THE INTERPLAY BETWEEN OBESITY, TASTE, AND THE MICROBIOME

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The interplay between obesity, taste, and the microbiome

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Our taste system plays the role of gatekeeper for whatever we ingest, warning us of aversive tasting foods, indicating the dangers of rotten or rancid food, and potentially poisonous bitter compounds. It also stimulates reward circuits in the brain when we ingest compounds triggering the appetitive tastes of sweet, salty, fat, and umami, indicating calories, minerals, lipids and protein. In the modern environment, where hyper palatable foods are bountiful, an altered sense of taste can lead to overconsumption of these energy dense appetitive compounds. Indeed, in the obese, researchers have noted altered taste responses, which may contribute altered eating behaviors, and drive the worldwide epidemic of overnutrition.

Work in our own lab has implicated certain proinflammatory cytokines as associated with a damaged taste phenotype in mice, with obese mice having fewer taste buds than lean littermates. Where the initial inflammatory insult comes from remains a mystery, but recent work on the microbiome has associated obese phenotypes with altered microbial communities. We conceived this work to test whether an altered microbiome may be the ultimate culprit in damage to the taste system observed with obesity.

We found that prebiotic supplementation to a high fat diet, which altered the composition of the microbiome, did protect mice from taste damage associated with obesity. However, we were unable to recapitulate the microbial dysbiosis present in obese mice through gavages to lean mice. This was partially due to the presence of a consortia of colonization resistant microbes that blunted the effectiveness of fecal transplants. As most fecal transplants are carried out in germ-free mice, the discovery of this consortia will be of use to other researchers without access to germ-free models and may be of interest to the burgeoning microbiome field as a whole.

BIOGRAPHICAL SKETCH

Jason Goodman was born in Philadelphia, Pennsylvania and obtained a B.S. in Microbiology with a minor in Biochemistry from Pennsylvania State University. While there he completed a co-op at GlaxoSmithKline (GSK) and Merck Inc., being named the Co-Op student of the year for work at Merck. There he developed a novel high throughput assay to assess the viability of Chlamydia in a mouse model of bacterial infection. At GSK he helped develop and execute immunohistochemical assays to quantify the effectiveness of therapeutics in models of cardiovascular disease and cancer.

After graduating, he began work at Integral Molecular, a Philadelphia based biotech company, as a research associate. He credits a great deal of his scientific and leadership maturity to escalating levels of responsibility at Integral. Specifically, he developed novel assays to map the epitopes of commercially developed antibodies for clients, and lead team meetings for the group responsible for that project. Here he was also exposed to taste research for the first time, developing and executing a functional screen in feline bitter taste to de-orphan several receptors.

After Integral, Jason worked as a research associate at Avid Radiopharmaceuticals, a subsidiary of Eli Lilly to develop, optimize and execute assays with the goal of finding a diagnostic for Parkinson's Disease.

After Avid, Jason joined the Dando lab at Cornell in the department of Food Science to further his growing passion for food, health, and nutrition. Here he worked at the intersection of taste, microbiology, and immunology, attempting to understand the complexities of three very complicated fields.

At this time, he also co-founded Antithesis Foods, where he currently serves as CEO, working to make better-for-you food that tastes great backed by cutting edge food science. Antithesis was awarded a Phase-I Small Business Innovation Research grant from the National Science Foundation to build a portfolio of crunchy ingredients based on chickpeas. Their first product is Grabanzos, a chocolate coated crunchy snack.

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"If you want to go fast, go alone. If you want to go far, go together."

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To my concurrent lab members, Fiona Harnischfeger and Anna Koh, there is no way to finish a Ph.D. without the support of your lab mates, and I could not have done it without your help. The journey was better because it was together.

To my friends who I never got to see enough, thanks for being there when I had time.

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Introduction and Background on Taste, Inflammation, and Obesity

Background and Significance: As food intake is primarily based on sensory factors[1], and excess food intake is a primary contributor to obesity[2–4], understanding the mechanisms that underly these factors is crucial to human health. More than 65% of the population of the U.S. is overweight or obese[25]. A major driver of intake is taste[6], with changes in taste acuity altering the perceived palatability of foods, and thereby impacting consumption behaviors[78]. Although taste perception at the behavioral level is relatively well studied[8], diverse and often contradictory results argue for a deeper investigation into the underlying physiological mechanism underpinning these perception alterations.

Work investigating taste buds (TBs) and the taste receptor cells (TRCs) of which they are composed, has identified a diverse slate of effectors which can alter TB and TRC number and cell type[91011], with additional alterations to signaling[12]. Our lab directly studies TBs and has implicated various effects of obesity on taste bud abundance, function, and gene expression[1314] primarily in animal models. These effects are often associated with pro-inflammatory cytokines and are otherwise attenuated in immune deficient animals. Specifically, we find damage to the taste system is likely due to a reduction in the proliferative capacity of taste cells in the obese, leading to fewer taste buds in mice made obese through consuming a HFD, (Figure i1A-C).

The mechanism responsible for such a change is likely the aforementioned inflammatory response, as TNF-α is upregulated in a HFD induced model of obesity[13], particularly in the taste buds (Figure i1D). Several immune pathways are implicated in the obese state, generally becoming overactive in these individuals, contributing to a persistent low grade inflammation hallmarked by increased IL-6 and TNFα[1516].

Recent work has implicated the human microbiome as a potential mediator of significant inter-subject variability in metabolism. In one study, subjects eating an identical meal had highly variable blood-glucose responses, partially explainable by their microbial composition[17]. Gut microbes are heavily implicated in the modification of hormone balance in obesity, altering levels of circulating hormones such

as Leptin, that contribute to appetite control[18]. These receptors are also highly expressed in, and may modulate taste buds[19]. Recent exploratory work in a large genome wide association study (GWAS) showed taste and olfactory genes were associated with specific heritable microbial taxa[20].

As in mice, the human taste system is influenced by obesity[21,22], with the obese having a weakened sense of taste. A study in High Fat Diet (HFD) fed mice showed a rescue of taste dysfunction by a prebiotic, building off other work suggesting that diets designed to alter the microbiome can alter taste perception in mice[23]. A HFD can also induce "dysbiosis", a pathological alteration in gut flora in humans and non-human animals[24][25]. While taste cells exist in the lingual epithelium in buds, they also line the gastrointestinal tract as Solitary Chemosensory Cells (SCCs)[26], in contact with commensal bacteria and the intestinal lumen. It is not surprising then, that taste cells might be acutely sensitive to the balance of microbes and their relative abundance in the microbiome. An indicator of dysbiosis, persistent low-grade inflammation, particularly acting through the cytokines Tumor Necrosis Factor alpha (TNF-α) and IL-6, potentially triggered by microbially derived lipopolysaccharides[27][28], have been demonstrated to reduce taste bud abundance in both humans and rodents[29][13].

Taken together, this suggests a potential effect of **diet on taste cells, mediated directly through pro-inflammatory cytokines, and triggered via dysbiosis of the microbiome**. The following work was conceived to test whether such a relationship exists.

Key background Figures

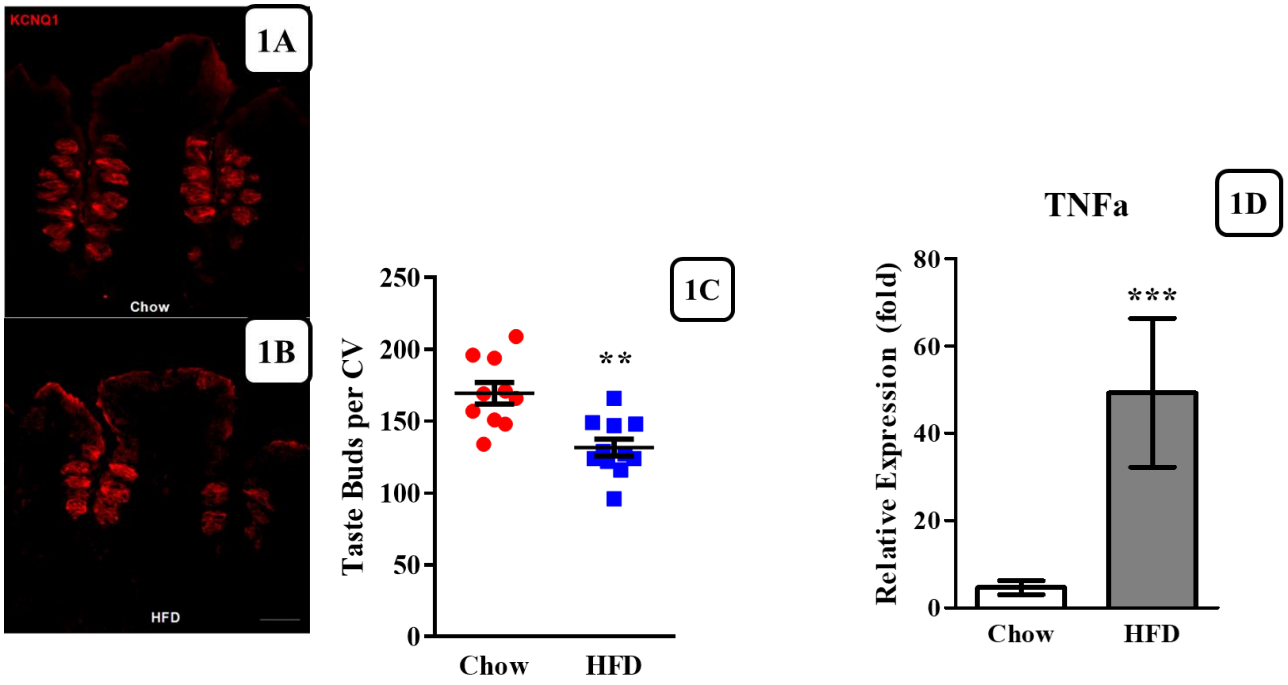

Figure i1: Experimental design scheme. C57 or TNFαKO mice were placed on Chow or HFD for 12 weeks. Post diet, they were subjected to assays to determine taste, weight, and microbiome phenotype. For the prebiotic study, a similar scheme was designed, though carried out for 8 weeks of diet. Representative images of Taste Buds of mice on i1A) Chow Diet, i1B) High Fat Diet. I1C) Enumeration of TBs in the CV of mice on Chow or HFD and i1D) the relative expression of TNFα of mice on these diets.

Chapter 1: To detect and reject, parallel roles for taste and immunity[30]

Running title: Taste and immunity

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Abstract

Purpose of Review: From single cells to entire organisms, biological entities are in constant communication with their surroundings, deciding what to 'allow' in, and what to reject. In very different ways, the immune and taste system both fulfill this function, with growing evidence suggesting a relationship between the two, through shared signaling pathways, receptors, and feedback loops. The purpose of this review was to explore recent reports on taste and immunity in model animals and in humans to explore our understanding of the interplay between these systems.

Recent Findings: Acute infection in the upper airway, as with SARS-CoV-2, are associated with a proinflammatory state, and blunted taste perception. Further, recent finding highlight taste receptors working as immune sentinels throughout the body. Work in humans and mice also points to obesity impacting taste, altering taste bud abundance and composition.

Summary: There is accumulating evidence that taste cells, and particularly their receptors, play a role in airway and gut immunity, responsive to invading organisms. Inflammation itself may further act on taste buds and other taste receptor expressing cells throughout the body as a form of homeostatic control.

Introduction

Taste is fundamentally linked to quality of life and is vital in determining food choice. As well as to assay the appeal of foods with appetitive characteristics, the taste system chemically interrogates the food we eat, serving as a gatekeeper, protecting against the ingestion of aversive, unpalatable items that may cause harm if taken into the body. The immune system serves an analogous function, detecting things that are harmful, or that bear the molecular signals of those that have been harmful in the past, acutely activating against systemic insult as a first line of defense. In the time necessary for the adaptive immune response to be generated, a pathogen may have grown to a problematic degree within the host, and therefore the innate immune response gives an "always on" protection to the body from novel pathogens. The innate immune response utilizes an array of cells, signaling factors and proteins to isolate, destroy and recycle potential pathogens before they can reproduce to the point where their progeny may become harmful to the host. Without an index of specific pathogens to identify, the innate immune response instead relies of on detecting common molecules which are absent in the host, but which are often signs of a pathogenic entity, the classical example being lipopolysaccharides, molecules commonly found in the external membranes of Gram-negative bacteria. Upon detection, the innate immune system responds to neutralize a perceived threat with both phagocytosis, whereby a potential intruder is engulfed and ingested, and inflammation.

As the basic roles of immunity and taste share some overlap in function, it is unsurprising then that recent evidence has begun to suggest that these systems may interact, and further may influence one another. Most visibly in the past year during the Covid-19 pandemic, infection with the novel coronavirus was strongly and publicly associated with anosmia, a loss of smell[31], in a manner that may correlate with an activated inflammatory response[32] as well as with ageusia, a loss of taste, and further loss of oral perception of irritants[33]. Taste cells and their canonical signaling cascades are commonly implicated in auto and hyperimmune diseases. Many genes associated with the innate immune response are over-expressed in taste cells, particularly in those isolated from taste cells bearing the molecular signals of type

II taste cells [34], the cells responsible for sweet, umami and bitter taste detection. In the taste buds of humans, inflammation and innate immune-associated genes are readily up-regulated in obese compared to lean individuals[35]. Being overweight or obese (a condition affecting over 65% of adults in the U.S), is associated with chronic low-grade inflammation, and is recently linked to alterations in taste or food intake patterns, usually promoting overconsumption, often through a loss of taste function. Taken together, evidence points toward a feedback relationship between the immune and taste systems, that may have implications for how the body functions to keep us safe from pathogens, and for our metabolic health in general.

In this review, we will concentrate on recent findings (primarily those from the past 5 years) regarding immunity, inflammation, and taste to provide a snapshot of our current understanding of how these systems interact, and highlight further interesting questions that future work may explore.

Search Strategy and Selection Criteria

PubMed and Google Scholar were used to search for primary research articles, over the previous 5 full years from 2016 to 2020, with a small number of important articles from earlier than this also included for context. Search terms included “taste”, “inflammation”, “immunity”, “autoimmune”, “obesity”, “cancer”, and various combinations thereof. Summaries and reviews were excluded to concentrate on most recent primary literature from the lab or clinic.

Inflammation and taste

Inflammation as a term encompasses the activation of the immune system toward an insult. This is characterized on the macroscale by an increase in blood flow leading to redness, swelling, and an increase in temperature, along with pain and potentially a temporary loss of function[36], and on the cellular scale by the recruitment of neutrophils, macrophages, and monocytes, as well as proinflammatory cytokines like Tumor Necrosis Factor alpha (TNFα)[37] and Interluekin-6 (IL-6)[38], and other markers of inflammation like

C-Reactive Protein (CRP)[39] and chemokines like Monocyte Chemoattractant Protein-1 (MCP-1) [38]. This contrasts with the more specific actions of the adaptive immune system acting post antigen presentation via B or T-Cells, to an already familiar threat.

While in many cases these acute phase effectors resolve after insult, a chronic low-grade inflammation, or metaflammation[40], has been observed in overnutrition and obesity[16]. Overfeeding also leads to hyperinsulemia and hyperleptinemia[41], as resistance to the effects of insulin or leptin necessitates their overproduction. Evidence points to proinflammatory cytokines directly acting on the insulin[42] receptor, and leptin receptors (among other mechanisms of leptin resistance[4344]) to affect this change. Interestingly, many proinflammatory signaling factors associated with inflammation also have their cognate receptors expressed in taste buds [454647], proferring a potential mechanism for their up- or down-regulation being associated with a modulation of taste function. TNFα Knock out (KO) mice are less sensitive to bitter stimuli than wild type animals [48], and exogenous TNFα blunts sodium taste by inhibiting sodium flux in taste buds[12]. Lipopolysaccharide (LPS), an inducer of acute inflammation of bacterial origin, has been utilized by multiple groups to model the influence of systemic inflammation on the taste system. Exogenous LPS resulted in a reduction in the proliferative capacity of taste buds[29] which are constantly renewing in nature, and altered licking response to NaCl in mice[4912], supported by reduced activity in the *chorda tympani* nerve which takes taste information from the anterior tongue to the brain, in response to various taste stimuli[49]. Serum-LPS is also elevated in mice experiencing systemic inflammation through consuming a high-fat diet (HFD)[50]. In one intriguing study, the bitter tastant (of recent infamy), chloroquine, was found to exhibit protective effects on pre-term birth in mice, when induced through LPS injections (inflammation is a key risk factor for pre-term labor)[51]. Protective effects were much weaker in animals lacking the G-protein alpha-subunit Gustducin (originally thought to be taste-specific, although now associated with many chemosensory cells throughout the body), suggesting protective effects were mediated in a manner analogous to taste signaling, via bitter receptors which were found expressed in myometrial cells. Interestingly, the primary receptor for LPS, TLR4[47], is also expressed in taste buds,

whereby taste preference is altered in mice deficient in TLR4, with KO mice displaying a reduced preference for sugars, lipids, and umami[52], further suggesting that an innate inflammatory response may alter taste function. Taken together with the above work, this suggests a dual function for the LPS/TLR4 pathway; 1) as sensor of inflammation which can damage taste buds, and 2) as an integral part of the sensory transduction pathway, without which taste sensations are altered. A critical recent finding that will doubtlessly accelerate our understanding of the nexus between innate immunity and taste is that taste organoid cultures are also able to model an inflammatory response such as that in bacterial infiltration *in vivo*, with rapid induction of TNF and IL-6 observed in these *in vitro* cultures after stimulation with LPS[53]. These taste organoids also expressed many other classical markers of the immune response, including all members of the NF-κB protein complex (NF-κB1, NF-κB2, RelA, RelB, c-Rel,) and multiple Toll-like receptors, most markedly TLR2, 3, 4, and 5.

Several autoimmune diseases are also linked to taste dysfunction, notably Sjögren's Syndrome (SS)[54], Inflammatory Bowel Disease (IBD)[55], and Systemic Lupus Erythematosus (SLE) in mice[56]. Each of these diseases impact taste signaling in their own way. For example, in SS, patients experience an infiltration of macrophages, plasma, and T Cells into their lacrimal and salivary glands[57]. This leads to a reduction in salivary production, which itself reduces the ability to detect taste compounds, though not necessarily through a direct action on taste cells. In a mouse model of autoimmune disease with a phenotype akin to that of lupus or Sjögren's syndrome, taste buds were smaller, and fewer taste cells were regenerated from taste stem cells in the native turnover process the taste bud relies on to maintain fidelity[58]. In humans with IBD, taste sensitivity was generally blunted versus healthy controls, save for sour taste, which was elevated in IBD. Sour is thoughts to be transduced through Type III taste cells[59], however most of the receptors associated with the immune response that are reported to be present in the taste bud tend to be expressed in Type II cells [604661]. In human colonic mucosa, the number of bitter-receptor (T2R38) expressing cells is higher in those who are obese than lean, and is confined to cells seeming to fit an enteroendocrine phenotype[62]. These cells would presumably be responsible for the chemical sensing of

luminal contents, with their abundance strongly correlating with BMI in this sample. Interestingly, the stimulation of T2R108 receptors in enteroendocrine cells in the guts of diet-induced obese mice with a bitter extract from hops was linked to GLP-1 release, and an improvement in multiple metabolic measures including fat mass, glucose homeostasis and insulin sensitivity[63].

When encountering microbes in the airway, the innate immune response must detect and respond to potentially harmful invaders, in a manner thought to be partially dependent on the T2R family of bitter receptors (for review see [60]). A recent report suggested that cells in the trachea expressing bitter taste receptors also expressed the tuft cell (a chemosensory cell located in the epithelium, linked to type 2 immunity through the taste-linked signaling channel TRPM5 (Transient receptor potential cation channel subfamily M member 5) [64], marker DCLK1 (microtubule-linked protein kinase 1), comprising around 4% of epithelial cells[65]. When isolating mRNA from these cells, sequencing revealed RNA for multiple additional taste signaling elements, as well as for multiple cytokines implicated in the immune response.

Interestingly, many of the antibiotics commonly used to treat airway infections (including levofloxacin, tobramycin, and azithromycin) are also capable of activating the T2Rs (T2R1, T2R4, T2R14, and T2R20) present in the airway, validated in an in vitro FLAG tagged HEK cell system[66]. These receptors are expressed in smooth muscle cells within the airway walls, functioning in a manner that seems to counter the inflammatory response, to relax smooth muscles and aid in bronchodilation[67]. Using cultured human sinonasal epithelial cells, Lee et al[68] were able to demonstrate that solitary chemosensory cells in the airway express the same T1R2/T1R3 receptor heterodimers present in taste buds used for sweet taste detection. While amino acids have a range of tastes, some D-amino acids are able to activate the T1R2/T1R3 receptor, where in the mouth this would correlate to the eliciting of a sweet taste[69]. Lee et al found that not only do bacterial isolates, for example Staphylococcus, from human airways produce several such T1R-activating D-amino acids (D-Leucine, D-Isoleucine, D-Phenylalanine), but D-amino acids taken from these isolates were able to inhibit biofilm formation, in a T1R-dependant manner.

Cancer, the inflammatory state association with it, and the treatment side-effects thereof are all associated with changes in taste in humans[7071]. In mice, the effects of cyclophosphamide, a common chemotherapeutic agent, are acutely damaging to taste cells, in particular type II and III cells[11]. This loss of taste cells seems to operate through apoptosis, and be particularly damaging to the progenitor cells responsible for resupplying the taste bud. When fractionating doses of cyclophosphamide, a practice common in cancer treatment that can alleviate some of the more negative side effects commonly encountered, loss of taste was in fact prolonged, and more severe[72]. As taste loss can discourage eating, leading to broad negative outcomes, and further result in a significant reduction in quality of life, careful consideration should be made of the implications of these findings.

As cancer patients are at risk of cachexia (a wasting disorder characterized by severe extreme weight loss along with loss of muscle and body fat, and marked by a loss of appetite) and its associated morbidity and mortality, a recent study worked to untangle the attribution of each [73]. Counter to the hypothesis that treatment which damages rapidly dividing cells would impact taste cell renewal, the authors found no impact of chemotherapy on taste detection in hospitalized patients. They did find altered taste perception in both the chemotherapy and acute inflammatory group versus healthy controls, however the marker CRP, and leukocyte counts did not correlate with dysfunction. This suggests a broader inflammatory state may be affecting both populations. Care should be taken interpreting these results as the study populations were, by the nature of the study, not standardized.

Obesity, Inflammation and Taste

While acute inflammation in the form of infection is well characterized, a chronic low-grade inflammation associated with obesity[74] is a remarkably widespread manifestation of long-term activation of the immune system. In obesity, white adipose tissue (WAT) is broadly remodeled, and is itself a source

of cytokines[75], and harbor for macrophages that promote systemic inflammation[76]. Further, pathologies associated with obesity such as atherosclerosis have an immune component themselves, with associated plaques composed of several types of immune cells, including macrophages and neutrophils[77]. Obesity is associated with lower levels of adiponectin, and higher levels of TNFα [78], IL-6, and C-Reactive Protein (CRP) [79]. IL-6 and CRP are associated with disease complications [8081], while TNFα is associated with insulin resistance and hyperleptinemia[8283]. One recent study also implicated various signaling elements of taste (T1R2, T1R3, Gα-Gustducin, phospholipase C-beta 2, and TRPM5 channels) expressed in renal tissues in stimulation of the inflammasome, in a diabetic mouse model, where activation of the inflammasome could further be partially mediated by the sweet taste blocker lactisole[84].

A great deal of studies associate taste changes with obesity, with the preponderance of evidence supporting a reduction taste acuity[22148586], although some work does show the opposite effect[87] or no change at all[88]. Recent work from our own group suggested a reduction in taste bud abundance in both mice [13] and humans [14] with weight gain, alongside a reduction in expression of various taste-linked signaling elements in mice [14]. The latter result is paralleled in humans in work from Archer et al[35], where a pioneering RNA sequencing experiment examined differential expression patterns of obese and lean Caucasian women, from isolated fungiform taste papillae. In fact, 2 of 3 ontological groupings of genes found to vary significantly between subjects were those associated with the immune response, and with sensory signaling.

Interestingly, in regions of the brain related to energy homeostasis (the hypothalamus and brainstem), both taste receptors (T1R3, T2R116) and taste signaling elements (Gα14, TRPM5) are down-regulated by obesity in mice[89], in a manner which we might speculate seems itself homeostatic in nature. Although in an acute model of taste loss panelists tend to select foods of a higher sensory impact, and thus caloric content[90], a large genome wide association study found limited evidence for an association between taste response and polymorphisms in taste genes, although GNAT3 alleles associated with greater sweet taste

response were negatively associated with sugar intake in one sample[91]. Studies of patients undergoing bariatric surgery point toward rapid changes in taste after extreme weight loss[929394], which are likely inflammation-linked, as well as depending on the hormonal remodeling common in such interventions. Further work even implicates dampened CNS reward circuity linked to taste with an increase in obesity in adults [9596], which may differ from that seen in children[97].

Taste buds, or more specifically the population of specialized epithelial cells that make them up, are constantly renewed from a population of stem cells, which in turn produce taste progenitor cells that further differentiate into 3 functionally and morphologically distinct taste cells[98], each cell type having a distinct half-life[99], or supporting keratinocytes. This complex and constant state of development requires a finely tuned balance of developmental and transcriptional regulators within the taste bud to ensure the judicious turnover of taste cells within the bud. Recent work by Archer[35] showed a downregulation of genes expressed in the Type II taste cells responsible for sweet, bitter or umami detection in the fungiform papillae of obese versus lean women, as well as a reduction of expression of sonic hedge-hog (*SHH*), a morphogen vital for taste cell development[10010]. Kaufman et al showed a similar decrease in taste cell developmental markers in obese mice[14], with a concomitant increase in inflammatory markers and fewer taste buds in obese mice from earlier work[13].

Conclusions and future work

Taste perception in humans is sensitive to disease states, including obesity and acute infection. Circulating factors arising from an inflammatory state can also interact with taste cells themselves, their progenitor cells, innervating nerves, and with processing within the brain.

Recent work has made it clear that the taste system is plastic and responds to inflammatory insult, whether acute or chronic, and thus inflammation has a role in taste function and intake which cannot be

discounted as we work toward behavioral approaches to treat metabolic diseases. Further, the identification of cells strikingly similar in phenotype to those we think of as taste cells throughout the body, which are seemingly fulfilling an immune sentinel role, highlight further links between immunity and taste. Future work should take advantage of the expanding array of molecular techniques available to study taste cells *in vitro*, and aim for longitudinal studies of taste and immunity pre- and post-obesity, to further elucidate the complex relationship between our perception of foods, and the consequences of over consumption.

Table 1 Studies in non-human models of inflammation and their consequences for taste from 2016 to 2020

Reference	Study Objective	Study Design	Model	Measures	Results	Conclusions
Ahart et al. (2020) [101]	Determine whether HFD or obesity itself induced taste deficits	Mice on HFD or Chow with or without Captopril (CAP) in water for 6 weeks	C57BL/6 mice Diet Induced Obesity (DIO)	Sucrose licking, calcium imaging of taste cells, taste gene expression.	HFD but not Obesity reduced licking of sucrose. Diet independently inhibited TRC activity. No decrease of TBs in HFD. Decrease in PLCB2	Both diet itself *and* obesity independently impact taste.
Kaufman et al (2020)[14]	Taste stem cell associated markers were measure in obese and lean mice	Mice on control diets or HFD for 8 weeks.	C57BL/6J Male mice, circumvallate papillae gene expression	qRT-PCR, genes expressed in TBs isolated from CV of mice.	Reduction of expression in HFD-fed mice of markers of proliferation->Ki-67 and β-catenin, and markers of all taste cell types- PLCβ2, KCNQ1, NPTDase2, PKD2L1	Gene expression of proliferation markers significantly reduced in the TBs of obese mice.
Bernard et al. (2019) [102]	Explore fat detection in obese rodents	1) Mice on chronic HFD and 2) 4 week treatment with LPS through osmotic pump to recreate a chronic inflammation.	C57Bl/6 male mice age 8 weeks	Two bottle preference with oil solution, LPS detection, taste gene expression,	DIO mice had a decreased preference for oil. Plasma LPS increased in DIO mice. LPS alone did not change preference for oil. Proinflammatory genes increase in Circumvallate Papillate (CVP) of LPS mice with NFκB a central mediator	Orosensory perception of fat is affected by a fatty diet, but not by a non-diet LPS induced proinflammatory state

Bernard et al. (2019) [103]	Determine whether prebiotic supplementation reverses DIO taste changes in mice	Mice on Chow or HFD alone and supplemented with 10% prebiotic for 12 weeks.	C57 Bl / 6 males. Age 7 weeks to start.	Two bottle taste preference, gustometer, blood draw, gut microbiota	DIO group reduced response to sucrose in two bottle and gustometer. Partially rescued by prebiotic (P). Proglucagon increase in DIO+Probiotic group	Though markers weren't measured, the authors indicate anti-inflammatory effects of prebiotic might partially explain sweet taste rescue
Kaufman et al. (2018) [13]	Investigate association of obesity with taste abundance in mice	Wild type C57, TNFαKO and Sel 1L adipocyte KO fed HFD or Chow. Acute injection of TNFα or vehicle into tongue.	8-week-old C57 Bl/6, B6.129S-Tnftm1Gkl/J, Sel 1L-/- ,all male	Taste bud and Taste Receptor Cell and taste progenitor cell counting, mRNA of stem cells and taste transduction markers.	Obese mice have elevated TNFα and concomitant taste bud loss. TNFα null mice and obese-resistant mice are protected. Taste progenitor (Ki67+) cells also reduced with bud loss.	Inflammation arising from obesity, associated with adipose tissue reduces the # of taste buds through a reduction in progenitor cells.
Djezriri et al. (2018) [50]	Mediterranean diets high in polyunsaturated fats exert anti-obesity effects. Mechanism explored in mice.	Mice on control diets or HFD with poly unsaturated fat in water or vehicle.	C57BL/6J Female mice on Chow, HFD, or HFD + oleanolic acid (OLA) in water for 16 weeks.	Plasma LPS, Taste Preference, Cytokine mRNA, fatty acid composition of tissue, blood glucose tolerance test.	OLA decreases weight gain of HFD mice, rescues CD36 expression, improves Glucose response, and reduces plasma LPS. Reduces TNFα, IL-1β, and IL-6 vs HFD alone. Ca^{2+} flux to OLA was abolished in HFD but rescued with HFD + OLA mice in a through CD36 independent pathway.	Reduction in bodyweight, pro-inflammatory cytokines, glucose response, and restoration of taste acuity all through addition of OLA to diet.

