THE ROLE OF PREBIOTICS ON ALTERTING SMALL INTESTINAL NUTRIENT SENSING MECHANISMS REGULATING FOOD INTAKE AND OBESITY

By

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Abstract

Manipulations of the gut microbiota have been implicated as potential therapeutic options for treatment of metabolic disorders. The ingestion of prebiotics, like oligofructose (OFS), represent a non-invasive way to beneficially alter the gut microbiome. Previous studies have shown that OFS lowers body weight and food intake in humans and rodents, although the mechanisms driving these benefits remain poorly understood. Further, no one has examined whether prebiotics alter the small intestinal microbiota, and whether these alterations impact small intestinal nutrient sensing mechanisms. In this study, high-fat diet (HFD)-fed Sprague Dawley rats were treated with OFS for 6 weeks and food intake, body weight, and adiposity were measured, and rats were tested for nutrient-induced intestinal satiation. Furthermore, small intestinal nutrient receptor and transporter expression was measured, and the small intestinal microbiota composition was analyzed. We found that OFS supplementation decreased body weight gain, food intake, and adiposity; and increased intestinal nutrient-sensing mechanisms during HFD. Lastly, we observed a shift in the composition of the small intestinal microbiota in prebiotic-treated HFD rats. Collectively, these results demonstrate the importance of small intestinal nutrient sensing in mediating the beneficial effects of OFS, and highlight the importance of the small intestinal microbiome in energy regulation.

Introduction

Obesity is a serious metabolic disorder which is increasingly affecting populations worldwide. The World Health Organization (WHO) reports that from 1975 to 2016, global obesity has almost tripled, with nearly 2 billion adults (39% of the world's population) classified as overweight and 650 million (13% of the world's population) categorized as obese in 2016. Alarmingly, the prevalence of obesity continues to rise, with childhood obesity rates having increased from 1% in 1975 to about 7% in 2016. In 2016, the estimated number of obese children (aged 5 to 19) worldwide was a staggering 340 million[1]. Obesity is defined as having a ratio of weight to height, called a body mass index (BMI), of 30 or greater[2] and is characterized by an abnormally increased presence of fat in the body, which typically has detrimental effects on health. As such, with obesity comes increased risk of developing other complications and diseases such as diabetes, cardiovascular disorders, and even cancer[3]. The development of obesity has often been linked to genetic and environmental factors, such as a lack of physical activity and overconsumption of a diet rich in fat and sugars[4] as well as to socioeconomic determinants[5]. One emerging factor for the development of obesity that is often resultant from gene and environmental interactions is the gut microbiota, which comprises all the microorganisms occupying the gastrointestinal tract.

These microbes, which are in populations as large as 10^{11} to 10^{12} cells per gram in the colon[6] are known to impact a plethora of crucial metabolic processes linked to the development of obesity and related metabolic diseases.

In obesity, the composition of the intestinal microbiota differs markedly from that of healthy adults in ways that suggest changes in microbiota contribute to the development of this disease[7]. Initially, it was thought that obesity was linked to alterations in the ratio of members of the phylums *Firmicutes* and *Bacteroidetes* [8–10]. Those changes were thought to increase energy extraction from the diet in the form of short-chain fatty acids (SCFAs), which were taken up by the host and contributed to increased energy intake[8,9,11]. However, many more recent studies have not been able to replicate these changes, and most researchers now agree that increases in SCFAs are actually beneficial to the host[11,12]. In fact, one study has confirmed that changes in the *Firmicutes* to *Bacteroidetes* ratio are not indicative of changes in energy extraction; microbial changes leading to obesity are now thought to be most likely due to alterations in more specific taxa[13]. In addition, a larger contribution to the development of metabolic disorders may due not the presence or absence of bacteria themselves, but broad changes in the genes expressed by the gut microbial community and overall genetic functional capacity of the microbial community. This collection of all the genes encoded in the gut microbiota is called the microbiome, and alterations in it have been implicated in obesity. For instance, in obesity there are known changes in carbohydrate and lipid metabolism (with decreases in the sensitivity to glucose and increases in fatty acid production and storage in adipocytes), as well as increases in essential amino acid metabolism pathways and choline synthesis, and decreases in bile acid production[14]. All of these pathways are affected by the gut microbiome and are implicated in the development of obesity. The use of germ-free (GF) mice further demonstrated the role of the gut microbiota in the development of obesity, as GF mice, those devoid of a gut microbiota are lean and resistant to diet-induced obesity. Further, GF mice colonized with the microbiota of lean or obese donors (murine or human) develop the phenotype of their donors, despite similar amounts of chow consumed by each group[15]. These results showed that differences in the gut microbiome differentially altered host metabolism. One prevailing mechanism to explain how the gut microbiome alters host metabolic processes towards an obese phenotype is the development of low-grade systemic inflammation seen in obesity and its related metabolic disease, type 2 diabetes (T2D). This inflammation is thought to be due to an excess of lipopolysaccharide (LPS), a Gram negative bacterial surface molecule. Indeed, it has been shown that there are higher concentrations of Gram-negative bacteria and LPS in obese versus lean individuals. LPS is a ligand for the cellular receptor toll-like receptor-4 (TLR4), which acts as part of the innate immune system. Binding of LPS to TLR4 is taken as a sign of host infection by Gram negative bacteria, and thus immune cells are sent to the gut and release proinflammatory cytokines. This results in the low-grade inflammation seen in

