Characterization of Fiber Intake, the Gut-Brain-Axis, and Glycemic Control in NH Bhutanese Refugees

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CHARACTERIZATION OF FIBER INTAKE, THE GUT-BRAIN-AXIS, AND GLYCEMIC CONTROL IN NH BHUTANESE REFUGEES

BY

BRANDY MOSER
BA, BS, Boston University, 2021

THESIS

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On June 15, 2023

Approval signatures are on file with the University of New Hampshire Graduate School.
Chapter 1


Chapter 2


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ABSTRACT

The gut microbiome has a significant influence on chronic diseases, potentially through mediating inflammation and the gut-brain axis, yet underlying pathways remain unclear. Microbiomes are highly variable and can be influenced by a variety of lifestyle and environmental factors, like diet and medication use. The study of both the composition and function of the gut microbiome is warranted, yet many gaps remain particularly in our knowledge about the gut microbiome function in the context of chronic diseases. Refugee groups in the US, such as the Bhutanese refugee population in New Hampshire, are at higher risk for chronic diseases but underrepresented in current research. Therefore, this project sought to characterize the gut microbiome composition and functional potential among Bhutanese refugee adults in NH in regard to glycemic status, inflammation, satiety regulation, fiber intake, and medication use. Data on health information and dietary intake was previously collected from (n=50) Bhutanese refugee adults, with a high prevalence of overweight, obesity and type 2 diabetes (T2D). Inflammation and satiety biomarkers were measured in plasma samples using electrochemiluminescence immunoassays. Microbiome data was examined via shotgun sequences of fecal samples and analyzed using validated bioinformatic pipelines and R packages. Compositional richness and diversity were lower in those with T2D, while functional diversity was not significantly different. Protein-coding genes related to the biosynthesis of lipopolysaccharide (LPS) were predictive of plasma C-reactive protein, lipopolysaccharide binding protein (LBP, a marker of intestinal permeability), fasting plasma glucose, and insulin. Further, LBP was positively associated with Hemoglobin A1c, fasting plasma glucose, and Homeostatic Model Assessment for Insulin Resistance. While there were no significant associations between gut microbiome composition or function with satiety regulation and fiber
intake, findings suggest an association between microbially produced LPS, inflammation, and glycemic regulation that could be a potential target of future therapeutics or interventions.
Chapter 1

Introduction

Bacteria, archaea, viruses, phages, fungi, protists, and nematodes among other microorganisms colonize the digestive tract to form the human gut microbiome, which has been highlighted for its profound role in human health. The gut microbiome has approximately 100 times the genes of the human genome (1). Although evidence is inconclusive, most frequently a healthy gut microbiota is characterized by higher microbial diversity and richness, which can be important for metabolic function (1,2). Beginning at birth, an individual’s signature microbiota composition is influenced by a variety of factors including mode of birth and genetics, and continues to change over time with the influence of diet, environment, and lifestyle (1). To this point, alterations in the gut microbiota, known as dysbiosis, are increasingly evident in the development and severity of metabolic disorders such as obesity and type 2 diabetes (T2D) (3). Additionally, seminal studies highlight the transferability of an obese phenotype to germ-free mouse models via transfer of human gut microbiota (3). Despite evidence for the role of the gut microbiome in metabolic disorders and the transfer of obese phenotypes, the exact connection between gut microbiota and metabolic phenotypic outcomes in humans is largely unknown. The microbiota-gut-brain axis theory suggests gut microbiota and bacteria-derived metabolites influence metabolic health via modulation of the gut-brain axis (4).

The microbiota-gut-brain axis refers to bidirectional signaling between the gastrointestinal tract and the brain. It is thought to be heavily influenced by the gut microbiota and involved in a multitude of homeostatic biological processes including digestive function, hunger and satiety, and eating behavior. Gut microbiota and bacteria-derived metabolites interact with the gut-brain
axis efferent and afferent pathways, while also exhibiting a high degree of interconnection and crosstalk (Figure 1) (5). The three predominant signaling pathways for gut-brain axis communication are neuronal, endocrine, and immune.

**Neuronal**

Nutrients in the gastrointestinal tract signal transmit information to the hypothalamus via the vagus nerve of the autonomous nervous system (ANS). In response, the hypothalamus serves an essential role in host homeostatic control, including regulation of appetite and energy balance, by sending feedback to the gut (3).

**Endocrine**

Non-digestible carbohydrate fermentation by the gut microbiota produces short chain fatty acids (SCFAs) which stimulate the secretion of anorexigenic factors such as glucagon-like peptide 1 (GLP-1) and peptide YY (PYY). Secretion occurs as a result of food ingestion but can continue long after meals are finished to prolong the feeling of satiety. To that end, individuals with obesity typically display decreased levels of GLP-1 and PYY, suggesting their essential role in the regulation of body weight, food intake, and metabolism (6). In addition to the anorexigenic hormones secreted following SCFA production, other microbial products and metabolites influence the production of ghrelin, leptin, and serotonin through enteroendocrine cells (3,5). Collectively, the gut microbiota and derived metabolites, like SCFAs, are key regulators in the production and secretion of hunger and satiety hormones.
Immune

The gastrointestinal tract is home to the densest population of immune cells (4). Thus, the gut microbiota interacts with the innate and adaptive immune systems, both of which contribute to the maintenance of the intestinal barrier, as well as acute and chronic inflammatory processes. Markers of high intestinal permeability have been found in obesity and type 2 diabetes, suggesting endotoxemia (or elevation of lipopolysaccharide binding protein in circulation stemming from increased gut permeability and leading an activated inflammatory response) may be a mechanistic link between the microbiome, low-grade chronic inflammation, and development of cardiometabolic diseases (3,7). As with the endocrine pathway of the gut-brain axis, SCFAs are also key players in the immune pathway, by enhancing the integrity of the intestinal barrier through the stimulation of mucus production and tight junction assembly. Evidence on the concentrations of SCFAs in metabolic disease outcomes has been inconsistent, however, most studies have suggested that SCFAs hold anti-inflammatory properties that can influence immune cells and reduce pro-inflammatory signaling by cells of the immune system (4).

This review will examine our knowledge thus far about the potential practical applications of the microbiota-gut-brain axis in obesity prevention and treatment, highlighting opportunities for future research.

Practical & Clinical Considerations

Ever-increasing obesity rates pose a threat to public health, emphasizing a need for the continued exploration of sustainable and cost-effective interventions targeting obesity (Figure 2). Obesity treatments incorporate a blend of surgical and non-surgical strategies including bariatric
surgery, behavioral interventions, dietary changes, physical activity (PA), and pharmacotherapies (8). Despite the variety of strategies for obesity treatment and prevention, each incur challenges and present unique barriers to success. For example, bariatric surgeries are life altering, burdensome, and costly. Changes to behavior, diet, and PA can be unsustainable, difficult to implement, and/or ineffective, particularly in weight loss maintenance (8). Despite challenges in feasibility, most weight management strategies have been shown to influence the gut microbiota and promote healthy alterations to gut-brain axis signaling factors (9,10). Thus, an opportunity exists to maximize the potential benefit of current therapies or develop novel therapies that harness the microbiota-gut-brain axis to address obesity.

Lifestyle Factors

Physical Activity

Exercise prescription is a common, evidence-based treatment for both psychological and gastrointestinal disorders (9). Specifically, moderate to high-intensity mixed aerobic and resistance training has been seen to significantly reduce inflammation in individuals with T2D and obesity, as well as to promote microbial diversity, though the reason for this is unclear (11,12). In addition to improving gut health, regular exercise has also been found to ameliorate and promote cognitive function, improve outcomes in psychopathology, and reduce risk of brain atrophy in older adults (13).

By contrast, overly vigorous or strenuous exercise has been found to negatively impact health, such that prolonged high-intensity endurance activities were associated with increased propensity for endotoxemia (11). Further, the disruptive impact of exercise-induced stress, though intentional (e.g., high-intensity training such as long-distance running), is
indistinguishable in its negative impacts on gut health from stress in response to dangerous stimuli (e.g., running to avoid harm) (13). The biological response to such stress activates the hypothalamic-pituitary-adrenal (HPA) axis. Interestingly, hyperresponsiveness of the HPA can be mediated by the gut microbiota and bacteria-derived metabolites, given that the HPA is a main communication pathway along the microbiota-gut-brain axis (13).