Feng et al. (2018) [104]	As TRCs have been found in the gut, group investigated whether α-Gustducin has a role in IBD, a gut inflammatory disorder	Induced colitis in either C57Bl/6 or their α-Gust null counterparts.	Animal model of IBD, using dextran sulfate sodium (DSS) to induce inflammation in α-gust-KO mice. M /F both used. 3% DSS in water for 7 days, then mice were euthanized.	Weight, cytokine measures in colon tissue, immunohistochemistry (IHC)	α-Gust null mice had more severe colitis than WT, lost more weight, had higher tissue injury score, more inflammatory cell infiltration in colons plus an increase in TNF and IFN-γ. Decrease in IL-5, IL-13, IL-10	α-Gust serves a critical role in protecting the colon from inflammation.
Sharma et al. (2017)[105]	GPCRs in Asthma are targets for treatment. As new evidence implicates T2R bitter receptors, which are GPCRS, in inflammatory pathways, bitter taste receptor agonists were tested as asthma treatments.	Mice treated with T2R agonists chloroquine (CQ) or quinine (Q) and challenged with Ovalbumin (OVA) or House Dust Mites (HDM) Used human neutrophils to determine mechanism	Male FVB/N mice 8 weeks old; Injected with OVA Intraperitoneally then challenged with or without agonists. Female BALB/c mice 8 weeks old challenged with HDM. Two models; Prophylactic: pre-treated with agonists coincides with initial HDM challenge	Neutrophil Migration, cytokines (IL-4,5,9,13,17,10), lung mechanics, Bronchoalveolar lavage cell counts (Eosinophils, neutrophils, macrophages, lymphocytes), various IHC staining for mucus accumulation via PAS	**Inflammation:** T2R Agonists reduced induction of IL-4,5,13,17, eotaxin, and keratinocyte chemoattractant. Failed to repress TNFα, IP-10, and RANTES. **Remodeling**: lower collagen deposition and fibronectin expression by taste receptors,. Differential effects in inhibiting matrix metalloproteinases **Mechanism** Dose dependent reduction in immune cell recruitment by Q and CQ.	CQ and Q differentially inhibited most chemokines and cytokines, Q was overall more effective than CQ at suppressing inflammation.

Howitt et al. (2016) [64]	Tuft cells, a gut immune cell that can detect parasites, contain taste receptors. Here their downstream signaling pathway is investigated in detail.	WT and *TRPM5*$^{-/-}$ mice challenged with *Tritrichomonas Muris* (Tm) in a model of helminth infection. Tuft cells were analyzed by IHC + Intestinal organoids cultured to measure Interleukin production. *Rag2*$^{-/-}$*,Ilr2*$^{\gamma -/-}$ Mice lacking T_h2 and ILC2 cells	WT and *TRPM5*$^{-/-}$ mice, DCLK1+ (tuft cells), intestinal organoids	Interleukin gene expression Flow cytometry cell counting	Upon infection with Tm, *TRPM5*$^{-/-}$ had reduced IL-25, IL-13, fewer Type2 innate lymphoid cells, and concomitant tuft cell expansion. Additionally,	TRPM5 taste transduction signaling may be used to detect Tm, which excited innate lymphoid type 2 cells (ILC2s), producing IL-13 and thereby promoting their own proliferation

Table 2 Studies with human models of inflammation and their taste consequences from 2016 to 2020

Reference	Study Objective	Study Design	Model	Measures	Results	Conclusions
Kaufman et al. (2020)[14]	Investigating changes in human taste bud abundance in increased bodyweight individuals non-invasively.	Taste buds counting from humans enrolled in a longitudinal study designed to measure college weight gain were counted	n=49 (39 F, 10 M) subjects originally aged 17-18. Measurements obtained during freshman year and again 4 years later	Neck circumference measured as a proxy for bodyweight along with BMI, fungiform TBs counted..	Change in fungiform density inversely correlated with neck circumference. R=-0.374, p=0.008	Although not a direct measure of taste buds per se`, taste papillae were diminished with increasing neck circumference, an accepted measure of adiposity.
Schalk et al. (2018) [73]	Investigating the contribution of cancer, its treatment, inflammation resulting from either, and concomitant taste-dysfunction on malnutrition	Measure taste, inflammation and effect of chemotherapy on each in patients with cancer.	138 patients, mean age 65.2 in three groups. Group 1: 42 patients hospitalized due to cancer undergoing chemotherapy Group 2: 57 patients hospitalized for an acute inflammatory disease without malignancy	Sniffin' Sticks for olfaction, recognition taste test for 5 basic tastes. Detection and recognition threshold recorded.	No significant difference between patients with or without chemotherapeutical treatment. Recognition and detection thresholds were significantly different between hospitalized patients and healthy controls. Cancer patients and hospitalized patients with inflammatory disease both exhibited taste dysfunction versus healthy controls. Inflammatory status as measured by CRP and	Inflammation, not chemotherapy, impacted taste function. As specific measures of inflammation did not correlate with taste dysfunction, mechanism is likely mediated through other proinflammatory cytokines (not CRP / leukocytes).

			Group 3: 39 healthy study participants		leukocyte count did not directly associated with changes in taste	
Archer et al. (2018) [35]	Determine whether there is a difference in molecular makeup of taste tissue in lean and obese individuals.	RNA transcript analysis of fungiform TB biopsies	Human Female Caucasians, lean (*n*=23) and obese (*n*=13) BMI >30	Fungiform papillae counting, RNA-seq	No difference in papillae count. Highly divergent gene expression: PLCβ2& Sonic Hedge Hog (SHH) ↓in obese vs lean Top ↑pathways associated with immune and inflammatory response. Generally ↓ expression of taste genes in obese, mostly type II.	Evidence in humans that immune and taste gene expression is altered in obese vs lean individuals.
Noel et al. (2017)[90]	Investigate relationship between reduced sweet taste function and sweet solution intake	Participants had sweet taste inhibited and performed ad-libitum mixing task to an optimal sweetness level, along with tasting various sweet foods	Repeated session with *Gymnema sylvestre(GS)* to inhibit sweet taste function to varying levels versus blank in 51 participants	gLMS ratings of sweetness with various levels of GS, compared with liking of sweet foods, plus ideal concentration of sugar in beverage from ad lib mixing task.	GS reduced sweet taste significantly vs control. Reduction in intensity perception was associated with an increase in desired sucrose in the ad-libitum mixing task. An incremental 1% reduction in rated sweetness intensity corresponded to a 0.4 g/L increase in desired sucrose.	Weakened sense of sweet taste was associated with an increased desire for sweeter beverages. Taste damage may thus lead to the desire for more energy dense foods.
Adappa et al. (2016) [106]	Investigate T2R38 phenotype status on biofilm formation in patients with chronic	Recruit CRS positive patients with evidence of immune activation, genotype, taste tests,	Human subjects >18 yrs (M and F), immune competent CRS patients with evidence of sinonasal	13 point PTC taste test, biofilm formation assay, T2R38 genotyping	PTC taste score correlates with biofilm formation in patients without polyps (p=.0026). Increasing PTC taste score was inversely correlated with biofilm formation in nonpolyploid patients.	Increased T2R38 expression in the airway may be associated with a reduction in biofilm formation.

	rhinosinusitis (CRS)	and biofilm assay.	inflammation. 59 subjects			

Chapter 2:

Damage to Taste Buds in diet-induced obesity is attenuated in mice lacking critical inflammatory cytokines, and by prebiotic fiber supplementation designed to alleviate inflammation.

Abstract

The taste system interacts with food, sending signals to the host that can be interpreted to indicate a food's palatability, evidence of spoilage, or sensations related to potentially harmful components. Taste receptor cells located in taste buds on the tongue are chiefly responsible for the chemical sensing of a food's taste properties. Signals sent from taste cells can be modulated by intrinsic and extrinsic factors working at the level of the taste cell itself, the innervating nerves, or within the CNS. Among such factors are disease[107108], age[109–111], obesity[112113], and diet[114115]. Modulations associated with disease can act through the inflammatory response, either actively, as with neutrophil influx[116117], or more passively, via cytokines altering taste bud development and renewal[139]. Here, we induce obesity in wild type C57/Bl6 mice (WT) and in mice from the same genetic background lacking TNFα, to investigate changes related to obesity and inflammation in the taste bud. We further aimed to rescue diet induced changes to taste behavior with pre-biotic supplementation, aimed at disrupting the inflammatory response. We found that HFD feeding and concomitant obesity caused taste bud loss in WT mice, but did not in prebiotic supplemented, or TNFαKO mice. HFD feeding also attenuated behavioral response to sucrose broadly, however this was regardless of taste damage, suggesting the HFD itself could also alter feeding behavior alone. Reduced taste bud abundance was accompanied by an increase in neutrophils in the taste tissues of HFD-fed obese mice. Interestingly, prebiotic fed mice, though displaying increases in neutrophils, did not show a concomitant decrease in taste buds. Together, this work suggests an inflammatory milieu of TNFα and neutrophil activation are associated with obesity-induced taste bud loss.

Introduction:

Studies in humans exploring the relationship between obesity and taste perception have generally shown an inverse relationship between BMI and suprathreshold taste detection[115], recognition thresholds [94], and detection threholds[85] i.e. a general weakening of taste function in the obese[86]. Explanations for this relationship are varied, including hedonic attenuation[114], nucleus accumbens activity inhibition[95], hormonal impact on taste structures[19], and inflammation[35] and its consequences for taste bud renewal[13]. It should be mentioned that some work finds the opposite[87], that the obese are *more* sensitive to sweet stimuli, although such reports are in the minority. Many such studies in humans are hampered by small sample sizes, and non-standardized sensory measurement methods. Recent work in human taste buds studied the genes of obese and lean subjects, finding an increase in the expression of pro-inflammatory genes[35]. The pro-inflammatory state brought on with obesity is associated with a dysbiotic microbiome[118]. Recent work in Diet Induced Obese (DIO) mice, known to have a decreased preference for sucrose (at a specific level, likely implying weakened detection), showed a reversal in that phenotype with a prebiotic supplementation[119] of Inulin. Inulin is a term for long chain oligofructose, also referred to as dietary fiber. It is non-digestible in the stomach and can only be broken down by commensal bacteria. Several studies indicate that supplementation with Inulin increases levels of butyrate[120], resulting in a number of positive downstream metabolic consequences. Inulin supplementation can result in an increase in colonic mucus, which is readily digested by *Akkermansia*. The by-products of mucin degradation are anti-inflammatory short chain fatty acids (SCFAs)[121] linked with positive health outcomes.

This intriguing result suggests that by reversing a dysbiotic microbiome through dietary treatment, damage to the taste system may also be reversed. In this set of experiments, we used a high fat diet to induce obesity and its associated dysbiosis in mice. This treatment has been shown to cause a reduction in the abundance of taste buds in mice previously[13] through a method we proposed to be associated with inflammation. We counted neutrophils as a biomarker for a pro-inflammatory state. Humans and many non-human model animals with obesity have an increased number of circulating neutrophils, key in innate

immunity [122]. In HFD-fed obese mice, adipose tissue and liver tissue display an increased number of neutrophils[123].

In this study we aimed to uncover the mechanism associated with a prebiotic reversal of taste dysfunction in a similar DIO model.

Methods

Overview and experimental design

Study Overview. In experiment 1, C57 Bl/6 (B6) and TNFα knockout mice (KO) consumed either a HFD or normal chow for 3 months, beginning at 2 months of age. Mice were assessed for the contributions of diet to taste preference and immune system activity. After 12 weeks of their respective diets, each group was tested for behavioral response to taste, then sacrificed for detailed analysis of their taste and immune systems as described below. In Experiment 2, C57 Bl/6 mice on HFD or Chow were supplemented with 10% Inulin at age 8 weeks and for 8 weeks thereafter and subjected to identical measures as in Experiment 1. 8 weeks was chosen for this experiment to compare behavioral data with recent work in a similar experimental setup by Bernard et al[119].

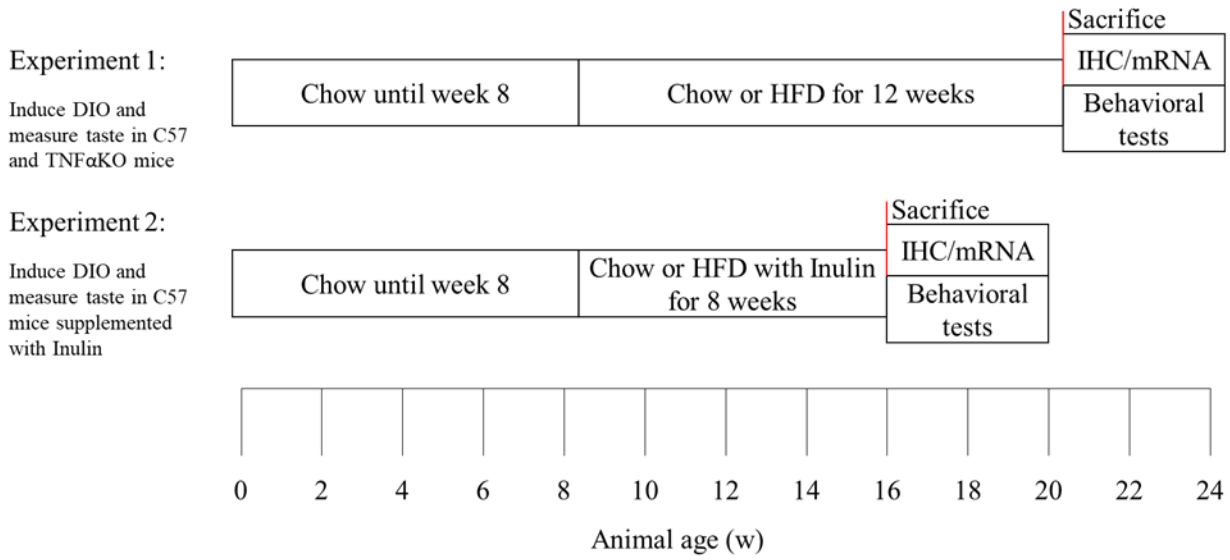

Figure 1: Experimental design scheme. C57 or TNFαKO mice were placed on Chow or HFD for 12 weeks. Post diet, they were subjected to assays to determine taste, weight, and microbiome composition. For the prebiotic study, a similar scheme was designed, though carried out for 8 weeks of diet.

Animals:

All methods were reviewed and approved by the Cornell University institutional animal care and use committee. C57Bl/6 & TNFα KO mice obtained from Jackson Labs (Bar Harbor, ME) were bred in house, with litters divided, receiving either chow or HFD for a period of 12 weeks, beginning when mice were 8 weeks of age. Sucrose sensitivity was tested using a Davis Lickometer prior to starting dietary treatment (baseline). After 12 weeks of respective dietary treatment, mice underwent further Lickometer testing, then were assayed for sucrose intake behavior via two-bottle testing. The duration of dietary treatment was chosen to mirror other work on diet-induced gut dysbiosis[124125], and therefore allow direct comparisons to previous studies. There is evidence that acute dysbiosis develops as early as after only 4 weeks on such diets[125], however, for this work, we intended to induce a model of chronic, low grade inflammation in our animals more consistent with that observed in those who are obese through diet.

Taste Bud (TB) Counting

Circumvallate (CV) papillae were excised from mice after euthanasia, with tongues fixed in 4% paraformaldehyde, then cryoprotected in 30% sucrose before being frozen in Optimal Cutting Temperature (O.C.T.) Compound (Sakura, Torrance, CA) at -80C. Sections were cut at 10 µm thickness, and then stained with primary antibodies. TBs were counted by staining for the Potassium Voltage-Gated Channel Subfamily Q Member 1 (KCNQ-1)(AB5932, Millipore Sigma, Darmstadt, Germany. 1:1000), a voltage gated potassium channel found in all taste cells. Every fifth section was counted, to ensure no taste cells were double counted.

Mouse Diet Composition

Macronutrient comparison			
	HFD (TD.03584)		**Chow (Envigo 2018)**
	% by weight	**% kcal from**	**% kcal from**
Protein	**20.4**	**15**	**24**
Carbohydrate	**36.1**	**26.6**	**58**
Fat	**35.2**	**58.4**	**18**

Neutrophil Counting

Sections were prepared as described above and stained with Anti-Myeloperoxidase (MPO) (AF3667, R&D Systems, Inc. Minneapolis, MN. 1:1000). Only MPO^+ cells that surrounded a $DAPI^+$ nucleus and were adjacent to the taste buds were counted.

Lickometer

Sucrose Responsivity: Mice were tested via the Davis lickometer, which measures the number of licks per second of a specific tastant an animal takes of a stimulus at varying concentrations, building up a dose/response curve for that tastant which is fit to a logistical regression model[126]. Licks are counted electronically and normalized on a per mouse basis. Mice were trained and acclimated to various licking tasks for 3 days prior to sucrose testing.

Sucrose Licks: To attain a broader measurement of "liking" and to compare with results from literature[119], we tallied individual licks to any concentration of sucrose.

Two bottle testing

Sucrose Responsivity: A two-bottle preference test presented water and a test solution to mice for 48 hours after a 24-hour training period. Bottles were weighed before insertion into cages and locations swapped after the first 24 hour period to control for side-preference. Bottle weights were recorded after 48 hours total and a % preference calculated by dividing the amount of the test solution consumed by the total amount of liquid (test + water) consumed.

Weights

Mouse weights were recorded 1 week prior to commencing dietary treatments, and then each week thereafter. Each mouse's % weight gain (the individual mouse's weight before dietary treatment, subtracted from their weight post treatment, divided by pre-treatment) corrects for individual mouse pre weight variability.

Taste Bud Isolation

Mice were euthanized as described previously and their tongues excised and placed into Normal Tyrode's solution. An enzyme cocktail (Dispase II 2.5 mg/ml, Collagenase A 1mg/ml, Elastase 0.25 mg/ml, and DNaseI 0.5mg/ml in Normal Tyrode's solution) was injected sublingually and then tongues were incubated in Ca^{2+} free Tyrodes solution for 15 minutes at RT. The lingual epithelium was cut free of the surrounding tissue and then peeled away, removing the taste structures from the CV on the underside of the lingual epithelium. This structure was incubated further in Enzyme Cocktail and washed in Normal Tyrode's. A borosilicate glass micro pipette coated in 0.2% PVP (Polyvinylpyrrolidone) was used to extract individual taste buds from the epithelium while visualized under a dissection microscope. Collected taste buds were then transferred to lysis buffer and processed for RNA extraction (Absolutely

RNA Nano/Microprep kit, Agilent Technologies). RNA was immediately reverse transcribed into cDNA via qScript cDNA Supermix (Quantabio, Beverly MA), and stored at -20.

Taste gene primers.

Primer	Forward	Rev
βactin	caccctgtgctgctcacc	gcacgatttccctctcag
T1R1	ctggaatggacctgaatggac	agcagcagtggtgggaac
T1R2	aagcatcgcctcctactcc	ggctggcaactcttagaacac
T1R3	gaagcatccagatgacttca	gggaacagaaggacactgag
PLCβ2	gagcaaatcgccaagatgat	ccttgtctgtggtgaccttg
TNFα	acgtggaactggcagaagag	gaggccatttgggaacttct
IL-6	gctggagtcacagaaggagtggc	ggcataacgcactaggtttgccg
Gα Gustducin	gcaaccacctccattgttct	agaagagcccacagtctttgag

Inulin Preparation

Mouse diets (HFD or Chow) with 10% Inulin (XL Long Chain Inulin, Cargill, Wayzata, MN). Chow diet was ground into a powder in a high-speed grinder and mixed with Inulin at a 9:1 (Chow/Inulin) ratio. Food was refreshed in mouse cages at the longest every 10 days, or when the cages were observed to be low on food.

HFD was crushed manually in thick plastic food safe bags until powderized. Inulin was added at the same ratio as chow, 9:1 (HFD/Inulin), and mixed to ensure proper dispersion. Diet was similarly refreshed at longest every 10 days.

Results

C57 BL/6 Mice on HFD gain more weight, have fewer TBs, more neutrophils in taste tissues, and reduced response to sucrose versus chow-fed controls.

After 12 weeks of eating a HFD, C57 BL/6 mice gained more weight (29.6% vs 16.9% weight gain from 8-week baseline point, P=0.001) than their chow fed controls (Fig. 2).

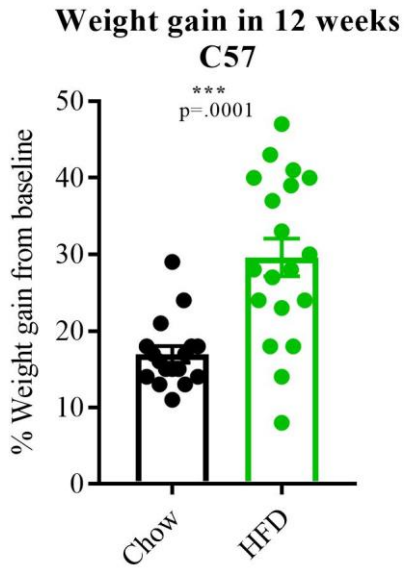

Figure 2. C57 Bl/6 Mice on HFD gain significantly more weight than their chow controls after 12 weeks on HFD.
Chow n=17, HFD n=19

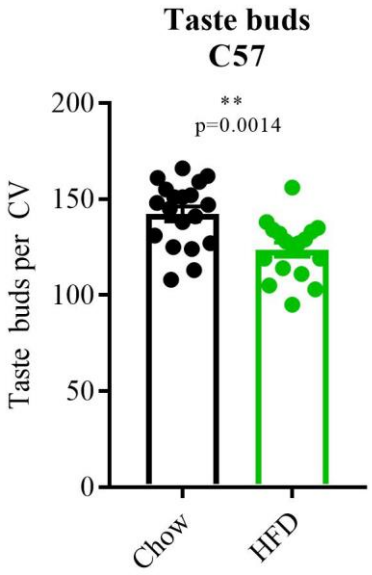

Figure 3. C57 Bl/6 Mice on HFD have significantly fewer taste buds in their circumvallate papillae than their chow controls after 12 weeks on HFD. Chow n=19, HFD n=17

These mice had significantly fewer TBs (mean 124 TBs vs 142 TBs, p=0.001) (Fig.3), confirming an important result previously shown previously by our lab at 8 weeks on the HFD.

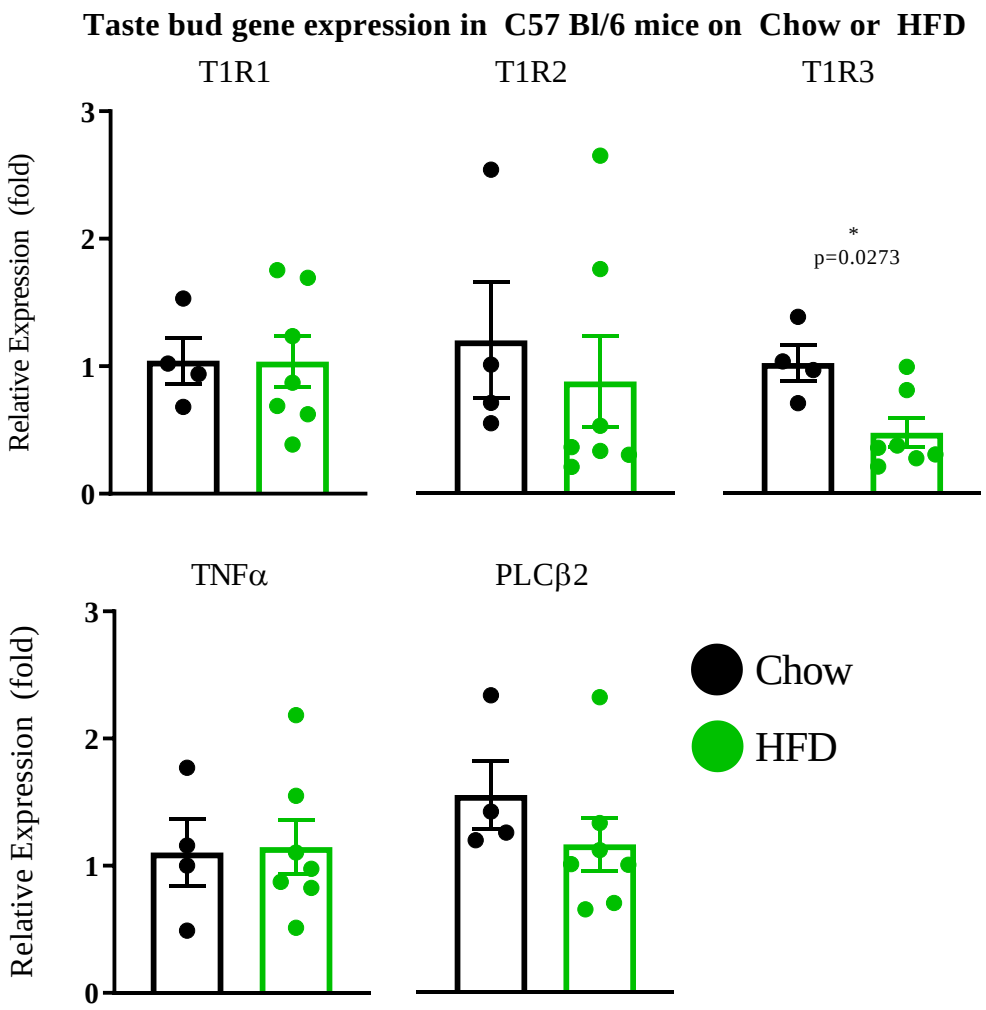

Figure 4 Taste gene expression of transduction machinery largely unchanged in C57 mice after 12 weeks on Chow or HFD. T1R3 significantly reduced. Chow n=4, HFD n=7, unpaired t-test.

TB's were isolated from the CV (Circumvallate) papillae of mice, with mRNA quantification by qPCR using B-Actin as an endogenous control gene. Expression was broadly similar across tested genes with only T1R3 expression reduced in mice eating HFD (p=0.027) (Fig.4).

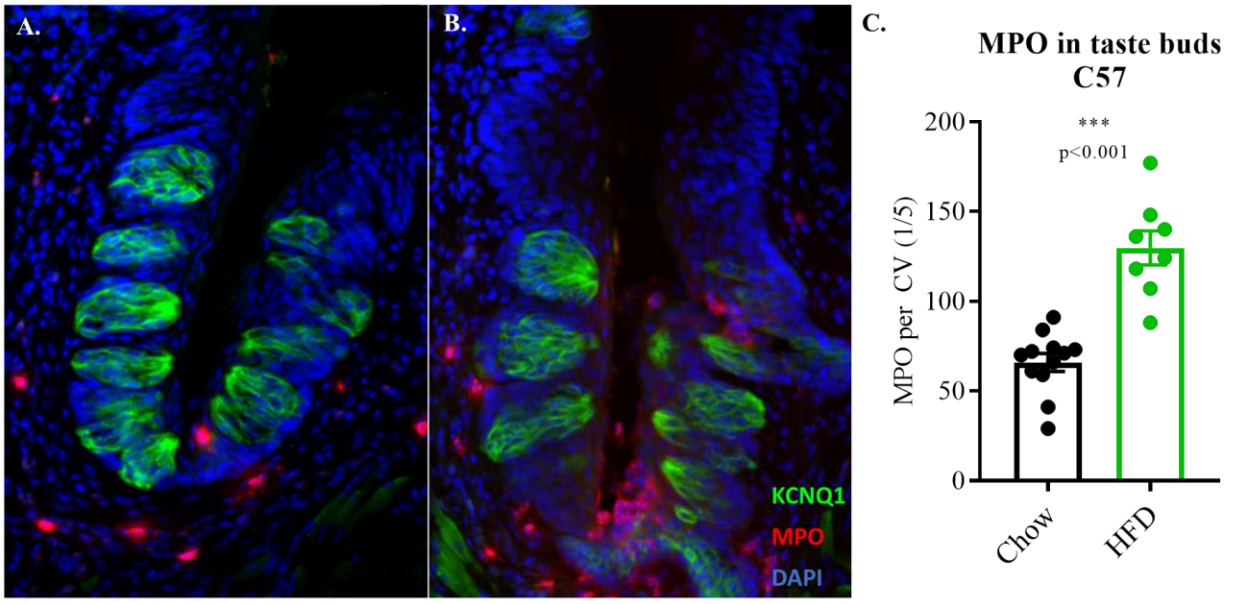

Figure 5. Representative image of right side of Circumvallate Papillae of C57 Bl/6 Mouse on A) Chow or B) HFD for 12 weeks. C) Counting of MPO+ cells shows C57 Bl/6 Mice on HFD have significantly more MPO in their taste Buds.
Chow n=12, HFD n=8

Immunohistochemical (IHC) staining of TBs for MPO positive cells was performed to ascertain the degree of immune infiltration of neutrophils into the taste field. HFD fed mice exhibited significantly more neutrophils than mice maintained on the chow diet, around double in the HFD-fed animals (mean 65.92 chow vs 129.8 HFD, $p<0.001$, Fig. 5), supporting a hypothesis that innate immune cells may be associated with taste dysfunction.

