6 microbiota on fructose-induced metabolic phenotypes in DBA mice. (A) B6 and DBA mice were provided by water or fructose water for 12 weeks. In the end of experiment, metabolic phenotypes were collected. Figures show the study design, body weight gain, fat mass, and glucose values during IPGTT in B6 and DBA mice. (B) Followed by clearing gut microbiota by antibiotics treatment, donor fecal supernatant from B6 or DBA was transplanted to the recipient mice by oral gavage. B6(DBA) mice are the B6 mice receiving DBA feces, DBA(B6) mice are the DBA mice receiving B6 feces. After 1 week of fecal transplant, B6(DBA) and DBA(B6) mice were challenged by fructose water for 12 weeks. Control group was provided by water. In the end of experiment, metabolic phenotypes were collected. Figures show fecal transplant experimental design (Fig. S1 describes in detail), body weight gain, fat

mass, and glucose values during IPGTT in B6 (DBA) and DBA (B6) mice. *$p < 0.05$; **$p < 0.01$ by Student's t test. Data are mean ± SEM; n = 8 except B6 (DBA) water group (n = 5).

Suppl. figure legends

Suppl. Fig. 1. Study design of fecal transplant.

Suppl. Fig. 2. Relative abundance of microbiota. Six major microbiota phyla identified in cecal and fecal contents from B6, DBA, and FVB mice. Only water group microbiota was analyzed to see the baseline microbiota composition.

Suppl. Fig. 3. Identification of differentially abundant taxa in the gut of three strains using Linear discriminant analysis (LDA) effect size. Cladogram shows the comparison of cecal (A) and feces microbiota of three strains (B). (C) Cecal or feces (D) microbial genera which are the most differentially enriched in either one of mouse strains. Each ring of the cladogram represents a different taxonomic level, starting with kingdom in the center and ending with genus in the outer ring. Colors indicate the group with the highest mean of differential features in which significant differences were found. Taxa colored yellow does not statistically differ in abundance between groups. n = 30-32 each mouse strain, only water groups were analyzed to examine the baseline microbiota composition.

Suppl. Fig. 4. Correlation between significant DBA microbiota in family level and metabolic phenotypes. The relative abundance of the differentially abundant microbiota by fructose treatment (in Table 1) were correlated with metabolic phenotypes.

r = Biweight midcorrelation, p = adjusted p value corrected with using the Benjamini-Hochberg procedure.

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Table 1. Differential abundance of gut microbiota by fructose drink at the family level

Strain	Bacterial family (Level 5)	Relative abundance (%) (Mean ± SD) Fructose	Water	p-value
(Cecum)				
DBA	Erysipelotrichaceae	0.007 ± 0.003	0.13 ± 0.07	0.038
(Feces)				
B6	Rikenellaceae	11.6 ± 2.86	18.70 ± 5.27	0.043
	S24-7	59.33 ± 4.53	49.89 ± 5.54	0.03
	Dehalobacteriaceae	0.026 ± 0.008	0.053 ± 0.01	0.02
	Lachnospiraceae	3.56 ± 1.19	6.027 ±1.36	0.03
	[Mogibacteriaceae]	0.022 ± 0.005	0.057 ±0.02	0.02
	Ruminococcaceae	2.28 ± 0.45	3.23 ±0.59	0.032
	Turicibacteraceae	0.64 ± 0.29	1.84 ± 0.76	0.03
	Pseudomonadaceae	0.019 ± 0.012	0.006 ± 0.002	0.043
	Verrucomicrobiaceae	4.93 ± 1.95	0.68 ± 0.48	0.002
DBA	Rikenellaceae	6.50 ± 3.04	17.38 ± 2.78	0.023
	Pseudomonadaceae	0.2 ± 0.17	0.006 ± 0.003	0.023

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Table 1. Differential abundance of gut microbiota by fructose drink at the family level

Strain	Bacterial family (Level 5)	Relative abundance (%) (Mean ± SD) Fructose	Water	p-value
(Cecum)				
DBA	Erysipelotrichaceae	0.007 ± 0.003	0.127 ± 0.073	0.038
(Feces)				
B6	Rikenellaceae	11.6 ± 2.861	18.701 ± 5.271	0.043
	S24-7	59.336 ± 4.534	49.892 ± 5.544	0.03
	Dehalobacteriaceae	0.026 ± 0.008	0.053 ± 0.014	0.02
	Lachnospiraceae	3.56 ± 1.194	6.027 ±1.358	0.03
	[Mogibacteriaceae]	0.022 ± 0.005	0.057 ±0.02	0.02
	Ruminococcaceae	2.278 ± 0.454	3.234 ±0.589	0.032
	Turicibacteraceae	0.644 ± 0.29	1.837 ± 0.763	0.03
	Pseudomonadaceae	0.019 ± 0.0121	0.006 ± 0.002	0.043
	Verrucomicrobiaceae	4.925 ± 1.925	0.678 ± 0.475	0.002
DBA	Rikenellaceae	6.495 ± 3.037	17.375 ± 2.776	0.023
	Pseudomonadaceae	0.2 ± 0.169	0.006 ± 0.003	0.023

Taxa within an individual strain at 12 weeks of the study were compared between the fructose and water treatment using the White's non-parametric T-test with the Storey false discovery rate.