the gut of obese and diabetic individuals[16]. The immune system is only able to attack LPS once it crosses the epithelial layer of the gut; as such, the microbiota are thought to first induce an increase in intestinal permeability which allows for the LPS to cross the intestinal barrier. This may be due to the degradation by harmful bacteria of glycoproteins in the mucous that normally lines and protects the intestinal epithelial cells from the contents of the intestine. Increased intestinal permeability is also thought to be caused by a reduction of the expression of tight junction genes, stimulated by changes in the gut microbiota. With a decrease in tight junction proteins, the intestinal epithelial cells are more permeable to foreign material entering the basal lamina of the intestine. Both of the aforementioned mechanisms would result in increased LPS uptake and thus increased inflammation. However, the inflammation seen in metabolic diseases is not restricted to the intestine alone. Due to the leakage of LPS through the gut, LPS increases in systemic circulation and is able to bind to TLR4 in the liver and adipose tissue, further driving chronic systemic inflammation, which is characteristic of obesity and diabetes[17]. All of these processes —degradation of the intestinal barrier, decreases in tight junction protein expression, and onset of systemic inflammation— are thought to be microbiota-directed, as high-fat diet fed rodents and (which tends to result in the development of obesity genetically obese (*ob/ob*) mice treated with antibiotics do not develop increased LPS, inflammation, and other symptoms of metabolic disease. As such, these mechanisms must have some dependence on gut microbial composition[4,18]. Beneficially altering the composition of the gut microbiota is thus a promising therapeutic target for obesity and related metabolic diseases. Interestingly, several studies have treated systemic low-grade inflammation with the prebiotic oligofructose (OFS), which stimulates growth of beneficial bacteria in the intestine, and is associated with increased gut barrier function and reduced LPS, and thus reduced LPS-induced metabolic endotoxemia[19–22]. Therefore, it is essential to uncover how prebiotic manipulations of the gut microbiota can ameliorate gut dysbiosis and, improve metabolic disease.

As such, prebiotics are a promising and non-invasive method for changing the gut microbiota. Prebiotics are typically defined as non-digestible polysaccharides which are selectively fermented by certain members of the gut microbiota whose presence is known to be beneficial for the health of the host[23]. Some of the most studied prebiotics are inulin-type fructans, such as inulin, fructo-oligosaccharides, and oligofructose; and galacto-oligosaccharides[24]; but others less commonly used are resistant starches, lactulose, and varieties of oligosaccharides including isomalto-oligosaccharides, soybean oligosaccharide, and arabinoxylan saccharides[12]. The Cani lab have conducted many elegant studies using prebiotic treatment in genetically obese (*ob/ob*) as well as high-fat fed mice. Their group has demonstrated that prebiotic treatment in these mice increases the abundance of beneficial bacteria, driving decreased LPS and intestinal permeability, through increased expression of tight junction proteins[19]. These

changes are associated with decreased food intake and body weight, as well as increased insulin sensitivity[18,25]. Decreased circulating LPS via prebiotic treatment was also observed in obese women supplemented with prebiotics[26] demonstrating a clinical therapeutic potential. As such, prebiotics have a great potential for the treatment of obesity and prevention of developing obesity-related health complications.

Prebiotics are believed to at least partially enact their benefits on host metabolism through improvements in nutrient-sensing mechanisms that regulate the release of gut peptides known to control food intake and regulate glucose homeostasis[25]. These peptides, including the incretin glucagon-like peptide 1 (GLP-1), the fat and protein induced satiation hormone cholecystokinin (CCK), and the satiety-inducing protein YY (PYY), are released from enteroendocrine cells (EECs), which constitute a small portion (~1%) of the total intestinal epithelial cells in the gut[27,28]. Release of gut peptides occurs in response to EEC sensing of nutrients in the intestine via appropriate nutrient receptors and/or transporters on these cells[27,29].