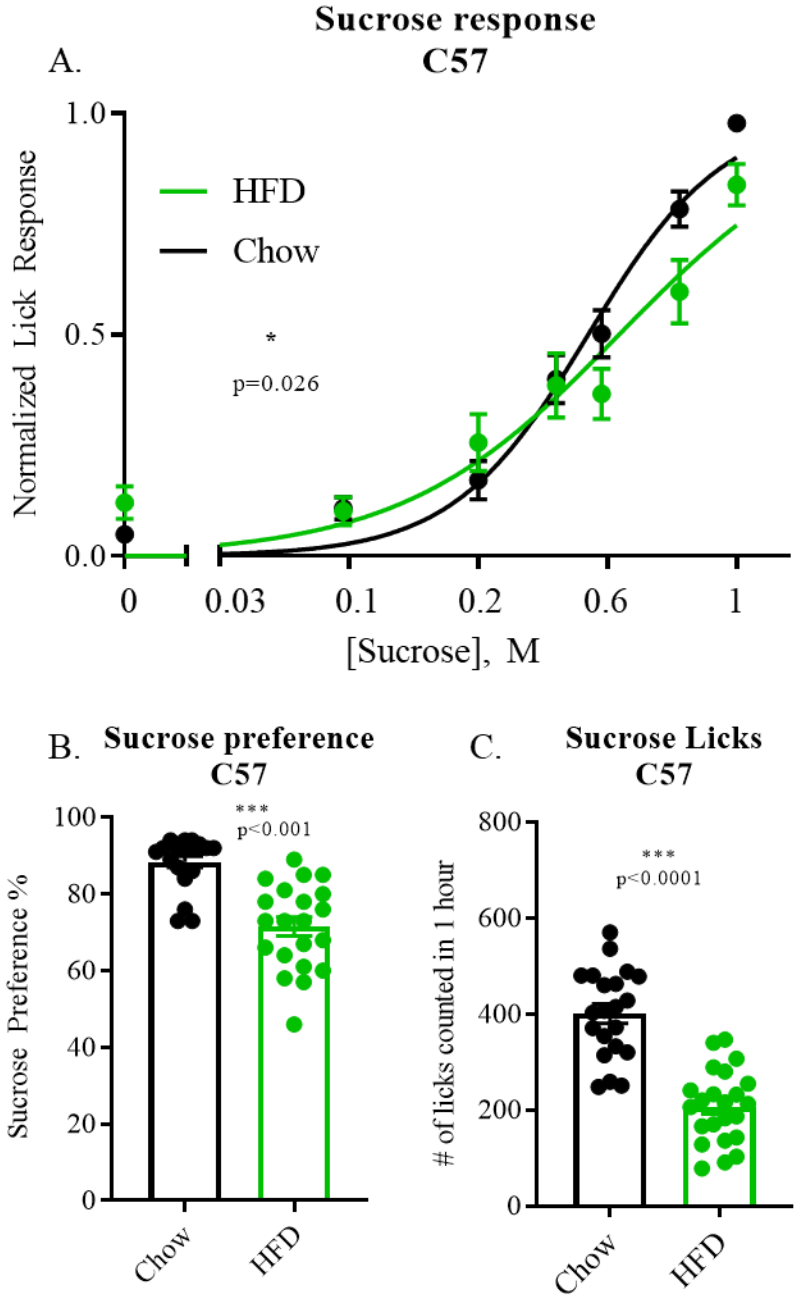

Figure 6. A) C57 Bl/6 Mice on HFD have a significantly reduced response to sucrose at multiple concentrations by Lickometer Chow n=21, HFD n=23. B) By two-bottle testing at 0.025M sucrose, preference is significantly reduced in C57 Bl/6 Mice on HFD (n=21) versus mice on Chow (n=21). C) C57 Bl/6 Mice on HFD lick sucrose fewer times at multiple concentrations versus mice on Chow. Chow n=21, HFD n=23

Interestingly, these mice had a broadly attenuated response to sucrose as assessed via Lickometer (EC_{50} 0.266 chow vs 0.350 HFD, p=0.026, Fig.6A), two bottle preference testing (mean preference 88.38% chow vs 71.52% HFD, p<0.001, Fig. 6B), and reduction in 'liking' sucrose as defined by Bernard[119] as a reduced total # of sucrose licks compared to chow controls (mean 401.4 chow vs 207.1 HFD, p<0.001, Fig. 6C), providing behavioral evidence for taste damage to complement earlier evidence of a morphological shift in obese mice.

TNFαKO mice, made obese through HFD do not display taste bud damage, but exhibit a reduction in response to sucrose similar to that of their immune competent counterparts.

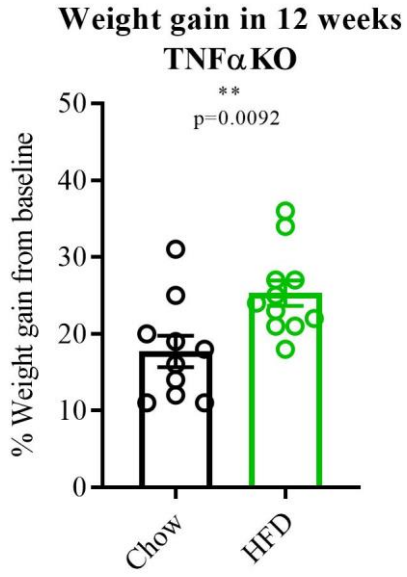

Figure 7. TNFαKO mice on HFD gain significantly more weight compared with chow controls after 12 weeks on HFD.
Chow n=10, HFD n=11

While TNFαKO mice on HFD may suffer fewer negative metabolic side effects due to their diet than wild type controls[127], in our experiments TNFαKO mice eating HFD did gain more weight than control TNFαKO mice on chow (chow 17.7% vs HFD 25.3%, p=0.009, Fig. 7).

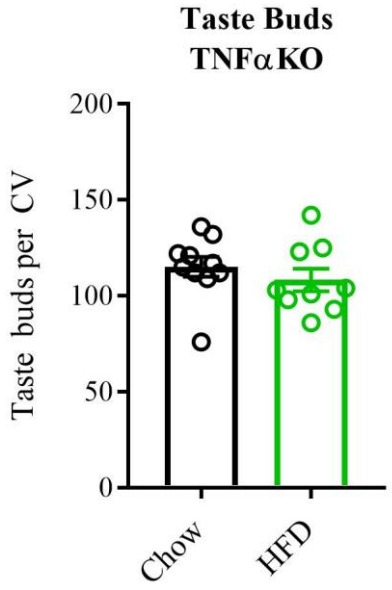

Figure 8. TNFαKO mice on HFD have a similar abundance of taste buds in their circumvallate papillae versus chow -fed controls after 12 weeks on HFD. Chow n=9, HFD n=10

However, while C57 mice on HFD had fewer taste buds than when on regular chow (Fig.3), TNFαKO mice on HFD did not see such a loss (chow 108.3 vs HFD 115.2, p=0.30, Fig.8), as has been previously reported[13]. Despite this, these mice still exhibited some behavioral attenuation to sweet taste, which may have been an effect of some sweetness present within the HFD, which was higher than that of the chow diet (HFD sucrose 15.0%, vs 0% sucrose in chow diet).

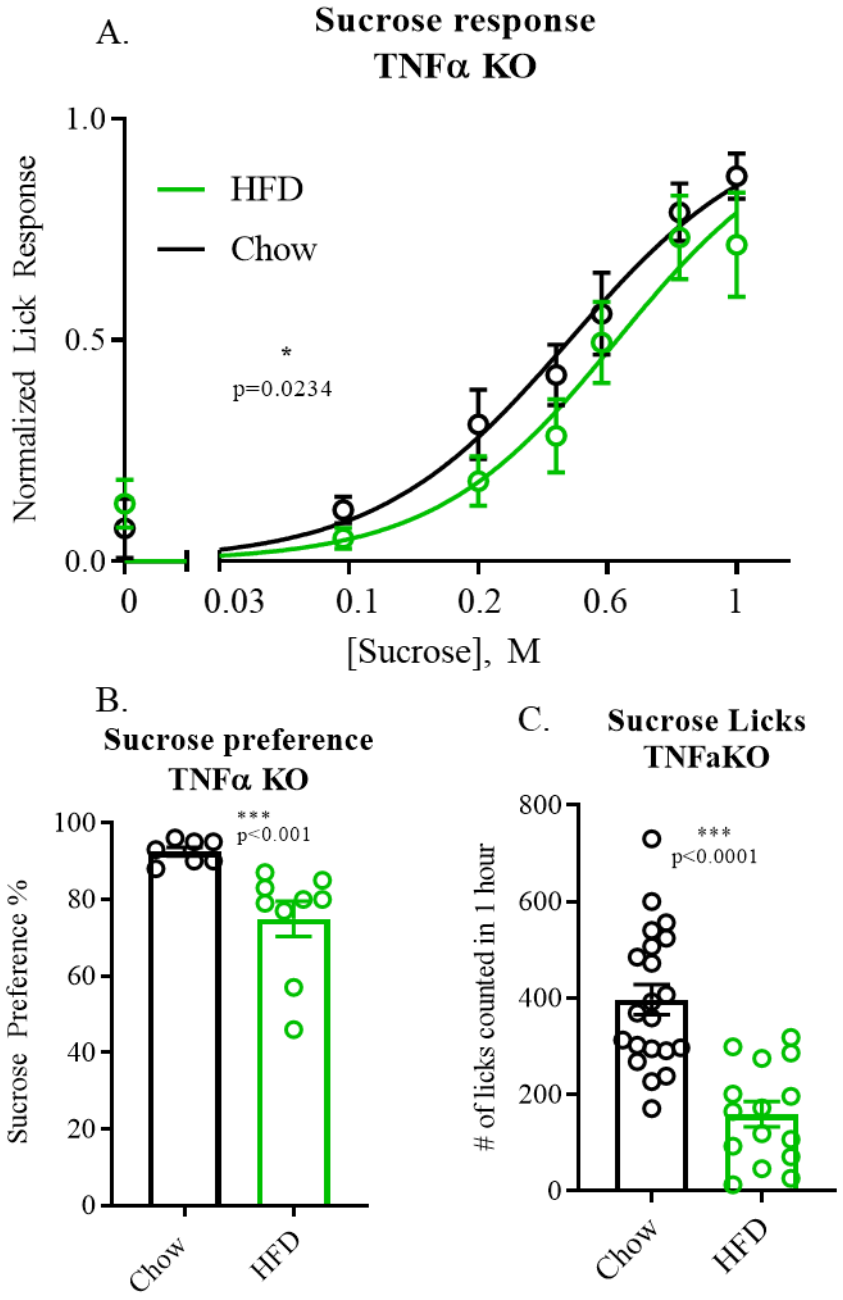

Figure 9. A) TNFαKO mice on HFD have a significantly reduced response to sucrose at multiple concentrations by Lickometer Chow n=12, HFD n=11. B) By two-bottle testing at 0.025M sucrose, preference is significantly reduced in Mice on HFD (n=9) versus mice on Chow (n=7). C) TNFαKO mice on HFD lick sucrose fewer times at multiple concentrations versus mice on Chow. Chow n=21, HFD n=15

TNFαKO mice made obese through a HFD have decreased sensitivity to sucrose as measured by Davis

Lickometer (Fig. 9A), shown in the figure by a shift in EC50 to higher concentrations in these animals

(EC_{50} 0.225 chow vs 0.344 HFD, p=0.023). In addition, these mice also exhibited a reduced preference for 0.025 M sucrose in a two-bottle test (74.89% HFD vs 94.43% Chow, p<.001, Fig.9B). TNFαKO mice eating HFD mice also display a reduced number of total sucrose licks throughout the entire test Lickometer test period (159 HFD vs 397 Chow, p<0.0001, Fig. 9C)

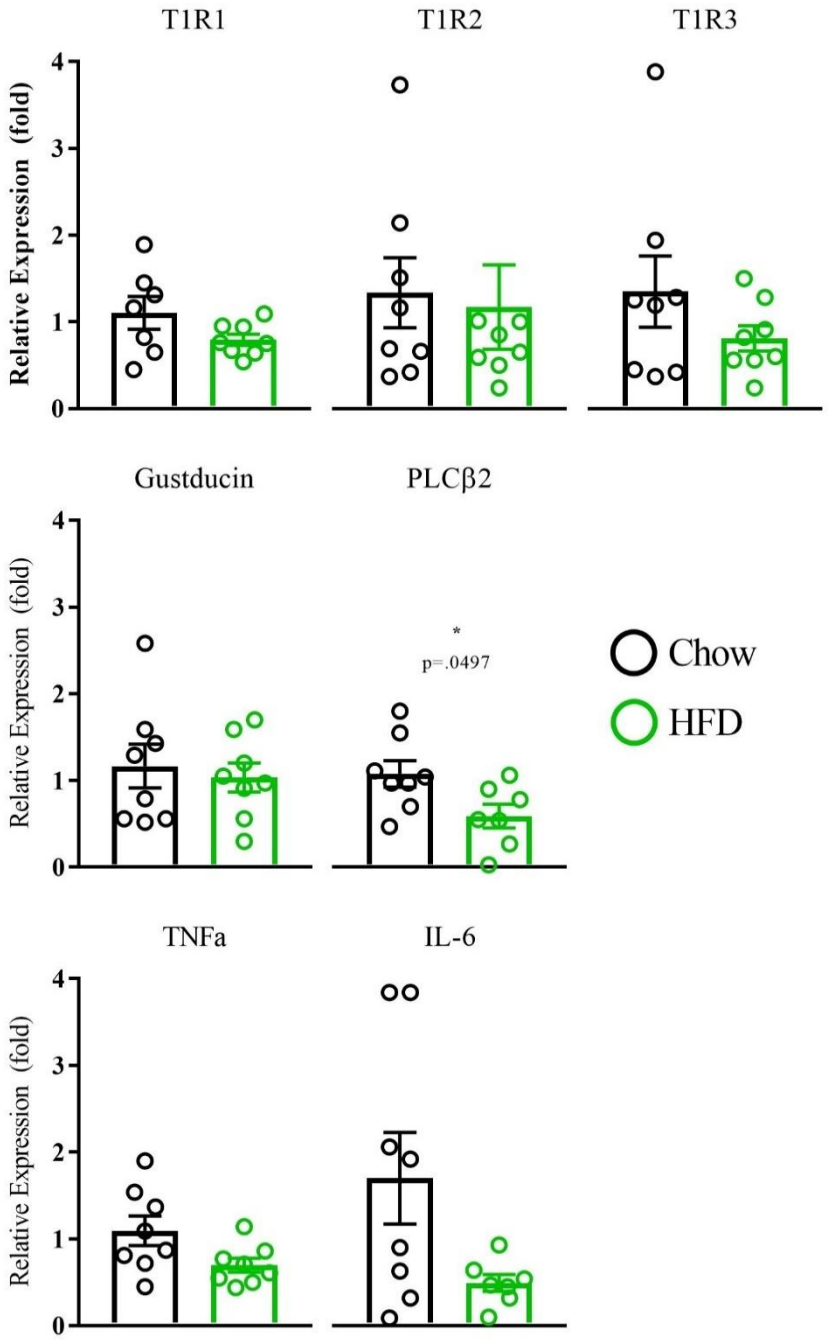

Figure 10 Gene expression of taste transduction machinery largely unchanged in TNFαKO mice after 12 weeks on Chow or HFD. PLCb2 is significantly decreased.
Chow n=8, HFD n=8

Broadly, gene expression was similar in the treatment groups of these mice (Fig.10), with PLCβ2 expression decreased (p=0.049) in HFD fed TNFα KO animals.

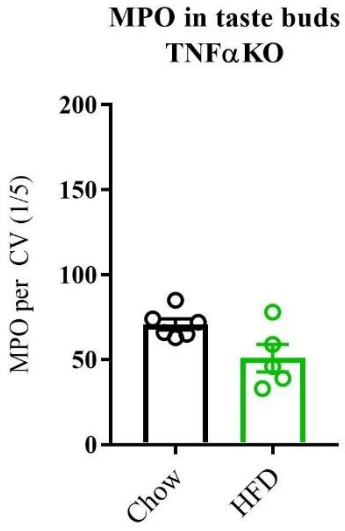

Figure 11. TNFα KO Mice have fewer MPO^+ cells in their taste buds. In this study, TNFα KO mice on HFD tended had fewer MPO^+ cells than their Chow controls, though not significantly.
Chow n=6, HFD n=5

While WT C57 mice eating a HFD exhibited an increase in MPO^+ cells in the taste field (Fig.5), the immune attenuated mice discussed here did not display a difference between HFD or chow fed littermates (Fig. 11), indicating that neutrophil induction may be dependent on TNFα.

C57 Bl/6 mice fed a prebiotic in addition to an obesogenic diet were protected from TB damage but displayed reduced liking for sucrose and increased Neutrophil infiltration into taste tissues.

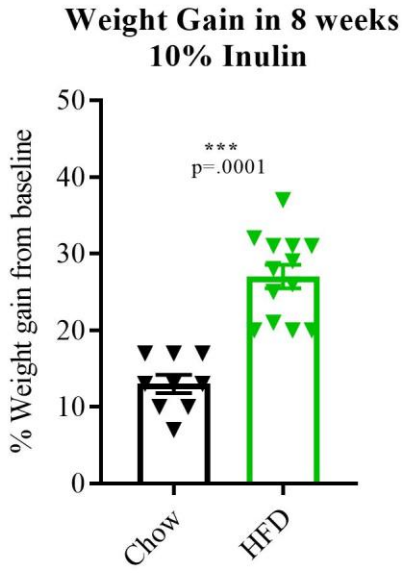

Figure 6. Mice eating HFD with 10% Inulin gain significantly more weight than mice on Chow with 10% Inulin.
Inulin Chow n=9, Inulin HFD n=13.
Unpaired T-Test.

C57 Bl/6 mice were fed Chow or HFD supplemented with 10% Inulin for 8-weeks to determine whether a prebiotic could rescue taste damage displayed in mice on HFD alone (Fig.3). After 8-weeks, High Fat Diet-Inulin (HFD-I) fed mice gained significantly more weight than Chow Inulin (Chow-I) controls (27% HFD-I vs 13% Chow-I, $p < 0.0001$) Fig.12).

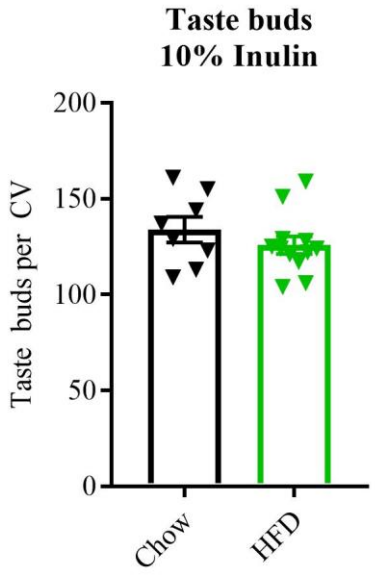

Figure 7.
After 8-weeks of eating Chow or HFD with 10% Inulin, there is no difference in TBs
Inulin Chow n=8, Inulin HFD n=12
Unpaired T-Test.

However, in contrast to results in HFD alone mice, the prebiotic fed mice on HFD did not lose taste buds when compared to control animals (125.8 HFD-I vs. 133.9 Chow-I, p=0.315 Fig 13.)

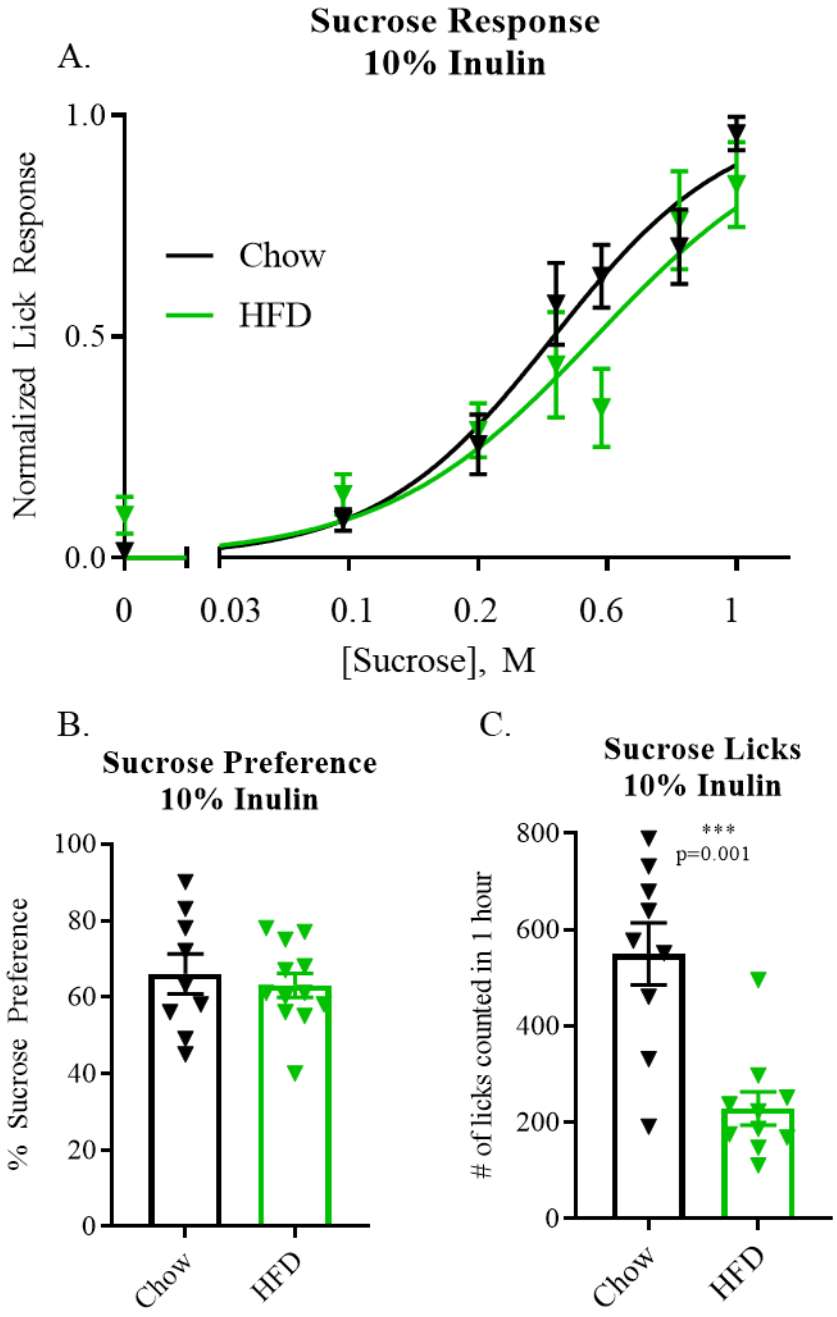

Figure 14. A) Mice eating diets with 10% Inulin do not show a significant difference in their response to sucrose via Lickometer Inulin Chow n=9 Inulin HFD n=9, or B) a difference in sucrose preference at 0.025M Inulin Chow n=9 Inulin HFD n=9, but C) C57 mice eating HFD with 10% Inulin lick sucrose less at multiple concentrations versus mice eating Chow with 10% Inulin, Inulin Chow n=9, Inulin HFD n=10.

These mice also did not show a significant difference in sucrose response by Lickometer (EC_{50} 0.196 Chow-I vs 0.285 HFD-I, p=0.069, Fig. 14A) or 0.025M sucrose preference by two-bottle test (66% Chow-I vs 63% HFD-I, p=0.608, Fig. 14B), indicating no behavioral shift in taste responses were evident when mice were supplemented with prebiotic. However, the lickometer test did show a trend toward a higher EC_{50}, and when all sucrose licks were summed to ascertain 'liking' of sucrose in HFD-I mice, we found that these animals scored lower than Chow-I mice (577 Chow-I, vs 204 HFD-I, p=0.001 Fig. 14C). This 'liking' attenuation is consistence with all mice in these experiments on HFD regardless of genotype or Inulin supplementation.

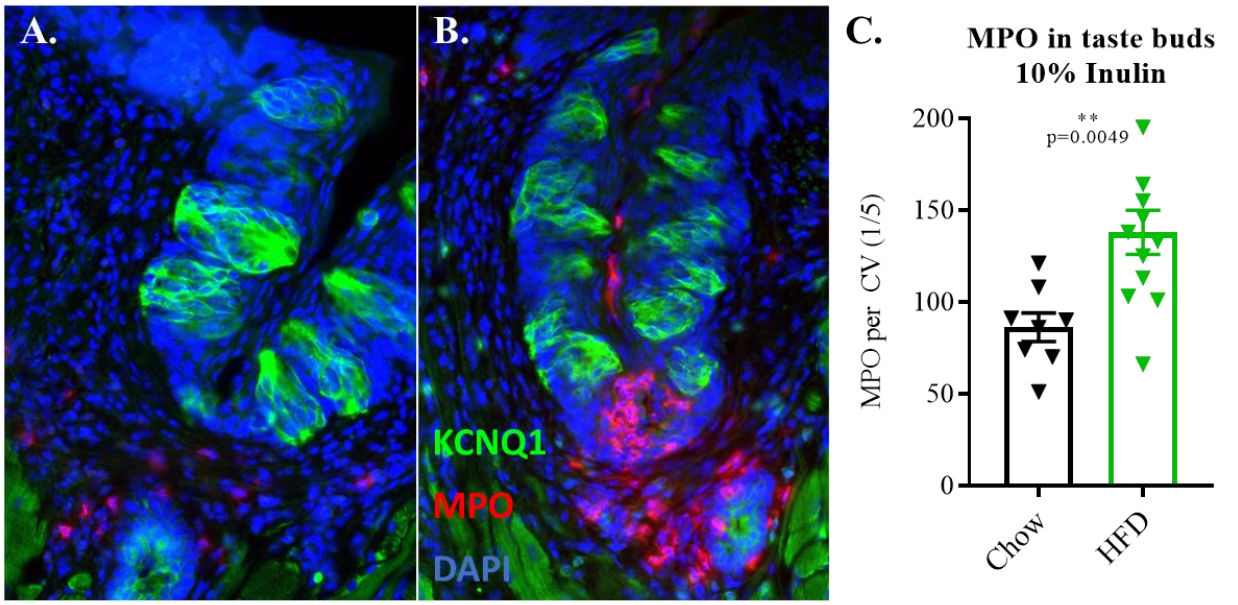

Figure 15. Representative image of left side of Circumvallate Papillae of C57 Bl/6 Mouse on A) Chow + 10% Inulin or B) HFD + 10% Inulin for 12 weeks. C) Counting of MPO+ cells shows Mice eating HFD with 10% Inulin have significantly more neutrophils in their taste buds.
Chow n=8 HFD n=12.

HFD + Inulin fed mice showed an increase in positivity for Myeloperoxidase (MPO), a component of neutrophil granules (Fig. 15), indicative of some level of activation of an innate immune response, as well as a decrease in sucrose 'liking' (Fig 14.C) as with all other mice in experiments consuming HFD. While we did see an increase in MPO in HFD-I fed mice (Fig.15 C), as in regular HFD feeding (Fig. 6) this did not correlate with a behavioral change as measured by Lickometer (Fig. 14A) or 2 bottle preference (Fig. 14B) testing with sucrose.

Discussion

In this study, we saw taste bud loss in DIO mice but not in TNFαKO and prebiotic fed DIO mice. This result accords with an inflammatory hypothesis for taste bud damage, which is supported by evidence in the literature. However, the precise mechanism remains unknown, and the behavioral impacts of such taste damage, in this mouse model, are somewhat complicated due to the composition of the HFD itself, formulated to approximate a western diet, and thus containing some level of sucrose as well as high levels of lipids.

Previous work from our group showed a similar decrease in TB abundance in DIO mice[13], but this work did not attempt to discern whether differences observed were behaviorally impactful. Other groups studying a DIO animal model of taste have shown various effects of HFD on the taste system. Ahart et al, showed a differential effect of the diet itself in addition to concomitant obesity on modulating taste[101]. In that study HFD fed mice which become obese showed a reduced number of licks to sucrose, saccharin, and acesulfame k which we were able to replicate here for sucrose. However, they also show that regardless of obesity status, those mice eating HFD had reduced lick scores for sucrose. The HFD used there was not specifically disclosed, but likely contained between 9-12% sucrose. Bernard et al[103] also showed a reduction in the number of licks toward sucrose in mice fed a HFD, which contained 5.6% sucrose, and a difference in sucrose preference as we were also able to show here. However, we were unable to reproduce the restoration of sucrose preference by prebiotic as in Bernard, which may be due to difference in experimental setup, critically that our sucrose preference test concentration was lower (0.86% vs 1%) and our HFD source was lard versus palm oil. Taken together, sweet taste response attenuation is likely partially explained by sucrose in the western diet used to induce obesity in the majority of diet-induced obese mouse studies, as there is evidence for a reduction in liking of sucrose after repeated exposure[128114129].

Weiss et al, fed a “High Energy Diet” (HED) to rats to induce obesity, which was similarly formulated to the diet we used here, and demonstrated an overall blunted neural response across taste stimuli in these

rats[130]. Maliphol et al showed an attenuated response to sweet stimuli in harvested taste receptor cells (TRCs) from obese C57 Bl/6 mice via calcium flux and by two bottle testing, again analogous to results we were able to show here[131]. Generalized attenuation of taste signals with obesity was also observed by Hardikar et al in obese humans[132], with obese subjects response to sweet taste degrading faster than lean subjects, with a lower peak signal. Interestingly, the signal onset intensity was no lower in obese vs. lean humans, indicating that signals from the lingual taste system should be similar. This would seem to contradict a hypothesis based on signal degradation from damage to the periphery as suggested here and in other animal models of DIO above. However, the signal peak strength and intensity duration are likely each affected independently by TB or TRC numbers, receptor gene expression, as well as hormones like Leptin[19], the circulating level of which is altered in obesity[133] and may be correlated with sweet taste perception[86134].

As obesity is associated with a chronic proinflammatory state[16], we used an immune deficient mouse model, B6.129S-Tnftm1Gkl/JTNFα and measured neutrophils in the taste field to assess the immune contribution to taste damage with obesity. MPO is a classical histological marker of neutrophils and has some association with obesity, as mice expressing human MPO display an increase in blood lipid markers for atherosclerosis and increased weight gain on a high fat diet vs control[135]. In this study more MPO+ labelled neutrophils were observed in the taste field of mice fed a HFD, but not in TNFαKO mice, therefore it is likely that TNFα is required for the accumulation of neutrophils. Although neutrophils are key in responding to acute damage, they are known to circulate at higher concentrations in the morbidly obese[122], as a component of the persistent low-grade inflammation associated with obesity.
Here we show taste bud loss in C57 Bl/6 mice eating HFD was associated with an infiltration of neutrophils (Fig.5). However, taste bud loss, likely through a reduction in the proliferative capacity of taste buds, was observed only in HFD fed mice (Fig.3), not in HFD + Inulin fed mice (Fig.13). This was

despite an accumulation of neutrophils in these animals (Fig.15), suggesting that such a response may not be essential for degradation of taste cells. Why then might Inulin be protective?

After ingestion, Inulin is fermented to SCFAs by microbes in the colon[136]. SCFAs, chiefly acetic, propionic, and butyric acids have a broad impact on the functioning of the immune system. They are taken up by intestinal epithelial cells and generally display anti-inflammatory properties[137120138].

Weitkunat et al. fed C57 Bl/6 mice a HFD supplemented with Inulin or with various SCFAs and ratios thereof, finding that SCFAs had a similar anti-inflammatory effect as Inulin[139], indicating that Inulin's fermentation products, and not Inulin itself, are immune effectors. Feeding effects in that study included changes in body temperature, mitochondrial activity, and fatty acid metabolism. This conclusion was supported by Besten et al. and mechanistically expanded by demonstrating that beneficial effects are dependent on the peroxisome proliferator-activated receptor-γ (PPARγ).

In humans, Inulin has been shown to reduce inflammatory markers (endotoxin, CRP, proinflammatory cytokines) generally[138]. Inulin feeding may also function by inhibiting the translocation of bacterial contents, primarily LPS, from the gut lumen, which may also be occurring in HFD fed animals[125140] and humans with type 2 diabetes (T2D)[141]. In a model of intestinal inflammation using LPS supplementation, mice displayed reduced licking and neural response to sweet stimuli, and elevated neutrophils in the small intestine and colon[142]. The prebiotic effects of Inulin may act via resident microbes like *Akkermansia muciniphila*[143], which can increase intestinal barrier strength, thereby reducing generalized inflammation due to diet.