Table 2. Correlation between differentially abundant microbiota and host signature genes

Mouse strain	Fructose-responsive microbiota		Correlation with host fructose responsive genes			
	Family	Genus	Tissue	Gene #	Relevant genes	Canonical pathway
B6 (feces)	Rikenellaceae		Hypothalamus	37	Emg1, Rpl37a, Oat, Rpl32, Psmb4, Ctsz, Ttc9b , Svop	Protein localiation in endoplasmic reticulum, membrane, Pathways involving ribosomal RNA, Interspecies interaction between organisms, Protein targeting to menbrane, peptide metabolic process, NCRNA processing
	S24-7			11	Eef1a1, Pef1, Gnb2, Slc25a17, Numbl	
	Dehalobacteriaceae			7	Atp2b2, Slc6a3, Nefh, Nrarp, Hint2, Aqp4, AW209491	Lactation, Response to cocaine, Transmembrane transport of samll molecules
	Dehalobacteriaceae	Dehalobacterium		41	Atp2b2, Slc6a3, Aqp4, Nefh, AW209491, PLIN3, ATG3, Nrarp	Neuron development, Response to inorganic substance (phenol containg compound), Response to alcohol, Regulation of heart contract, Response to corticosterone
	Lachnospiraceae			2	Rbm39, Maneal	
	[Mogibacteriaceae]			54	Timp3, Slc6a15, Tmem199, Snrpe, Igfbp7, Rps15a, Psmb4, Mogs	Organonitrogen compound metabolic process, Metabolism of proteins, Protein targeting to membrane, Cellular catabolic process, Metabolism of RNA
	[Mogibacteriaceae]	Mogibacteriaceae (F)		130	Timp3, Slc6a15, Tmem199, Mogs, Stard3nl	Multiorganism metabolic process, Metabolism of protein, Protein localization to endoplasmic reticulum, Metabolism of RNA,
	Ruminococcaceae			12	Trappc2l, Gnb2, Uck1, Mid1ip1, Itm2a	
	Pseudomonadaceae			2	Cxx1c, Numbl	
	Verrucomicrobiaceae			13	Scarna2, AW209491, Gja1, Rps9 , Ptov1, Aqp4	
	Verrucomicrobiaceae	Akkermansia		44	Rps9, Aqp4, Slc11a2, Oxt, Gja1, Th	Ribosome, Protein localization to endoplasmic reticulum, Mulitorganism metabolic process, neuron development, Response to steroid hormone
DBA (cecum)	Erysipelotrichaceae	Erysipelotrichaceae (F)	Liver	5	Gsdmd, Wdr82, Pgk1, S100a10, Tmem62	
	Erysipelotrichaceae	Clostridum		5	Epha2, Cyp8b1, Ero1l, Pus10, Mad2l2	
	Lachnospiraceae	Anaerostipes		4	Cyp8b1, Rogdi, Mad2l2, Baiap2	
DBA (feces)	Rikenellaceae		Adipose tissue	448	Arsj, Ccr1, F11r, Stab2, Faim, Gpr183, Fas, Hif1a, Apobec1, CDC73, LEP, Oxtr, Abhd3, , Msr1, Sema3E	Response to small molecule (lipid, cytokines, bacterial origin molecule), Lipid metabolic process, Immune system process, Vesicle mediated transport, Cell communication
	Rikenellaceae	Rikenellaceae(F)		433	F11r, Stab2, Faim, Gpr183, Apobec1, Nr2c2, Oxtr, Abhd3, , Msr1, Sema3E, Fas	Response to small molecule (organic compound, lipid, cytokines, bacterial origin molecule) , Lipid metabolic process, Immune system process, Vesicle mediated transport, Cell communication
	Pseudomonadaceae			73	FAS, S100a8, Sfrp4, Ceacam1, Nr2c2, Abhd3, , Msr1, Sema3E	Cell differentiation and cell death, Regulation of lipid metabolic process, Autophagy, Response to stress, Regulation of lipid metabolism
	Pseudomonadaceae	Pseudomonadaceae(F)		235	Nr2c2, Gbp6, Creb1, Irf5, Fabp3, Msr1, Sema3E, Fas	RNA processing, Response to organic molecules, lipid, Response to stimulus (bacterium), Catabolic process, Immune system process
	Pseudomonadaceae	Pseudomonas		43	Ppp1r13b, Jmjd1c, Msr1, Mir493, Wars2, Fbxw7, Slc25a10, Fabp3	

1. Fructose responsive microbiota which was decreased in relative abundance were expressed in blue.
2. Relevant genes which were negatively correlated with microbiota were expressed in blue.

3.Correlation was analyzed using Biweight midcorrelation with the P value ($p < 0.05$) corrected with Benjamini-Hochberg procedure.