Thus, moderate exercise prescription serves as an effective intervention to promote the improvement of microbial diversity and cognitive function as well as to reduce inflammation in adults with T2D and obesity (11–13). Although human clinical evidence lacks a complete understanding of the relationship between exercise and the microbiota-gut-brain axis in treating or preventing obesity, the HPA appears intertwined in this approach. Furthermore, additional research is required to identify safe and appropriate clinical applications of exercise and the points at which exercise intensity, duration, and frequency may become detrimental to the microbiota-gut-brain axis.

**Stress**

Beginning in utero, the impacts of stress on gut microbial diversity can be seen throughout the lifetime. Prenatal stress is associated with long-term modulations to microbial richness and chronic exposure to psychosocial stress relates to altered microbial profiles in adults (14). Concurrently, early-life stress has been seen to increase risk of obesity in adulthood and longitudinal analyses have found exposure to life stressors (e.g., financial stress) to correlate with higher BMI and waist circumference (14). However, there is no evidence to date demonstrating a causal relationship between stress-induced microbial changes and weight gain nor obesity (15).
Stress is further associated with disruptions in gut-brain function, such that stress elevates ghrelin levels, which influence hypothalamic satiety and stress centers via the neuronal and endocrine pathways. Despite this physiological response, pharmacological interventions targeting ghrelin are not recommended because the amount by which a medication may lower ghrelin levels is imprecise and may result in abnormally low levels (15).

Chronic stress can additionally increase gut permeability, allowing the microbiota-gut-brain axis’ neuronal and endocrine pathways to influence hypothalamic satiety and stress centers, which may contribute to dysregulated appetite and uncontrolled eating behavior associated with obesity (14,16). Specifically, eating behaviors associated with overweight, such as emotional eating, credited increased levels of ghrelin and other peptides following acute periods of stress (17). Given the role of ghrelin in appetite and metabolic regulation, concentrations of ghrelin may impact the gut microbiome to increase the risk of obesity. Thus, interventions targeting stress tolerance and emotion regulation may be useful in reducing stress-induced overeating and microbiome dysbiosis, though further investigation is required.

**Dietary patterns**

Diet is a critical determinant of gut microbiota composition and function (4). Changes in diet have consistently been found to alter gut microbial composition in as little as 24 hours (4). Dietary patterns associated with core compositional traits of the gut microbiome provide substrates for the production of a wide array of bacteria-derived metabolites in the microbiota-gut-brain axis (18,19). It is important to note the gut microbiota exhibits a high level of resiliency, such that short term diet modifications may result in some microbiota compositional changes, but post-diet microbiota composition often remains stable with regard to dominant taxa.
and falls within the same pre-diet enterotype classification (20). This evidence may have important implications when developing dietary interventions for populations with obesity who may require a more personalized approach for best results.

Western-style diets, generally high in salt, sugar, and/or saturated and trans fats, exhibit a similar gut microbiota profile as individuals with obesity and are often characterized by dysbiosis and negative metabolic health outcomes (21). Notably, Hildebrandt and colleagues found a high fat diet (HFD) increased Proteobacteria and Firmicutes, accompanied by decreased Bacteroides in mice (22). High animal-derived saturated fat was also associated with an increase the B. wadsworthia, which induces systemic inflammation (4). Given the detrimental impacts of various diets, such as the Western diet and HFD, on gut composition and metabolic health, dietary intake serves as a salient prospective target for obesity treatment and prevention. Many dietary recommendations may positively impact the microbiota-gut-brain axis and overall health. The Mediterranean diet emphasizes fruits, vegetables, legumes, nuts, whole grains, and healthy fats, and is associated with reductions in cardiovascular disease (4). Among other characteristics, the Mediterranean diet induces changes in the composition and inflammatory potential of the gut microbiota. A randomized feeding trial in adults with overweight and obesity found whole grain (WG) and fruit/vegetable (FV) intervention diets to decrease levels of inflammatory markers such as Lipopolysaccharide-binding protein (LBP). WG diets led to decreased tumor necrosis factor alpha (TNF-alpha) while FV diets related to both decreased IL-6 and increased alpha diversity. Thus, a diet rich in whole grains, fruits, and vegetables may be protective against obesity through its various anti-inflammatory roles (23). In addition to dietary quality, dietary diversity has been associated with fecal microbiota stability (24).
Similarly, to the effects of increasing WG and FV intake, diets with high protein intake can increase SCFA and branch-chain amino acid (BCAA) production associated with anti-inflammation, as well as increase *Bacteroides* to support amino acid proteolysis (4). As a result, high-protein diets low in carbohydrates are commonly employed in weight loss interventions. However, despite their potential anti-inflammatory benefits, such diets have also been associated with gastrointestinal consequences from increased fermentation of undigested protein and lowered gut microbial diversity (25). Furthermore, an increased potential for BCAA production, particularly by *Prevotella copri* and *Bacteroides vulgatus*, along with increased serum BCAA were associated with increased insulin resistance in non-diabetic individuals (26).

**Calorie restriction**

Apart from interventions targeting macronutrient composition such as fiber or protein, caloric restriction has long been employed as a primary weight loss tactic. Calorie restriction has been shown to increase microbial richness in individuals with low richness prior to the intervention (27). However, despite initial weight loss from lower calorie intake, interventions involving considerable caloric restriction, specifically very-low calorie diets (VLCD; ~800 calories/day), may be detrimental to the gut microbiome. For example, a liquid form VLCD in a clinical trial of women with overweight and obesity showed weight loss, decreased adiposity, and improved glucose regulation but also contributed to gut microbiota (reversible) compositional restructuring and an overall loss in bacterial abundance (28). Further, when post-intervention microbiota from women in this clinical trial were transplanted to mice, it induced weight loss while also impairing nutrient absorption, resulting in decreased bile acids (28), which may ultimately increase risk of *Clostridium difficile* colonization. Additionally, there was a
reversible increase in abundance of genes involved in SCFA biosynthesis but a decrease in SCFAs, potentially attributed to decreases in colonization during the VLCD period (28). It has yet to be elucidated whether the weight loss and metabolic outcomes of a restrictive diet outweigh the potentially negative impacts of the gut microbiota and gut-brain axis (28). Though changes in the gut microbiome from calorie restriction have been studied, their direct role in human physiology remains to be fully described (29). Furthermore, the resiliency of these changes and their potential role in weight regain and weight loss maintenance is a priority, given that low calorie diets often require dramatic and unsustainable changes in dietary intake that frequently lead to subsequent weight regain. For example, a 10-week weight loss program for adults with overweight or obesity found that after the intervention satiety signaling factors decreased and appetite-inducing factors increased for one-year after program completion (30). A generalizable relationship between dietary changes in gut composition and the gut-brain-axis has yet to be delineated (4). Monitoring and targeting microbiota-gut-brain axis endocrine factors in weight management may present novel approaches to modulate hunger and satiety signaling and metabolism, thereby preventing weight regain and increasing the effectiveness of weight loss treatment.

Importantly, hunger and satiety signaling, as well as traits of eating behavior such as uncontrolled eating and restraint, are associated with dietary intake and gut microbiota composition. The existing evidence is reviewed in a later section.

**Eating behavior**

In addition to dietary intake, eating behaviors, such as eating frequency, are also targets for obesity prevention and treatment. For example, intermittent fasting (IF), a process in which food
intake is restricted for 16-24 hours at a time, is credited with inducing weight loss, improving insulin response, and reducing cardiometabolic disease risk (31). These beneficial effects are considered only partially attributable to calorie restriction and is thought to also result from restructuring and remodeling of the gut microbiome from fasting behavior (32,33). Other forms of IF include Ramadan Intermittent Fasting (RI), an annual faith-associated fasting period prone to investigation for its regularity (32). One such investigation found fecal samples from RIF participants exhibited an increased microbiome diversity from gut remodeling, ultimately upregulating SCFA-producing capacity/species (butyric acid) (32). Zouhal and colleagues found RIF, in a sample of males with obesity, to improve microbiota-gut-brain factors, including pre-to-post fasting leptin, GLP-1, PYY, and cholecystokinin (CCK), but found no effect on ghrelin (34). Given its support of SCFA production and effect on microbiota-gut-brain factors, IF may be an effect strategy at improving body composition in populations with obesity by targeting the microbiota-gut-brain axis, however, more evidence is needed to understand what types of intermittent fasting are most beneficial.