Taken together, this suggests a protective effect of inulin supplementation and indicates that while neutrophils may be a marker of taste damage, they are not sufficient or causative of such pathophysiology alone.

What then might be the mechanism for TB loss? Taste buds are composed of 50-100 individual specialized epithelial cells[144], which are further subdivided into three cell types (reviewed by Finger and Simon, 2000). Type 1 cells, which are the most abundant cell type, are supporting, NTPDase containing

cells[145]. Type II 'receptor' cells sense sweet, umami[146], or bitter compounds[147], depending upon receptor expression[148]. Finally, type III cells which respond to sour tastes[59], and are the only taste cells which form classical synapses[149]. Each cell type within the bud has a distinct abundance and longevity[99], with an average life span of 10-20 days. TCs are regenerated from keratinocyte stem cells[150] adjacent to taste buds which migrate to TBs where their gene expression profiles are altered, triggering differentiate into one of the three cell types described[151].

Here we see differences in the total number of TBs between obese and lean mice, with relatively similar expression of taste related genes. Cancer is associated with dysgeusia (reviewed[71]), which can either be caused directly by acute treatment of the disease, which is typically chemotherapeutic or radiation based, or by effects of the disease itself. In one study of 51 patients undergoing radiation therapy (RT), a significant decrease in response to all tastes was found after five weeks of an initial RT[152] dose which recovered 11 weeks after the first dose. In the same study, the authors subjected rats to a single dose of RT and showed TB loss after 8 days which eventually recovered. Nerves were unaffected, causing the authors to conclude that the RT affected TBs specifically. A similar design was tested in mice using roughly half the RT dose (8 vs 15 Gy) and measuring Ki-67 as an indicator of proliferation by Nguyen et al. with similar findings[153].

In a chemotherapeutic pathway, taste progenitor cells may fail to differentiate into receptor cells, remaining as keratinocytes. Chemotherapeutics that targets stem cell renewal via Hedgehog (HH) signaling are deployed against basal cell carcinoma. Sekulic et al. reported >30% of patients treated with Vismodegib, a HH signaling inhibitor, reported dysguesia[154]. In mice, HH inhibitors almost completely ablated typical TB morphology and taste signaling through the innervating nerve[155]. However, Keratin-8 positive cells did remain in TBs, where in fact some were hyperkeratinized, suggesting that cellular renewal in lingual tissues was proceeding away from gustatory cells and toward keratinocytes in the absence of HH.

Interestingly, Cancer itself is also associated with an imbalance of proinflammatory cytokines[156] which have an impact on feeding[15715873]. Inflammatory diseases and their effect on taste are well known and reviewed in a recent article by the author[30].

TB loss is also observed in acute nerve injury or denervation of the tongue. While innervation is not required for TB development, it is required for TB maintenance in adulthood[159]. Denervation of taste buds through severing either the Glossopharyngeal or Chorda Tympani nerve leads to rapid apoptosis of TBs in the CV[160] and Fungiform Papillae (Guth L. 1971) respectively. McCluskey et al showed an influx of neutrophils and macrophages to the denervated and non-denervated control sides of tongues, with taste deficits occurring on both sides. Interestingly, using diet to inhibit neutrophil accumulation protected mice from taste dysfunction[116], implicating these immune cells as effectors.

Work by Zhu et al[28] expanded on this by inducing acute inflammation through feeding rats LPS in a single overnight session. 7 days later, sweet nerve recording response was significantly reduced. Further work by Pittman et al[49] used chronic exposure to LPS, and showed similar sweet taste deficits in nerve recording and lick testing, with a concomitant increase in gut neutrophils, though without a change in taste transduction genes. Interestingly, intraperitoneal (IP) injection of LPS by Cohn et al[29]. increased proinflammatory cytokines in TBs, and reduced TB abundance and markers for taste progenitor cells. Aguliar-Valles et al. injected LPS intra-peritoneally in mice and observed ‘depression like behavior’ and reduced physical activity[44]. As we describe here, mice eating HFD were less motivated to lick sucrose by behavior test. Aguliar-Valles implicated brain transmigrating neutrophils in this behavior, as it was reversible with neutropenia or by leptin inhibition.

Neutrophils act to phagocytose microbes (reviewed [161162]) but also secrete effector serine proteases, such as neutrophil elastase (NE) through neutrophil extracellular traps (NETs)[162]. Recent work on this secreted elastase implicated it as an effector in several proinflammatory pathways in host cells, not just in the intended microbial target[163]. Talukdar et al.[123] showed NE increasing in DIO mice after 3d of HFD. In the same paper, the authors treated WT or TLR4 $^{-/-}$ macrophages with NE or LPS, finding NE and LPS had a

directionally identical TLR4 dependent effect (↑ Tnfα, Il1b,Cxcl1, Il6). Other groups have similarly found NE inducing inflammation through the TLR4 pathway[164165]. As demonstrated by Cohn, activation of the TLR4 pathway reduces TB number via taste progenitor cells[29].

As we show empirically here, neutrophils are increased in HFD and HFD + Inulin C57 Bl/6 mice, but not in TNFαKO mice. While there is some evidence implicating TNFα in neutrophil recruitment[166–168], the role of Inulin supplementation had no effect on their accumulation. However, despite infiltrating neutrophils (Fig. 15), Inulin fed mice did not show a significant loss in taste buds (Fig. 13). As described above, Inulin is fermented to SCFAs in the colon after ingestion. Two studies report that SCFAs (chiefly butyrate and propionate) reduces the production of TNFα, as well as other proinflammatory cytokines, from neutrophils stimulated by LPS[169170]. As HFD feeding can acutely increase the level of transmigrating LPS[171172] from the gut, and the obese have higher levels of circulating LPS[173], we proffer the following mechanism; HFD induces inflammation acutely, or chronically through obesity, via activated neutrophils secreting TNFα and NE, in addition to circulating LPS itself from the gut. These effectors can be partially attenuated by the mechanism of prebiotics; gut barrier strengthening to reduce LPS translocation and the anti-inflammatory effect of SCFAs on neutrophils, which can explain the protective effect of Inulin on taste buds in these mice.

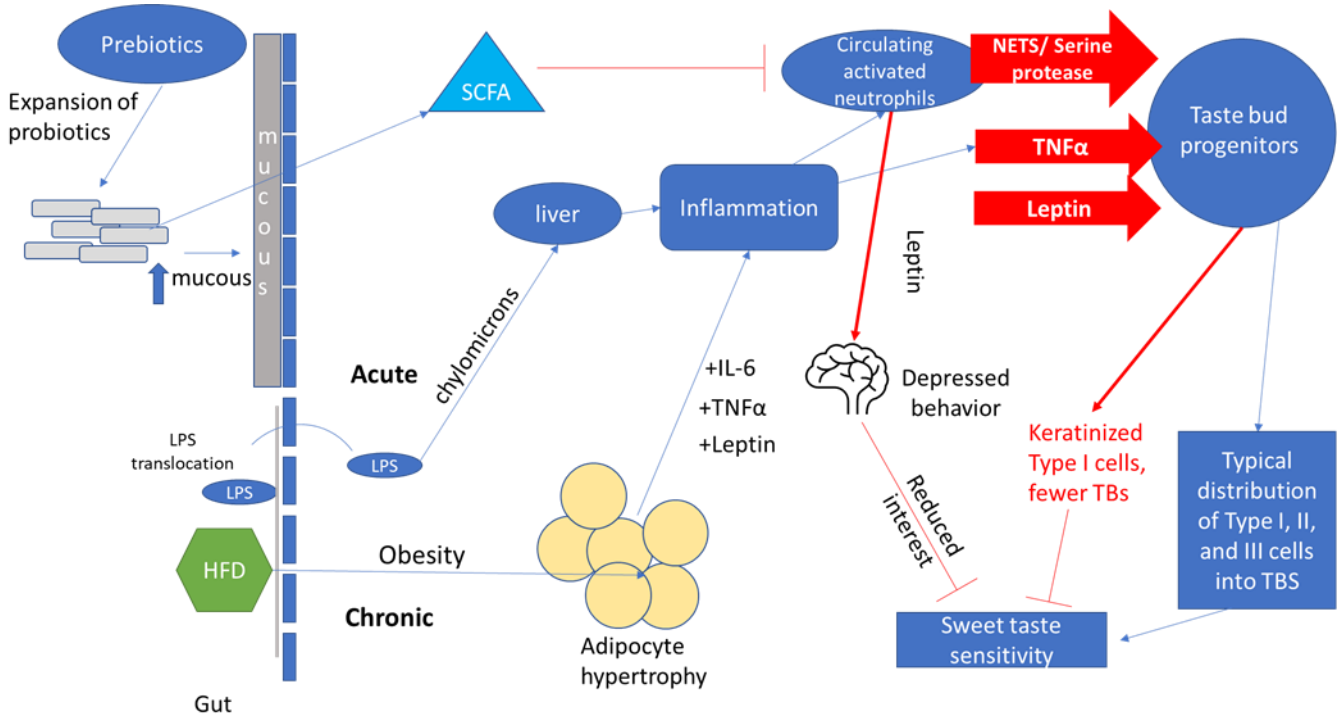

Figure 16. Proposed mechanism: Taste bud loss and sweet behavioral changes proceed along two pathways, acute, and chronic. In the acute pathway, a High Fat Diet can reduce the thickness of the mucous membrane in the gut, and lead to LPS translocation which causes acute inflammation. This increases circulating proinflammatory cytokines like TNFα, and neutrophils which by the effector action of NETS, can contribute to the polarization of taste progenitor cells toward keratinization and away from a taste receptor cell fate. In the chronic pathway, inflammation is primarily due to the action of proinflammatory cytokines released by adipose tissue. The effects of these and other circulating hormones, like leptin, on signal processing areas in the brain on taste behavior also likely contribute substantially to taste perception.

Conclusions

Here we describe a series of experiments that seek to uncover the differential effects of diet and inflammation on the taste system. We find that taste bud abundance is likely impacted by inflammatory tone, which can be modified by prebiotics, and that this can impact taste preference. However, taste preference was also influenced by the type of diet used to conduct experiments. Further, the motivation to lick sweet substances may be affected by eating a High Fat Diet, a finding now supported across several papers. Future work should aim to more deeply understand the effect of inflammatory mediators of neutrophils in the taste field and elucidate mechanisms to reduce their activation.

Chapter 3

Population of naïve mice with a unique commensal composition are resistant to exogenous colonization by Fecal Microbiota Transplant

Abstract

A well-documented effect of a high-fat diet (HFD) is a shift in the gut microbiome toward a 'dysbiotic' phenotype, with an increased relative abundance of *Firmicutes* over *Bacteroides* when compared to littermates maintained on regular chow. It has been demonstrated previously that such a dysbiotic gut phenotype can be transplanted to naïve mice and is sufficient to induce weight gain in the host. In this study, we transferred the cecal contents of obese mice into naïve, conventionally housed (non germ-free) mice maintained on chow to test whether these animals would recapitulate taste deficiency observed in obese mice. While taste phenotype was inconclusive, measurements of recipient microbiomes post transfer gave new insights into the stability of fecal microbiota transfer (FMT) in conventionally raised mice. We further identified *Akkermansia muciniphila, Parabacteroides distasonis, Clostridium citroniae* and others as highly overrepresented in a subgroup of mice which best resisted shifts toward the dysbiotic donor animals. As this work was performed in conventionally raised mice, rather than germ-free animals, it affords an opportunity to record changes in an otherwise stable commensal population acutely challenged by a divergent population. Recipient mice flora remained more similar to control mice maintained on chow than donor mice on HFD after three gavages, with those presenting high levels of *Akkermansia muciniphila*, *Parabacteroides distasonis*, *Clostridium citroniae* notably more resistant to shifts. Taken together, these results suggest a strong bias toward diet over acute transfer in altering the host microbiome, and further identifies marker species associated with a unique phenotype resistant to dysbiotic FMT.

Introduction:

Fecal transplants into immune naïve animals have been used to show the sufficiency of donor microbiota to recapitulate metabolic phenotypes in otherwise healthy animals. There are striking examples where insulin resistance[124], and obesity[174] are initiated in a donor animal via diet, but transferred to recipient mice by microbiota transplant alone. Most studies of this type utilize germ free mice, which have never been exposed to bacteria, more readily allowing only the transplanted microbiota to take hold. However, the unique state of germ free animals results in an underdeveloped immune system and other developmental deficits[175] that complicate the drawing of broader conclusions from such animals. Likewise, there remains some difficulty in extrapolating results to humans, where an active microbiome would be the norm. Work in rats has shown that fecal transplants from donors into conventional, non-germ-free animals is sufficient to shift the microbiome of recipients to resemble that of the donor in the short (1 month), and long (3 months) term[176].

Several thousand papers[177] have been published showing the effects of various compounds on the microbiome. As the human diet directly impacts the fitness of gut microbes, diet and its consequences, particularly obesity, are of high interest. Several papers demonstrate an association between obesity and metabolic syndrome with changes in the microbiome [140,178,179]. Some of the most compelling evidence of such associations rests on the transfer of cecal contents of obese mice into germ-free (GF) lean mice. Interestingly, despite consuming a relatively healthy diet, these mice go on to develop obesity and its concomitant metabolic comorbidities[179].

The striking connection between obesity and the microbiome was first laid out in leptin deficient mice[180]. Leptin, a hormone which inhibits hunger and promotes satiety, causes obesity in these knockout mice via overeating. Compared with lean mice, leptin deficient obese mice showed a significant increase in gut composition of *Firmicutes*, and a decrease in *Bacteroidetes*. Further work showed that these obese associated microbial communities can harvest additional caloric energy from the same dietary intake, thus making weight loss more difficult, and further entrenching an obese state[174]. When an obese mouse's

microbiota is transferred to germ free mice, the obese phenotype is recapitulated in mice consuming a regular, healthy diet[174]. Obesity frequently co-occurs with a chronic low grade inflammation[15], with adipose tissue harboring immune cells and generating proinflammatory cytokines like IL-6 and TNFα (reviewed[79]). Interestingly, triggering inflammatory pathways in mice using Lipopolysaccharides (LPS), a large surface exposed molecule on certain bacteria, as one might observe in an acute inflammatory event, inhibits taste bud renewal[29]. TNFα upregulation has been linked to a decrease in taste bud abundance and life span[29], obesity[27], and insulin resistance (reviewed[15]). We have shown in a diet induced obesity model that TNFα expression is inversely proportional to taste bud abundance[14].

As inflammatory disorders impact the taste system (Reviewed by Goodman et al. 2021) and are associated with dysbiotic microbiomes, we postulated that the two might be linked. This work was conceived to test whether transferring the microbiota of taste deficient, obese mice to lean mice would accordingly transfer such a phenotype and further elucidate the effect of microbial transfer to non-germ free mice.

Methods

Animals / Experimental Design

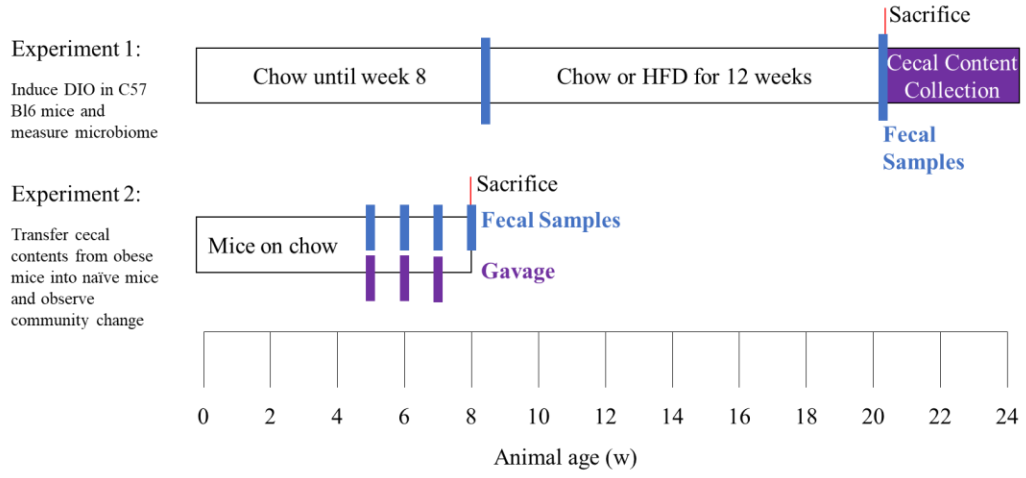

Figure 17. Experimental design detailing the induction of dysbiotic diet in mice in Experiment 1 and the scheme for transferring microbes to conventionally raised mice over 3 gavages in Experiment 2. Mice were fed either a HF or Chow diet starting at age 8 weeks and for 12 weeks thereafter. Cecal contents from each group was collected, pooled, and gavaged three times into conventionally raised mice

maintained on chow at weeks 5,6, and 7 of their lives. Fecal samples were obtained to monitor success of microbiota transfer and taste phenotypes were measured post gavage.

Cecal content collection

A 50% Glycerol solution in PBS was prepared and autoclaved for 35 minutes to ensure sterility. Aliquots of 500 µL were prepared in sterile Eppendorf tubes. After sacrifice and dissections, Ceca were isolated from the rest of the gut, excising the connected small and large intestines, then moved to a sterile plastic disposable dish and washed with PBS to remove external detritus. Clean ceca were moved to another clean dish and 500 µL PBS/Glycerol added. A scissors, pre-cleaned in 70% Ethanol, was used to open the cecum by cutting across its lateral face. Cecal contents were washed out by continually pipetting PBS/Glycerol solution in and around the organ. Finally, contents were collected, put on ice, and rapidly moved to -80 within 60 minutes.

Cecal content gavage

Extracted cecal contents from HFD treated mice was thawed on ice and diluted 1:5 in autoclaved PBS/Glycerol prior to gavage. 150 uL of this diluted material was gavaged via an 18 Gauge, 5 cm needle with ball tip (Kent Scientific Corp, Torrington, CT).

Mice were monitored for evidence of aspiration and observed for 7 minutes post gavage to ensure a successful procedure without harm.

Fecal samples were collected immediately prior to each gavage to ascertain the extent of successful donor colonization.

16S rRNA gene sequencing and Microbiome analysis pipeline

The 16s rRNA gene was used to determine gut flora composition, isolated in the following manner: Fecal samples were collected and frozen at -80◦C before processing for microbial DNA isolation (PowerFecal DNA isolation kit, MO BIO laboratories Inc, Carlsbad CA). Post extraction, PCR was performed (PCR stages detailed in appendix) using primers specific for the V4 variable region (515F[181] and 806R[182]) and containing illumina nextera adapter overhangs to generate genera/species specific amplicons. A second step PCR reaction appended sample specific barcodes which was performed by Biotechnology Resource

Center (BRC) Genomics Facility at the Cornell Institute of Biotechnology (http://www.biotech.cornell.edu/brc/genomics-facility). The facility normalized samples by concentration, pooled, and run on the MiSeq sequencing platform utilizing 250 x 250 paired end sequencing. Sequencing files were run through the QIIME2[183] (Quantitative Insights for Microbial Ecology) analysis pipeline. Briefly, files were de-multiplexed by barcode identifier and quality filtered for ambiguous base calls and low-quality reads. Sequences were identified and placed into ASVs using the using the DADA2[184] algorithm and taxonomically matched using the GreenGenes database[185]. Prior to downstream analysis, samples were rarefied to the lowest abundant sample (24,000 reads) to correct for variation in sequencing depth. Sequences were aligned using the mafft[186] function and filtered for variable positions using mask[187]. The aligned sequences were joined to a tree using fasttree[188] and then mid-point rooted using 'phylogeny'. β-diversity was determined using the weighted and unweighted Unifrac[189] distances between samples. Unifrac uses the evolutionary distance between two identified taxa, adding up all of the evolutionary branchpoints pairwise between compared samples. Weighted unifrac takes into account species relative abundance when generating distances while unweighted unifrac does not.

Overhang adapter for use with Illumina Nextera sequencing
TCGTCGGCAGC GTCAGATGTGTATAAGAGACAG

515F Primer (Parada et al. 2016**)**
GTGYCAGCMGCGCGGTAA

Overhang adapter for use with Illumina Nextera sequencing
GTCTCGTCGGGCTCGGAGATGTGTATAAGAGACAG

806R Primer (Apprill et al. 2015**)**
GGACTACNVGGGTWTCTAAT

16s rRNA Gene PCR for microbiome sequencing amplicons
2.5 uL Microbioal DNA (5 ng/uL) extracted via PowerFecal DNA isolation kit (MO BIO laboratories Inc, Carlsbad CA).
5 uL Forward Primer (515F)
5 uL Reverse Primer (806R)
12.5 uL 2x KAPA Hifi HotStart Ready Mix

1. 94°C 3 minutes

2. 94°C 45 seconds
3. 60°C 60 seconds
4. 72°C 90 seconds
5. Repeat steps 2-4 35 times
6. 72°C 10 minutes
4°C HOLD

Predictive genomics via PICRUSt2. We used the PICRUSt2(Phylogenetic Investigation of Communities by Reconstruction of Unobserved States)[190–192] plugin embedded in Qiime2 to predict the metagenome of our samples and ascertain whether observed differences in community structure also differ in the presence or absence of certain metabolic pathways. A PcoA plot was created using a Bray Curtis dissimilarity matrix generated from estimated metacyc pathway abundance for each sample. ASVs were aligned to a reference tree using SEPP (SAT´e-Enabled Phylogenetic Placement)[193].
Caution is warranted as these metagenomes are calculated based on abundance data from 16S amplicons. Any errors or biases in these data will be present in this extrapolation

Statistics

Clustering Algorithm

To determine whether specific clusters of samples were distinct from other clusters, a Permanova test was performed via QIIME2 with 999 permutations[173].

Distance metrics significance

To determine whether movement toward or away from donor clusters were significant, we used weighted unifrac distances generated as described above. Distances from individual mice in each subgroup were compared to HFD donors at baseline, and then after each first gavage. We then subtracted the distances of each subsequent points to generate a ‘change in distance toward donor’ metric. A 0 on this metric would indicate no movement toward the position of the donors. We performed a one sample Wilcoxon signed rank test of each group for each gavage timepoint compared to a hypothetical median 0 value.

Differential Abundance

To surface the specific microbes which differed significantly between groups, we used the Qiime2 package analysis of composition of microbiomes (ANCOM)[173]. ANCOM corrects for the compositional effect of using relative abundance data when comparing across groups and reports which taxa are differentially abundant and how different that abundance is.

Faiths Phylogenetic Diversity.

Phylogenetic diversity was calculated in Qiime2 using the metric described by Faith in 1992[194] and compared between groups using a one-way ANOVA.

Results

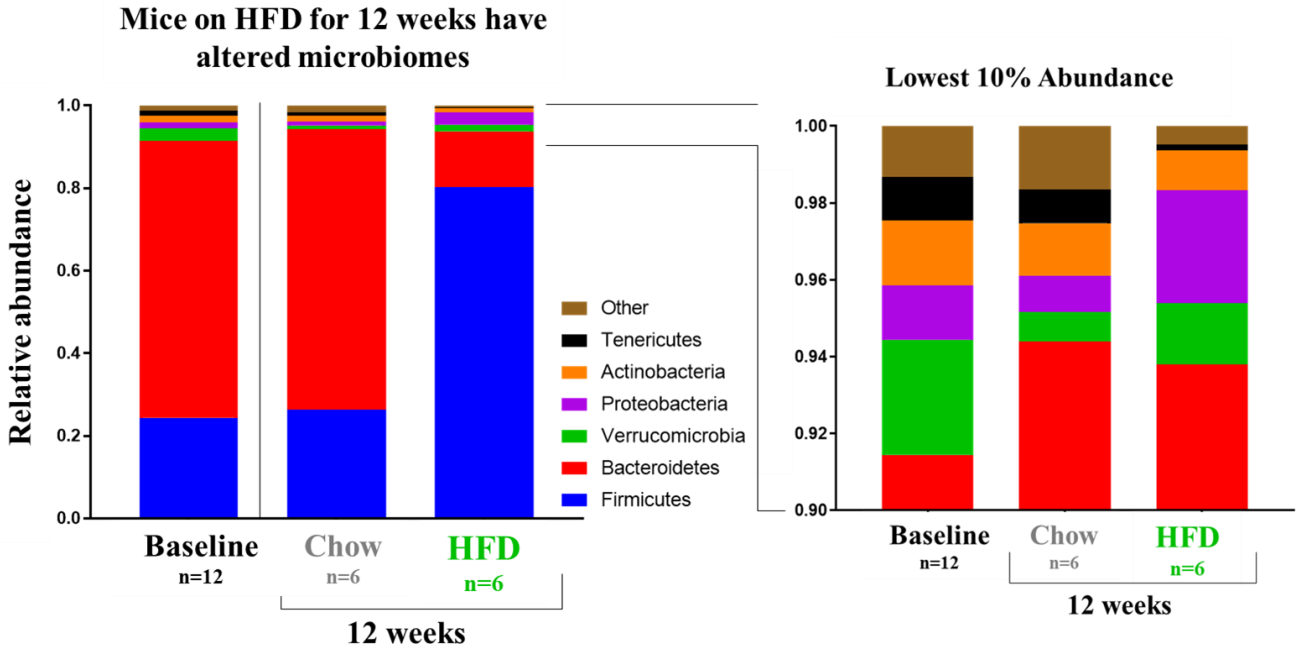

Figure 18. After 12 weeks, mice on chow and HFD have distinct microbiomes, with mice on HFD changing to overemphasize *Firmicutes* over *Bacteroidetes*.

HFD Feeding alters the microbiome of mice. After eating a HFD for 12 weeks, obese mice over emphasized *Firmicutes* (80.24% relative abundance versus chow mice at 26.36%.). HFD feeding also reduced *Bacteroidetes* to 13.54% versus 68.02% in mice fed chow (Fig.18). Chow fed mice did not deviate significantly from baseline in the 3-month period between sequencing timepoints.

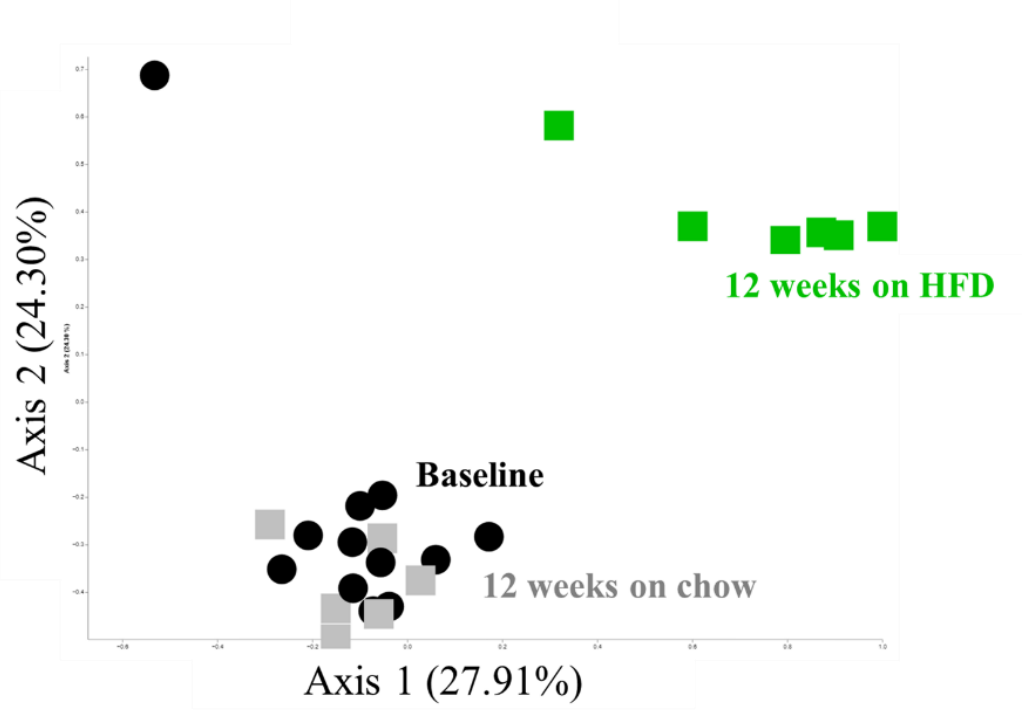

Figure 19. PCoA plot of weighted unifrac of individual mice microbiomes at baseline (black circles), after 12 weeks of chow (grey square), and after 12 weeks of HFD (green squares). Cecal contents of these dieted mice were used as input gavages in

HFD fed mice(green squares) plotted on PcoA using a distance matrix derived from weighted unifrac clustered significantly differently from baseline (PERMANOVA p=0.001, test statistic=17.475, 999 permutations) while the mice maintained on a chow diet (grey squares) were not significantly different than when at baseline (black circles) (Fig. 19) (PERMANOVA p=0.287, test statistic=1.203, 999 permutations).

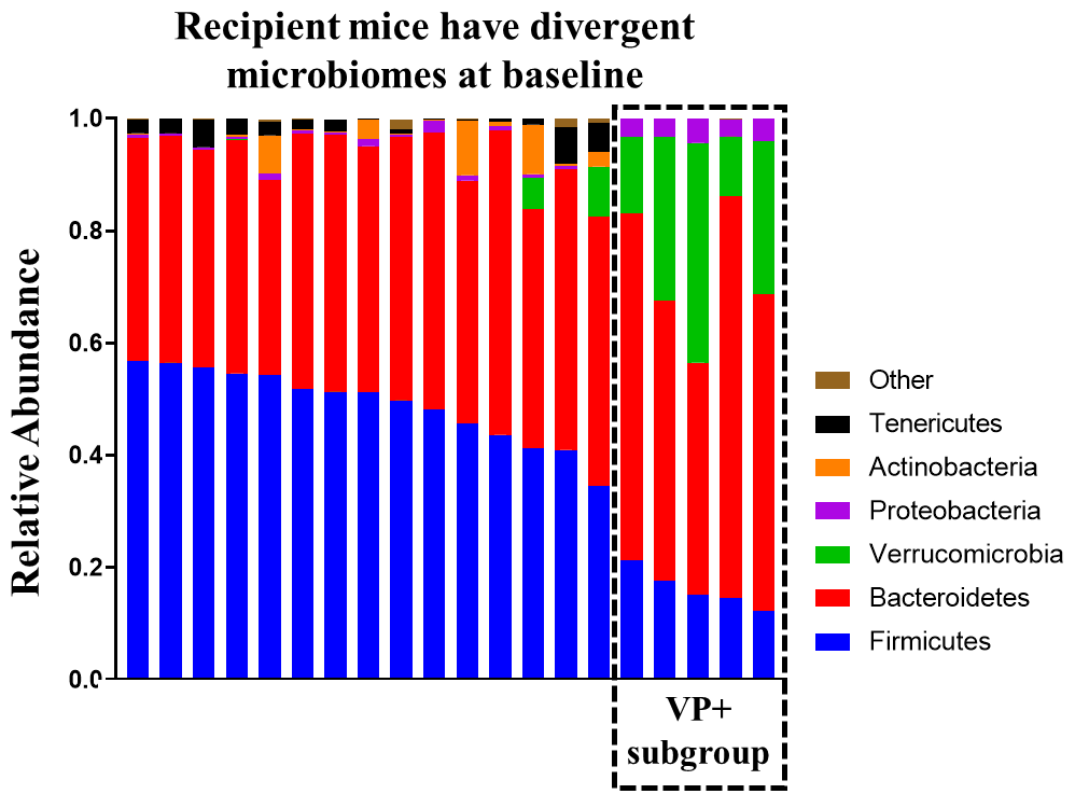

Figure 20. Relative abundance at the Phylum level at baseline for naïve mice set to receive gavage from either HFD or Chow fed mice. A subset of mice which have elevated abundance of *Verrucomicrobia* and *Proteobacteria* stand out and our labeled as the VP+ subgroup.