Fig. 1

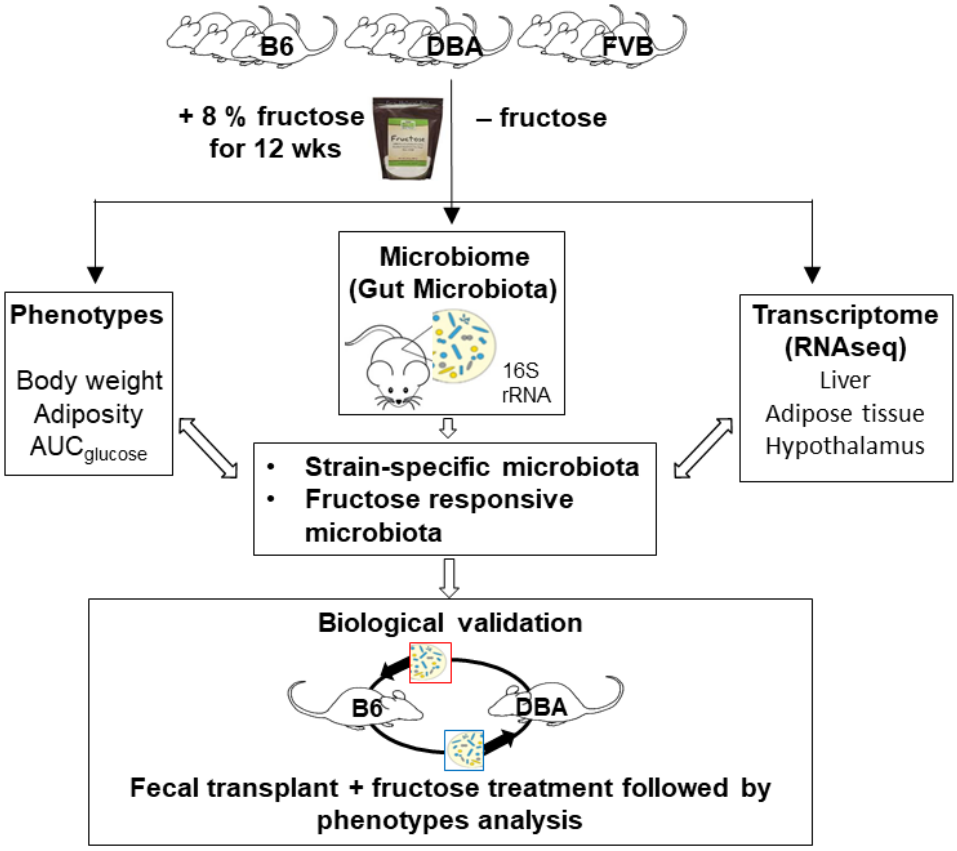

B6
DBA
FVB
+ 8 % fructose
for 12 wks
– fructose
Phenotypes
Body weight
Adiposity
AUCglucose
Microbiome
(Gut Microbiota)
16S
rRNA
Transcriptome
(RNAseq)
Liver
Adipose tissue
Hypothalamus
• Strain-specific microbiota
• Fructose responsive microbiota
Biological validation
B6
DBA
Fecal transplant + fructose treatment followed by phenotypes analysis