Probiotics, prebiotics, and symbiotics

**Probiotics**

Probiotics are live microorganisms that, when consumed in appropriate quantities, are expected to confer beneficial effects such as glycemic control through the introduction of beneficial species to the gastrointestinal tract (35). Research suggests specific bacterial strains may reduce inflammation, leptin levels, and endotoxemia implicated in the microbiota-gut-brain axis pathology of metabolic diseases (3). *Bifidobacterium, Lactobacillus*, and *Akkermansia*
*muciniphila* are among the most promising species to have been tested as probiotics and to influence host metabolism (3).

A comprehensive systematic review of randomized control trials investigating the anti-obesity properties of probiotic supplementation in overweight and obese populations concluded high-dose probiotics are a promising intervention, with the most common significant outcome as a moderate but significant reduction in BMI (on average about half a kilogram across interventions) (35). Potential underlying mechanisms include strengthening of the intestinal barrier, modulation of chronic inflammation, and production of metabolites that influence the microbiota-gut-brain axis (35). There is also evidence, particularly from rodent studies, of probiotics having an effect in reducing anxiety and depressive symptoms (4). The question of a potential effect on behaviors and psychological symptoms associated with obesity warrant investigation in humans. Additional factors that may be involved in the potential anti-obesity properties of probiotics include supplement amount, duration of usage, and strain specificity (35). Since most studies use a mix of species in probiotics supplements administered such as VSL #3 (combination supplement of *Lactobacilli, Streptococcus thermophilus*, and *Bifidobacterium,* further research is needed to identify the best practice of probiotic supplementation for improving metabolic diseases (36). Future investigations will need a heavier focus on human populations as findings from mouse models are not readily applicable to key human components including the intestinal mucosa. Additionally, there are unknown long-term effects of probiotic supplementation use and often contradictory evidence.
Prebiotics

Prebiotics are defined as “non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already established in the colon, and thus improve the host health” (8). Fiber intake, and specifically intake of microbiota-accessible carbohydrates (MACs), has been identified as a key dietary driver of gut microbiota composition and metabolite production. Dietary fiber includes prebiotics such as inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), resistant starch, and other soluble dietary fibers, unhydrolyzable or unabsorbable by the small intestine. Common sources of dietary fiber include fruits, vegetables, and grains (4).

Dietary fiber serves as a primary energy source for gut microbiota which drives production of short chain fatty acids (SCFA), primarily acetate, propionate, and butyrate, through fermentation. SCFAs have anti-inflammatory, immunomodulatory, and metabolic effects; however, there remains conflicting evidence regarding the role of SCFAs in metabolic diseases (2). For example, studies in both rodents and humans have shown on the one hand that acetate suppresses appetite and is beneficial for metabolic health but on the other hand may also have obesogenic and hyperglycemic effects (2). These diverging effects may be related to the mode of acetate administration, but further research is needed to fully elucidate the role of microbe-produced acetate in human metabolic health. Although the role of SCFA is not fully understood, fiber intake and SCFAs potentially influence metabolic functions in the human host including glycemic control, hunger and satiety signals to the brain, and inflammatory pathways. Broadly, prebiotic intake is protective against metabolic diseases and results in favorable gut microbiota outcomes. Prebiotics have been shown to alter the gut composition by reducing *Firmicutes* and *Bacteroidetes* and increasing *Lactobacillus* and *Bifidobacterium*. Compositional
changes correlated with improvements in microbiota-gut-brain factors (entero-endocrine cell activity, glucose homeostasis, leptin sensitivity) important in addressing obesity (8). Specifically, FOS (oligosaccharides commonly found in fruit) used in a double-blind intervention study in women with obesity and found increased levels of *Bifidobacterium* and *Faecalibacterium prausnitzii*, which are often reduced in populations with obesity (37). However, such studies examining metabolic health outcomes and microbiota compositional changes from fiber supplementation have yielded conflicting results and are scarce (38). Inconsistent results of responses to fiber may be attributable to gut composition (39). Thus, further evidence is needed to support recommendations of specific prebiotics for clinical applications.

Fiber intake is additionally associated with microbiota-gut-brain axis factors that could reduce inflammation and improve hunger and satiety signaling in obesity. The RESOLVE study involved participants with metabolic syndrome to a 3-week intensive diet-exercise residential intervention followed by a 1-year free-living period. Dietary fiber was the only nutritional component that was significantly predictive of health outcomes and decreased serum CRP levels, indicative of reduced inflammation. Therefore, its adequacy should be prioritized in future diet-weight reduction interventions (40). Fiber may also play a role in increased brain-derived neurotrophic factor (BDNF) and appetite suppression, however, this is largely supported in mouse models with limited evidence in humans (4). Existing human evidence stems primarily from observational research, thus, there remains a need to investigate and identify specific microbial metabolites and pathways involved in prebiotic impacts on the microbiota-gut-brain axis.
**Symbiotics**

Symbiotics are probiotic supplements that also contain prebiotic components (25). A randomized control trial of participants with obesity and T2D found 24-weeks of symbiotics supplementation produced no significant changes in inflammatory markers when compared with the control group. The supplementation did induce changes in the gut microbiome by increasing counts of specific beneficial bacteria and altering the concentrations of acetic and butyric acids (41). Furthermore, a randomized clinical trial (RCT) exploring symbiotic supplementation in individuals with T2D found significant reductions in bacterial translocation through the intestinal barrier, proposing combined symbiotic/probiotic and prebiotic supplement regimen as a potential therapeutic strategy to control low-grade inflammation (41). However, studies on symbiotics are limited and evidence suggests other factors, including symbiotic administration timing, may be related to success.

**Polyphenols**

Polyphenols are noted for their anti-inflammatory and antioxidant capabilities, among many other properties (cardioprotective, cancer chemopreventive, and neuroprotective) (42). These beneficial compounds contribute to human health through a bidirectional phenolic-microbiota pathway, producing bioactive metabolites and modulating gut microbiota composition. The functions of the gut microbiome and polyphenols are intertwined, as the colon’s microbiota provides enzymes essential for polyphenol metabolism, prior to their absorption (42). Polyphenols impact microbiota composition by increasing *Bifidobacteria*, *Lactobacillus*, and *F. prausnitzii*, a butyrate producer, among others. Furthermore, polyphenols can increase *Akkermansia muciniphila* which plays a role in insulin sensitivity (7). Given their
ability to selectively reduce the growth of particular species, polyphenols have been found to reduce the abundance of LPS producers and thus decrease metabolic endotoxemia (7). Though evidence remains inconclusive, specific phenolic compounds have shown promise in their anti-obesogenic effects targeting the gut brains axis (43).

Notably, resveratrol, a natural polyphenol often found in grapes and berries, can mediate the microbiota-gut-brain axis through anti-inflammatory properties, GLP-1 secretion promotion, regulation of serotonin 5-hydroxytryptamine (5-HT) signaling, and modulation of gut microbiota composition. For example, resveratrol improves the integrity of intestinal tight junction proteins by influencing gut microbiota diversity, thus enhancing gut permeability function. Resveratrol stands as a promising future natural therapeutic for obesity-related intestinal dysfunction, but more evidence is necessary (43). One investigation found ferulic acid (FA), a polyphenolic compound, prevented weight gain and attenuated dyslipidemia in mice. FA exerts hypolipidemic abilities by suppressing cholesterol synthesis and increasing the HDL/LDL ratio. Although slight changes in gut microbiota structure were observed, the anti-obesity effects of FA were not associated with the changes in gut microbiota and diversity (44). Therefore, FA may be a useful therapeutic target in treating obesity, however its involvement in the microbiota-gut-brain axis still requires further research.

Polyphenols also play a role in SCFA production and their beneficial effects on metabolic health. Production of fecal succinate is induced by dietary polyphenols such as curcumin and dietary fiber (in rats) (45). Succinate induces intestinal gluconeogenesis which activates portal glucose signaling, which in turn decreases hunger and promotes insulin sensitivity. This
phenomenon had also been described with propionate, showing that activation of intestinal 
gluconeogenesis was necessary for the beneficial effects of SCFA (46).