Genetically identical mice have distinct microbiomes at 5 weeks of age. Interestingly, mice bred in identical conditions displayed highly variable abundances of microbes (Fig 20.). A subset of mice, all born in the same litter, displayed a well differentiated microbiome marked by an elevated abundance of *Verrucomicrobia* and *Proteobacteria*.

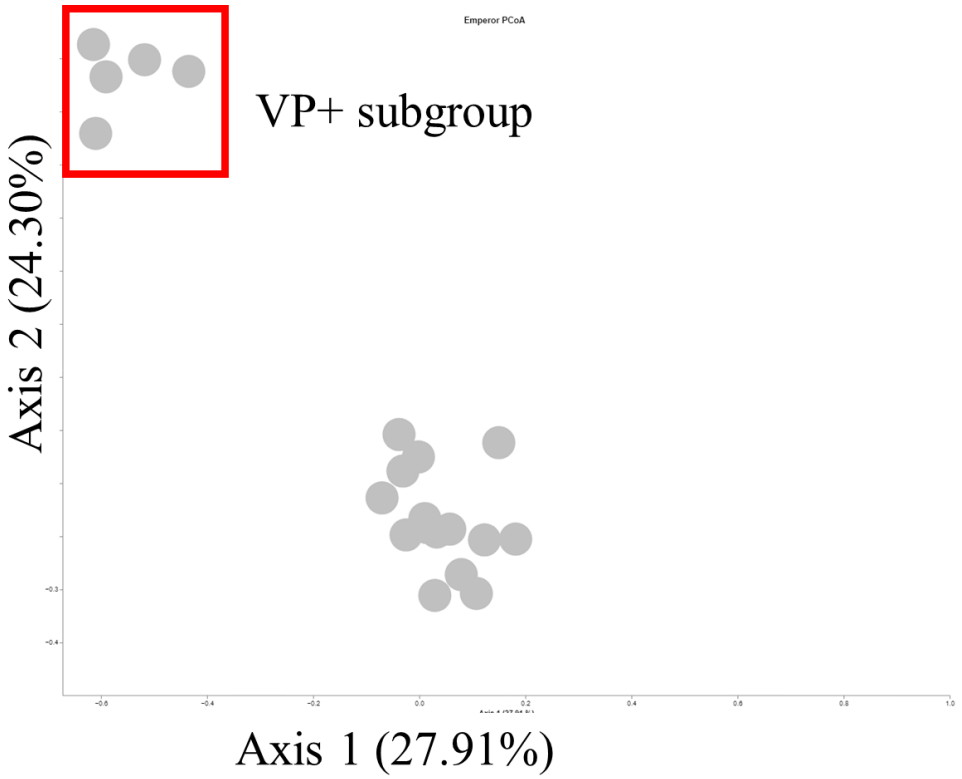

Figure 21. Baseline differences in mice prior to gavage plotted on a PcoA using weighted unifrac to generate the underlying distance matrix. Top left cluster is described by several differentially abundant microbes, with the top two being from the families *Verrucomicrobia* and *Proteobacteria* phylum

When plotting these mice on a PcoA using weighted unifrac distances, two distinct unique clusters formed. The community described in Fig. 20 is also labelled here as the VP+ subgroup (Fig.21).

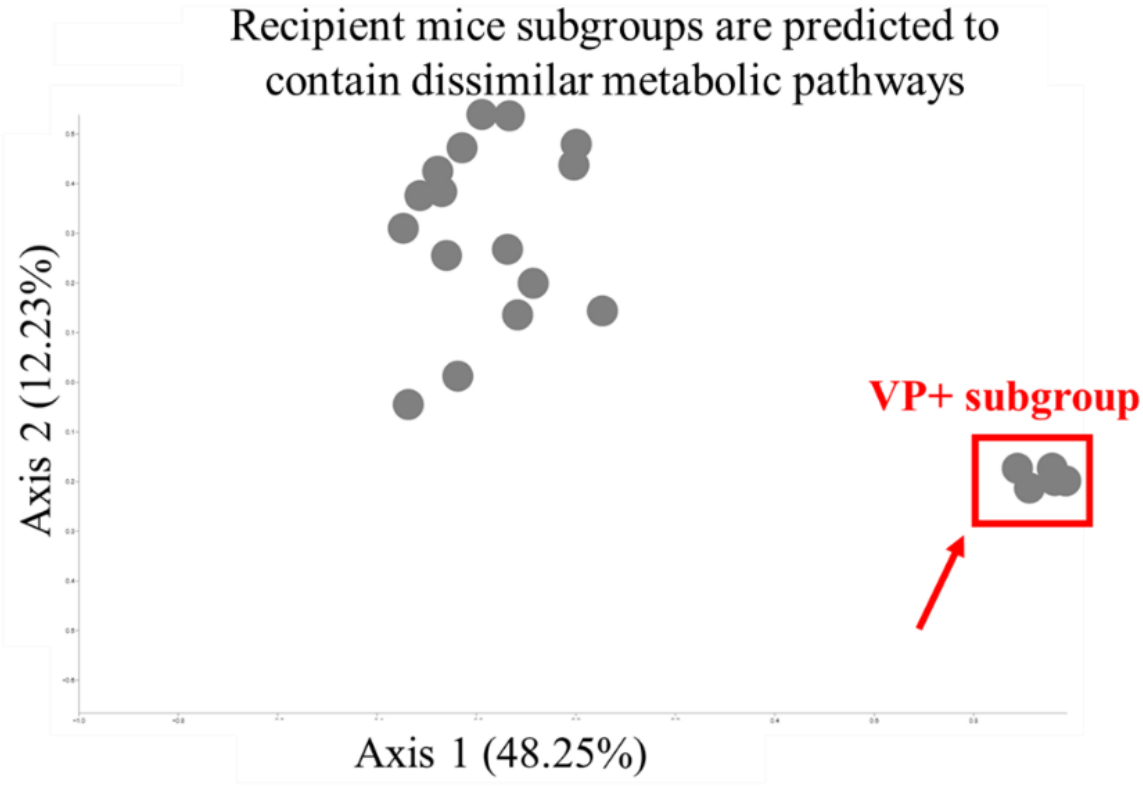

Figure 22. PcoA of Bray Curtis Dissimilarity generated from PICRUSt2 predicted metabolic pathways for gavage recipient mice. VP+ cluster is annotated.

We estimated the abundance of metabolic pathways of recipient mice using the PICRUSt2 software package and observed a similar divergent clustering of this subgroup (Fig 22.), indicating that the genomes of VP+ are likely functionally distinct in addition to being phylogenetically distinct.

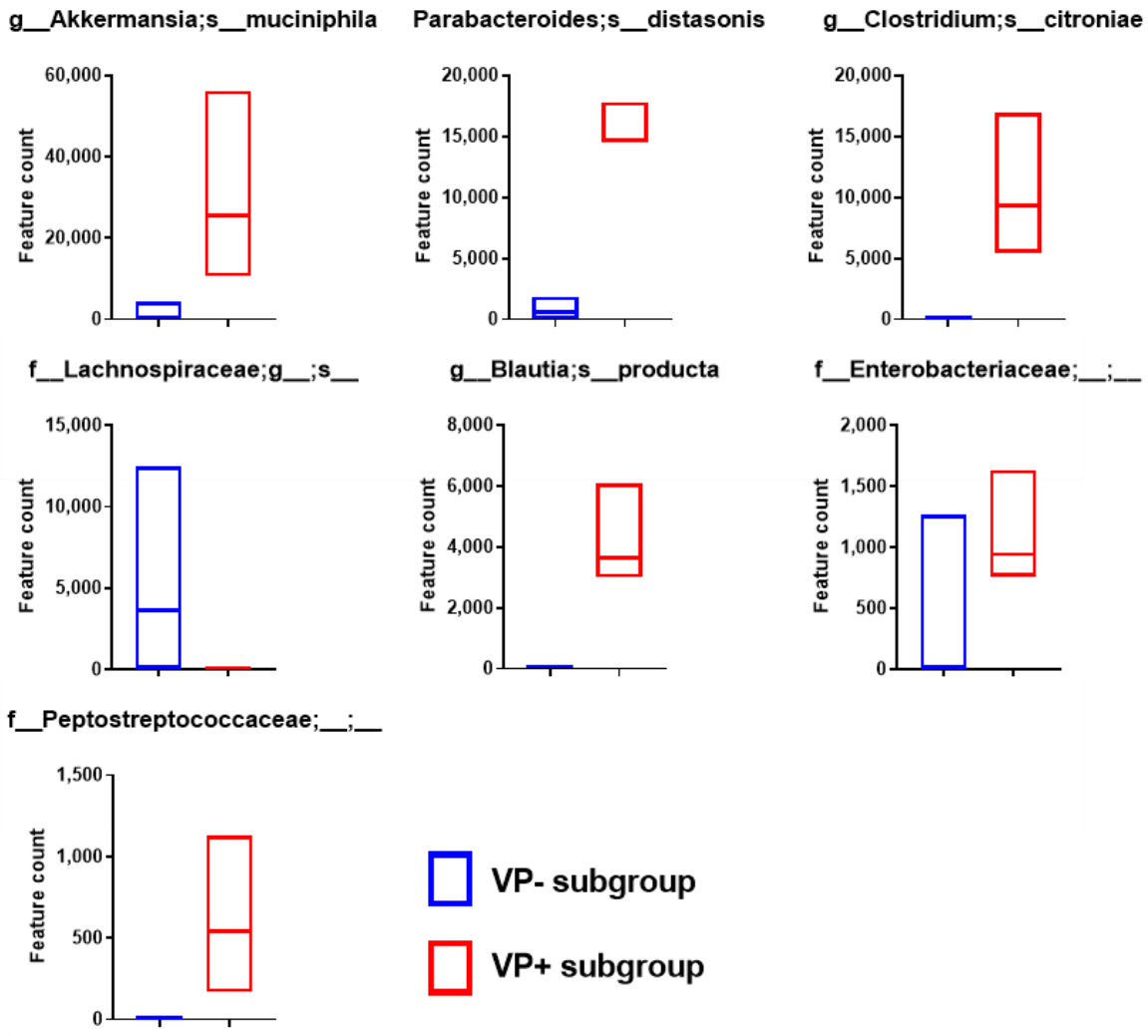

Figure 23. Differentially abundant species of bacteria when comparing subgroups between mice who had an abundance of microbes in the families *Verrucomicrobia* and *Proteobacteria*.

Using the ANCOM QIIME2 package, we determined the top seven differentially abundant taxa in the VP+ and VP- subgroup, sorting by largest w value (Fig 23.). These 7 taxa were all w>85, well separated from with the next species at w=68. In this package, the w value is the count of times that the null hypothesis (that this specific taxon has the same abundance across the tested groups) is rejected. A large w value means that this specific taxa was very often found to have a differential abundance between samples. The VP+ subgroup was marked by the differential abundance of *Akkermansia muciniphila, Parabacteroides distasonis, Clostridium citroniae,* and *Blautia producta*. The VP- subgroup tended to

lack all the above, though *Lachnospiraceae*, a family within *Firmicutes*, dominated. When comparing the overall abundances in Fig.20, the VP+ subgroup also tended to have a lower abundance of *Firmicutes*.

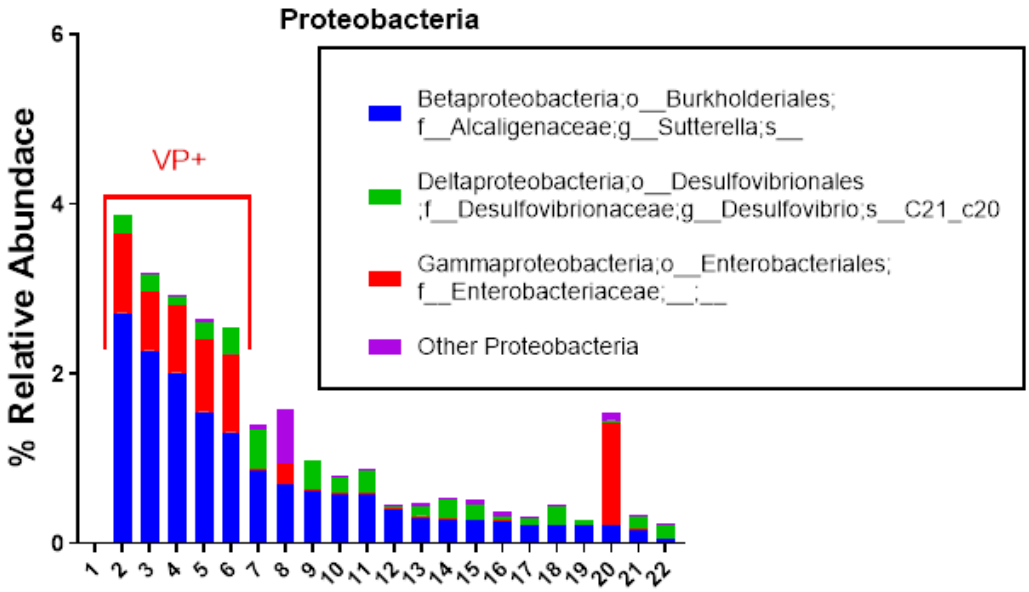

Figure 24. VP+ *Proteobacteria* are distinguished by the presence of *Enterobacteriaceae* and elevated *Sutterella spp.*

***Proteobacteria* differences are concentrated in the genus *Sutterella* and in the family *Enterobacteriaceae*.**

While the ANCOM analysis above looks at the differential abundance between ASVs in provided subgroups (Fig. 23), it does not consider the evolutionary differences between groups in contrast to a metric like weighted Unifrac, which explains the large distances between VP+/- groups in Fig. 21. When looking at % abundances of just the *Proteobacteria* phylum, the VP+ subgroup saw a relatively higher abundance of the genus *Sutterella*, and an unnamed ASV in the family *Enterobacteriaceae* (Fig. 24).

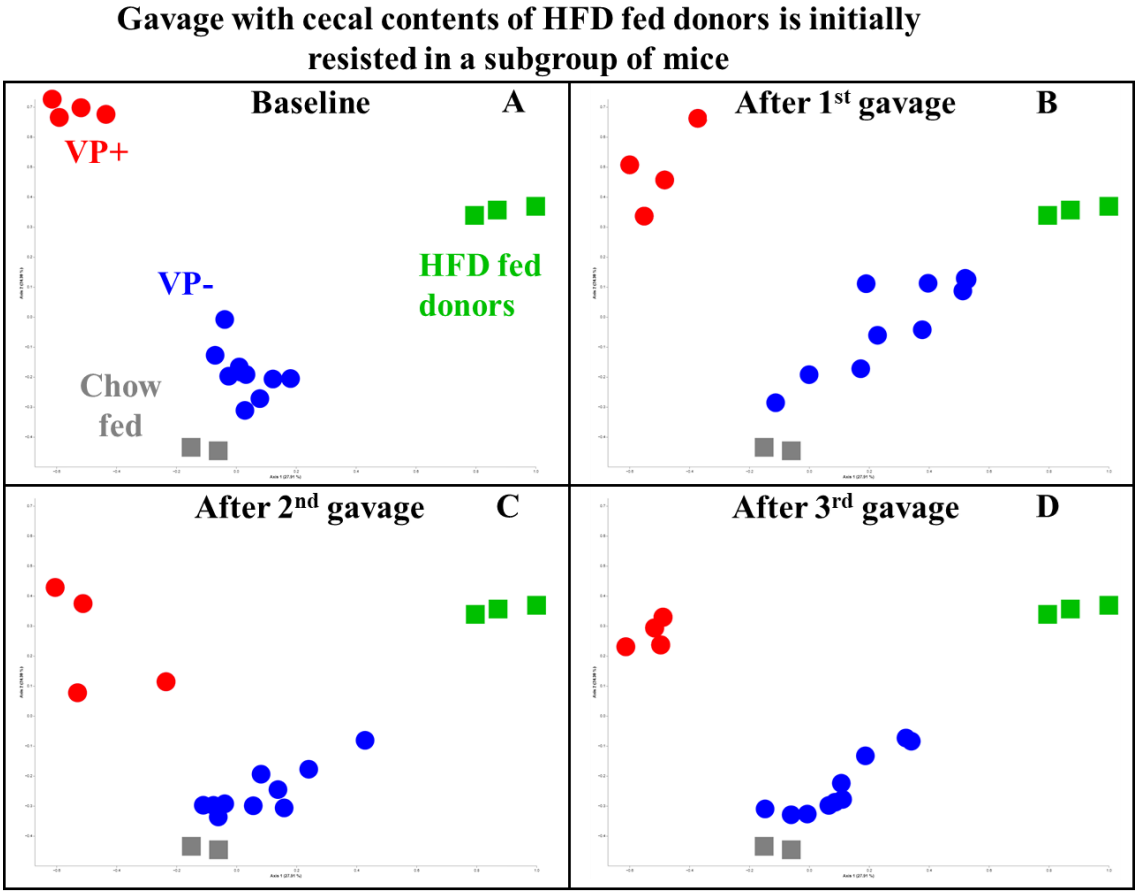

Figure 25. Weighted unifrac PcoA of HFD donors (green squares), recipients (red and blue circles), and chow fed mice (grey squares) for comparison. Similarity prior to gavage in A), one week after the first gavage B), a week after the second gavage C), and a week after the final gavage D).

Mice with VP+ microbiota are resistant to exogenous changes from gavage. VP+ and VP- mice were gavaged three times from a subset of HFD fed donors described in Fig.18. Donors overemphasized *Firmicutes* and clustered in the top and right of PC1 and PC2 of Figure 25 (green squares). The same multidimensional space was used to analyze changes in recipient mice. As discussed above, at baseline, recipient mice formed two unique clusters corresponding primarily to presence or absence of *Verrucomicrobia* and *Proteobacteria*, VP+ and VP- respectively (Fig. 25 A red and blue circles). After

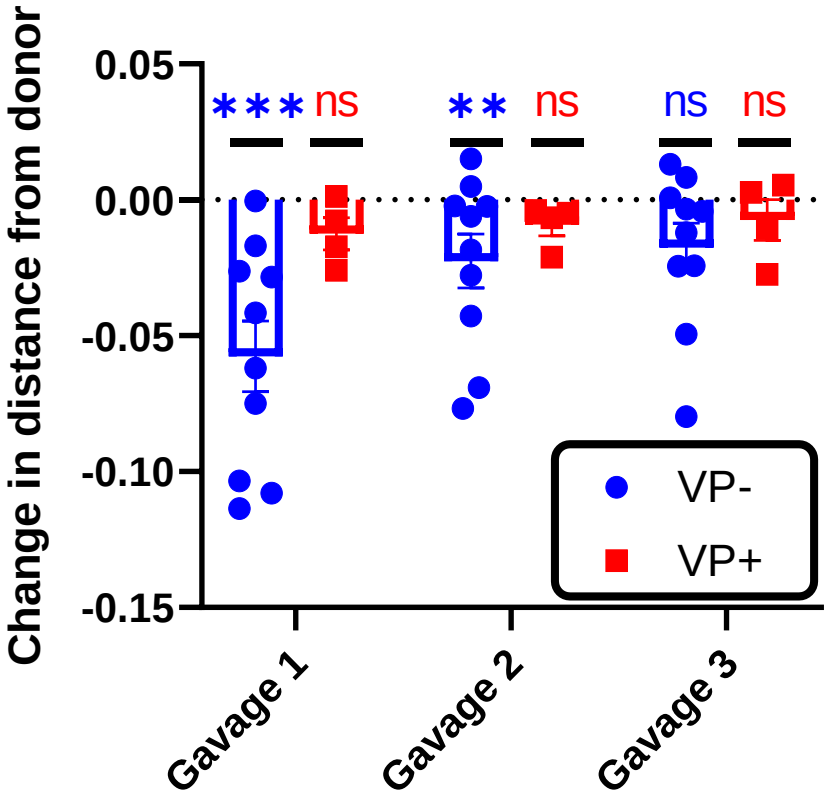

Figure 26. VP+ Subgroup is resistant to changes induced by gavage with HFD donor ceca, compared to the VP- subgroup which is resistant after gavage three, but shows movement toward donors after the first two gavages. Distances from individual mice in each subgroup were compared to HFD donors at baseline, and then after each first gavage. We then subtracted the distances of each subsequent points to generate a 'change in distance toward donor' metric.

the first gavage, VP- mice moved toward their donors (Fig 25 B. Toward green squares), while VP+ mice did not.

These distance changes were quantified in Fig. 26 by taking the average distance of each recipient at baseline from all of the donors and then quantifying the change in that distance after each gavage. A change greater than 0 indicates a move away from the donor microbiome, while a change less than 0 indicates a change which reduces this distance, i.e. begins to more closely approximate that of the donor microbiome. For each timepoint, VP+ mice had nonsignificant changes versus donors, whereas VP- mice moved toward donors significantly after the first and second gavage.

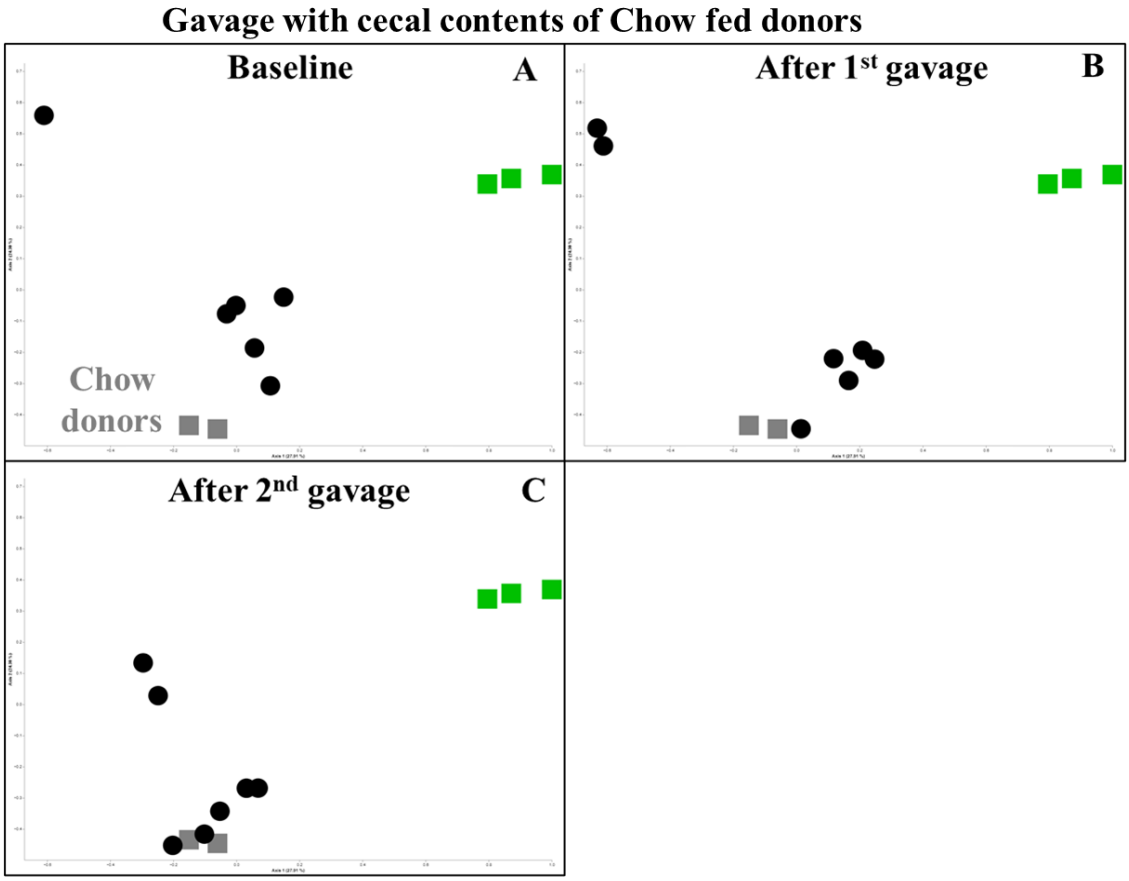

Figure 27. Weighted unifrac PcoA of HFD fed mice (green squares), chow donors (grey squares) and recipient mice (black circles). Similarity prior to gavage in A), one week after the first gavage B), a week after the second gavage C), and a week after the final gavage. VP+ Mice gavaged with fecal samples from chow donors are also resistant after initial gavage. However, they move toward donors after the second gavage. Low n in this group make comparisons difficult.

VP+ mice gavaged with cecal contents from chow fed mice also displayed initial resistance toward shifts in their microbiota (Fig 27. B). While they received gavages from ceacal contents of mice that were maintained on chow for 12 weeks, the recipient mice *were also* eating a chow only diet. Therefore, shifts toward chow donors (grey squares) cannot be adequately explained with this experimental setup.

Analysis of gavage for explicit maternal influence

As the VP+ group described herein were birthed from a single litter, we re-analyzed the data by sub segmenting not by VP status, but by mothers (Fig. 28).

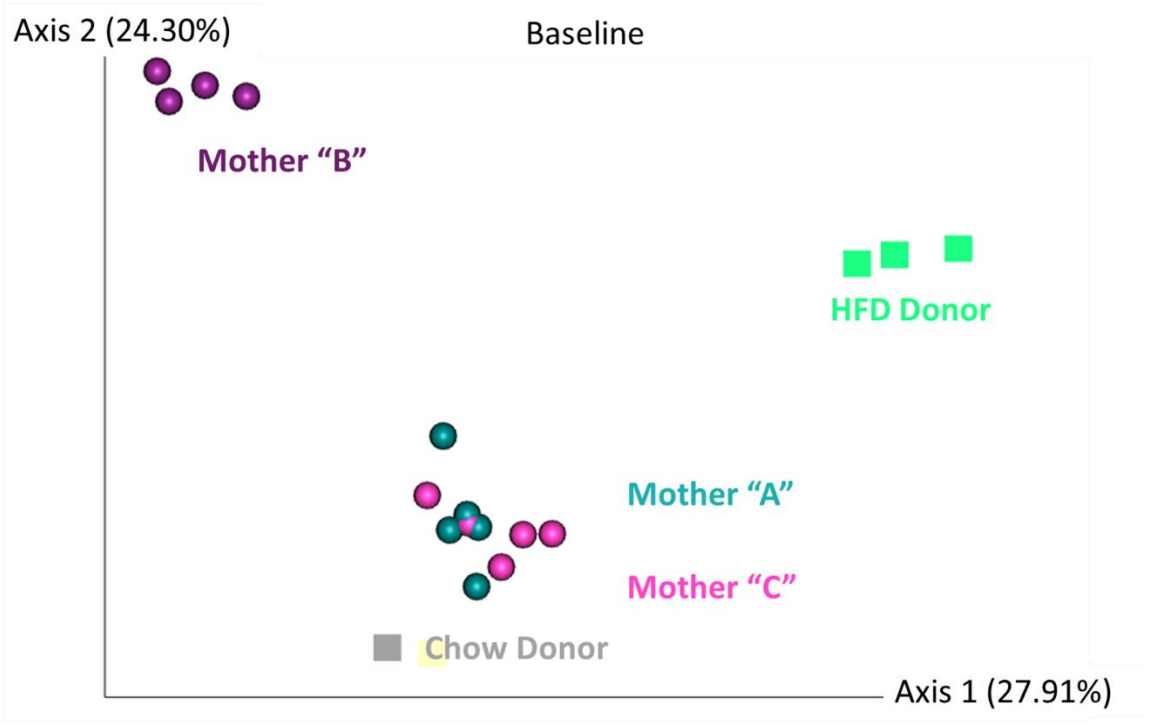

Figure 28. Weighted unifrac PcoA of HFD donors (green squares), and recipients colored by their mother/litter (hunter green, pink, purple), and chow fed mice (grey squares) used as a proxy for 'diet'. All mice are displayed at baseline prior to any gavages to indicate the initial distances that are subtracted out to calculate baseline.

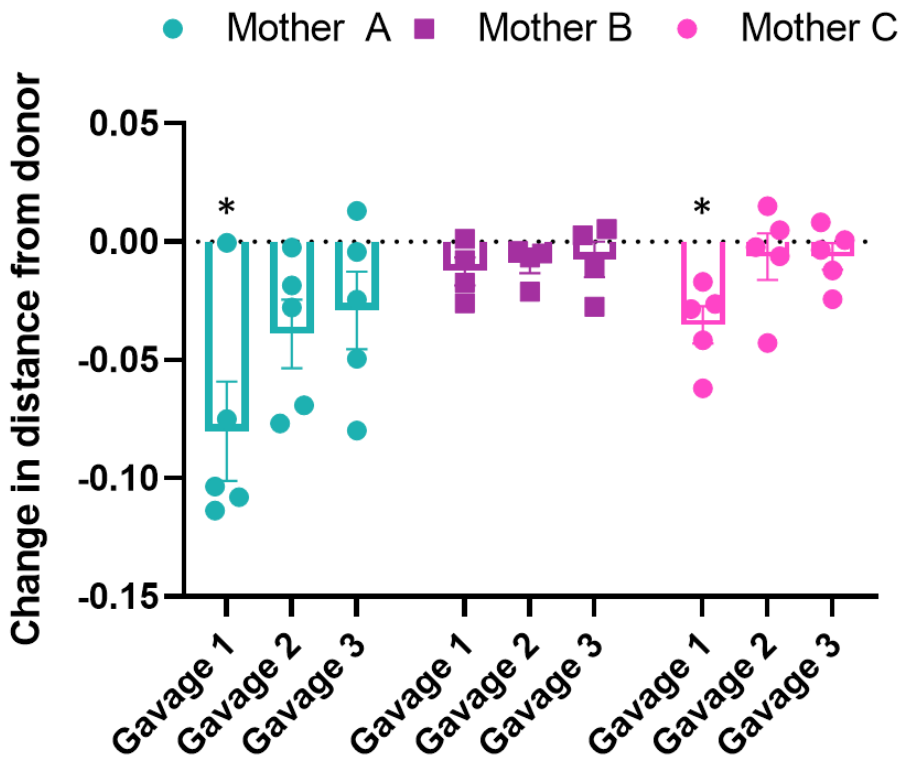

Figure 29. VP+ Subgroup (mother B) is resistant to changes induced by gavage with HFD donor ceca, compared to the VP- subgroup(s) of mother A and C, which are amenable to the first gavage. * indicated $P<0.05$ using a one sample T-Test versus 0.