Fig. 2

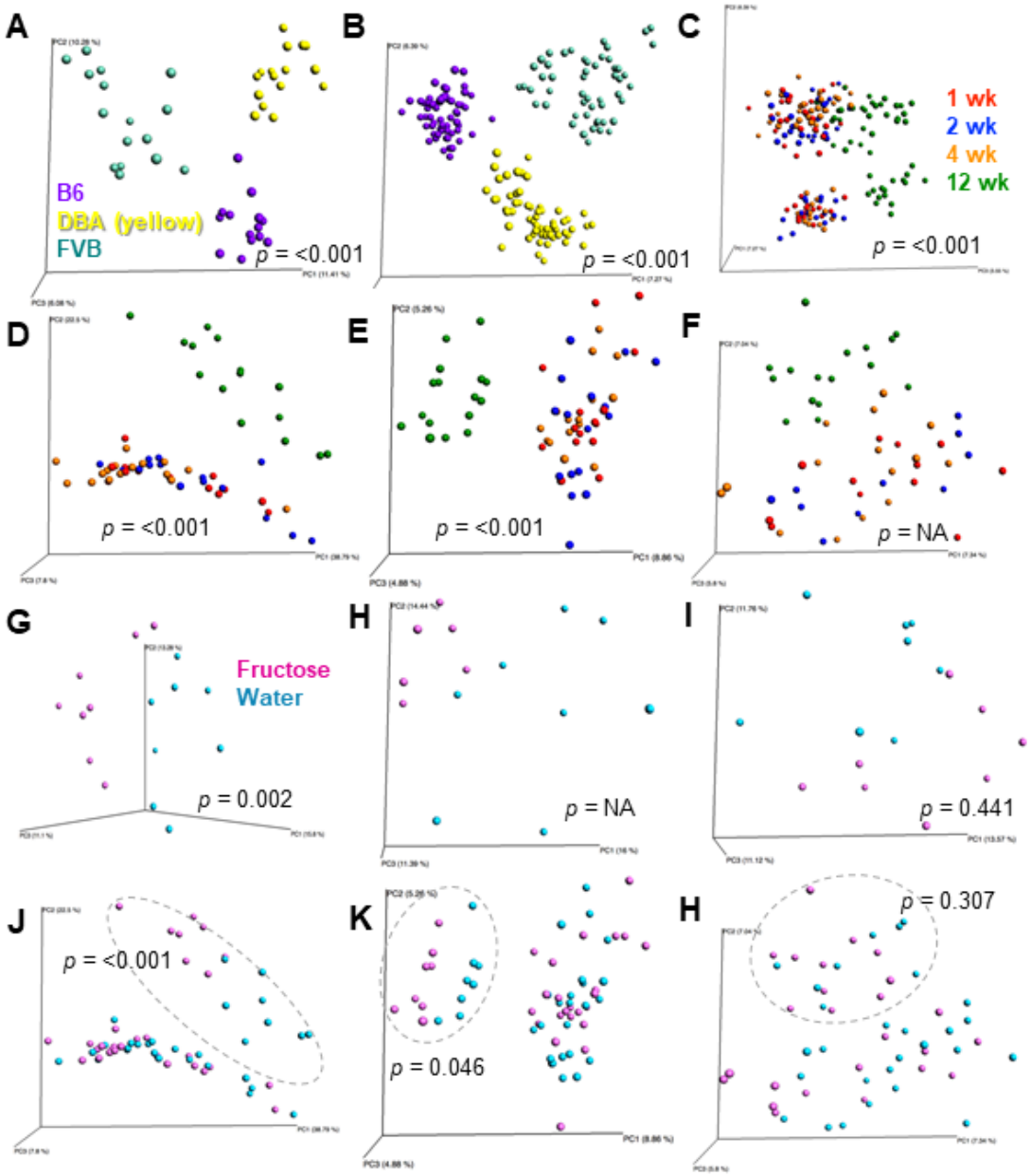

A
B6
DBA (yellow)
FVB
p = <0.001
B
p = <0.001
C
1 wk
2 wk
4 wk
12 wk
p = <0.001
D
p = <0.001
E
p = <0.001
F
p = NA
G
Fructose
Water
p = 0.002
H
p = NA
I
p = 0.441
J
p = <0.001
K
p = 0.046
H
p = 0.307