Detection of incoming nutrients results in the release of gut peptides from EECs. These peptides can act locally, as well as distally via stimulation of the vagal nerve which links the gut to the hindbrain, ultimately leading to a reduction in food intake or a decrease in hepatic glucose production via subsequent vagal efferent signaling to the liver[27]. Detection of lipids in the intestine is thought to be mediated by GPR40 and GPR120 which bind to medium- and long-chain fatty acids, as well via transport into the cell via CD36 and subsequent lipid metabolism. Lipid detection primarily results in the release of the hormone CCK, which acts via the vagal nerve to stimulate decreases in food intake and gluconeogenesis in the liver. The detection of carbohydrates in the intestine leads to EEC depolarization from cotransport of sodium and glucose via the sodium glucose transporter 1 (SGLT1), initiating release of GLP-1 which suppresses food intake and regulates plasma glucose via insulin secretion and suppression of hepatic glucose production via vagal signaling[30]. Interestingly, obesity and high-fat feeding are associated with a decrease in both the fasting levels and the nutrient-stimulated secretion of these gut peptides[25,27], potentially driving metabolic dysregulation due to inadequate gut-brain negative feedback. Indeed, disruption of these processes is observed in obesity and high-fat feeding, and is thought to be due to the decreased levels of gut peptides and the decreased sensitivity to those peptides in obese individuals. These changes in gut peptide signaling and sensitivity have recently been associated with alterations in the gut microbiota during these conditions. As such, there is a possible role for the gut microbiota in driving changes in nutrient sensing[28]. Indeed, many recent studies have shown strong evidence that the gut microbiota can impact nutrient sensing capabilities, such as altered gut peptide release from EECs, ultimately altering host metabolic regulation[27–29]. This alteration in host metabolism via the microbiota is thought to be at least partially due to the microbial breakdown of indigestible carbohydrates, resulting in the release of short chain fatty acids (SCFAs).

SCFAs in rodents and humans to decrease food intake and reduce body weight; these benefits are thought to result from SCFA-induced stimulation of intestinal signaling pathways, likely via activation of the G-couple protein receptors GPR43 and GPR41 which bind preferentially to SCFAs, resulting in the release of gut peptides like GLP-1 and PYY. Besides SCFAs, the gut microbiota can also influence host metabolism by altering the expression of chemosensory machinery that regulates nutrient-induced gut peptide release, like SGLT1[31]. Though the mechanisms for this process remain to be elucidated, studies in GF mice strongly support the direct connection between host gut microbial composition and intestinal nutrient-sensing. For example, one study showed that GF mice transplanted with the microbiota of obese-prone or obese-resistant rats exhibit changes in nutrient receptors and gut peptides similar to donors[32]. It has also been shown that certain strains of bacteria can change the levels of GPR120 and GLP-1 expression[30]. All of these findings give evidence for the influence of the gut microbiota in host intestinal nutrient-sensing, and thus in the regulation of energy homeostasis.

One limitation is that the vast majority of studies have looked at the microbiota colonizing the large intestine, however, nutrient sensing and absorption primarily takes place in the small intestine. As such, more research needs to be done to elucidate how alterations in small intestinal microbial composition impacts host metabolism. Recently, two studies have demonstrated the capacity of the small intestinal microbiota to regulate host energy nutrient sensing. The first study demonstrated that the intestinal sensing of glucose, demonstrated to regulate hepatic glucose production, is perturbed during high-fat feeding, but that metformin (the number one drug used for treatment of T2D) can restore this intestinal glucose-sensing pathway. This was due to metformin altering the SI microbiota, which subsequently increased intestinal epithelial expression of SGLT1, thus improving glucose-sensing[31]. In the second study, Bauer *et al* showed that similar to glucose, lipid sensing in the intestine is abolished during high-fat feeding, and that transplantation of the SI microbiota from chow to HF rats can restore these sensing pathways, whereas transplantation of the SI microbiota from HF to chow rats can abolish them. The researchers also demonstrated that changes in the small intestinal microbiota can alter glucose homeostasis[33], thus providing support for small intestinal gut microbiota in regulating metabolic processes related to obesity and type 2 diabetes. These results demonstrate the importance of the gut microbiome in the regulation of host metabolism via alterations of EEC driven nutrient-sensing, particularly those in the small intestine. Thus, given that: 1) obesity and high-fat feeding is associated with a decrease in nutrient-induced satiation and changes in the gut microbiota composition, 2) prebiotics increase circulating gut peptide levels, and 3) the small intestinal microbiota can regulate nutrient-sensing mechanisms, we hypothesized that prebiotics improve energy homeostasis during HF-feeding by reducing food intake via improved nutrient-induced satiation from interactions of the host intestinal epithelium with the SI microbiota. Here, we demonstrate that HF-fed obese rats treated with oligofructose for

6wks exhibited reductions in food intake, body weight gain, and adiposity. This was associated with a change in the SI microbiota as well as alterations in small intestinal nutrient receptors. We also found that short-term prebiotic treatment improved nutrient-induced satiation, raising the possibility that prebiotic-induced changes in the small intestinal microbiota drive improvements in small intestinal nutrient sensing to normalize food intake and adiposity.