When categorizing by mother and interrogating the impact of gavage (Fig.29), mother B (the VP+ group) remains the only group resistant to all gavages. The other groups are susceptible to change after gavage 1, but resistant after subsequent inducements.

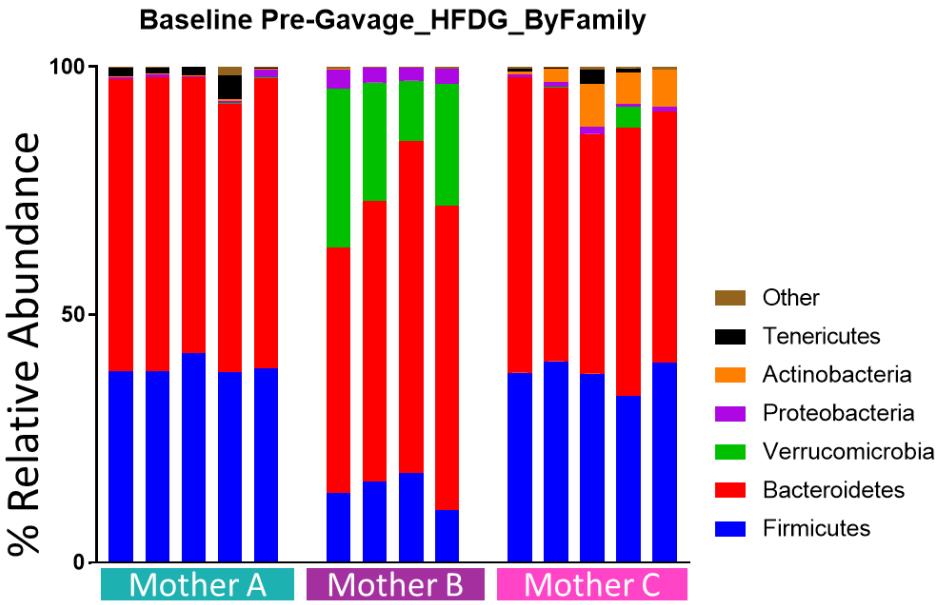

Figure 30. Relative abundance of Phyla level microbes, organized by mother.

Maternal effect had an impact on the relative abundance of microbes in each litter. Notably, when merely observing the relative abundance data, *Verrucomicrobia* and *Proteobacteria* are overrepresented in offspring of Mother B, *Actinobacteria* in Mother C, and neither of these in Mother A (Fig. 30).

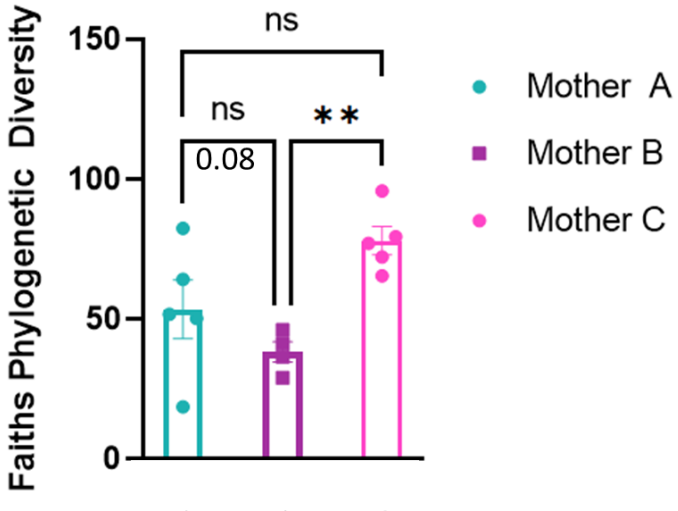

Figure 31. Faiths Phylogenetic Diversity of litters organized by mother. Mother B scientifically different vs. C, and approaching significance vs. Mother A.

The overall diversity of each of group was described using Faiths Phylogenetic diversity and compared between groups (Fig.31). Mother B is the only group with a significant difference in diversity on this metric, however, this litter had the lowest overall diversity compared to the other groups, with mother C having the greatest overall diversity.

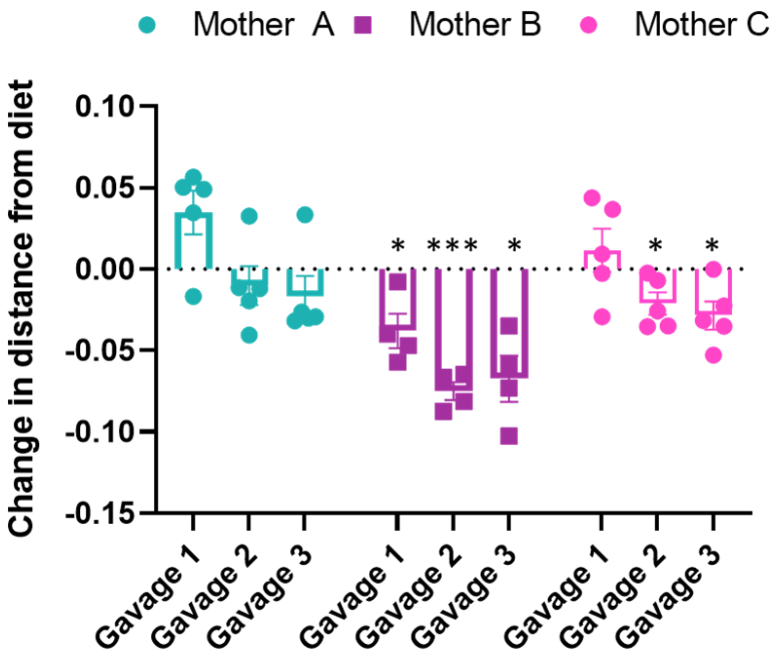

Figure 32. HFD gavaged mice categorized by mother plotted against their change toward or away from mice on a chow diet for 12 weeks. One sample T-Test versus 0 was used to determine if the microbiome changed from baseline.

*=p<0.05
**=p<0.01
***=p<0.001

Mice resistant to gavage move significantly toward a proxy of chow diet. We used the microbiomes of mice fed a chow diet for 20 weeks as a proxy for the effect of diet on shifting the microbiome of gavaged mice. As the mice were undergoing gavage treatments, they were maintained on chow and therefore may have been affected by that diet which is high in fermentable carbohydrates. Mice from mother B moved significantly toward the diet proxy at each time point, with mice from mother C moving after gavage 2 and 3.

Discussion

We sequenced the gut microbes from seemingly near-identical, littermate mice, and found a notable divergence in microbiomes, with some mice displaying a unique phenotype, primarily harboring *Verrucomicrobia* and *Parabacteroides* (VP+). Further, the subgroup was also well separated (Fig. 22) on a Bray Curtis Dissimilarity PcoA built off PICRUst2 reporting of metabolic pathway abundances. This software package uses the relative abundance data of microbes to predict which genes are present in the sampled population.

Using HFD feeding, we altered donor mice microbiomes, making changes consistent with literature [180195196], although these changes may not replicate completely in humans[197],and gavaged their cecal contents into naïve mice, maintained on chow. Post gavage, we found the VP+ recipient subgroup resisted changes toward these exogenous microbes. This subset of mice were born in the same litter and thus likely acquired their microbiome from their mother or early living environment. This distinct microbiome, while changing slightly during our study window, maintained a unique cluster versus other mice when challenged with divergent exogenous microbiota.

Exogenous colonization. To establish and test the function of singular microbes in animals, it is common to introduce them via FMT in mice raised in germ free (GF) environments. However, these mice display unique immunopathology's due to this method of breeding[198–200] and access to these models remains limited. Antibiotic treatment followed by colonization has also been employed successfully[201–203], although antibiotics alone induce their own phenotype[176]. Barba et al. transferred microbes three times, three weeks apart, without the use of antibiotics in a C57 model of kidney failure[204]. These transfers were able to significantly alter the microbiome of recipients toward control, indicating that transfer is possible in the absence of antibiotics. Here we chose to adopt a similar protocol, and choose not to use antibiotics to remove commensals, which might closer mimic a more typical wild-type process of colonization. Wrzosek et al. used polyethylene glycol (PEG) to 'cleanse' the bowels of recipient mice prior to various strategies of FMT[205]. They found that even in the mildest transfer condition (a single human FMT into

mice), donor OTUs were found 4 weeks later. These studies used gavages from rodents of one species into another, allowing them to track specific species associated OTUs. In our study, donor and recipient mice were genetically identical and therefore we relied on changes in community structure to determine dissimilarity. In VP+ mice receiving gavages from HFD fed mice, there were no time points post FMT where a movement toward these donors indicated an increase in community similarity.

Finally, Freitag et al. directly tested FMT with and without antibiotics from Balb/c to C57BL/6 mice. They found donor transfer was effective with or without antibiotics with a single gavage at week 0 and an 8-week endpoint. In our work, some, though not all, of the VP- mice were more similar to donors at our endpoint than they started, as would be predicted from the literature. However, none of the VP+ mice made any significant movement towards the donor phenotype, indicating that sequencing, isolating and excluding such mice from future studies of FMT without antibiotics may be essential to the transfer's success.

VP- were in general more susceptible to changes in their microbial ecology than VP+ mice. Maldonado-Gómez et al. studied the predictors of persistence of a *B.longum spp* in adult humans in 2016, finding that a subset of subjects had *B. Longum* persisting for 200 days after initial treatment[206]. The authors find that establishment was best correlated to specific traits or genes, noting that the absence of these allowed these specific *B. Longum spp.* to fill a niche. Other work by Li et al. found that FMT success, defined by longer term persistence of donor strains in recipients, was correlated to the presence of the donor species, but not the donor strain[207]. While we lack strain level resolution, VP- mice were more similar to donors than VP+ mice in our study, with an average of 0.26 vs 0.32 units in weighted unifrac distance matrix multidimensional space. Interestingly *Parabacteroides distasonis*, observed in this study, was among the top 5 strains found in resistant recipient microbiomes found by Li et al. above.

It is likely that a complicated dynamic exists between the recipient microbial community, the composition of the donors microbiome, the existence of a niche` to be exploited in the recipient, and the recipients immune system. Further, the presence of unique combinations of microbes may exist with specific colonization resistant traits which inhibit engraftment of a donors microbiome.

Colonization resistance. Here we show VP+ commensals inhibiting colonization from donors. This 'colonization resistance'(CR) is a known phenomenon, with several mechanisms including nutrient competition, direct bacteriocin defense, and colonization of the mucous layer (reviewed in detail by Ducarmon et al.[208]). Pathogenic bacteria can utilize an altered immune system to overwhelm commensals, as reported by Stecher et al. in 2007[209]. The authors showed that commensals outcompeted a pathogenic *Salmonella* but were themselves outcompeted after inflammation was induced via other microbes or by pro-inflammatory T-Cells. A highly differentially abundant species in the VP+ group was *Akkermansia muciniphila* (Am) (Fig. 23), a mucin degrading microbe[210] adhering to enterocytes[211] in the mucous layer of the gut, which serves as a barrier between the foreign contents of the lumen, and the endothelial cells making up the human intenstine[210]. A decrease in barrier thickness is implicated is several diseases associated with insulin sensitivity and inflammation[27]. It is theorized that a thinner mucous membrane brings potentially pathogenic bacteria into closer contact with the endothelial lining, leading to activation of the innate immune system, and TNF-α elevation. Several commensal bacteria inhabit this mucous layer, with *Akkermansia muciniphila* being a major resident, compromising 1-4% of our total gut composition[210,212] .

Further work on this species indicates an inverse relationship between its abundance and several measures of metabolic health, including weight gain, gut barrier function, and inflammation [213,214]. Additionally, dietary interventions aimed at improving metabolic health, and diabetes treatments like metformin have been shown to associate with *Akkermansia* abundance[215].

Less is known about *Parabacteroides distasonis* (Pd), with a general search on Google scholar listing 1,750 results since 2017 versus *Akkermanisa muciniphila* at 9,560 (authors search in April 2021). However, recent work implicates this commensal as associated with health. In 2018, Wang et al. carried out a detailed study administering live or heat killed Pd into DIO or genetically obese mice and tracking metabolic outcomes. Among other outcomes, Pd decreased weight gain and hepatic steatosis, through a mechanism associated with secondary bile acids[216]. Recent work by Koh et al. in a mouse model of colorectal cancer showed administration of freeze dried Pd could attenuate inflammation (↓IL-4,

↓TNFα,↑IL-10,↑TGF-β, by qPCR) and its associated tumorogensis[217]. The authors also observed an increase in gut barrier associated gene expression (ZO-1). A recent study in humans by Deehan et al. looked at the impact of different types of resistant starch on the microbiome and its associated metabolites. They found a significant increase in Pd in humans fed tapioca starch, which was associated with an increase in the SCFA propionate, in a dose-dependent manner[218]. As described earlier, propionate is itself associated with anti-inflammatory effects (↑gut barrier[219],↓IL8[220]), though many of these studies are in-vitro or in mice, with mild benefits in humans. Further, butyrate[221] may also be the component driving many of these effects, in contrast to propionate.

Future work should focus on uncovering the mechanisms associated with these changes, but along with Am, Pd tends to be identified in the literature as a health-associated commensal. A strikingly relevant study by Caballero et al. in 2017 identified Pd as associated with a group of commensals which exhibited colonization resistance toward *Enterococcus faecium (*Ef*).* Here they showed a consortium of commensals including *Blautia Producta*, another VP+ microbe which we identified, able to repel exogenous colonization with Ef[222].

While *Clostridium citroniae* (Cc) is less well studied even than Pd, the same study by Caballero above noted a commensal, *Clostridium bolteae* (Cb), which along with *B. producta* is part of the group conferring colonization resistance. Cb and Cc shared 98% sequence homology and were, until 2006, part of the same group[223]. Therefore, our finding here may be in accord with Caballero's vis a vis a group of commensals conferring colonization resistance.

We also showed a relatively increased abundance of *Sutterella spp (*Ss*).* In VP+ mice (Fig 24.). There is evidence of some *Sutterella spp,* particularly *Sutterella wadsworthensis* (Sw). being associated with inflammatory diseases like Crohn's disease (CD)[224], and in children with autism[225]. However, other investigations show no difference in Sw prevalence versus controls in IBD[226]. A recent report in 2020 by Kaakoush suggested a role for Ss in inhibiting successful FMT's[227] from earlier results from the group. Specifically, Paramsothy et al. in 2019 investigated the association between remission and lack thereof in

in-patients receiving FMT for Ulcerative colitis with specific microbes. They found *Sutterella* associated with lack of remission.[228] In that report, Kaakoush also referenced a study by Moon et al in 2015 which associated Ss with a population of microbes able to degrade IgA in B6 mice[229]. IgA production from the host might be hypothesized to be associated with a stable commensal microbiome, and therefore its degradation would allow for opportunistic colonization by FMT. In our work here, it's possible that IgA degradation is allowing for the colonization and proliferation of other commensals which themselves contribute to colonization resistance. This may be a role for the *Enterobacteriaceae spp.* associated with increase Ss (Fig.27). Recent work in 2020 by Wang et al found Ss increased in rats after bariatric surgery with a concomitant increase in *Enterobacteriaceae spp*[230]. It's unclear which specific *Enterobacteria* are enriched in these samples, as this species is difficult to identify solely using 16S rRNA gene sequencing of the V4 region as we did here[231].

IgA degradation described above is one of several examples of host immune and microbiome crosstalk. Microbes living symbiotically in the intestinal tract of another organism are in constant contact and communication with their hosts immune system, starting at, or before birth. While there is controversy over the microbial sterility of the placenta[232][233], there is consensus that stable colonization is a result of an immune – microbial partnership[234][235]. Mazmanian et al. in 2005 showed that a molecule, "polysaccharide A (PSA)" produced by *Bacteroides fragilis* could correct an imbalanced immune response in germ-free mice[236], showing the benefits of this crosstalk. Work by Han et al. in 2013 which ablated the expression of a signaling molecule (TRAF-6) in dendritic cells (DCs) which sample the gut, showed a loss of tolerance, with increased markers of enteritis in the small intestine[237]. A key mediator of microbial tolerance is immunoglobulin A (IgA), the most abundant Ig in mammals, and induced by the introduction of commensals to GF mice[238]. Work by Lindner et al. in 2012 pointed to the existence of IgA memory cells[239] which might explain the initial toleration and persistence of a select group of commensals (reviewed[240]). In addition to IgA, Regulatory T cells ($Foxp3^+$, $CD4^+$) also appear after intestinal colonization of commensals[241]. Lawson et al in 2011 introduced a synthetic microbiome of non-pathogenic commensals into GF mice and measured the distribution of T_{regs}, finding that colonization of

microbes is 'essential' for immune homeostasis[242]. Without commensals, the immune response did not include T_{regs} and developed an activated effector response. It is therefore not surprising that an offspring's mother has a significant impact on their progeny's microbiome.

Maternal influence on initial microbiota. This maternal impact on the heterogeneity of gut communities on offspring is well known, being reported by Ley in 2005[180]. Schloss et al. in 2012 collected fecal samples from C57 BL/6 mice for a year post weaning[243], suggesting that communities stabilize 20 days post weaning, showing little change up to a year later. Hansen et al. found that exogenous colonization at 3-weeks of age was particularly stable[244], suggesting immunomodulation as a cause. Mice in our study received their first gavage at age 5 weeks, seven days post weaning. We chose this time point as we expected the communities to be more amenable to change at this younger age, versus adult mice, 20 days post weaning according to Schloss, with more stable and therefore imperturbable microbiomes.

While we discuss at length the impact of gavage on microbiome change, it should be emphasized that during the entire period of gavaging, these mice are maintained on a chow diet. This diet is a good source of soluble and insoluble fiber and is likely exerting its own effect. To test this, we performed the same distance measurements as described in Ch.3, but used 20-week old mice maintained on chow for 12 weeks (Fig. 28 grey square). as proxies for the effect of chow diet on the microbiome (Fig.32). In stark contrast to changes induced by gavage, the Mother B group changed significantly toward the proxy of diet at each time point. This was not the case for Mother A or C mice. Mother A mice never significantly moved toward diet, while Mother C mice did at later time points.

We should note that as we are measuring change from baseline, mice that were already more similar to mice on chow (Mother A and Mother C) have less distance to travel (Fig.28). There may also be an age effect on the microbiome, as the proxy for chow mice are 20 weeks old, versus these mice at gavage 3 who are 8 weeks old. Therefore, using 'chow donor' as a proxy is useful, but not a completely sufficient method for explaining the impact of diet.

We also analyzed overall phylogenetic diversity of each litter using Faiths Phylogenetic diversity (Fig.31). This metric sums the distances of all branch lengths in a sample. The more diverse the sample, the more branches and therefore the higher the numbers.

When comparing overall diversity to data on resistance to gavage, in this case, the lack of diversity in mother B was more predicative of resistance to colonization. However, the intermediate diversity level of mother A, a group that was *most* susceptible to colonization, casts doubt on this metric on its own as an indicator of colonization resistance.

When sub-categorizing mice by their mothers, similar trends that we described using VP+/- measures held. However, when we looked the effect of diet, using mice on chow for 12 weeks as a proxy, we did see a differential impact by mother. Further, mice who were resistant to any change by HFD gavage were very susceptible to change by diet, indicating that even this microbiome is not static. Future work should continue to explore the impact of pre and probiotics on the microbiome, as this work indicates that prebiotics may be more impactful.

Conclusions

Here we show a subset of commensal microbes which resist acute changes from exogenous microbes. These commensals are associated with mice born of the same mother and mirror a similar group with unique properties reported in literature. This work gives support for communities of microbes which co-occur to create emergent phenotypes.

Remarks and future direction

While it is tempting and useful to study single microbes and their functions independently, this work underscores the importance of looking at the community as a whole. The interaction between microbes, their environment, what they metabolize, excrete, and how they support or inhibit each other is a complex milieu. Deeper study of these complex communities will improve our ability to fight pathogens, improve gut and immune health, and may yield insights which allow for the creation of novel therapeutics.

Appendix

Introduction

Mice from Ch.2 who underwent treatment with 10% Inulin had unique changes to their microbiome which are analyzed and discussed herein. Initially, this was among a list of treatments taken to induce the growth of *Akkermansia*, and therefore these other treatments will also be discussed.

Microbiome analysis of 10% Inulin mice from Ch. 1

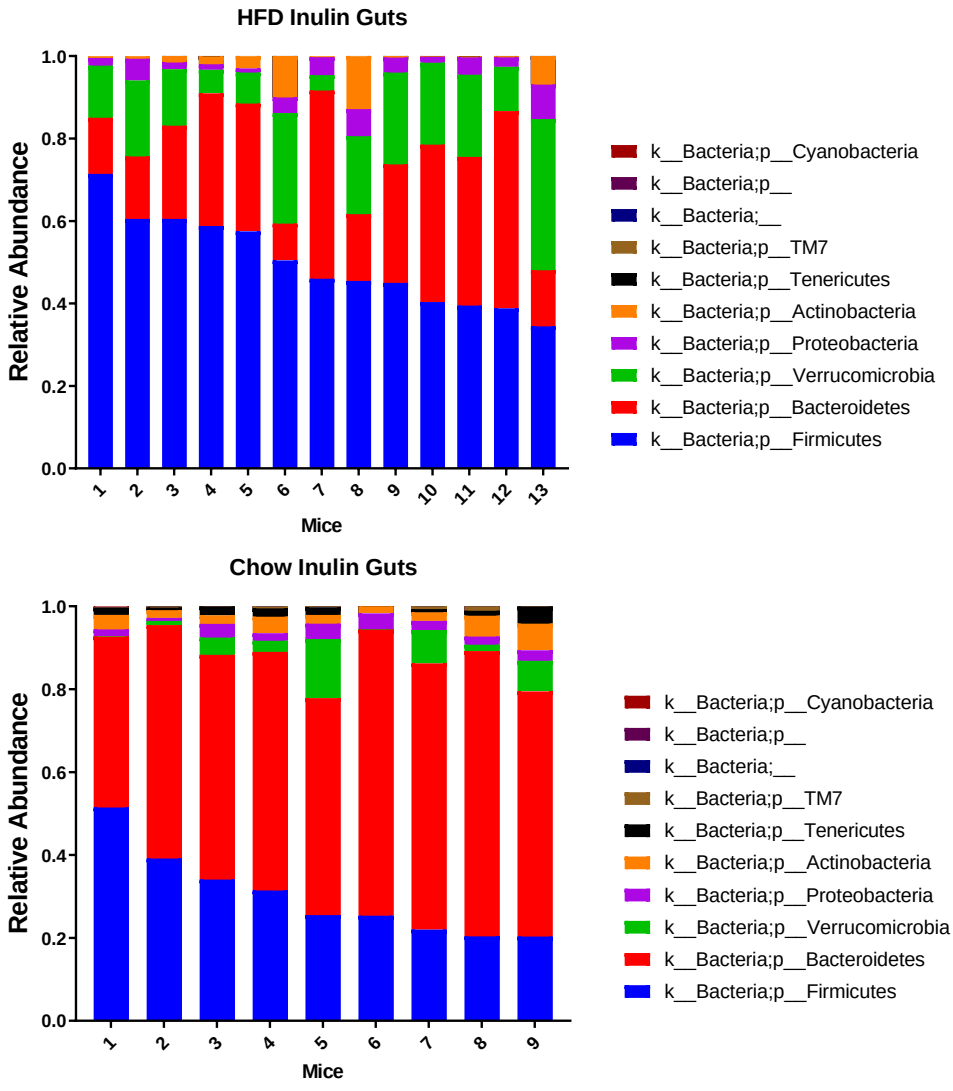

Figure 33. C57 Bl/6 mice on HFD or Chow supplemented with 10% Inulin for 8 weeks.

C57 Bl/6 mice from Ch.1 that were fed Chow or HFD + Inulin for 8 weeks had their microbiomes sequenced and analyzed as reported in Ch.2 (Fig.33). Here we intended to increase *Akkermansia muciniphila* in order to protect from certain metabolic and taste dysfunction, as outlined in Ch.1.

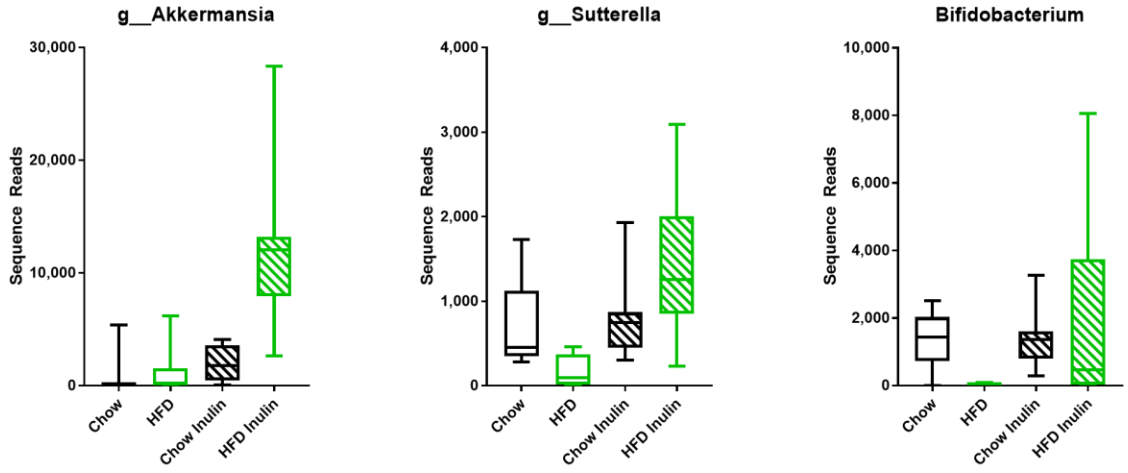

Figure 34. Differentially abundant species of bacteria elevated in mice fed HFD + 10% Inulin for 8 weeks.

HFD + Inulin did increase *Akkermansia*, along with *Sutterella* and *Bifidobacterium* as compared with other treatments (Fig 34).

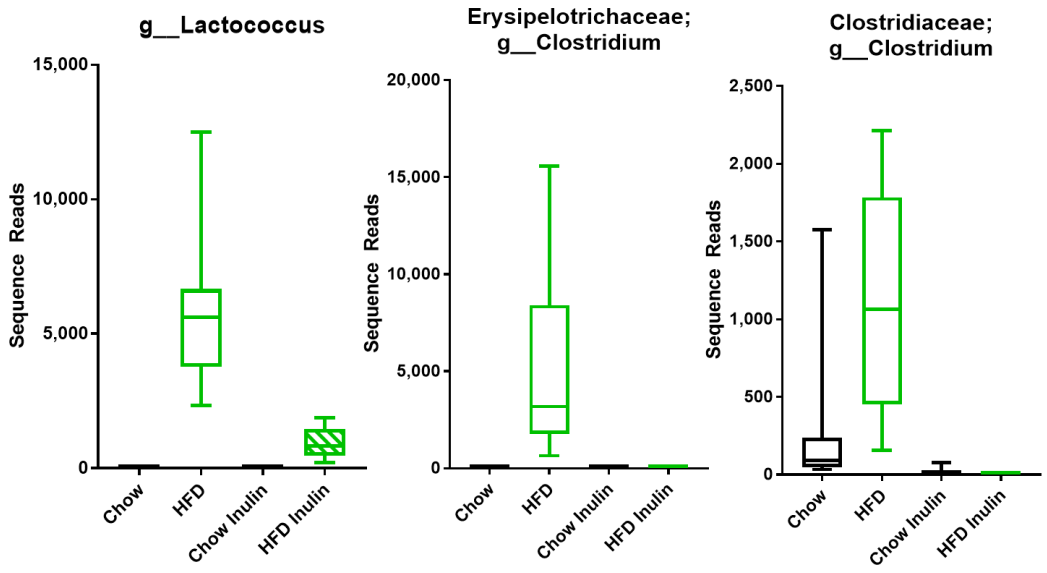

Figure 35. Differentially abundant species of bacteria elevated in mice fed HFD for 12 weeks versus mice fed Chow for 12 weeks, or HFD / Chow supplemented with 10% Inulin for 10 weeks/

When compared to mice on HFD alone for 12 weeks HFD + Inulin mice have significantly less of the phylum *Firmicutes*, specifically *Lactococcus* and '*Clostridium'* from *Erysipelotrichaceae*, and *Clostridiaceae* families (Fig. 35). It should be noted that Chow + Inulin was much less effective at inducing *Akkermansia* versus HFD + Inulin (Fig.33). It is likely that a high fiber environment, as in chow feeding, provides a stable niche for the *Bacteroidetes* phylum, which resists the expansion of *Akkermansia*, even in the presence of Inulin. However, in the context of HFD, they lose fitness versus *Firmicutes*, opening a niche for *Akkermansia* to expand in the presence of Inulin.

Akkermansia Induction via Berberine

Per the report of Roopchand et al. in 2015, we intended to use Berberine to induce the growth of *Akkermansia* in our mice[245]. At 0.5g Berberine /L H_2O, mice did not prefer it versus water (Fig.31).

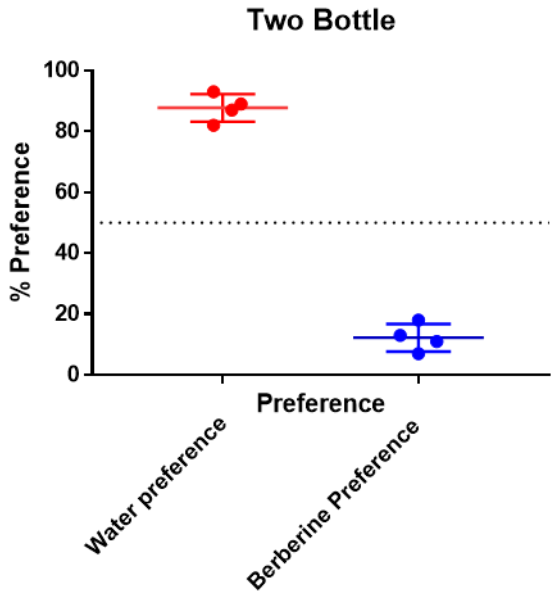

Figure 36. Two bottle preference test of H_2O vs 0.5g/L Berberine in H_2O.