Fig. 3

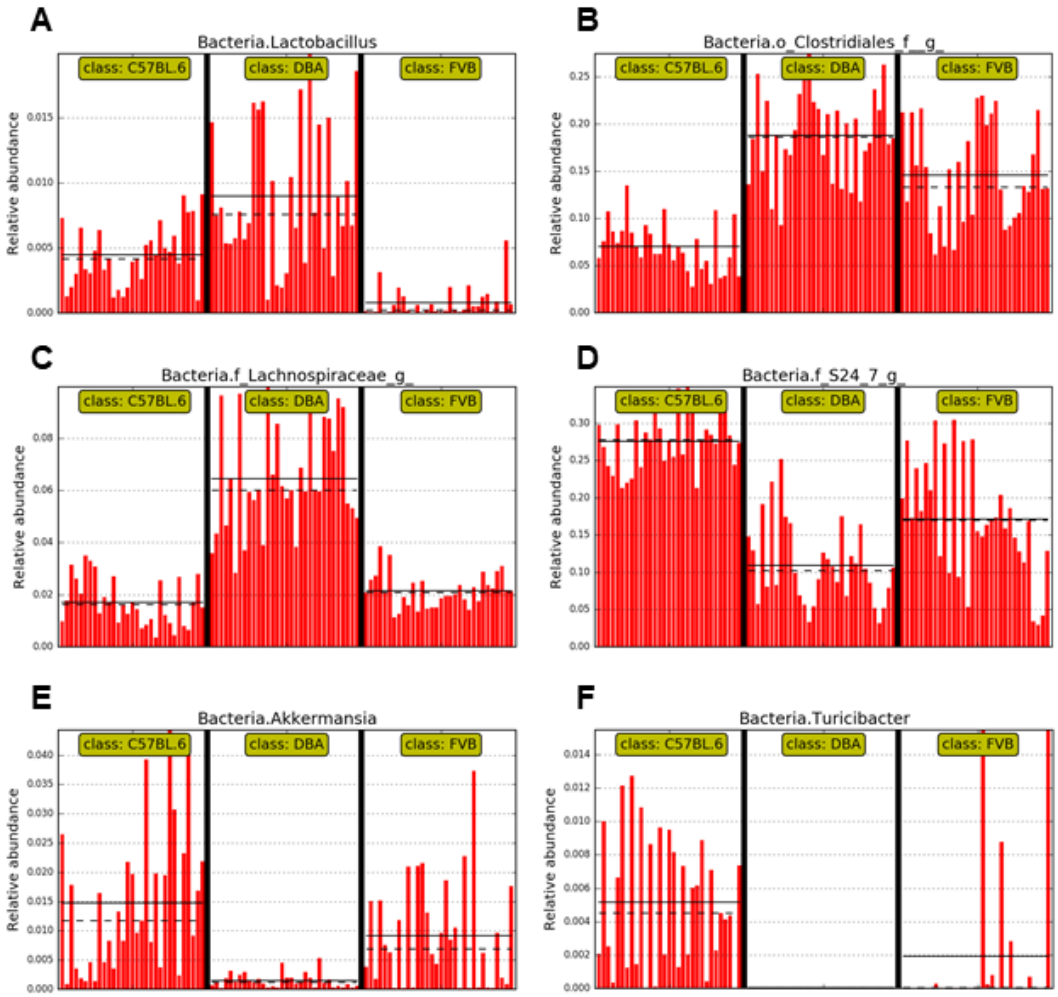

A
Bacteria.Lactobacillus
class: C57BL.6
class: DBA
class: FVB
Relative abundance
B
Bacteria.o_Clostridiales_f__g_
C
Bacteria.f_Lachnospiraceae_g_
D
Bacteria.f_S24_7_g_
E
Bacteria.Akkermansia
F
Bacteria.Turicibacter

Fig. 4

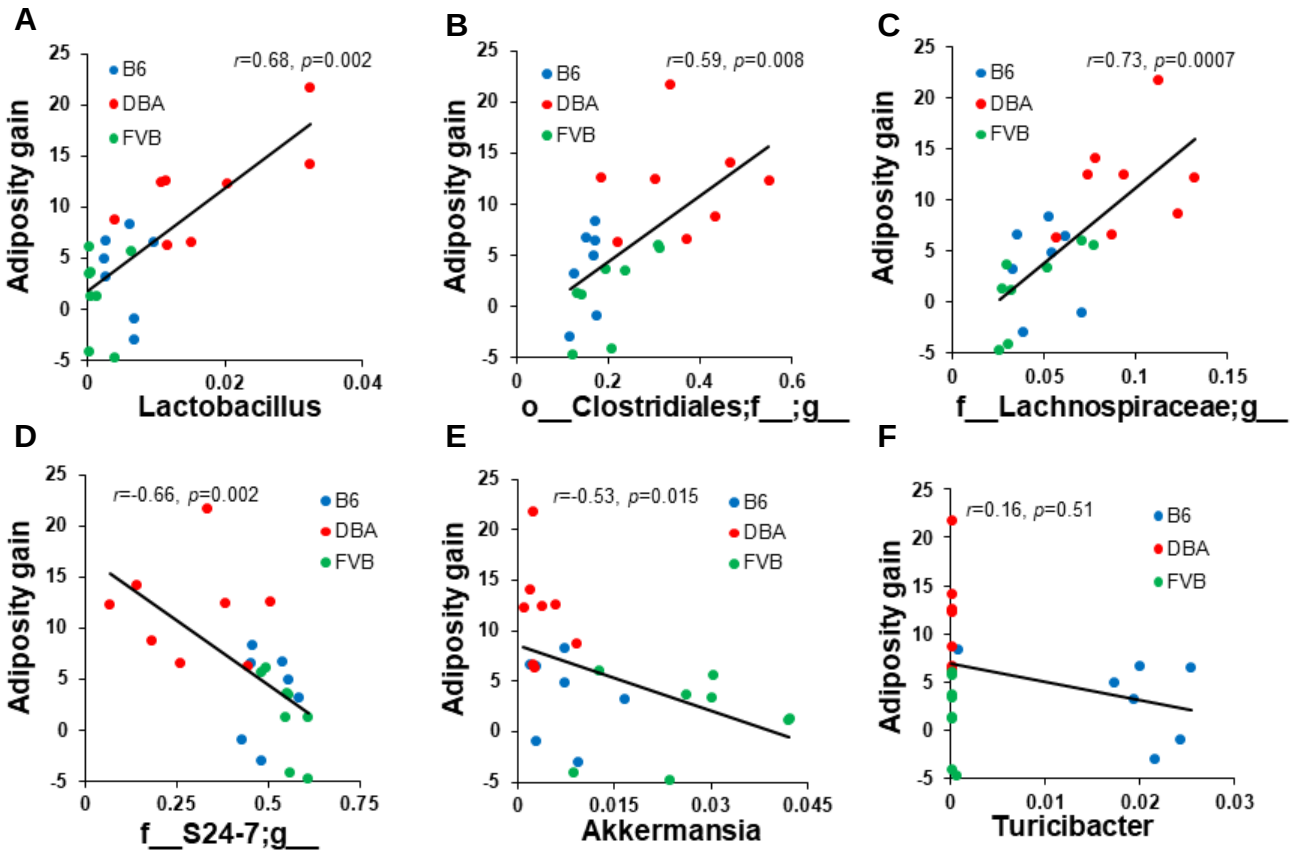

A
B6
DBA
FVB
r=0.68, p=0.002
Adiposity gain
Lactobacillus
B
r=0.59, p=0.008
o__Clostridiales;f__;g__
C
r=0.73, p=0.0007
f__Lachnospiraceae;g__
D
r=-0.66, p=0.002
f__S24-7;g__
E
r=-0.53, p=0.015
Akkermansia
F
r=0.16, p=0.51
Turicibacter