Methods

Subjects

A total of 24 male Sprague Dawley rats (8 weeks old) were split into 3 groups and one was maintained on chow diet (n=6), while the other two groups (n=9) were placed on a HF-diet (Research Diets D12451; 45% fat, 35% carbohydrates, 4.7 kcal/g) for three weeks to promote weight gain and adiposity. After 3 weeks of HFD, one weight-matched group began supplementation with 10% oligofructose (OFS, Beneo P95) in drinking water (designated as being in the HFD + OFS group), while the other was maintained on the HFD diet. OFS treatment was based on calculations to ensure that the amount of daily OFS received equals 10% of daily food intake (in grams; ~2.5g OFS), similar to previous studies[34]. Food intake and body weight were measured weekly. Prebiotic treatment was administered for 6 weeks (Fig 1A), with an oral glucose tolerance test (OGTT) performed at 4 weeks. At the conclusion of the study, adiposity pads were removed (retroperitoneal, visceral, and epididymal) and weighed, small intestinal contents were collected for analysis of their microbial composition, and the proximal small intestine was scraped and the mucosal layer snap frozen for qPCR analysis.

Oral glucose tolerance test (OGTT)

Rats were tested for glucose tolerance after 4wks of prebiotic treatment by administering 50% glucose solution (2 g glucose/kg body weight) via oral gavage following an overnight fast. Tail blood was sampled at 0, 15, 30, 60, 90, and 120, 180 min post gavage, and blood glucose was analyzed with a glucometer (One-Touch)[35].

RT-qPCR: Was run as we have done previously. Briefly, total RNA will be extracted from mucosal scrapings using a commercially available kit from Ambion (PureLink RNA Mini Kit). For complementary DNA (cDNA) synthesis, a total of 5-10 µg RNA was reverse transcribed in a reaction volume of 25 µL using the SuperScript VILO MasterMix kit (Invitrogen). Quantitative real-time PCR was performed in triplicate with a reaction volume of 20 µL using a QuantStudio 7 Real-Time PCR System with TaqMan Universal PCR Master Mix and TaqMan primers (for Slc5a1, Cd36, Ffar4) per the manufacturer's

instructions. Relative mRNA expression was quantified using the 2−ΔΔCT method with 18S as an internal control.

Small intestinal (SI) microbiome analysis

The extraction, sequencing, and analysis and interpretation of the small intestinal microbiota contents was conducted in collaboration with Dr. Greg Caporaso's lab at Northern Arizona University. Briefly, DNA was extracted using commercially available kits designed for this purpose (DNA Soil Kit). A 16S V4 rRNA library for MiSeq Illumina platform was prepared following the Earth Microbiome Project protocol (a widely used protocol for microbiome profiling that collaborator Dr. Caporaso was involved in developing)[62]. Microbiome sequencing data was analyzed using the QIIME 2 microbiome bioinformatics pipeline for sequence quality control (using DADA2) and subsequent data analysis, statistics and visualization.

Intestinal nutrient infusion

A separate set of Sprague-Dawley rats (n=24) were used to measure conduct the intestinal nutrient infusions. Rats were initially split into two groups, chow (n=8) or HFD (n=16), and maintained on their respective diet for 2 weeks, and then intestinal catheterization surgery was performed. Briefly, rats were anesthetized and a midline incision was made and the proximal small intestine was exposed. A puncture was made ~10cm from the pyloric sphincter and a catheter was inserted and supported with a suture and hernia mesh with vetbond tissue glue. The catheter was exited subcutaneously behind the neck and the incisions were closed. The catheter was flushed everyday with saline to ensure patency. After a full recovery of 7-10d, rats were habituated to the infusion procedure before testing began, with saline (SAL) infusion being conducted every other day. For the infusion, animals were overnight fasted (1700–0800) and the catheter was connected via PE-50 tubing to a 10-ml syringe mounted in a syringe pump. A total of 6 ml infusate was given every other day at a rate of 0.4 ml min−1, after which rats were presented with a pre-weighed amount of their respective diet and intake was measured after 2hrs[36]. Following stable baselines, rats received intraintestinal infusions of ensure (ENS), or saline solutions (6 kcal for ENS and IL; diluted with saline). Following this testing, half of the HFD rats (n=8) were switched to 10% OFS in drinking water for the reminder of the experiment. Three days after starting treatment, rats were again subject to the intestinal infusion study every other day, receiving either ENS, intralipid (IL, isocaloric), or saline. It should be noted that during overnight fasting, 10% OFS water was replaced with normal drinking water to limit the possibility of consumption of the OFS which could have been consumed for energy. Percent suppression was calculated according to the following formula: percent suppression = 1 − (experimental/saline baseline) × 100, with the baseline being the average of the saline prior and after the experimental test.