We evaluated these mice for presence of *Akkermansia* by qPCR, never detecting the microbe despite 8 weeks of Berberine treatment.

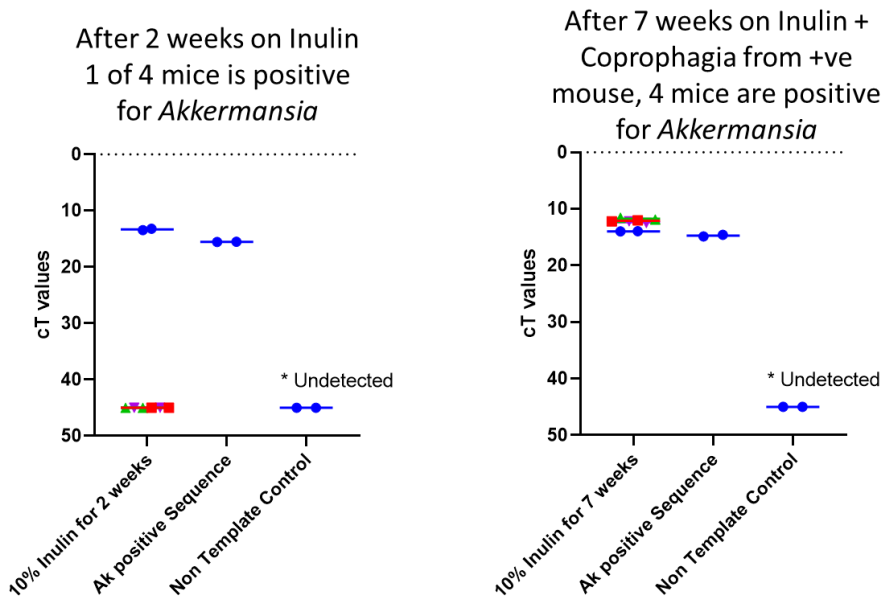

Figure 37. qPCR on DNA extracted from fecal samples of mice maintained on Inulin for two weeks (Left). Then maintained on Inulin and co-housed with feces from positive mouse over 7 weeks (right). Each color represents a different sample. Samples were run in duplicate, so each color has two points. Mice are in the far-left column, positive control sequence in center, and non-template control on right. Primers were adapted from Roopchand et al.
"AM1: 5'-CAGCACGTGAAGGTGGGGAC-3'; AM2: 5'-CCTTGCGG TTGGCTTCAGAT-3')", amplification protocol was determined experimentally and is described in the appendix.

Akkermansia Induction via Inulin and Coprophagia

The above 4 mice were then switched to eating 10% Inulin in their chow and after two weeks, one was positive for *Akkermansia*. We used fecal droppings from that cage to induce *Akkermansia* in the remaining mice, achieving *Akkermansia* induction in all four 5 weeks later (Fig. 36).

Gavage effects on taste behavior in mice from Ch. 3

Mice from the gavage experiments in Ch.2. were also assayed for taste behavior as in the groups from Ch.1. However, analysis revealed that the gavage itself seemed to have a negative impact on Lickometer testing, causing a majority of mice to fail a quality control (QC) check prior to stimulus testing. A mouse will pass QC if it licks 1M sucrose more than water. In the groups receiving gavages, 4 of 7 mice receiving gavage from chow mice failed, 8 of 14 from HFD mice failed, and 3 of 8 receiving a control PBS/Glycerol mix failed. Typically, 90% of mice will pass this test.

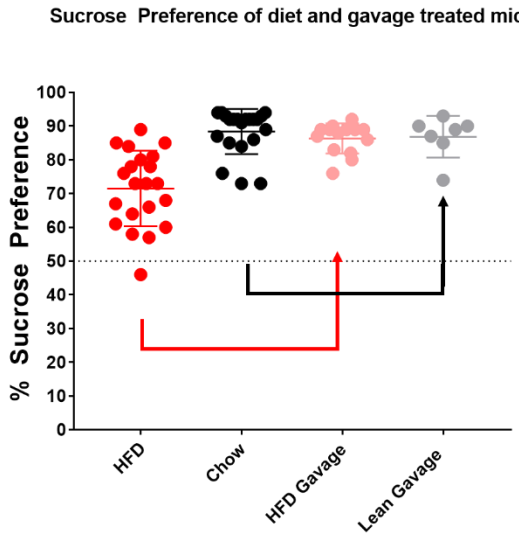

Figure 38. Two bottle sucrose preference test (0.025M sucrose) on mice receiving gavages from either HFD or Chow fed mice for 12 weeks.

We were able to complete a two-bottle sucrose preference test (Fig. 37) and saw no impact on sucrose preference from either donor.

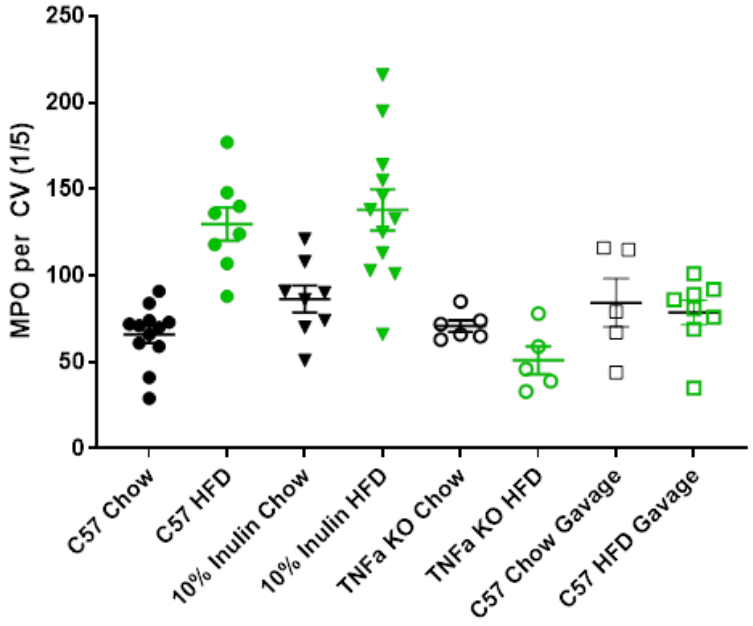

Figure 39. MPO positivity across all groups, with the gavage groups in the last two columns from right.

We suspected increased inflammation or injury due to the trauma of repeated gavage as a cause for an increase in mice failing to complete the lickometer tasks. To that end, we sectioned their tongues and stained for MPO, as described throughout this document, as an indicator of inflammation. We found no difference in MPO positivity between mice receiving gavage from HFD versus chow, nor between mice who received gavages versus mice maintained on various diets. Comparisons are complicated due to the younger age of these mice, but acute inflammation did not seem to be a factor, at least as measured by elevated MPO in the CV of taste buds (Fig. 38). However, the gavage needle is engineered as to not damage any lingual surfaces, and any acute inflammation would therefore be more likely to occur in the esophagus or further down the gastrointestinal tract.

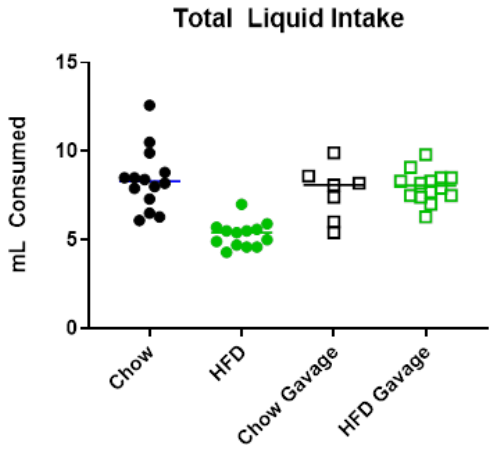

Figure 40. Total liquid intake in a two bottle test of mice on Chow, HFD (12w) and mice receiving cecal contents from Chow and HFD mice via gavage. Liquid intake is sucrose + H_2O.

We did not detect a difference in total liquid intake through two-bottle testing, which might have indicated a more general esophageal damage to the animals (Fig. 39). It should be noted that the two bottle tests occurred ~7-10 days after lickometer and behavioral testing and could therefore miss any acute damage sustained during gavage.

Conclusions and remarks

Inducing the growth of *Akkermansia* was not as straightforward as adding Inulin. As shown in figure 28, Inulin alone was not sufficient to up regulate *Akkermansia* in all subjects when mixed with the chow diet, yet it was sufficient when mixed with High Fat Diet. These mice did not receive fecal transplants or coprophagia, yet *Akkermansia* did develop. It is possible that the microbes were introduced through contaminated HFD, as this diet is not irradiated or autoclaved prior to use. However, in mice fed HFD without Inulin for 12 months, we generally see a reduction in *Akkermansia* abundance. HFD is likely working to clear away commensals, perhaps in the *Bacteroidetes* Phylum, that would otherwise compete with *Akkermansia*, allowing it to thrive in a high Inulin niche. Even with high inulin, this is not enough to make *Akkermansia* completely competitive in an the otherwise adequate fiber environment as shown in the Inulin Chow mice.

Miscellaneous reagent compositions and technical information

Reagents:

Normal Tyrode's
135 mM NaCl
5 mM KCl
2 mM CaCl2
1 mM MgCl
5 mM NaHCO3
10 mM HEPES
10 mM Glucose
10 mM Sodium Pyruvate
pH 7.4

Ca^{2+} Free Tyrode's
135 mM NaCl
5 mM KCl
20mM EGTA
10 mM HEPES
5 mM BAPTA (1,2-Bis (2-aminophenoxy) ethane-N, N, N, N-tetraacetic acid tetrapotassium salt)
10 mM Glucose
10 mM Sodium Pyruvate
pH 7.4

Enzyme cocktail
In Normal Tyrode's solution
Dispase II 2.5 mg/ml
Collagenase A 1mg/ml
Elastase 0.25 mg/ml
DNaseI 0.5mg/ml

Methods

Real time *Akkermansia* tracking via qPCR.

Fecal samples were processed into DNA as described above via PowerFecal DNA isolation kit (MO BIO laboratories Inc, Carlsbad CA). Primers for the qPCR reaction were as follows, F, 5'->3' (CAGCACGTGAAGGTGGGGAC), R (CCTTGCGGTTGGCTTCAGAT). qPCR was performed as described in the supplemental protocol section that follows this manuscript.

TNFα ELISA.

Serum collection

Mice were euthanized with CO_2 followed by cervical dislocation, following protocols approved by Cornell CARE (Center for Animal Resources and Education) and IACUC (Institutional Animal Care and Use Committee). Immediately after euthanizing, mice were dissected longitudinally, revealing the abdominal cavity and visualizing the abdominal aorta. A 30-gauge needle was inserted into the artery and ~300 µL of arterial blood was collected in non-heparanized Eppendorf tubes. The blot was left to clot for 1hr at room temperature before being centrifuged at 3000g at 4 C for 15 minutes to separate serum from blood. Serum collected from the supernatant was immediately frozen and stored at -80 C for later analysis.

Induction of *Akkermansia*: We first attempted to induce Akk by supplementing drinking water with 0.5 g/L Berberine chloride hydrate. According to the literature, berberine boosts *Akkermansia* growth after a 12 week treatment from ~0% to 8-13%[246]. Mice began Berberine treatment immediately after being single housed at 4 weeks of age, with high fat diet starting at 8 weeks and continuing for 12 weeks, as in Aim 1. We intended to test mice both consuming berberine throughout dietary treatment, and with berberine treatment suspended after Akk positivity is conferred, to deconvolute any downstream drug effects. However, Akk abundance was not increased in our animals (However, *Akkermansia* was successfully induced by supplementing with 10% Inulin and coprophagia from already positive mice)

Berberine. Berberine, an alkaloid found in foods such as turmeric, has long been employed as a traditional remedy in China. More recently, berberine has been investigated as a treatment for several metabolic diseases, in lowering cholesterol levels, alleviating inflammation, and ameliorating symptoms of insulin resistance[247–249]. A more recent study on the mechanism of Berberine activity suggests that it exerts these effects by increasing the abundance of *Akkermansia* in the gut of the host[246]. This increase

leads to the multiple downstream effects listed above, with authors positing the effects are due to increases in butyrate production in the gut.

We treated mice with Berberine to encourage the expansion of *Akkermansia* in mice fed either a control or a HFD, which we predicted would attenuate its downstream effects and protect mice from taste damage. This was not successful.

Primers and PCR:

qPCR amplification of *Akkermansia*
1. 95°C 10 minutes
2. 95°C 15 seconds
3. 66°C 60 seconds (Fluorescence reading ongoing at this step)
4. Repeat steps 2-3 40 times

***Akkermansia* primers from Roopchand et al.**[245]
AM1: 5'-CAGCACGTGAAGGTGGGGAC-3'
AM2: 5'-CCTTGCGG TTGGCTTCAGAT-3'

qPCR amplification of 16S rRNA to determine total microbial amount

1. 95°C 10 minutes
2. 95°C 15 seconds
3. 50°C 40 seconds
4. 72°C 30 seconds

5. Repeat steps 2-4 40 times

qPCR amplification of cDNA for taste and gut gene expression
1. 95°C 10 minutes
2. 95°C 15 seconds
3. 60°C 60 seconds
4. Repeat steps 2-3 40 times

References

1. Mccrickerd, K. & Forde, C. G. Sensory influences on food intake control: Moving beyond palatability. *Obes. Rev.* **17**, 18–29 (2016).
2. Martinez, J. A. Body-weight regulation : causes of obesity. 337–345 (2021).
3. Jeffery, R. W. & Harnack, L. J. Evidence implicating eating as a primary driver for the obesity epidemic. *Diabetes* **56**, 2673–2676 (2007).
4. Wright, S. M. & Aronne, L. J. Causes of obesity. *Abdom. Imaging* **37**, 730–732 (2012).
5. Caballero, B. The global epidemic of obesity: An overview. *Epidemiol. Rev.* **29**, 1–5 (2007).
6. Drewnowski, A. Taste Preferences and Food Intake. *Annu. Rev. Nutr.* **17**, 237–253 (1997).
7. Nasser, J. Taste, food intake and obesity. *Obes. Rev.* **2**, 213–218 (2001).
8. Han, P., Bagenna, B. & Fu, M. The sweet taste signalling pathways in the oral cavity and the gastrointestinal tract affect human appetite and food intake: a review. *Int. J. Food Sci. Nutr.* **70**, 125–135 (2019).
9. Cohn, Z. J., Kim, A., Huang, L., Brand, J. & Wang, H. Lipopolysaccharide-induced inflammation attenuates taste progenitor cell proliferation and shortens the life span of taste bud cells. *BMC Neurosci.* **11**, 9–14 (2010).
10. Yang, H., Cong, W. na, Yoon, J. S. & Egan, J. M. Vismodegib, an antagonist of hedgehog signaling, directly alters taste molecular signaling in taste buds. *Cancer Med.* **4**, 245–252 (2015).
11. Mukherjee, N., Pal Choudhuri, S., Delay, R. J. & Delay, E. R. Cellular mechanisms of cyclophosphamide-induced taste loss in mice. *PLoS One* **12**, 1–23 (2017).
12. Kumarhia, D., He, L. & McCluskey, L. P. Inflammatory stimuli acutely modulate peripheral taste function. *J. Neurophysiol.* **115**, 2964–2975 (2016).
13. Kaufman, A., Choo, E., Koh, A. & Dando, R. Inflammation arising from obesity reduces taste bud abundance and inhibits renewal. *PLoS Biol.* **16**, 1–14 (2018).
14. Kaufman, A., Kim, J., Noel, C. & Dando, R. Taste loss with obesity in mice and men. *Int. J. Obes.* **44**, 739–743 (2020).
15. Wellen, K. E. & Hotamisligil, G. S. Inflammation, stress, and diabetes. *J. Clin. Invest.* **115**, (2005).
16. Gregor, M. F. & Hotamisligil, G. S. Inflammatory Mechanisms in Obesity. *Annu. Rev. Immunol.* **29**, 415–445 (2011).
17. Zeevi, D. *et al.* Personalized Nutrition by Prediction of Glycemic Responses. *Cell* **163**, 1079–1095 (2015).
18. Hussain, S. S. & Bloom, S. R. The regulation of food intake by the gut-brain axis: implications for obesity. *Int. J. Obes.* **37**, 625–633 (2012).
19. Shigemura, N. *et al.* Leptin Modulates Behavioral Responses to Sweet Substances by Influencing Peripheral Taste Structures. *Endocrinology* **145**, 839–847 (2004).
20. Goodrich, J. K. *et al.* Genetic Determinants of the Gut Microbiome in UK Twins Correspondence. *Cell Host Microbe* **19**, 731–743 (2016).
21. Donaldson, L. F., Bennett, L., Baic, S. & Melichar, J. K. Taste and weight: Is there a link? *Am. J. Clin. Nutr.* **90**, 800–803 (2009).
22. Drewnowski, A., Brunzell, J. D., Sande, K., Iverius, P. H. & Greenwood, M. R. C. Sweet tooth reconsidered: Taste responsiveness in human obesity. *Physiol. Behav.* **35**, 617–622 (1985).
23. Bernard, A., Ancel, D. & Neyrinck, A. M. A Preventive Prebiotic Supplementation Improves the Sweet Taste Perception in Diet-Induced Obese Mice. (2019). doi:10.3390/nu11030549
24. Carabotti, M., Scirocco, A., Maselli, M. A. & Severi, C. The gut-brain axis: Interactions between enteric microbiota, central and enteric nervous systems. *Ann. Gastroenterol.* **28**, 203–209 (2015).
25. Clemente, J. C., Ursell, L. K., Parfrey, L. W. & Knight, R. The impact of the gut microbiota on human health: An integrative view. *Cell* **148**, 1258–1270 (2012).
26. Bezen??on, C., le Coutre, J. & Damak, S. Taste-signaling proteins are coexpressed in solitary intestinal epithelial cells. *Chem. Senses* **32**, 41–49 (2007).

27. Cani, P. D. *et al.* Metabolic Endotoxemia Initiates Obesity and Insulin Resistance. doi:10.2337/db06-1491
28. Zhu, X., He, L. & Mccluskey, A. L. P. Ingestion of bacterial lipopolysaccharide inhibits peripheral taste responses to sucrose in mice. *Neuroscience* **258**, 47–61 (2014).
29. Cohn, Z. J., Kim, A., Huang, L., Brand, J. & Wang, H. Lipopolysaccharide-induced inflammation attenuates taste progenitor cell proliferation and shortens the life span of taste bud cells. *BMC Neurosci.* **11**, 72 (2010).
30. Goodman, J. R. & Dando, R. To Detect and Reject, Parallel Roles for Taste and Immunity. *Curr. Nutr. Rep.* 137–145 (2021). doi:10.1007/s13668-021-00355-3
31. Boscolo-Rizzo, P. *et al.* Evolution of Altered Sense of Smell or Taste in Patients with Mildly Symptomatic COVID-19. *JAMA Otolaryngol. - Head Neck Surg.* **146**, 1–5 (2020).
32. Cazzolla, A. P. *et al.* Taste and smell disorders in COVID-19 patients: Role of interleukin-6. *ACS Chem. Neurosci.* **11**, 2774–2781 (2020).
33. Parma, V. *et al.* More Than Smell-COVID-19 Is Associated With Severe Impairment of Smell, Taste, and Chemesthesis. *Chem. Senses* **45**, 609–622 (2020).
34. Sukumaran, S. K. *et al.* Whole transcriptome profiling of taste bud cells. *Sci. Rep.* **7**, 1–15 (2017).
35. Archer, N. *et al.* Obesity is associated with altered gene expression in human tastebuds. *Int. J. Obes.* **43**, 1475–1484 (2019).
36. Medzhitov, R. Origin and physiological roles of inflammation. *Nature* **454**, 428–435 (2008).
37. S, Gautamethi, Bokyung Sung, B. B. A. [Frontiers in Bioscience 5094-5107, May 1, 2008] TNF: A master switch for inflammation to cancer Gautam Sethi, Bokyung Sung, Bharat B. Aggarwal. *Front. Biosci.* 5094–5107 (2008).
38. Tanaka, T., Narazaki, M. & Kishimoto, T. IL-6 in Inflammation, Immunity, and Disease. **6**, (2014).
39. Marnell, L., Mold, C. & Du Clos, T. W. C-reactive protein: Ligands, receptors and role in inflammation. *Clin. Immunol.* **117**, 104–111 (2005).
40. Christ, A. & Latz, E. The Western lifestyle has lasting effects on metaflammation. *Nat. Rev. Immunol.* **19**, 267–268 (2019).
41. Wang, J. *et al.* Resistance. (2001).
42. Hotamisligil, G. S. & Erbay, E. Nutrient sensing and inflammation in metabolic diseases. *Nat. Rev. Immunol.* **8**, 923–934 (2008).
43. Myers, M. G., Cowley, M. A. & Münzberg, H. Mechanisms of leptin action and leptin resistance. *Annu. Rev. Physiol.* **70**, 537–556 (2008).
44. Aguliar-Valles, A., Kim, J., Jung, S., Woodside, B. & Luheshi, G. N. Role of brain transmigrating neutrophils in depression-like behavior during systemic infection. *Mol. Psychiatry* **19**, 599–606 (2014).
45. Wang, H., Zhou, M., Brand, J. & Huang, L. Inflammation activates the interferon signaling pathways in taste bud cells. *J. Neurosci.* **27**, 10703–13 (2007).
46. Feng, P., Zhao, H., Chai, J., Huang, L. & Wang, H. Expression and secretion of TNF-?? in mouse taste buds: A novel function of a specific subset of type II taste cells. *PLoS One* **7**, (2012).
47. Wang, H., Zhou, M., Brand, J. & Huang, L. Inflammation and taste disorders: Mechanisms in taste buds. *Ann. N. Y. Acad. Sci.* **1170**, 596–603 (2009).
48. Feng, P. *et al.* Regulation of bitter taste responses by tumor necrosis factor. *Brain. Behav. Immun.* **49**, 32–42 (2015).
49. Pittman, D. W. *et al.* Behavioral and neurophysiological taste responses to sweet and salt are diminished in a model of subclinical intestinal inflammation. *Sci. Rep.* **10**, 1–13 (2020).
50. Djeziri, F. Z. *et al.* Oleanolic acid improves diet-induced obesity by modulating fat preference and inflammation in mice. *Biochimie* **152**, 110–120 (2018).
51. Zheng, K. *et al.* Bitter taste receptors as targets for tocolytics in preterm labor therapy. *FASEB J.* **31**, 4037–4052 (2017).
52. Camandola, S. & Mattson, M. P. Toll-like receptor 4 mediates fat, sugar, and umami taste

preference and food intake and body weight regulation. *Obesity* **00**, 1–9 (2017).
53. Feng, S., Achoute, L., Margolskee, R. F., Jiang, P. & Wang, H. Lipopolysaccharide-Induced Inflammatory Cytokine Expression in Taste Organoids. *Chem. Senses* **45**, 187–194 (2020).
54. Gomez, F. E., Cassí-Nosthas, L., Morales-de-León, J. C. & Bourges, H. Detection and recognition thresholds to the 4 basic tastes in Mexican patients with primary Sögren's syndrome. *Eur. J. Clin. Nutr.* **58**, 629–636 (2004).
55. Steinbach, S. *et al.* Smell and Taste in Inflammatory Bowel Disease. *PLoS One* **8**, (2013).
56. Ballok, D. A., Szechtman, H. & Sakic, B. Taste responsiveness and diet preference in autoimmune MRL mice. *Behav. Brain Res.* **140**, 119–130 (2003).
57. Jonsson, R., Bolstad, A. I., Brokstad, K. A. & Brun, J. G. Sjögren's syndrome - A plethora of clinical and immunological phenotypes with a complex genetic background. *Ann. N. Y. Acad. Sci.* **1108**, 433–447 (2007).
58. Kim, A. *et al.* Defects in the peripheral taste structure and function in the MRL/lpr mouse model of autoimmune disease. *PLoS One* **7**, (2012).
59. Kataoka, S. *et al.* The candidate sour taste receptor, PKD2L1, Is expressed by type III taste cells in the mouse. *Chem. Senses* **33**, 243–254 (2008).
60. Carey, R. M. & Lee, R. J. Taste receptors in upper airway innate immunity. *Nutrients* **11**, 1–17 (2019).
61. Lee, R. J. & Cohen, N. A. Taste receptors in innate immunity. *Cell. Mol. Life Sci.* **72**, 217–236 (2015).
62. Latorre, R. *et al.* Expression of the Bitter taste receptor, T2R38, in enteroendocrine cells of the colonic mucosa of overweight/obese vs. Lean subjects. *PLoS One* **11**, 1–16 (2016).
63. Kok, B. P. *et al.* Intestinal bitter taste receptor activation alters hormone secretion and imparts metabolic benefits. *Mol. Metab.* **16**, 76–87 (2018).
64. Howitt, M. R. *et al.* Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut. *Science (80-.).* **351**, 1329–1333 (2016).
65. Liu, S. *et al.* Members of bitter taste receptor cluster Tas2r143/Tas2r135/Tas2r126 are expressed in the epithelium of murine airways and other non-gustatory tissues. *Front. Physiol.* **8**, 1–17 (2017).
66. Jaggupilli, A. *et al.* Chemosensory bitter taste receptors (T2Rs) are activated by multiple antibiotics. *FASEB J.* **33**, 501–517 (2019).
67. Deshpande, D. A. *et al.* Bitter taste receptors on airway smooth muscle bronchodilate by localized calcium signaling and reverse obstruction. *Nat. Med.* **16**, 1299–1304 (2010).
68. Lee, R. J. *et al.* Bacterial D-amino acids suppress sinonasal innate immunity through sweet taste receptors in solitary chemosensory cells. *Sci. Signal.* **10**, 1–12 (2017).
69. Bassoli, A., Borgonovo, G., Caremoli, F. & Mancuso, G. The taste of D- and L-amino acids: In vitro binding assays with cloned human bitter (TAS2Rs) and sweet (TAS1R2/TAS1R3) receptors. *Food Chem.* **150**, 27–33 (2014).
70. Spotten, L. E. *et al.* Subjective and objective taste and smell changes in cancer. *Ann. Oncol. Off. J. Eur. Soc. Med. Oncol.* **28**, 969–984 (2017).
71. Murtaza, B., Hichami, A., Khan, A. S., Ghiringhelli, F. & Khan, N. A. Alteration in taste perception in cancer: Causes and strategies of treatment. *Front. Physiol.* **8**, 1–10 (2017).
72. Delay, E. R. *et al.* Cyclophosphamide and the taste system: Effects of dose fractionation and amifostine on taste cell renewal. *PLoS One* **14**, 1–23 (2019).
73. Schalk, P. *et al.* Influence of cancer and acute inflammatory disease on taste perception: a clinical pilot study. *Support. Care Cancer* **26**, 843–851 (2018).
74. Lumeng, C. N. & Saltiel, A. R. Review series Adipose tissue remodeling and obesity. *J. Clin. Invest.* **121**, 2111–2117 (2011).
75. Karastergiou, K. & Mohamed-Ali, V. The autocrine and paracrine roles of adipokines. *Mol. Cell. Endocrinol.* **318**, 69–78 (2010).
76. Strissel, K. J. *et al.* Adipocyte death, adipose tissue remodeling, and obesity complications.