The therapeutic potential of probiotics, prebiotics, symbiotics, and polyphenols, remains 
highly promising, yet underexplored in necessary experimental studies. Not only do they 
maintain the potential to treat metabolic disorders but also prevent their development, through 
mechanisms implicating the microbiota-gut-brain axis. However, there is a great need for well-
designed RCTs in humans to further understand their role and effectiveness in targeting 
metabolic disorders and better characterize the underlying role of the microbiota-gut-brain axis 
in these processes.

Precision and Personalized Nutrition

Dietary interventions for obesity often fail to consider inter-individual heterogeneity in 
their design. Studies assessing fecal microbiota prior to and following intervention have found on 
the one hand resilience in the core microbiome composition throughout time, and on the other 
vastly different responses to identical dietary interventions, such that two individuals following 
identical dietary plans may experience drastically different physiological responses and changes 
to their gut microbiota (47). Thus, dietary interventions tailored to individual gut microbiota 
composition may maximize metabolic benefits. To this point, Hjorth and colleagues found a 
Nordic diet yields greater body fat loss among individuals characterized by dominant Prevotella 
genera compared to Bacteroides-dominant microbiomes, illustrating differences in 
responsiveness to diets based on dominant taxa (39). In view of this, Precision or Personalized 
Nutrition (PN) seeks to customize dietary interventions for obesity by accurately predicting 
metabolic responses. PN utilizes dietary interventions predominantly focused in (1) increasing
fiber intake, (2) restricting caloric intake, or (3) adding pre- and pro-biotics (47). These tactics resemble generalized interventions, however, PN evaluates individual past responses to dietary interventions to develop a new diet plan, rather than the conventional one-size-fits-all approach. PN often evaluates microbial response (e.g., increased abundance of \textit{Prevotella}), changes in body weight, body composition, BMI, fat percentage, and other indicators of health such as glycemic response (48). Prior works have documented the accuracy and efficiency of algorithms and machine learning in large-scale implementation of personalized dietary interventions (49). One such study developed an algorithm that significantly predicted blood sugar levels in response to prospective diet plans, over and above predictions formulated from dietary assessments such as glycemic index (50). Though microbial response is measure of PN intervention success, PN is still widely unexplored within the realm of the microbiota-gut-brain axis. Additionally, more long-term studies are needed to examine long term dietary change impacts on the core gut microbiota composition and corresponding biochemical profile regarding the gut-brain axis components (e.g., hunger hormone levels and inflammatory cytokines) in the context of obesity and T2D.

Additional Practices: FMT, Bariatric Surgery, Pharmacology

\textbf{Fecal Microbial Transplant}

Standard fecal microbiota transplant (FMT) in humans involves transferring intestinal microbiota from a donor to a recipient, typically via colonoscopy. A successful FMT is defined as the establishment of a donor-like microbiome in the recipient. FMT from a research perspective has enabled “humanization” of rodent models for mechanistic investigations of the human gut microbiome. This method has elucidated transferrable behavioral phenotypes and has
linked gut microbial composition to metabolic disorders (4). Hartstra and colleagues found donor FMTs from Roux-en-Y gastric bypass (RYGB) donors were capable of mediating the microbiota-gut-brain axis in humans with obesity. Those receiving the FMT exhibited altered dopamine and serotonin transporters and alterations in gut microbiota composition, underscoring the potential role of FMT in treating obesity by targeting the microbiota-gut-brain-axis (1).

However, FMT has primarily been used thus far in the treatment of *Clostridium difficile* infection, though it holds significant promise for the treatment of other GI-related diseases and conditions. Further, autologous FMT, in which a patient has their fecal matter saved prior to surgery and receives their own “healthy” microbiota during their recovery, is considered a potential tactic to mediate individual changes in gut microbiota composition (4). Despite its currently limited clinical application, optimized FMT interventions remain a research focus for future use in the medical field. Examples of such optimization is the use of broad-spectrum antibiotic cocktail pretreatments, which provide FMT-administered microbes a less competitive environment by depleting the recipient’s gut microbiota and increasing FMT efficacy (4). The impact of FMT on microbiota-gut-brain axis components needs to be established.

**Bariatric Surgery**

Bariatric surgery is currently regarded as the most effective treatment for significant and sustained weight loss in severe obesity (20). Multiple bariatric surgeries currently exist with RYGB, adjustable gastric banding, and sleeve gastrectomy being the most common (3). The BRAVE effect (alterations in bile flow and gastric size, anatomical changes, vagal nerve
adaptations, and enteric gut hormone modifications) results from each type of bariatric surgery through a combination of anatomical rearrangements and changes to the digestive tract (20). Evidence suggests bariatric surgery can impact host metabolism, gut hormone secretion, and insulin sensitivity via alterations in gut microbiota composition and resulting alterations in SCFA production (51). Moreover, bariatric surgery can modify hormonal secretion and inflammation, thereby reducing adiposity, improving insulin sensitivity, and increasing microbiota diversity (20). Human bariatric surgeries show increases in Gammaproteobacteria and Verrucomicrobia (Akkermansia), while abundance of Firmicutes has consistently decreased (20).

In addition to compositional changes in the gut microbiota, the modulation of enteric hormones significantly influences the microbiota-gut-brain axis. Common modifications include reductions in ghrelin and increases in GLP-1 and PYY. RYBG has been shown to specifically increase GLP1 and PYY levels, while sleeve gastrectomy is associated with decreased ghrelin (20).

Antibiotic use prior to bariatric surgery is a common practice but has the potential for both short-term and long-term impacts on the gut microbiota (20). Additionally, targeting the gut microbiota with probiotics, post-surgery, is thought to increase bacterial diversity and further benefit the host (51). Mouse models have shown a connection between improved outcomes in diabetes and obesity post bariatric surgery and have suggested probiotic may be an effective strategy to improve obesity-related disease after bariatric surgery. However, this connection is understudied and has not yet been shown in humans (20).

Studies examining the impact of bariatric surgery on the gut microbiome are often confounded by the changes in diet accompanied by such procedures. Bariatric surgeries have been associated with changes in dietary intake and food preferences. Furthermore, RYGB has
been associated with a reduced preference for high-fat or high-sugar foods, yet the cause of these changes remains inconclusive (20).

Therefore, not only does bariatric surgery provide the most significant weight loss outcomes, but it can also influence the microbiota-gut-brain axis. Bariatric surgery remains an important tool in addressing obesity and may provide a new perspective into the role of the gut microbiome in metabolic health.

**Pharmacology**

In addition to antibiotics, many non-antimicrobial drugs also influence gut microbiota composition, such as various hormones, antidepressants, antihistamines, among others. Forty-four drug categories were associated with impacting the gut microbiota, including metformin, statins, and laxatives (52). Furthermore, the largest variance in fecal microbiome in healthy populations was attributed to medications in a combined analysis of the Belgian Flemish Gut Flora Project (N = 1106) and the Dutch LifeLines-DEEP (N = 1135) cohorts (53). Current anti-obesity medications have also shown profound impacts on the gut microbiome composition, but they remain underexplored in regard to the microbiota-gut-brain axis. Medications that often cause weight gain also cause variations in the gut microbiota (4). Probiotic/prebiotic interventions could be explored as avenues for circumventing the negative side effects of medications on the gut microbiota and weight status (4). While the microbiota-gut-brain axis remains a promising target for polypharmacy and targeted drugs to intervene in the pathology of obesity, current evidence is generally limited to animal models. Thus, well designed clinical trials targeting the microbiota-gut-brain axis with pharmacological approaches is warranted and the impact of current anti-obesity drugs on the gut microbiome should be further explored.
Conclusion