Statistical Analysis

All statistics were computed with Statistical Analysis Software (SAS, version 9.1.3 Cary, NC). Differences in food intake at 1wk and 6wk, gene expression, and percent suppression of solid intake following nutrient infusion were analyzed for each nutrient infused with one-way ANOVA followed by post hoc Bonferroni. Differences in body weight was analyzed by repeated measures ANOVA (rmANOVA).

Results

Effect of OFS on body weight, food intake, OGTT, and adiposity

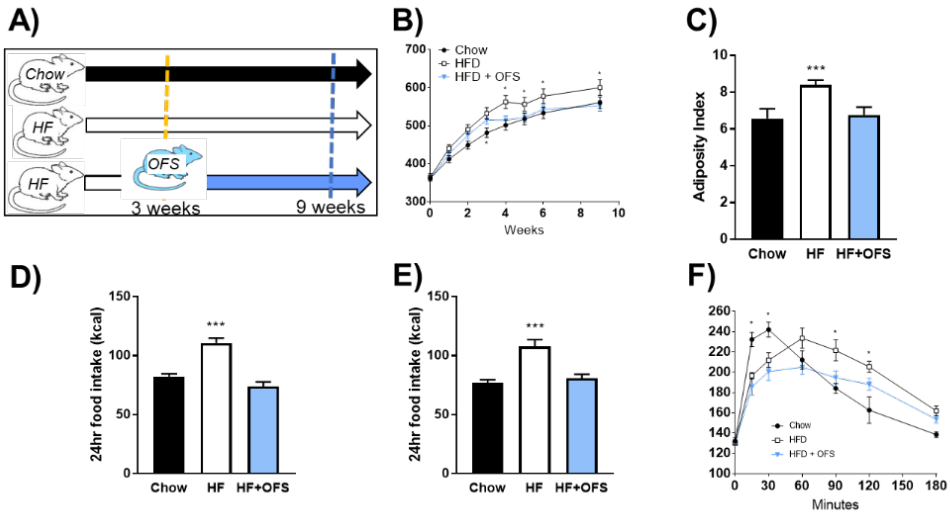

Figure 1. Timeline of experiment with body weight and food intake over time, and OGTT and adiposity. (a) Timeline of experiment. All rats began on a chow diet, and then ⅔ were put on a HFD. After 3 weeks, half of the HFD group began supplementation with 10% OFS. (b) Body weight of rats over the course of the experiment. Prebiotic supplementation began after 3 weeks of HFD. Before prebiotic treatment, both HFD groups showed similar increases in body weight. After prebiotic treatment, the HFD + OFS rats had reduced body weight gain. (c) Total adiposity of all treatment groups at the end of the study at 9 weeks. Food intake measured in kilocalories for chow, HFD, and HFD + OFS rats (d) one week (e) and six weeks after prebiotic treatment. Food intake was significantly reduced in HFD + OFS rats (f) Results from the OGTT for all treatment groups. HFD + OFS showed improved glucose tolerance *p < 0.05; **p < 0.01; ***p<0.001

Three weeks after HFD, both HFD and HFD+OFS (before start of treatment) were significantly heavier than chow controls. However, one week after starting treatment, HFD+OFS rats had a significantly decreased body weight compared to HFD rats, with no difference compared to chow controls that was maintained throughout the experiment. One week after treatment, HFD+OFS rats had significantly decreased food intake compared to HFD rats, which was also maintained at 6wks of OFS treatment. At 4wks of treatment, rats were subject to an OGTT. Interestingly, chow rats had a differing blood glucose curve that was significantly increased at 15 and 30 min compared to the two other groups. However, at 60min, HFD rats exhibited an increased blood glucose level compared to HFD+OFS and chow rats that was maintained at the 90 and 120 min time points. When examining area under the curve (AUC), HFD rats exhibited a significantly increased AUC compared to both HFD+OFS and chow controls,

with no difference between HFD+OFS and chow rats. At the conclusion of the study, rats were sacrificed and fat depots were collected. HFD rats had increased total fat (g) compared to HFD+OFS and chow rats, and had a significantly increased adiposity index (g of fat/BW) compared to HFD+OFS and chow rats.