Diabetes **56**, 2910–2918 (2007).
77. Legein, B., Temmerman, L., Biessen, E. A. L. & Lutgens, E. Inflammation and immune system interactions in atherosclerosis. *Cell. Mol. Life Sci.* **70**, 3847–3869 (2013).
78. Hotamisligil, G. S., Atkinson, R. L. & Spiegelman, B. M. alpha in human obesity and insulin resistance . Obesity and Insulin Resistance. **95**, 2409–2415 (1995).
79. Ellulu, M. S. *et al.* Obesity and Inflammation : The Linking Mechanism and the Complications. *Arch. Med. Sci.* 851–863 (2016).
80. Den Hertog, H. M. *et al.* C-reactive protein in the very early phase of acute ischemic stroke: Association with poor outcome and death. *J. Neurol.* **256**, 2003–2008 (2009).
81. Danesh, J. *et al.* Long-term interleukin-6 levels and subsequent risk of coronary heart disease: Two new prospective studies and a systematic review. *PLoS Med.* **5**, 0600–0610 (2008).
82. Kirchgessner, T. G., Uysal, K. T., Wiesbrock, S. M., Marino, M. W. & Hotamisligil, G. S. Tumor necrosis factor-α contributes to obesity-related hyperleptinemia by regulating leptin release from adipocytes. *J. Clin. Invest.* **100**, 2777–2782 (1997).
83. Uysal, K. T., Wiesbrock, S. M. & Hotamisligil, G. S. Functional analysis of tumor necrosis factor (TNF) receptors in TNF-α- mediated insulin resistance in genetic obesity. *Endocrinology* **139**, 4832–4838 (1998).
84. Zhou, L. *et al.* Sweet taste receptors mediated ROS-NLRP3 inflammasome signaling activation: Implications for diabetic nephropathy. *J. Diabetes Res.* **2018**, (2018).
85. Pepino, M. Y., Finkbeiner, S., Beauchamp, G. K. & Mennella, J. A. Obese Women Have Lower Monosodium Glutamate Taste Sensitivity and Prefer Higher Concentrations Than Do Normal-weight Women. *Obesity* **18**, 959–965 (2009).
86. Fernandez-Garcia, J. C. *et al.* An increase in visceral fat is associated with a decrease in the taste and olfactory capacity. *PLoS One* **12**, 1–14 (2017).
87. Hardikar, S., Höchenberger, R., Villringer, A. & Ohla, K. Higher sensitivity to sweet and salty taste in obese compared to lean individuals. *Appetite* **111**, 158–165 (2017).
88. Frijters, J. E. & Rasmussen-Conrad, E. L. Sensory discrimination, intensity perception, and affective judgment of sucrose-sweetness in the overweight. *The Journal of general psychology* **107**, 233–247 (1982).
89. Chao, D. H. M. *et al.* Impact of obesity on taste receptor expression in extra-oral tissues: Emphasis on hypothalamus and brainstem. *Sci. Rep.* **6**, 1–14 (2016).
90. Noel, C. A., Sugrue, M. & Dando, R. Participants with pharmacologically impaired taste function seek out more intense, higher calorie stimuli. *Appetite* **117**, 74–81 (2017).
91. Hwang, L. D. *et al.* New insight into human sweet taste: A genome-wide association study of the perception and intake of sweet substances. *Am. J. Clin. Nutr.* **109**, 1724–1737 (2019).
92. Nielsen, M. S. *et al.* Bariatric Surgery Leads to Short-Term Effects on Sweet Taste Sensitivity and Hedonic Evaluation of Fatty Food Stimuli. *Obesity* **27**, 1796–1804 (2019).
93. Berthoud, H. R. & Zheng, H. Modulation of taste responsiveness and food preference by obesity and weight loss. *Physiol. Behav.* **107**, 527–532 (2012).
94. BURGE, J. C., SCHAUMBURG, J. Z., CHOBAN, P. S., DiSILVESTRO, R. A. & FLANCBAUM, L. Changes in Patients' Taste Acuity after Roux-en-Y Gastric Bypass for Clinically Severe Obesity. *Journal of the American Dietetic Association* **95**, 666–670 (1995).
95. Green, E., Jacobson, A., Haase, L. & Murphy, C. Reduced nucleus accumbens and caudate nucleus activation to a pleasant taste is associated with obesity in older adults. *Brain Res.* **1386**, 109–117 (2011).
96. Babbs, R. K. *et al.* Decreased caudate response to milkshake is associated with higher body mass index and greater impulsivity. *Physiol. Behav.* **121**, 103–111 (2013).
97. Bohon, C. Brain response to taste in overweight children: A pilot feasibility study. *PLoS One* **12**, 1–9 (2017).
98. Jones, R. Taste bud regeneration and the search for taste progenitor cells. *Bone* **23**, 1–7 (2014).
99. Perea-Martinez, I., Nagai, T. & Chaudhari, N. Functional Cell Types in Taste Buds Have Distinct

Longevities. *PLoS One* **8**, 1–9 (2013).
100. Castillo-Azofeifa, D. *et al.* Sonic hedgehog from both nerves and epithelium is a key trophic factor for taste bud maintenance. *Dev.* **144**, 3054–3065 (2017).
101. Ahart, Z. C. *et al.* Differential Effects of Diet and Weight on Taste Responses in Diet-Induced Obese Mice. *Obesity* **28**, 284–292 (2020).
102. Bernard, A. *et al.* A chronic LPS-induced low-grade inflammation fails to reproduce in lean mice the impairment of preference for oily solution found in diet-induced obese mice. *Biochimie* **159**, 112–121 (2019).
103. Bernard, A. *et al.* A preventive prebiotic supplementation improves the sweet taste perception in diet-induced obese mice. *Nutrients* **11**, (2019).
104. Feng, P. *et al.* Aggravated gut inflammation in mice lacking the taste signaling protein α-gustducin. *Brain. Behav. Immun.* **71**, 23–27 (2018).
105. Sharma, P. *et al.* Bitter Taste Receptor Agonists Mitigate Features of Allergic Asthma in Mice. *Sci. Rep.* **7**, 1–14 (2017).
106. Adappa, N. D. *et al.* Correlation of T2R38 taste phenotype and in vitro biofilm formation from nonpolypoid chronic rhinosinusitis patients. *Int. Forum Allergy Rhinol.* **6**, 783–791 (2016).
107. Schiffman, S. S. Taste and smell losses in normal aging and disease. *J. Am. Med. Assoc.* **278**, 1357–1362 (1997).
108. Henkin, R. I., Levy, L. M. & Fordyce, A. Taste and smell function in chronic disease:: A review of clinical and biochemical evaluations of taste and smell dysfunction in over 5000 patients at the Taste and Smell Clinic in Washington, DC. *Am. J. Otolaryngol. - Head Neck Med. Surg.* **34**, 477–489 (2013).
109. Mojet, J., Christ-Hazelhof, E. & Heidema, J. Taste perception with age: Generic or specific losses in threshold sensitivity to the five basic tastes? *Chem. Senses* **26**, 845–860 (2001).
110. Kaneda, H. *et al.* Decline in taste and odor discrimination abilities with age, and relationship between gustation and olfaction. *Chem. Senses* **25**, 331–337 (2000).
111. Puputti, S., Aisala, H., Hoppu, U. & Sandell, M. Factors explaining individual differences in taste sensitivity and taste modality recognition among Finnish adults. *J. Sens. Stud.* **34**, (2019).
112. Kure Liu, C. *et al.* Brain Imaging of Taste Perception in Obesity: a Review. *Curr. Nutr. Rep.* **8**, 108–119 (2019).
113. Ahmed, K., Penney, N., Darzi, A. & Purkayastha, S. Taste Changes after Bariatric Surgery: a Systematic Review. *Obes. Surg.* **28**, 3321–3332 (2018).
114. McCluskey, L. P., He, L., Dong, G. & Harris, R. Chronic exposure to liquid sucrose and dry sucrose diet have differential effects on peripheral taste responses in female rats. *Appetite* **145**, 104499 (2020).
115. Sartor, F. *et al.* Taste perception and implicit attitude toward sweet related to body mass index and soft drink supplementation. *Appetite* **57**, 237–246 (2011).
116. Steen, P. W., Shi, L., He, L. & McCluskey, L. P. Neutrophil responses to injury or inflammation impair peripheral gustatory function. *Neuroscience* **167**, 894–908 (2010).
117. Kumarhia, D., He, L. & McCluskey, L. P. Inflammatory stimuli acutely modulate peripheral taste function. *J. Neurophysiol.* **115**, 2964–2975 (2016).
118. Brown, K., DeCoffe, D., Molcan, E. & Gibson, D. L. Diet-induced dysbiosis of the intestinal microbiota and the effects on immunity and disease. *Nutrients* **4**, 1095–1119 (2012).
119. Bernard, A., Delzenne, N. & Besnard, P. A Preventive Prebiotic Supplementation Improves the. (2019). doi:10.3390/nu11030549
120. Simpson, H. L. & Campbell, B. J. Review article: Dietary fibre-microbiota interactions. *Aliment. Pharmacol. Ther.* (2015). doi:10.1111/apt.13248
121. Belzer, C. & De Vos, W. M. Microbes insidefrom diversity to function: The case of Akkermansia. *ISME J.* **6**, 1449–1458 (2012).
122. Nijhuis, J. *et al.* Neutrophil activation in morbid obesity, chronic activation of acute inflammation. *Obesity* **17**, 2014–2018 (2009).

123. Talukdar, S. *et al.* Neutrophils mediate insulin resistance in mice fed a high-fat diet through secreted elastase. *Nat. Med.* **18**, 1407–1412 (2012).
124. Suez, J. *et al.* Artificial sweeteners induce glucose intolerance by altering the gut microbiota. *Nature* **514**, 181–186 (2014).
125. Gil-Cardoso, K. *et al.* A cafeteria diet triggers intestinal inflammation and oxidative stress in obese rats. *Br. J. Nutr.* **117**, 218–229 (2017).
126. Glendinning, J. I., Gresack, J. & Spector, A. C. A high-throughput screening procedure for identifying mice with aberrant taste and oromotor function. *Chem. Senses* **27**, 461–474 (2002).
127. Moller, D. E. Potential role of TNF-α in the pathogenesis of insulin resistance and type 2 diabetes. *Trends Endocrinol. Metab.* **11**, 212–217 (2000).
128. Pepino, M. Y. & Mennella, J. A. Habituation to the pleasure elicited by sweetness in lean and obese women. *Appetite* **58**, 800–805 (2012).
129. Naneix, F., Darlot, F., Coutureau, E. & Cador, M. Long-lasting deficits in hedonic and nucleus accumbens reactivity to sweet rewards by sugar overconsumption during adolescence. *Eur. J. Neurosci.* **43**, 671–680 (2016).
130. Weiss, M. S., Hajnal, A., Czaja, K. & Di Lorenzo, P. M. Taste Responses in the Nucleus of the Solitary Tract of Awake Obese Rats Are Blunted Compared With Those in Lean Rats. *Front. Integr. Neurosci.* **13**, 1–16 (2019).
131. Maliphol, A. B., Garth, D. J. & Medler, K. F. Diet-induced obesity reduces the responsiveness of the peripheral taste receptor cells. *PLoS One* **8**, (2013).
132. Hardikar, S., Wallroth, R., Villringer, A. & Ohla, K. Shorter-lived neural taste representations in obese compared to lean individuals. *Sci. Rep.* **8**, 1–10 (2018).
133. Dielen, F. M. H. Van *et al.* Increased leptin concentrations correlate with increased concentrations of inflammatory markers in morbidly obese individuals. 1759–1766 (2001).
134. Umabiki, M. *et al.* The improvement of sweet taste sensitivity with decrease in serum leptin levels during weight loss in obese females. *Tohoku J. Exp. Med.* **220**, 267–271 (2010).
135. Castellani, L. W., Chang, J. J., Wang, X., Lusis, A. J. & Reynolds, W. F. Transgenic mice express human MPO -463G/A alleles at atherosclerotic lesions, developing hyperlipidemia and obesity in -463G males. *J. Lipid Res.* **47**, 1366–1377 (2006).
136. Rossi, M. *et al.* Fermentation of fructooligosaccharides and inulin by bifidobacteria: A comparative study of pure and fecal cultures. *Appl. Environ. Microbiol.* **71**, 6150–6158 (2005).
137. Corrêa-Oliveira, R., Fachi, J. L., Vieira, A., Sato, F. T. & Vinolo, M. A. R. Regulation of immune cell function by short-chain fatty acids. *Clin. Transl. Immunol.* **5**, 1–8 (2016).
138. Fernandes, R., do Rosario, V. A., Mocellin, M. C., Kuntz, M. G. F. & Trindade, E. B. S. M. Effects of inulin-type fructans, galacto-oligosaccharides and related synbiotics on inflammatory markers in adult patients with overweight or obesity: A systematic review. *Clin. Nutr.* **36**, 1197–1206 (2017).
139. Weitkunat, K. *et al.* Short-chain fatty acids and inulin, but not guar gum, prevent diet-induced obesity and insulin resistance through differential mechanisms in mice. *Sci. Rep.* **7**, 1–13 (2017).
140. Cani, P. D., Bibiloni, R., Knauf, C., Neyrinck, A. M. & Delzenne, N. M. Changes in gut microbiota control metabolic diet–induced obesity and diabetes in mice. *Diabetes* **57**, 1470–81 (2008).
141. Jayashree, B. *et al.* Increased circulatory levels of lipopolysaccharide (LPS) and zonulin signify novel biomarkers of proinflammation in patients with type 2 diabetes. *Mol. Cell. Biochem.* **388**, 203–210 (2014).
142. Pittman, D. W. *et al.* Behavioral and neurophysiological taste responses to sweet and salt are diminished in a model of subclinical intestinal inflammation. *Sci. Rep.* **10**, 1–13 (2020).
143. Li, J., Lin, S., Vanhoutte, P. M., Woo, C. W. & Xu, A. Akkermansia muciniphila protects against atherosclerosis by preventing metabolic endotoxemia-induced inflammation in Apoe-/- Mice. *Circulation* **133**, 2434–2446 (2016).
144. Chaudhari, N. & Roper, S. D. The cell biology of taste. *J. Cell Biol.* **190**, 285–296 (2010).

145. Bartel, D. L., Sullivan, S. L., Lavoie, É. G., Sévigny, J. & Finger, T. E. Nucleoside triphosphate diphosphohydrolase-2 is the ecto-ATPase of type I cells in taste buds. *J. Comp. Neurol.* **497**, 1–12 (2006).
146. Zhao, G. Q. *et al.* The receptors for mammalian sweet and umami taste. *Cell* **115**, 255–266 (2003).
147. Chandrashekar, J. *et al.* T2Rs function as bitter taste receptors. *Cell* **100**, 703–711 (2000).
148. Clapp, T. R., Yang, R., Stoick, C. L., Kinnamon, S. C. & Kinnamon, J. C. Morphologic Characterization of Rat Taste Receptor Cells That Express Components of the Phospholipase C Signaling Pathway. *J. Comp. Neurol.* **468**, 311–321 (2004).
149. DeFazio, R. A. *et al.* Separate populations of receptor cells and presynaptic cells in mouse taste buds. *J. Neurosci.* **26**, 3971–3980 (2006).
150. Kapsimali, M. & Barlow, L. A. Developing a sense of taste. *Semin. Cell Dev. Biol.* **24**, 200–209 (2013).
151. Barlow, L. A. Progress and renewal in gustation: new insights into taste bud development. *Development* **142**, 3620–3629 (2015).
152. Leoncini, E. *et al.* Adult height and head and neck cancer: A pooled analysis within the INHANCE Consortium. *Head Neck* **36**, 1391 (2014).
153. Nguyen, H. M., Reyland, M. E. & Barlow, L. A. Mechanisms of taste bud cell loss after head and neck irradiation. *J. Neurosci.* **32**, 3474–3484 (2012).
154. Sekulic, A. *et al.* Efficacy and Safety of Vismodegib in Advanced Basal-Cell Carcinoma. *N. Engl. J. Med.* **366**, 2171–2179 (2012).
155. Kumari, A. *et al.* Hedgehog pathway blockade with the cancer drug LDE225 disrupts taste organs and taste sensation. 1034–1040 (2021). doi:10.1152/jn.00822.2014
156. Grivennikov, S. I., Greten, F. R. & Karin, M. Immunity, Inflammation, and Cancer. *Cell* **140**, 883–899 (2010).
157. Argilés, J. M., Busquets, S., Stemmler, B. & López-Soriano, F. J. Cancer cachexia: Understanding the molecular basis. *Nat. Rev. Cancer* **14**, 754–762 (2014).
158. Watt, H., Scheede-bergdahl, C., Watt, H. L., Trutschnigg, B. & Kilgour, R. D. Is IL-6 the best pro-inflammatory biomarker of clinical outcomes of cancer cachexia ? Is IL-6 the best pro-in fl ammatory biomarker of clinical outcomes of cancer cachexia ? q. (2011). doi:10.1016/j.clnu.2011.07.010
159. Krimm, R. F., Thirumangalathu, S. & Barlow, L. A. Development of the Taste System. *Handb. Olfaction Gustation Third Ed.* 727–748 (2015). doi:10.1002/9781118971758.ch33
160. Huang, Y. J. & Lu, K. S. TUNEL staining and electron microscopy studies of apoptotic changes in the guinea pig vallate taste cells after unilateral glossopharyngeal denervation. *Anat. Embryol. (Berl).* **204**, 493–501 (2001).
161. Wright, H. L., Moots, R. J., Bucknall, R. C. & Edwards, S. W. Neutrophil function in inflammation and inflammatory diseases. *Rheumatology* **49**, 1618–1631 (2010).
162. Amulic, B., Cazalet, C., Hayes, G. L., Metzler, K. D. & Zychlinsky, A. Neutrophil function: From mechanisms to disease. *Annu. Rev. Immunol.* **30**, 459–489 (2012).
163. Pham, C. T. N. Neutrophil serine proteases: Specific regulators of inflammation. *Nat. Rev. Immunol.* **6**, 541–550 (2006).
164. Walsh, D. E. *et al.* Interleukin-8 Up-regulation by Neutrophil Elastase is Mediated by MyD88/IRAK/TRAF-6 in Human Bronchial Epithelium. *J. Biol. Chem.* **276**, 35494–35499 (2001).
165. Devaney, J. M. *et al.* Neutrophil elastase up-regulates interleukin-8 via toll-like receptor 4. *FEBS Lett.* **544**, 129–132 (2003).
166. Manna, S. K., Mukhopadhyay, A., Bharat, B. & Alerts, E. This information is current as of March 25, 2013. (2013).
167. Donnahoo, K. K. *et al.* rapid communication. (2021).
168. Ramos, C. D. L. MIP-1 [CCL3] acting on the CCR1 receptor mediates neutrophil migration in immune inflammation via sequential release of TNF- and LTB4. *J. Leukoc. Biol.* **78**, 167–177

(2005).
169. Vinolo, M. A. R. *et al.* Suppressive effect of short-chain fatty acids on production of proinflammatory mediators by neutrophils. *J. Nutr. Biochem.* **22**, 849–855 (2011).
170. Tedelind, S., Westberg, F., Kjerrulf, M. & Vidal, A. Anti-inflammatory properties of the short-chain fatty acids acetate and propionate: A study with relevance to inflammatory bowel disease. *World J. Gastroenterol.* **13**, 2826–2832 (2007).
171. Ghoshal, S., Witta, J., Zhong, J., de Villiers, W. & Eckhardt, E. Chylomicrons promote intestinal absorption of lipopolysaccharides. *J. Lipid Res.* **50**, 90–97 (2009).
172. Harte, A. L. *et al.* High fat intake leads to acute postprandial exposure to circulating endotoxin in type 2 diabetic subjects. *Diabetes Care* **35**, 375–382 (2012).
173. Creely, S. J. *et al.* Lipopolysaccharide activates an innate immune system response in human adipose tissue in obesity and type 2 diabetes. *Am. J. Physiol. - Endocrinol. Metab.* **292**, 740–747 (2007).
174. Turnbaugh, P. J. *et al.* An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* **444**, 1027–31 (2006).
175. Smith, K., McCoy, K. D. & Macpherson, A. J. Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. *Semin. Immunol.* **19**, 59–69 (2007).
176. Manichanh, C. *et al.* Reshaping the gut microbiome with bacterial transplantation and antibiotic intake. *Genome Res.* **20**, 1411–1419 (2010).
177. Maruvada, P., Leone, V., Kaplan, L. M. & Chang, E. B. Perspective The Human Microbiome and Obesity : Moving beyond Associations. *Cell Host Microbe* **22**, 589–599 (2017).
178. Ley, R. E. Obesity and the human microbiome. *Curr. Opin. Gastroenterol.* **26**, 5–11 (2010).
179. Ridaura, V. K. *et al.* Cultured gut microbiota from twins discordant for obesity modulate adiposity and metabolic phenotypes in mice. doi:10.1126/science.1241214
180. Ley, R. E. *et al.* Obesity alters gut microbial ecology. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 11070–5 (2005).
181. Parada, A. E., Needham, D. M. & Fuhrman, J. A. Every base matters: Assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environ. Microbiol.* **18**, 1403–1414 (2016).
182. Apprill, A., Mcnally, S., Parsons, R. & Weber, L. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat. Microb. Ecol.* **75**, 129–137 (2015).
183. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener, and C. J. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* **37**, 852–857 (2019).
184. Ombrello, A. K. Dada2. *Encycl. Med. Immunol.* **13**, 1–7 (2020).
185. McDonald, D. *et al.* An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME J.* **6**, 610–8 (2012).
186. Katoh, K. & Standley, D. M. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol. Biol. Evol.* **30**, 772–780 (2013).
187. Lane, D. *16s/23s rrna sequencing*. (1991).
188. Price, M. N., Dehal, P. S. & Arkin, A. P. FastTree 2 - Approximately maximum-likelihood trees for large alignments. *PLoS One* **5**, (2010).
189. Lozupone, C. & Knight, R. UniFrac : a New Phylogenetic Method for Comparing Microbial Communities UniFrac : a New Phylogenetic Method for Comparing Microbial Communities. *Appl. Environ. Microbiol.* **71**, 8228–8235 (2005).
190. Douglas, G. M. *et al.* PICRUSt2 for prediction of metagenome functions. *Nat. Biotechnol.* **38**, 685–688 (2020).
191. Louca, S. & Doebeli, M. Efficient comparative phylogenetics on large trees. *Bioinformatics* **34**,

1053–1055 (2018).
192. Ye, Y. & Doak, T. G. A parsimony approach to biological pathway reconstruction/inference for genomes and metagenomes. *PLoS Comput. Biol.* **5**, 1–8 (2009).
193. Mirarab, S., Nguyen, N. & Warnow, T. SEPP: SAT´ e-Enabled Phylogenetic Placement. 247–258
194. Faith, D. P. Conservation evaluation and phylogenetic diversity. *Biol. Conserv.* **61**, 1–10 (1992).
195. Du, H., Zhao, A., Wang, Q., Yang, X. & Ren, D. Supplementation of Inulin with Various Degree of Polymerization Ameliorates Liver Injury and Gut Microbiota Dysbiosis in High Fat-Fed Obese Mice. *J. Agric. Food Chem.* **68**, 779–787 (2020).
196. Anitha, M. *et al.* Intestinal Dysbiosis Contributes to the Delayed Gastrointestinal Transit in High-Fat Diet Fed Mice. *Cmgh* **2**, 328–339 (2016).
197. Finucane, M. M., Sharpton, T. J., Laurent, T. J. & Pollard, K. S. A taxonomic signature of obesity in the microbiome? Getting to the guts of the matter. *PLoS One* **9**, 1–5 (2014).
198. Bhattarai, Y. & Kashyap, P. C. Germ-Free Mice Model for Studying Host--Microbial Interactions. in *Mouse Models for Drug Discovery: Methods and Protocols* (eds. Proetzel, G. & Wiles, M. V) 123–135 (Springer New York, 2016). doi:10.1007/978-1-4939-3661-8_8
199. Round, J. L. & Mazmanian, S. K. The gut microbiota shapes intestinal immune responses during health and disease. *Nat. Rev. Immunol.* **9**, 313–323 (2009).
200. Olszak, T. *et al.* NIH Public Access. **336**, 489–493 (2012).
201. Sun, J. *et al.* Fecal microbiota transplantation alleviated Alzheimer's disease-like pathogenesis in APP/PS1 transgenic mice. *Transl. Psychiatry* **9**, (2019).
202. Bárcena, C. *et al.* Healthspan and lifespan extension by fecal microbiota transplantation into progeroid mice. *Nat. Med.* **25**, 1234–1242 (2019).
203. Zhou, D. *et al.* Total fecal microbiota transplantation alleviates high-fat diet-induced steatohepatitis in mice via beneficial regulation of gut microbiota. *Sci. Rep.* **7**, 1–11 (2017).
204. Barba, C. *et al.* Effects of Fecal Microbiota Transplantation on Composition in Mice with CKD. *Toxins (Basel).* **12**, 1–12 (2020).
205. Wrzosek, L. *et al.* Transplantation of human microbiota into conventional mice durably reshapes the gut microbiota. *Sci. Rep.* **8**, 1–9 (2018).
206. Knights, D. & Hutkins, R. W. Stable Engraftment of Bifidobacterium longum AH1206 in the Human Gut Depends on Individualized Features of the Resident Microbiome Article Stable Engraftment of Bifidobacterium longum AH1206 in the Human Gut Depends on Individualized Features of the Resid. 515–526 (2016). doi:10.1016/j.chom.2016.09.001
207. Li, S. S. *et al.* Durable coexistence ofdonor and recipient strains after fecal microbiota transplantation. **352**, 586–590 (2016).
208. Ducarmon, Q. R. *et al.* Gut Microbiota and Colonization Resistance against Bacterial Enteric Infection. *Microbiol. Mol. Biol. Rev.* **83**, 1–29 (2019).
209. Stecher, B. *et al.* Salmonella enterica serovar typhimurium exploits inflammation to compete with the intestinal microbiota. *PLoS Biol.* **5**, 2177–2189 (2007).
210. Derrien, M., Vaughan, E. E., Plugge, C. M. & Vos, W. M. De. a human intestinal mucin-degrading bacterium. 1469–1476 (2018). doi:10.1099/ijs.0.02873-0
211. Reunanen, J. *et al.* Akkermansia muciniphila adheres to enterocytes and strengthens the integrity of the epithelial cell layer. *Appl. Environ. Microbiol.* **81**, 3655–3662 (2015).
212. Collado, M. C., Derrien, M., Isolauri, E., De Vos, W. M. & Salminen, S. Intestinal integrity and Akkermansia muciniphila, a mucin-degrading member of the intestinal microbiota present in infants, adults, and the elderly. *Appl. Environ. Microbiol.* **73**, 7767–7770 (2007).
213. Brown, C. T. *et al.* Gut microbiome metagenomics analysis suggests a functional model for the development of autoimmunity for type 1 diabetes. *PLoS One* **6**, 1–9 (2011).
214. Png, C. W. *et al.* Mucolytic bacteria with increased prevalence in IBD mucosa augment in vitro utilization of mucin by other bacteria. *Am. J. Gastroenterol.* **105**, 2420–2428 (2010).
215. Shin, N. R. *et al.* An increase in the Akkermansia spp. population induced by metformin treatment improves glucose homeostasis in diet-induced obese mice. *Gut* **63**, 727–735 (2014).

216. Wang, K. *et al.* Parabacteroides distasonis Alleviates Obesity and Metabolic Dysfunctions via Production of Succinate and Secondary Bile Acids. *Cell Rep.* **26**, 222-235.e5 (2019).
217. Koh, G. Y., Kane, A. V., Wu, X. & Crott, J. W. Parabacteroides distasonis attenuates tumorigenesis, modulates inflammatory markers and promotes intestinal barrier integrity in azoxymethane-treated A/J mice. *Carcinogenesis* **41**, 909–917 (2021).
218. Deehan, E. C. *et al.* Precision Microbiome Modulation with Discrete Dietary Fiber Structures Directs Short-Chain Fatty Acid Production. *Cell Host Microbe* **27**, 389-404.e6 (2020).
219. Tong, L. C. *et al.* Propionate ameliorates dextran sodium sulfate-induced colitis by improving intestinal barrier function and reducing inflammation and oxidative stress. *Front. Pharmacol.* **7**, 1–9 (2016).
220. Asarat, M., Vasiljevic, T., Apostolopoulos, V. & Donkor, O. Short-Chain Fatty Acids Regulate Secretion of IL-8 from Human Intestinal Epithelial Cell Lines in vitro. *Immunol. Invest.* **44**, 678–693 (2015).
221. Kelly, C. J. *et al.* Crosstalk between microbiota-derived short-chain fatty acids and intestinal epithelial HIF augments tissue barrier function. *Cell Host Microbe* **17**, 662–671 (2015).
222. Caballero, S. *et al.* Cooperating Commensals Restore Colonization Resistance to Vancomycin-Resistant Enterococcus faecium. *Cell Host Microbe* **21**, 592-602.e4 (2017).
223. Warren, Y. A., Tyrrell, K. L., Citron, D. M. & Goldstein, E. J. C. Clostridium aldenense sp. nov. and Clostridium citroniae sp. nov. isolated from human clinical infections. *J. Clin. Microbiol.* **44**, 2416–2422 (2006).
224. Mangin, I. *et al.* Molecular inventory of faecal microflora in patients with Crohn's disease. *FEMS Microbiol. Ecol.* **50**, 25–36 (2004).
225. Wang, L. *et al.* Increased abundance of Sutterella spp. and Ruminococcus torques in feces of children with autism spectrum disorder. *Mol. Autism* **4**, 12–15 (2013).
226. Mukhopadhya, I. *et al.* A comprehensive evaluation of colonic mucosal isolates of sutterella wadsworthensis from inflammatory bowel disease. *PLoS One* **6**, 1–10 (2011).
227. Kaakoush, N. O. Sutterella Species, IgA-degrading Bacteria in Ulcerative Colitis. *Trends Microbiol.* **28**, 519–522 (2020).
228. Paramsothy, S. *et al.* Specific Bacteria and Metabolites Associated With Response to Fecal Microbiota Transplantation in Patients With Ulcerative Colitis. *Gastroenterology* **156**, 1440-1454.e2 (2019).
229. Moon, C. *et al.* Vertically transmitted faecal IgA levels determine extra-chromosomal phenotypic variation. *Nature* **521**, 90–93 (2015).
230. Větrovský, T. & Baldrian, P. The Variability of the 16S rRNA Gene in Bacterial Genomes and Its Consequences for Bacterial Community Analyses. *PLoS One* **8**, 1–10 (2013).
231. Jovel, J. *et al.* Characterization of the gut microbiome using 16S or shotgun metagenomics. *Front. Microbiol.* **7**, 1–17 (2016).
232. Kuperman, A. A. *et al.* Deep microbial analysis of multiple placentas shows no evidence for a placental microbiome. *BJOG An Int. J. Obstet. Gynaecol.* **127**, 159–169 (2020).
233. Kliman, H. J. Comment on 'The placenta harbors a unique microbiome'. *Sci. Transl. Med.* **6**, (2014).
234. Round, J. L., O'Connell, R. M. & Mazmanian, S. K. Coordination of tolerogenic immune responses by the commensal microbiota. *J. Autoimmun.* **34**, (2010).
235. Chow, J. & Mazmanian, S. K. Getting the Bugs out of the Immune System: Do Bacterial Microbiota 'Fix' Intestinal T Cell Responses? *Cell Host Microbe* **5**, 8–12 (2009).
236. Mazmanian, S. K., Cui, H. L., Tzianabos, A. O. & Kasper, D. L. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell* **122**, 107–118 (2005).
237. Han, D. *et al.* Dendritic Cell Expression of the Signaling Molecule TRAF6 Is Critical for Gut Microbiota-Dependent Immune Tolerance. *Immunity* **38**, 1211–1222 (2013).
238. MacPherson, A. J., McCoy, K. D., Johansen, F. E. & Brandtzaeg, P. The immune geography of IgA induction and function. *Mucosal Immunol.* **1**, 11–22 (2008).

239. Lindner, C. *et al.* Age, microbiota, and T cells shape diverse individual iga repertoires in the intestine. *J. Exp. Med.* **209**, 365–377 (2012).
240. McCoy, K. D., Burkhard, R. & Geuking, M. B. The microbiome and immune memory formation. *Immunol. Cell Biol.* **97**, 625–635 (2019).
241. Ivanov, I. I. *et al.* NIH Public Access. **139**, 485–498 (2010).
242. Geuking, M. B. *et al.* Intestinal Bacterial Colonization Induces Mutualistic Regulatory T Cell Responses. *Immunity* **34**, 794–806 (2011).
243. Schloss, P. D. *et al.* Stabilization of the murine gut microbiome following weaning. *Gut Microbes* **3**, (2012).
244. Hansen, C. H. F. *et al.* Patterns of early gut colonization shape future immune responses of the host. *PLoS One* **7**, 1–7 (2012).
245. Roopchand, D. E. *et al.* Dietary polyphenols promote growth of the gut bacterium akkermansia muciniphila and attenuate high-fat diet-induced metabolic syndrome. *Diabetes* **64**, 2847–2858 (2015).
246. Zhu, L. *et al.* Berberine treatment increases Akkermansia in the gut and improves high-fat diet-induced atherosclerosis in Apoe−/−mice. *Atherosclerosis* **268**, 117–126 (2018).
247. Tillhon, M., Guamán Ortiz, L. M., Lombardi, P. & Scovassi, A. I. Berberine: New perspectives for old remedies. *Biochem. Pharmacol.* **84**, 1260–1267 (2012).
248. Habtemariam, S. Berberine and inflammatory bowel disease: A concise review. *Pharmacol. Res.* **113**, 592–599 (2016).
249. Tabeshpour, J., Imenshahidi, M. & Hosseinzadeh, H. A review of the effects of berberis vulgaris and its major component, berberine, in metabolic syndrome. *Iran. J. Basic Med. Sci.* **20**, 557–568 (2017).