The microbiota-gut-brain axis provides an untapped potential target for therapeutic interventions in obesity. From a genetic standpoint, the gut microbiome is vastly larger than the human genome and is modifiable by a wide array of factors (54). However, evidence is insufficient to identify the temporal associations between gut microbiota and obesity and the relationship appears bidirectional (20). Nevertheless, it is necessary to investigate the effects of predominant obesity prevention and treatment methods on the gut microbiota and gut-brain-axis to understand mechanistic links and increase efficacy, and eventually effectiveness, of interventions. Current microbiota research is too limited to independently translate into clinical applications and inform recommendations. The future goals for this field include expanding reach in diverse populations with a shift in focus to functional capacity of the gut microbiota to obtain a deeper characterization of the microbiota-gut-brain axis in obesity. Future research should also be proactive in including vulnerable populations often underrepresented in research, who bear the strongest burden of obesity-associated metabolic diseases.
Chapter 2

Abstract

South Asian refugees experience a high risk of obesity and diabetes yet are often underrepresented in studies on chronic diseases and their risk factors. The gut microbiota and gut permeability, as assessed through circulating lipopolysaccharide binding protein (LBP), may underlie the link between chronic inflammation and type 2 diabetes (T2D). The composition of the gut microbiota varies according to multiple factors including demographics, migration, and dietary patterns, particularly fiber intake. However, there is no evidence on the composition of the gut microbiota and its relationship with metabolic health in refugee populations, including those migrating to the United States from Bhutan. The objective of this study was to examine glycemic status in relation to LBP, systemic inflammation fiber intake, and gut microbiota composition in Bhutanese refugee adults residing in New Hampshire (n=50). We identified a substantial chronic disease burden in this study population, and observed a correlation between glycemic status, LBP, and inflammation, and a correlation between glycemic status and gut microbiome alpha diversity. Further, we identified a correlation between proinflammatory taxa and inflammatory cytokines. Short-chain fatty acid (SCFA)-producing taxa were found to be inversely correlated with age, while proinflammatory taxa were found to increase. The findings of this study highlight areas for future investigations of inflammation and glycemic impairment, in addition to informing potential future interventions targeting this vulnerable population.

Introduction

The gut microbiota has been identified as a key mediator of cardiometabolic health with implications in inflammation and glycemic control. Moreover, alterations in human gut
microbiota composition, known as dysbiosis, are associated with increased risk for type 2 diabetes (T2D) (55). Gut dysbiosis often leads to intestinal hyperpermeability and increased plasma levels of lipopolysaccharide (LPS), defined as metabolic endotoxemia (56,57). This pathway is likely responsible for low-grade inflammation exhibited in T2D, (58) which is characterized by increased secretion of pro-inflammatory cytokines, including tumor necrosis factor alpha (TNF-α) and interleukin (IL) 6, and acute phase proteins such as C-reactive protein (CRP) levels into systemic circulation (56). Fiber intake has been highlighted as a key modulator of gut microbiota composition and its production of metabolites, which can impact intestinal permeability, inflammation, and glycemic control (59). However, more evidence is necessary to fully elucidate specific microbe-host interactions that contribute to the pathology of metabolic diseases and potential modifiable risk factors, specifically in human models.

Microbial composition is population-specific and varies drastically across ethnic groups, demographics, lifestyles, (1) environmental factors, and migration (60,61). Chronic disease risk tends to be higher for refugee populations in the US compared to non-Hispanic White (NHW) populations (Kumar et al., 2021). The Bhutanese refugee population in NH not only faces higher rates and risk of T2D (63–65) but is underrepresented in current research on the gut microbiome. Moreover, South Asian adults, including those from Bhutan and Nepal, experience a higher prevalence of prediabetes and T2D as compared to non-Hispanic White, African American, and Hispanic/Latino adults (66). The objectives of this paper were to quantify the cross-sectional associations between glycemic status and inflammatory markers (IL-6, TNF-α, LBP), fiber intake, and the microbiome (richness, diversity, composition) among Bhutanese refugee adults. We hypothesized that T2D and poor glycemic control are characterized by higher inflammation and LBP concentrations, lower microbial richness, and lower fiber intake in this population. This
work addresses the significant burden of metabolic diseases among vulnerable populations and shows unprecedented results on the associations between the gut microbiota, inflammation, and glycemic status in this refugee population.

Materials and Methods

Study Population

Participants were recruited in collaboration with a non-profit community-based organization, Building Community in New Hampshire (BCNH), in the areas of Manchester and Concord, New Hampshire (Figure 3). Fifty-four individuals from a convenience sample of culturally insulated adult Bhutanese refugee were recruited as part of a previously completed study led by one of the co-authors (Bigornia). The original study criteria included Bhutanese refugee adults that were 18 years or older and resource limited. The latter was determined by eligibility to receive SNAP (Supplemental Nutrition Assistance Program) benefits. Participants were excluded if they indicated moving within 2 months, being pregnant or trying to become pregnant, or prescribed antibiotics within the past 6 months. After screening, 50 participants were included in this cross-sectional study. All the participants signed informed consent. Recruitment, consent, and data collection were conducted by a trained community health worker who identified as Bhutanese refugee and spoke Nepali fluently. The research protocol was approved by the University of New Hampshire Institutional Review Board (IRB #8042).
**Dietary Intake**

Three supervised 24-hour recalls were conducted on non-consecutive days by a bilingual and bicultural community health worker trained to collect dietary information. Participants were instructed in person on how to report their dietary intake and estimate portion sizes. The community health worker directly entered 24-hour recall data into Nutrition Data System for Research (NDSR) (version 2019, Minneapolis, MN). This software package and nutrient database were used to estimate nutrient intakes based on 24-hour recall food weights. Fiber consumption as well as other nutrients and foods were averaged across the three 24-hour recall days.

**Blood Sampling, Processing, and Biomarker Assessment**

After a 12-hour fast, a sample of approximately 35 mL of blood was collected by a trained phlebotomist, using EDTA (14 mL total), lithium heparin (10 mL), and serum (10 mL) vacutainers. All vacutainer samples were transported on ice to the University of New Hampshire (UNH) and centrifuged at 3,000 RPM for 10 minutes. Post centrifugation, samples were aliquoted into 0.5 mL portions into six 2 mL cryotubes and frozen at -80°C. TNF-α, IL-6, and insulin were measured via enzyme-linked immunosorbent assays (ELISA). High sensitivity CRP was calculated using a clinical chemistry analyzer. Glycosylated hemoglobin (HbA1c) was measured from whole blood samples using a Siemens DCA Vantage analyzer. Fasting glucose was measured using a clinical chemistry analyzer. Homeostasis model assessment-estimated insulin resistance (HOMA-IR), a measure of insulin resistance, was calculated by fasting serum insulin (μIU/ml) × fasting plasma glucose (mg/dL)/405, with a higher value indicating a higher degree of insulin resistance. Participants were classified as having T2D if they met any of the
following criteria: self-reported diabetes, HbA1c of 6.5% or higher, or use of a diabetes medication. Prediabetes was defined as $6.5\% \geq \text{HbA1c} \geq 5.7\%$ (67). LBP was measured using an electrochemiluminescence technology (MesoScale Discovery). Briefly, carbon electrodes of the assay plates bind biological reagents attaching biomarkers from participant serum samples. Biomarker concentrations are measured as light intensity emitted from electrochemiluminescence labels conjugated with detection antibodies as electricity is applied to the assay plate. This method allows for high sensitivity, broad dynamic range, and reduced time compared to the traditional ELISA method.

**Fecal Sampling and Processing**

Fecal samples were collected in DNA preservation solution and transported at room temperature. DNA from fecal samples was extracted using Zymo Research Quick-DNA Fecal/Soil Microbe kits. Extracted DNA was sequenced by the Hubbard Center for Genome Studies (University of New Hampshire) using shallow shotgun metagenomic sequencing (Illumina NovaSeq) (68).