Effect of OFS on small intestinal nutrient transporters and receptors

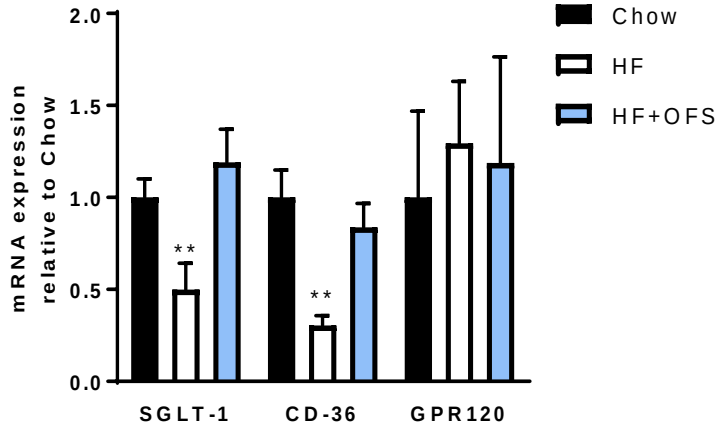

Figure 2. Concentrations of nutrient transporters detected by RT-qPCR. Expression of nutrient transporters in the small intestinal epithelial cells of chow, HFD, and HFD + OFS rats were measured using RT-qPCR. Abundance of SGLT1, a glucose transporter; CD36, a lipid transporter; and GPR120, a fatty-acid receptor. HFD rats have significantly lower levels of SGTL1 and CD36, but there was no significant difference between the levels of GPR120 for all three groups. **p < 0.01

At the termination of the experiment at nine weeks, HFD rats had significantly decreased expression of the small intestinal nutrient transporters SGLT-1 and CD36, for glucose and fatty acid transport, respectively, as compared to chow rats. In comparison to HFD rats, HFD + OFS rats showed significant increases in SGLT-1 and CD36. The expression of these nutrient receptors in HFD + OFS rats was nearly equivalent to that of chow rats. There was no difference between groups in the expression of the small intestinal fatty acid receptor GPR120.

Effect of OFS on small intestinal microbiota

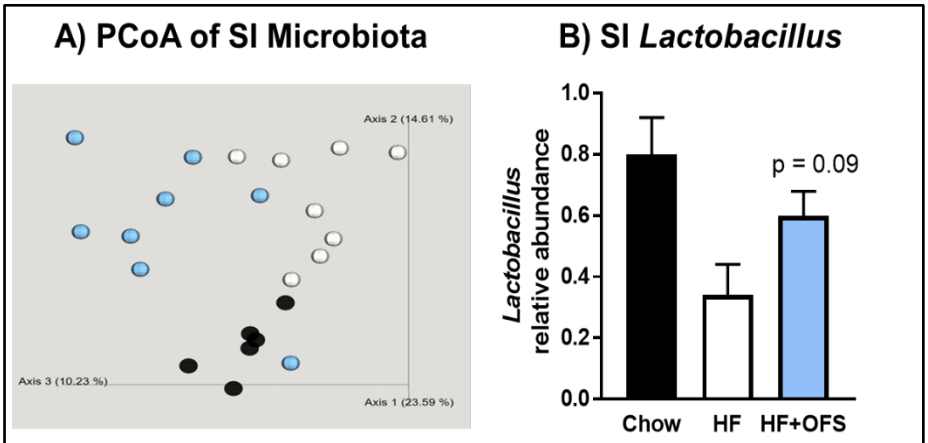

Figure 3. PCoA of small intestinal microbiota and abundance of genus *Lactobacillus*. (a) Unweighted Unifrac analysis of the small intestinal microbiota in chow (black), HFD (white), and HFD + OFS (blue). There is a distinct separation between chow and both HFD groups, and a clear trend towards different SI microbiota compositions between the HFD and HFD + OFS groups. (b) Changes in the genus *Lactobacillus* among the three treatment groups. There is a significant increase in the amount of *Lactobacillus* in HFD + OFS rats.

Rats were sacrificed and the luminal contents were collected from small intestine. For each sample, the variable region 3 (V3) of the bacterial 16S rRNA gene was amplified by PCR and sequenced using Illumina MiSeq platform. Principal coordinate analysis (PCoA) of unweighted Unifrac-based distances between the upper

small intestinal samples of each group showed a trend toward separation between chow and HFD and HFD+OFS communities. Importantly, HFD-feeding decreased the relative abundance of the genus Lactobacillus with a trend towards an increase following OFS treatment.

Effect of OFS on small intestinal nutrient sensing

Before OFS treatment, chow rats exhibited an increase in % suppression of food intake from baseline following an ensure infusions compared to HFD and HFD+OFS rats. However, five days after OFS treatment began, there was a significant reduction in % suppression of food intake in HFD rats compared to chow and HFD+OFS treatment following both an ensure or intralipid infusion, but there was no difference in % suppression of food intake compared to chow and HFD+OFS rats. This indicates that OFS treatment during HFD-feeding restores intestinal-sensing of a mixed meal and lipids comparable to chow-fed rats.