Fig. 5

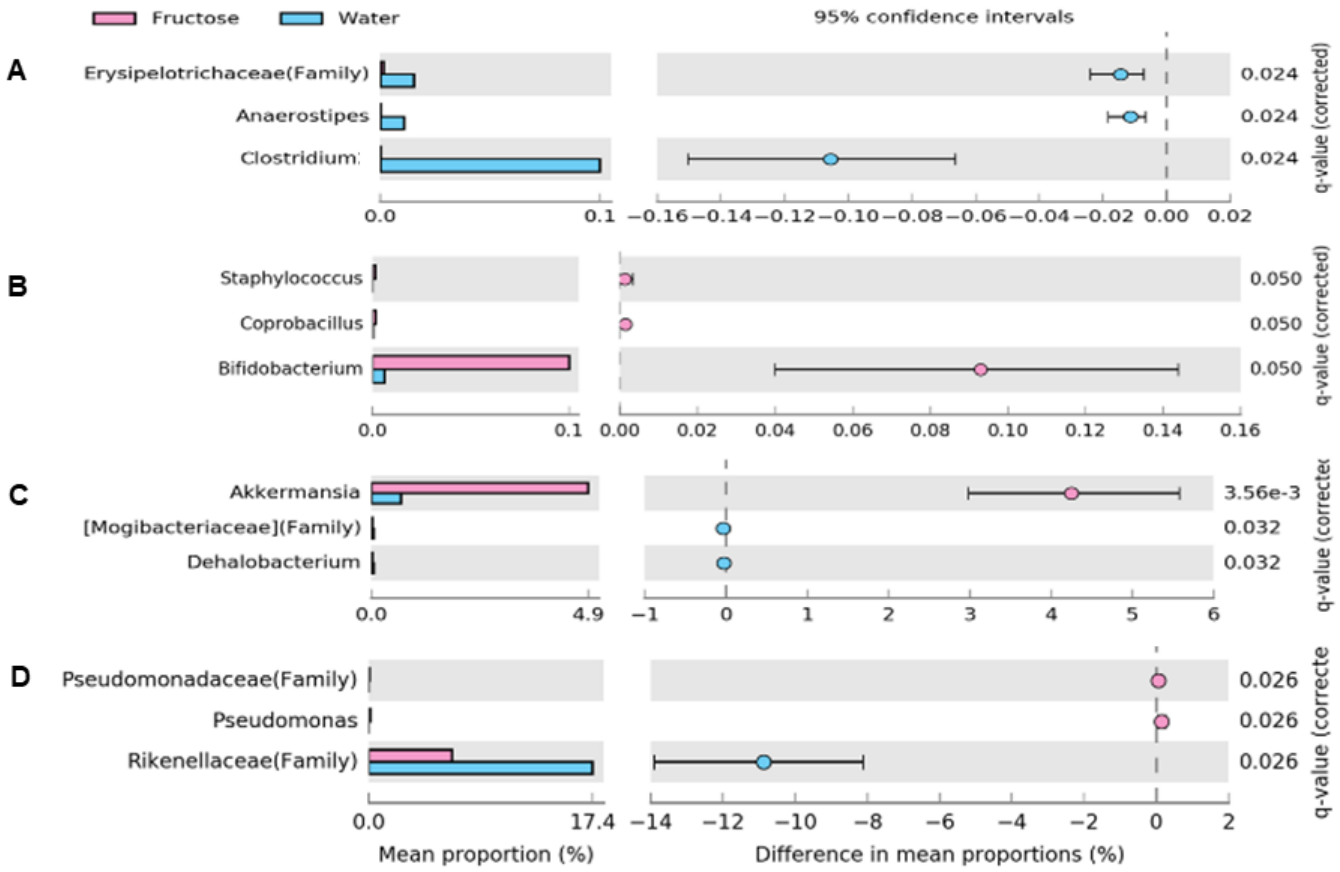

Fructose
Water
95% confidence intervals
A
Erysipelotrichaceae(Family)
Anaerostipes
Clostridium
0.024
0.024
0.024
q-value (corrected)
0.0
0.1
−0.16 −0.14 −0.12 −0.10 −0.08 −0.06 −0.04 −0.02 0.00 0.02
B
Staphylococcus
Coprobacillus
Bifidobacterium
0.050
0.050
0.050
q-value (corrected)
0.0
0.1
0.00 0.02 0.04 0.06 0.08 0.10 0.12 0.14 0.16
C
Akkermansia
[Mogibacteriaceae](Family)
Dehalobacterium
3.56e-3
0.032
0.032
q-value (correcte
0.0
4.9
−1 0 1 2 3 4 5 6
D
Pseudomonadaceae(Family)
Pseudomonas
Rikenellaceae(Family)
0.026
0.026
0.026
q-value (correcte
0.0
17.4
−14 −12 −10 −8 −6 −4 −2 0 2
Mean proportion (%)
Difference in mean proportions (%)