Raw sequences were processed using the Metagenome-atlas snakemake workflow (69). The workflow conducted quality control processes using the *BBtools* package. PCR duplicates were removed (clumpify). Adapters were removed and reads were trimmed and filtered (BBduk). Host contamination was removed (BBsplit) based on a masked HG19 reference. Quality controlled sequence files (fastq files) were used in subsequent components of the atlas workflow and in further pipelines. *Sourmash gather* (scaled 1000) was used to generate FracMinHash sketch from the samples and the GTDB rs207 full reference database (70). *Sourmash taxonomy* provided taxonomic lineage information for *Sourmash gather* results (71). Results were then
converted into a phyloseq object for further analyses and processing using the pipeline by Callahan (72). Rarefying or proportions were not used to normalize data for sequencing depth (73). All richness and diversity measures were calculated using the raw, unfiltered taxa data. The observed species richness was calculated by counting the number of unique species in each sample. Only phyla prevalent in 5% or more samples were kept in filtered count data. To calculate relative abundance of particular genus and species, the metagenomic species table was further filtered to only include species with a mean greater than $10^{-5}$ and two data frames were agglomerated, one to the species and one to the genus levels. Counts were converted to relative abundance and normalized to the median sequencing depth. Relevant taxonomic groups were identified, and the relative abundance of given groups were extracted. The species *Clostridium coccoides*, *Ruminococcus productus*, *Clostridium cocleatum*, *Bifidobacterium catenulatum*, *Eubacterium hallii*, and *Akkermansia municiphila* were excluded as they were not present in the filtered dataset.

**Other Variables**

Demographics and additional health information were collected via an in-person survey. Participants self-reported having a history of heart attack, heart disease (other than heart attack), and or stroke. Those who reported experiencing any of these health conditions were categorized as having cardiovascular disease (CVD). Validated methods were followed for calculating physical activity (PA) score and food security score (FSS) (74). PA scores are interpreted as low (<30), moderate (30–39), or high (40+) (74). FSS was dichotomized into < 3 food secure and $\geq$ 3 food insecure. Anthropometrics, height and weight, were obtained during the study visit and body mass index (BMI) was calculated as kg/m$^2$. Overweight was defined as a BMI $> 23$ kg/m$^2$. 
and obesity as a BMI ≥ 25 kg/m² (75). Medication use, smoking status, years in the US, household size, and high school completion were all obtained via in-person survey. Differences in age, sex, cardiovascular disease (CVD), BMI, smoking status, medication use, years in the US, PA score, household size, FSS, and high school completion were compared according to T2D status. Age and sex were used as covariates in all analyses.

**Statistical Analysis**

Analyses were conducted using SAS 9.4 (Cary, NC) and R. Between group differences were assessed using parametric (ANCOVA, t test) and non-parametric (Wilcoxon, Fishers Exact) analyses depending on data normality. HOMA-IR and HbA1c followed non-normal distributions and were log transformed for linear regression analyses. Spearman correlations were used to examine continuous variable associations. The Benjamin Hochberg method was used to adjust for multiple testing in the microbiome analysis. All models included the covariates age and gender. The rationale for this is that the adult gut is relatively stable until the process of aging and disruption of homeostatic control diminishes the stability. Older age is accompanied by increased proinflammatory status and decreasing adaptive immunity. Aging can influence the gut microbiota through its various impacts of gut function including gastric motility, hypochlorhydria, and changes in the enteric nervous system (4). Further, risk factors of chronic disease and microbiota composition vary by sex (76). Additionally, fiber consumption levels often vary between sexes (77). Further, linear regression was used to assess predictors of diabetes markers and logistic regression was used to predict T2D, with age and sex as covariates (Table 1).

Alpha diversity was compared according to T2D status using Wilcoxon rank sum test. Spearman correlation analysis was used to quantify the relationship between observed species
richness and other markers of glycemic status and inflammation. The relative abundance of fecal pro-and anti-inflammatory bacteria and SCFA-producing bacteria, identified through a literature review, was compared by glycemic status. Additionally, the relative abundance of fecal pro-and anti-inflammatory bacteria and SCFA-producing bacteria was correlated to multiple biomarkers and demographic characteristics using a spearman correlation matrix. All correlations were subsequently corrected using the Benjamini-Hochberg method.

Results

Population Characteristics and Health Behaviors

The median (interquartile range, IQR) age of the 50 participants (82% female) was 49.5 (24.0) years (Table 2). Forty-two percent of the participants were classified as having T2D and 10% as having cardiovascular disease (CVD), excluding hypertension. The prevalence of prediabetes was 34% in the non-T2D group, with individuals having an median (IQR) HbA1c of 5.6 (0.5) %. The median BMI was 27 (5.4) kg/m² and 92% of participants were categorized as being overweight or having obesity. Eight percent of participants reported habitual smoking. At the time of data collection, participants had spent a median of 8 (4) years in the US and 14% completed high school. The median physical activity score was calculated as 27 (3.3) (i.e., moderate, on average). Although all participants were SNAP-eligible, 90% of them reported a food security score of < 3, which corresponds to food security (74).

The prevalence of overweight and obesity for participants with T2D was 90% and 95.2% for participants without T2D, but the median BMI was not significantly different between both groups. The median age of the T2D group, 58 (21) years, was notably higher than the non-T2D group, 45 (22) years (p=0.014) (Table 2). Both groups showed comparable years lived in the US
and physical activity level. Fiber intake and whole grain consumption were significantly higher in the T2D group (p=0.035 and p=0.028 respectively). No misreporters were identified upon application of the Mifflin equation or cutoffs for implausible dietary intake (data not shown) (Willett, 1998).

**Glycemic Status and its Correlation with Inflammation and LBP**

Although the T2D group had significantly higher median HbA1c and glucose compared to the non-T2D (both p<0.001), 34.5% of participants without T2D qualified as having prediabetes (Table 3). Among all participants, 28%, 30%, 8%, and 26% reported taking metformin, statins, non-steroidal anti-inflammatory drugs (NSAIDs), or proton pump inhibitors (PPIs), respectively. Metformin and statin use were higher in the T2D group, 66.7% and 52.4% respectively, as compared to the non-T2D group 0% and 13.8%, respectively (Table 2).

Lipids, excluding triglycerides, were lower in the T2D group, potentially as a result of pharmacological therapy. Even though there were no differences in inflammatory cytokines, CRP, or LBP between the T2D and non-T2D groups (Table 3), CRP had a weak positive correlation with HbA1c (Rho=0.39, p=0.006) and FPG (Rho=0.31, p=0.033). LBP was also moderately correlated with HbA1c (Rho=0.42, p=0.003) and FPG (Rho=0.42, p=0.003) (Table 4), and only weakly correlated to HOMA-IR (Rho=0.35, p=0.016) (Table 4). Inflammatory cytokines and dietary intake measures were not associated with glycemic status (Table 4). Regression models used to assess predictors of T2D are shown in Table 5. Age was the only significant predictor of T2D in any of the logistic regression models (Table 5). A one unit increase in LBP was associated with a 27% increase in HOMA-IR (p=0.004) in model 4 and a
4% increase in HbA1c (p=0.010) in model 5 (Table 6). Both models included observed species richness, age, and sex as covariates.

**Association between gut microbiota composition and glycemic status**

Observed species richness and alpha diversity measures were significantly higher in the non-T2D group than the T2D group (Figure 3). Specifically, observed species richness, Shannon, and Fisher diversity measures were statistically significant (Table 7).

The most abundant phyla were *Firmicutes, Bacteroidetes,* and *Actinobacteria.* No significant differences in relative abundance of proinflammatory or SCFA-producing species were identified according to T2D status. Age was positively correlated with *Lactobacillus* (Rho=0.333, p=0.018), *Veillonella* (Rho=0.325, p=0.021), and *Bacteroides* (Rho=0.306, p=0.031) and negatively correlated with *Clostridium* (Rho=-0.292, p=0.040), *Faecalibacterium* (Rho=-0.386, p=0.006) (Figure 5A), and the species *Faecalibacterium prausnitzii* (Rho=-0.374, p=0.007) (Figure 5B). Given the differences in fiber intake, microbiome composition, and glycemic status according to age, a partial correlation analysis adjusting for age was conducted (Supplementary Figure 1).

Pro-inflammatory genera *Parabacteroides* (Rho=0.339, p=0.017) and *Bacteroides* (Rho=0.363, p=0.010) were positively correlated with the inflammatory cytokine TNF-α, while anti-inflammatory *Prevotella* was negatively correlated (Rho=-0.320, p=0.010) with TNF-α. After adjusting for age, all associations with TNF-α remained significant. Observed species richness was negatively correlated with FPG (Rho=-0.280, p=0.049) and HbA1c was positively
correlated with *Phascolarctobacterium* (Rho=0.293, p=0.039). These correlations between glycemic status and taxonomic groups remained significant after adjusting for age.