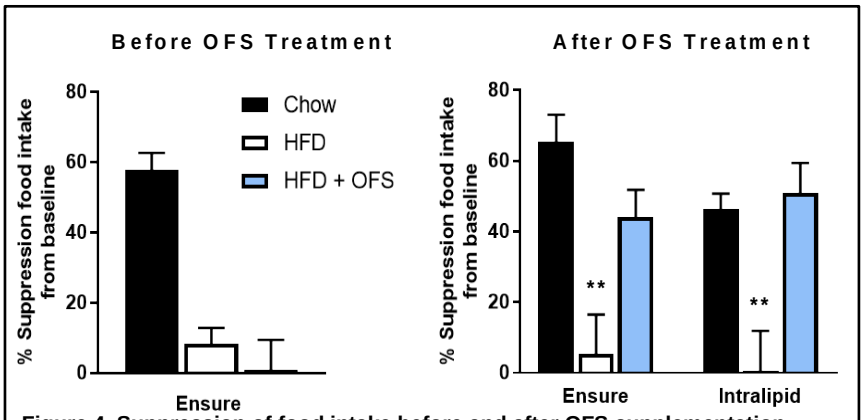

Figure 4. Suppression of food intake before and after OFS supplementation. (a) Suppression of food intake in chow and HFD rats following intestinal infusion of ensure (b) Suppression of food intake in chow, HFD and HFD + OFS rats following intestinal infusion of ensure and intralipid

Discussion/Conclusions

In this study, we found that prebiotic supplementation of rats on HFD decreased body weight, food intake, and adiposity, and increased glucose tolerance. We propose that these benefits occur in part due to increases in small intestinal nutrient transporters SGLT-1 and CD36 that mediate gut peptide release, which likely drive the observed improvements in small intestinal nutrient sensing in HFD rats treated with OFS. These nutrient-related changes may be the direct result of beneficial alterations in the small intestinal microbiota, which were observed in prebiotic-treated HFD rats.

Our findings show that prebiotic treatment decreases body weight and food intake, improves glucose tolerance, and regulates adiposity, similar to other studies conducted in rodents[18,25,34,37]. Similarly, in humans prebiotic supplementation has been shown in most cases to reduce body weight and energy intake, and increase satiety[38,39].

One of the main mechanisms behind these changes is thought to be gut microbiota-induced alterations in the intestinal nutrient sensing via EECs. Upon presence of luminal nutrients, EECs release gut peptides into the basal lamina[28]. These gut peptides act on vagal afferents innervating the intestine to signal to the hindbrain. In either case, appropriate signals are released to respond appropriately to changes in the intestinal environment[27]. Interestingly, both food intake and glucose homeostasis rely on similar gut-brain signaling pathways[36]. Given the drastic changes seen, we wanted to examine whether nutrient receptors or transporters are altered in rats given HFD versus HFD + OFS treatment.

SGLT1 is a glucose transporter which also acts as a signaling receptor for the release of peptides from EECs, thus stimulating the gut-brain axis[40]. CD36, a lipid transporter, has also been demonstrated to activate EECs and stimulate the release of gut peptides which contribute nutrient-induced satiation[41]. Additionally, there is some evidence that GPR120, a fatty acid receptor protein, can affect the gut peptide release by EECs[42]. As such, we wanted to examine expression of these important nutrient transporters and receptors during HFD and HFD + OFS treatment. We found significant increases in the expression of SGLT1 and CD36 in HFD + OFS rats as compared to HFD rats. The increase in SGLT1 in particular suggests that the gut is possibly better able to sense, and thus respond to, glucose levels in the small intestine. We postulate that this occurs by the SGTL-1-stimulated release of appropriate gut peptides that induce neural activity in the brain to directing processes for the maintenance of glucose homeostasis, such as reduced hepatic glucose production or increased insulin output from pancreatic β-cells[40]. However, we did not measure the concentration of gut peptides in the small intestine of these rats. It would be of interest for future studies to ascertain whether there are changes in gut peptide levels of prebiotic-treated HFD rats following nutrient infusion, which correlates with increases in these nutrient transporters and receptors, as well as increased nutrient induced satiation. Two recent studies[31,33] have shown that the small intestinal microbiota are able to alter small intestinal epithelial cell nutrient receptors and transporters, in line with other work which has shown that germ-free (GF) mice have alterations in their nutrient receptors[43]. Interestingly, in both GF mice and in the small intestinal transplant studies, SGLT1 was heavily influenced by the gut microbiota. This is in line with our study that found that prebiotic treatment altered small intestinal SGLT1 levels[31]. Given this, we decided to look at whether there were changes in the small intestinal microbiota of our chow, HFD, and HFD + OFS rats.