Fig. 6

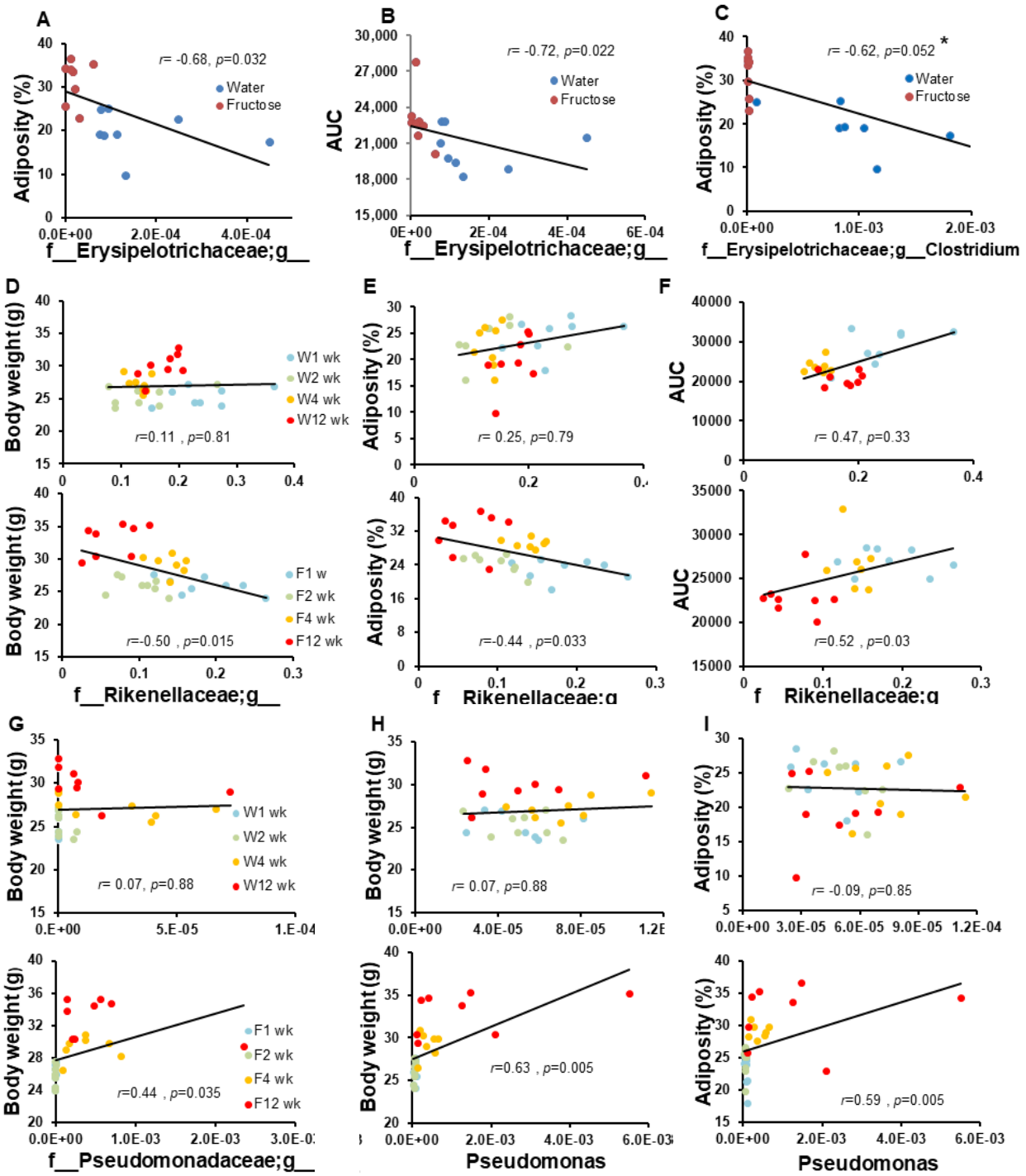
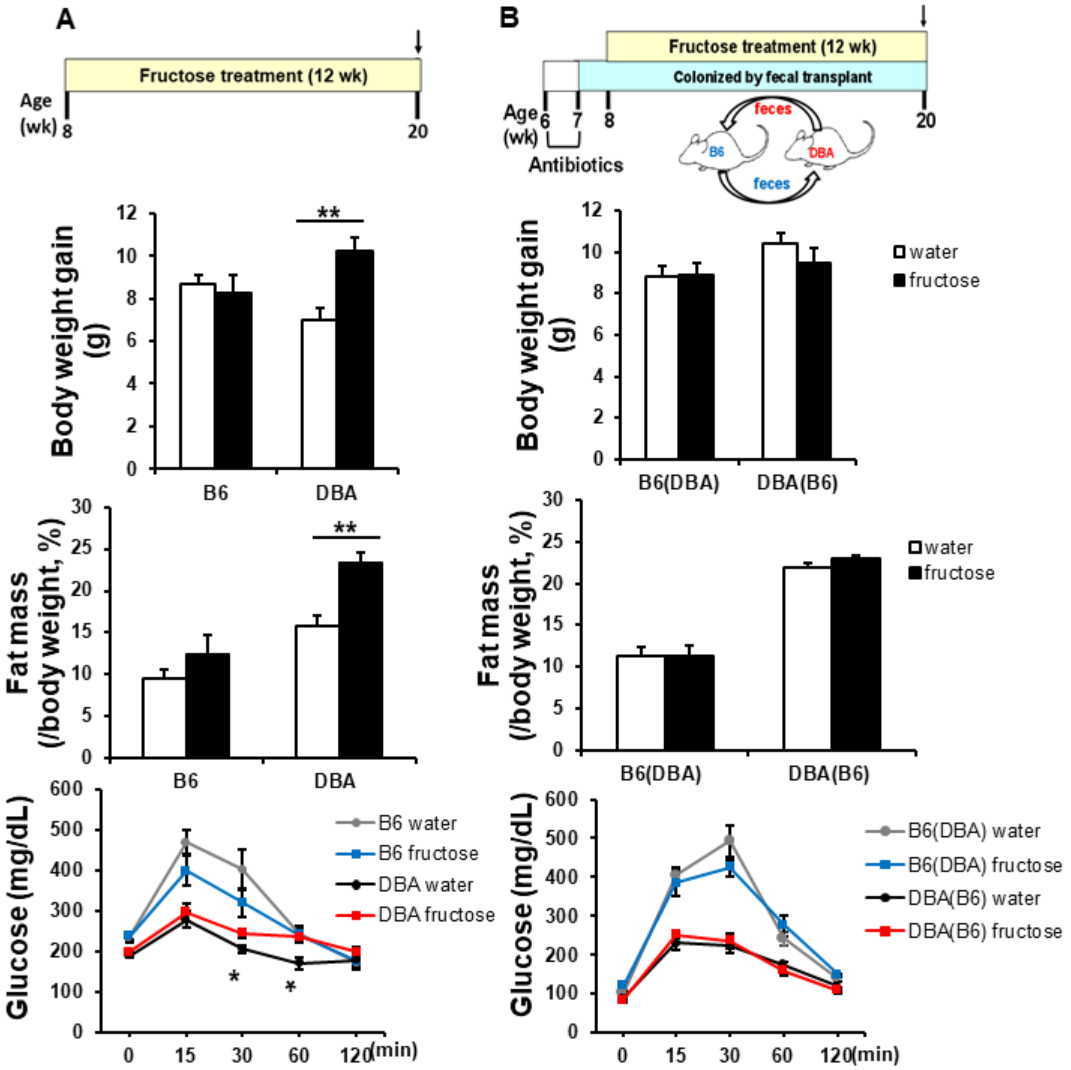