When considering dietary fiber, positive correlations were identified between *Lactobacillus* and whole grains (Rho=0.307, p=0.030), Bacteroides and whole grains (Rho=0.364, p=0.009), and *Clostridium* and insoluble fiber (Rho=0.295, p=0.037). Furthermore, *Clostridium* was negatively correlated to FPG (Rho=-0.293, p=0.039) and HbA1c (Rho=-0.300, p=0.035). However, after adjusting for age, *Clostridium* became positively correlated to insoluble fiber (Rho=0.356, p=0.013) and total fiber (Rho=0.323, p=0.025).

**Discussion**

This work shows the characterization of glycemic status in relation to clinical risk factors, with an emphasis on inflammation and metabolic endotoxemia, dietary intake, and gut microbiota composition which, to our knowledge, is unprecedented in Bhutanese refugee adults in the US. The chronic disease burden of this study population was substantial, with particularly high rates of overweight/obesity, prediabetes, and diabetes. Glycemic impairment (e.g., higher HOMA-IR and HbA1c) was characterized by higher levels of CRP and LBP, while diet and inflammatory cytokines were unrelated to glycemic markers. Observed species richness, Shannon, and Fisher alpha diversity were higher in the non-T2D group than the T2D group.

There is a strong impetus to better characterize the gut microbiota in relation to health outcomes in vulnerable populations that are underrepresented in research. Many Nepalese-speaking Bhutanese individuals have resettled in the United States due to political, social, and economic restrictions in the 1990s and a lack of successful integration in Nepal (Krause et al., 2021). This population qualified as living in an isolated social enclave. Eighty-six percent of the
convenience sample had not completed high school and all participants qualified for SNAP benefits based on household income. This situation has placed this population, and other similarly vulnerable populations in the US, at a particularly higher risk for malnutrition and chronic disease.

Limited studies exist on obesity and related chronic health conditions in Bhutanese refugee adults in the United States. Previous studies focused primarily on infectious diseases and dietary deficiencies (79). This study identified a substantial chronic disease burden among this convenience sample of Bhutanese refugees, with an alarming prevalence of overweight/obesity comparable to the US national average (80). To compound the comparable weight status measures, South Asian populations have a higher risk of cardiometabolic diseases at lower BMIs than other ethnic groups (79). Furthermore, the prevalence of overweight/obesity in this study (92%) was higher than other studies in Bhutanese refugee communities in the US (64). Given the high prevalence of obesity, greater attention to culturally relevant, economically feasible interventions and education are warranted in the Bhutanese refugee community, specifically for those who are among SNAP eligible groups. Evidence emphasizes the need for relevant lifestyle and dietary change education programs and interventions to address the overweight/obesity prevalence among this vulnerable population (79).

The proportion of diabetes was higher among the study participants (42%) than the general US population (8.2%), and surpassed prevalence measures in other US Bhutanese refugee communities (6-14%) (81,82). Additionally, a high prevalence of prediabetes was identified in the non-T2D group. South Asian populations are at a higher risk of insulin resistance and T2D than non-Hispanic white populations, yet to our knowledge this is the first study to have explored inflammatory markers in relation to diabetes in the Bhutanese refugee
population (75). Consistent with previous findings, CRP, an indicator of systemic inflammation, was correlated with diabetes markers (83). Additionally, LBP, was associated with HbA1c and FPG, and weakly with HOMA-IR. LBP was also found predictive of HOMA-IR and HbA1c, replicating trends in previous studies exploring LBP and diabetes (58). It is unclear whether LBP increases as a result of endotoxemia or systemic inflammation present in chronic diseases, but it is often used as a proxy for endotoxemia or impaired gut barrier integrity (58). Findings suggest a potential connection between gut function, inflammation, and glycemic markers. The variation in LBP concentrations in our study was small, which could be attributed to an insufficient number of metabolically healthy participants and high proportion of prediabetes in the non-T2D group. Studies with a greater range of metabolic profiles may find stronger correlations between inflammation indicators and diabetes markers.

Inflammatory cytokine concentrations were unexpectedly not associated with diabetes markers. Given that obesity is associated with low grade inflammation, characterized by elevated inflammatory cytokines, which in turn may induce systemic insulin resistance (84), a potential explanation for these finding may be that the high proportion of overweight and obesity in both the non-T2D group and T2D group, and the high prevalence of pre-diabetes in the non-T2D group (85,86) may have attenuated expected differences in cytokines according to glycemic status.

Dietary intake, including fiber and whole grain consumption, was not associated with glycemic impairment. Specific dietary components, including red meat consumption, have been shown to influence chronic disease in Bhutanese refugee adults in the US (79). Increasing fiber intake has been suggested to reduce the risk of T2D, however this relationship has not been studied extensively within the US Bhutanese refugee population (87). Median intake and whole
grain consumption were higher in the T2D group (p=0.0354 and p=0.0279 respectively). However, higher fiber intake may be due to reverse causality of a T2D diagnosis, which may translate into high motivation for nutrition and health education, providing a pivotal opportunity for nutrition interventions (88).

Studies suggest that at least 5 years after resettlement and subsequent acculturation precede higher risk of chronic disease (89). The median time spent in the US for the study population was 8 (4) years, providing sufficient time to see the impact of US acculturation among this population. However, previous studies, such as one including Ohio Bhutanese refugee women, have failed to find associations between chronic diseases and length of time in the US (79). Our findings indicate no correlation between years in the US and T2D prevalence. Kumar et al suggest that chronic disease risk factors may develop at refugee camps and associated lifestyle changes before relocation to the US (64). Irrespective of the origin of such chronic diseases, interventions are needed to address the significant proportion of Bhutanese refugee adults succumbing to risk factors of chronic disease and those already suffering from various chronic diseases.

Utilizing strengths of shallow shotgun metagenomic sequencing, this study provided a thorough characterization of the gut microbiota composition and its capabilities in relation to T2D and inflammation. The T2D group was expectedly characterized by lower average observed species richness and alpha diversity measures. Although inconsistently observed, low microbial richness has been associated with obesity, insulin resistance, and low-grade inflammation (90). Low richness and diversity have been mostly identified as markers of gut dysbiosis. However, we did not observe an association between richness, inflammation, or LBP (56,57). The
underlying explanation for these unexpected results could be also attributed to the high prevalence of overweight, obesity, and glycemic impairment in this population.

Additional outcomes were aligned with the hypothesis that inflammation and glycemic impairment are correlated with gut microbiome composition in this population. *Bacteroides* has been suggested to dominate gut profiles in chronic disease and inflammatory states, which is consistent with our observed correlation between the genera *Parabacteroides* and *Bacteroides* and TNF-α (90).

Age appeared to be the strongest characteristic associated with gut microbiota composition in this population. This study observed negative correlations with SCFA-producing genera and species (*Faecalibacterium* and *Faecalibacterium prausnitzii*). SCFA-producing potential typically decreases as individuals age and the gut composition alters over time (91). Additionally, *Bacteroides* and *Lactobacillus* were positively correlated with age (91). However, in this study the T2D was significantly older than the non-T2D group. Given that over a third of the participants in the non-T2D group have prediabetes, age may mask differences in composition attributed to the disease progression of T2D.

While culturally competent health treatments are often inaccessible to Bhutanese refugee communities in the US (88), Krause found that yoga and mindfulness activities were perceived as medicinal and therapeutic by US Bhutanese refugee adults (88). Stress and physiological states have been shown to influence the gut microbiota bidirectionally, suggesting a potential role for mindfulness interventions to influence this bidirectional relationship (92). Exercise broadly has also been well documented to influence the gut microbiota (92). Bhutanese refugee populations could benefit from these types of interventions, which would also support the cultural identity and social support of these communities (88,92). Further, studies highlight a
need for culturally appropriate and feasible dietary interventions that recognize the importance of dietary practices as part of cultural identity (79). An emphasis of the mind-body connection is essential in supporting the ideas and values that are the foundation of the Bhutanese refugee community (88). Thus, culturally appropriate interventions that emphasize mind-body connections should be explored in this and other underrepresented populations, particularly ones that value the synergies among the body, mind, and health.

**Strengths and Limitations**

To date, this study has been the most comprehensive examination of fiber intake, inflammation, and glycemic status in Bhutanese refugee adults in NH. Three 24-hr recalls, the gold standard for dietary assessment, were utilized to assess fiber intake, reducing the chance of misclassification errors. Additionally, participant-facing interactions and data collection were conducted by a bilingual and bicultural community health professional.