As previously mentioned, there is a clear role for the gut microbiota in the development of metabolic diseases. In particular, strong associations have been made between altered large intestinal microbial compositions and obesity and type 2 diabetes (T2D)[8–10,44–46]. However, few studies have explored the role of the small intestinal microbiota[47]. Though the concentration of microorganisms in the small intestine is much

less than that found in the large intestine, the small intestine is where the majority of nutrients are sensed and absorbed. As such, the small intestine is the primary place for negative gut-brain feedback to occur. This occurs via gut peptides which are secreted from small intestinal EECs into the basal lamina and contribute to the stimulation of the gut-brain axis via binding of its receptors on vagal afferent neurons[48]. Hence microorganisms which reside in the small intestine, having their own metabolites and other metabolic byproducts, may impact this pathway[47]. Recent work has shown that the small intestine can alter nutrient-induced gut-brain-liver feedback and glucose homeostasis[49,50] thus providing another mechanism through which the small intestinal microbiota may influence host metabolism. We found that high fat feeding alters the small intestinal microbiota, and that prebiotic treatment results in the differentiation between the microbiota of HFD and HFD + OFS groups. Though the differences in the small intestinal microbiota between these two groups was not significant, there was a clear trend towards a separation in their microbial compositions, which we believe would be more profound with a larger sample size. Specifically, it was determined that prebiotic supplementation during HFD increased the abundance of members of the genus *Lactobacillus* to levels almost comparable with chow rats. This could be an important genus in the small intestine for the regulation of nutrient sensing, as previous studies have also shown that high fat feeding correlates with decreased concentrations of the genus *Lactobacillus*, a prominent member of the small intestine microbial community[33,51]*.* This is the first time changes in small intestinal gut microbial composition by prebiotic treatment has been examined. It is likely that the majority of the prebiotics are broken down in the large intestine, but it is thought that some are initially broken down in the small intestine and thus lead to changes in the local microbial ecosystem[52–54]. Future studies will examine how and what specific small intestinal microbiota changes drive improvements in nutrient-induced satiation. For example, Bauer et al found that *Lactobacillus gasseri* was positively associated with intestinal lipid sensing, and that direct treatment with *Lactobacillus gasseri* improved lipid sensing[33]. This was due to the fact that *Lactobacillus gasseri* is a bile salt hydrolyse, and increased breakdown of bile salts in the intestine resulted in reduced FXR signaling (a nuclear receptor that binds bile acids) which improved intestinal sensing mechanisms. Whether *Lactobacillus gasseri* drives the changes in the current study remains unknown, but future work will begin to examine more specific shifts in the composition of the microbiota, as well as changes in the metabolites, like bile acids, in the small intestinal lumen.

Small intestinal nutrient sensing was improved with prebiotic treatment in HFD rats after both an intestinal infusion of the mixed meal Ensure and after infusion of intralipid. After both types of infusion, HFD rats had significantly decreased suppression of food intake as compared to chow rats, indicating an abolishment of intestinal nutrient-

sensing via HFD-feeding. Upon prebiotic supplementation, HFD rats exhibited similar levels of suppression of food intake as chow rats following intestinal nutrient infusions, and had increased levels of nutrient-induced suppression compared to HFD alone. Thus, treatment with OFS restores the ability of HFD rats to sense nutrients and respond accordingly, ultimately decreasing further food intake. This drastic change in small intestinal nutrient sensing upon prebiotic supplementation is thought to be due to either CCK or GLP-1 signaling, as both are released in response to lipids and induce satiation[50,55]. The activity of these signaling pathways were not measured in this study, and are left for examination in future studies. Furthermore, although we only examined lipid sensing, it is likely that carbohydrate sensing is also impacted by HF-feeding and prebiotic treatment, given that lipids and carbohydrates go through similar pathways[30]. For example, carbohydrate and lipids both reduce food intake via GLP-1 signaling and a vagal gut-brain pathway. In addition, in the current study we found that CD36, which mediates lipid sensing, is decreased during HF feeding and restored following prebiotics. This is identical to our observations with SGLT1, which is known to mediate GLP-1 release following glucose administration. Overall, improved nutrient-induced satiation via OFS treatment likely drives the observed reductions in daily food intake, and thus contributes to the reductions in body weight and adiposity.

Overall, we found that prebiotic supplementation of OFS in HFD rats decreased body weight, food intake, and adiposity, and improved glucose homeostasis and small intestinal nutrient-sensing mechanisms. The latter benefit is thought to be due to the metabolic byproducts and presence of certain microorganisms in the small intestine, which results in the alterations in nutrient transporters and receptors known to release satiation signals. As such, our findings support the hypothesis that changes in the small intestinal microbiota alter nutrient sensing mechanisms, and may be a potential target for therapeutic options for energy homeostasis. Given obesity is currently a pandemic, it is essential to continue conducting research aimed at ameliorating this serious metabolic disorder. Our promising results suggest prebiotic supplementation as a simple and non-invasive way to treat obesity and prevent the development of insulin resistance. However, more studies elucidating the mechanisms and effects of prebiotics are certainly warranted. A deeper understanding of those metabolic changes driven by prebiotics in the small intestine, which are responsible for the benefits seen in prebiotic supplementation, will no doubt aid in our understanding of the true impact the gut microbial community can have on host health.

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