A
Adiposity (%)
r= -0.68, p=0.032
Water
Fructose
f__Erysipelotrichaceae;g__
B
AUC
r= -0.72, p=0.022
Water
Fructose
f__Erysipelotrichaceae;g__
C
Adiposity (%)
r= -0.62, p=0.052 *
Water
Fructose
f__Erysipelotrichaceae;g__Clostridium
D
Body weight (g)
W1 wk
W2 wk
W4 wk
W12 wk
r=0.11 , p=0.81
E
Adiposity (%)
r= 0.25, p=0.79
F
AUC
r= 0.47, p=0.33
Body weight (g)
F1 w
F2 wk
F4 wk
F12 wk
r=-0.50 , p=0.015
f__Rikenellaceae;g__
Adiposity (%)
r=-0.44 , p=0.033
f Rikenellaceae;g
AUC
r=0.52 , p=0.03
f Rikenellaceae;g
G
Body weight (g)
W1 wk
W2 wk
W4 wk
W12 wk
r= 0.07, p=0.88
H
Body weight (g)
r= 0.07, p=0.88
I
Adiposity (%)
r= -0.09, p=0.85
Body weight (g)
F1 wk
F2 wk
F4 wk
F12 wk
r=0.44 , p=0.035
f__Pseudomonadaceae;g__
Body weight (g)
r=0.63 , p=0.005
Pseudomonas
Adiposity (%)
r=0.59 , p=0.005
Pseudomonas

Fig. 7


A
Fructose treatment (12 wk)
Age (wk)
8
20
Body weight gain (g)
**
B6
DBA
Fat mass (/body weight, %)
**
B6
DBA
Glucose (mg/dL)
B6 water
B6 fructose
DBA water
DBA fructose
*
*
0
15
30
60
120
(min)
B
Fructose treatment (12 wk)
Colonized by fecal transplant
Age (wk)
6
7
8
20
Antibiotics
feces
B6
DBA
feces
Body weight gain (g)
water
fructose
B6(DBA)
DBA(B6)
Fat mass (/body weight, %)
water
fructose
B6(DBA)
DBA(B6)
Glucose (mg/dL)
B6(DBA) water
B6(DBA) fructose
DBA(B6) water
DBA(B6) fructose
0
15
30
60
120
(min)