Despite the strengths of our study, several limitations should be noted. Utilizing a convenience sample was necessary for the feasibility of this study, however, it may limit the generalizability of results. Further, the relatively small sample size of n=50, could have contributed to type 2 error. A larger and more representative sample is recommended for future studies on the Bhutanese refugee population. Another limitation to this study was the inability to account for medication use in our statistical analysis, which may explain some unexpected findings and potentially confounded the analysis of the microbiome. Medication use was not an exclusionary factor in this study thus many participants were on a variety of medications targeting their chronic diseases or risk factors, including Metformin, Statins, NSAIDs, and PPIs. Unexpected results in lipid profiles are likely attributable to medication use, as a large proportion
of the participants with T2D were taking statins, which lower lipid levels (93). In addition to clinical biomarkers, medication use may have also confounded gut microbiome composition and function. Metformin, Statins, NSAIDs, and PPIs are known to potentially influence the gut microbiome and glycemic impairment (83,93,94). Accounting for medication use in the future is warranted to reduce any confounding and simplify interpretation of results. Shallow sequencing introduced additional bias in microbial analyses results through elimination of rare taxa and a focus on only the most abundant taxonomic groups (68). Utilization of deeper whole genome sequencing is recommended for future analyses of the gut microbiome composition and functional potential.

Conclusion

To date, this is the most comprehensive examination of metabolic health, diet, and the gut microbiome in a Bhutanese refugee population in NH. Bhutanese refugee adults are at an increased risk of chronic diseases, such as T2D and population specific interventions are necessary to mediate the risk. Findings from this study highlight the need to investigate culturally relevant interventions to address chronic diseases and their risk factors. Future studies and interventions should focus on approaches to reduce chronic inflammation among this population with culturally tailored dietary and lifestyle changes.
Chapter 3

Introduction

Obesity and type 2 diabetes (T2D) are complex diseases related to a multitude of risk factors stemming from lifestyle and environmental characteristics. Dysbiosis, or an altered gut microbiota composition with low richness and diversity as its main traits, has been consistently observed with obesity, but inconsistently with T2D. Chronic diseases are evidently intertwined with the gut microbiome, however, the exact mechanisms, particularly in regard to T2D, are not fully elucidated. Chronic inflammation, hunger and satiety regulation as part of the microbiota-gut-brain axis, and the structure of microbial communities are three pathways potentially underlying the development of T2D.

Low-grade inflammation characteristic in chronic diseases has been partially attributed to features of the gut microbiome as well as factors that may be indicative of gut permeability, such as decreased short-chain fatty acid (SCFA) production from fiber fermentation, and increased levels in circulation of lipopolysaccharide (LPS), which is a component of the gram negative bacterial wall (95). Further, lipopolysaccharide binding protein (LBP), a human acute phase protein that binds to LPS to initiate immune responses, is a biomarker of gut permeability previously associated with insulin resistance (96). However, the mechanisms linking gut permeability with chronic inflammation seen in T2D have not been fully elucidated.

The microbiota-gut-brain axis, a bidirectional relationship between the gut and brain that is mediated by gut microbes, plays a role in metabolic regulation, including glycemic control and modulation of hunger and satiety hormones (97). Leptin and brain derived neurotrophic factor (BDNF) are components of the microbiota-gut-brain axis pathway and are involved in appetite
suppression and energy regulation (98), but their association with gut microbiome composition and function in the context of human T2D has not been established.

Dietary intake is a driving force of microbiome composition and a modifier of inflammatory and gut-brain axis processes (99). Fiber-containing foods provide the fuel for microbial fermentation to produce SCFA and are important for intestinal barrier function and glycemic control (55,100,101). However, the intersections of fiber and T2D with respect to the functional potential of the gut microbiome has yet to be thoroughly explored.

Highlighting the taxonomic composition of the gut microbiome has become a standard practice, particularly using 16S sequencing, to characterize the human gut microbiome. However, with the advancement of multi-omics technology, new methods are emerging to expand upon the characterization and understanding of microbiomes (102). Whole genome sequencing enables the exploration of the functional potential of microbes, i.e., the genetic potential to produce proteins, through the processing and annotation of microbial DNA sequences (103). These new analytical approaches are enhancing our understanding of the gut microbiome beyond the presence of microbes to the functions and metabolic processes in which they may be participating.

While the relationship between the gut microbiome and T2D is multifaceted, it is also highly population-specific (104). South Asian populations in the US, and in particular refugee groups, are disproportionately impacted by chronic diseases and underrepresented in research on the gut microbiome. This burden extends to the Bhutanese refugee population in New Hampshire, which is one of the largest refugee populations in the state (66,79). Previously, in a convenience sample of 50 Bhutanese refugee adults, we determined that 92% had overweight or obesity and 42% had T2D (105). Our findings indicated significantly lower microbial richness
and species diversity (Shannon and Fisher indexes) among those with T2D (105). Markers of glycemic status, including hemoglobin-A1c (HbA1c), homeostatic model of insulin resistance (HOMA-IR), and fasting plasma glucose (FPG), were all correlated with lipopolysaccharide binding protein (LBP) (105). It is important to note that some individuals with T2D in this sample were taking metformin (n=14), a T2D medication known to impact the gut microbiome composition (94). These results highlighted the interconnectedness of glycemic status, inflammation, and diversity of the gut microbiome. However, a deeper characterization of the functional potential of the gut microbiome is warranted to provide key insights into the specific functional features that may play a role in observed associations with the gut microbiome and T2D.

The objective of this project is to explore the potential for LPS and SCFA production of the gut microbiome in relation to glycemic status and inflammation among Bhutanese refugee adults. This work will also address potential lifestyle mediators of these relationships, including fiber intake and metformin use. These findings will address the gap in representation of refugee groups in microbiome analyses via a thorough characterization of the genetic functional potential with whole genome sequencing. Further, this investigation will better elucidate the specific features of the gut microbiome involved in associations with glycemic status and inflammation.

Methods

Study Design & Population Characteristics

The study design and population characteristics are as described in Moser et al. (105). Briefly, this cross-sectional study included a convenience sample of 50 Bhutanese refugee adults residing in NH from a previously conducted study by one of the thesis committee members.
(Bigornia), which provided health questionnaire data, dietary intake, fecal samples and fasting blood samples.

**Biomarker assessment**

LBP, Leptin, and BDNF were measured via electrochemiluminescence immunoassays per Moser et al. (105). Samples were assessed in duplicate and standard curves were generated to determine concentrations in serum samples.

**Fecal microbiome sequencing and analysis of composition**

Fecal samples were collected, DNA was extracted, and sequences were processed as described in Moser et al. (105). This study included shallow whole genome shotgun sequences (NovaSeq 6000), with a median sequencing depth of 1.9M reads. The individual relative abundance of taxonomic groups at the family level was used. Beta diversity (Bray-Curtis, Aitchison) was calculated via the MicroViz R package (105–107) to compare microbial communities according to T2D status.

**Fecal microbiome functional Potential: PALADIN Processing**

The functional potentials of the gut microbial communities were produced using the Protein Alignment and Detection Interface (PALADIN) pipeline (108). Only post quality control R1 reads were utilized in this pipeline and outputs included all UniProt protein ID identified in each sample. PALADIN was run against the UniRef90 database and all samples aligned >88% total detected open reading frame sequences (109). UniProt protein ID outputs were then filtered for max quality of ≥ 20 and Eukaryotic proteins were removed. UniProt protein IDs, i.e.,
annotated protein-coding genes, were compiled for each sample and exported for further downstream analyses in RStudio.

**Fecal Microbiome Functional Richness and Diversity**

Functional richness and diversity measures (Shannon, Simpson, Fisher) were calculated in RStudio using the phyloseq pipeline and based upon unique UniProt protein IDs (110). Richness and diversity measures were compared according to T2D status via Wilcoxon rank sum test and inflammatory, glycemic, and satiety markers via Spearman correlations with BH correction.

**Fecal Functional Potential According to T2D Status: Corncob Analysis**

Lists of LPS and SCFA related UniProt proteins IDs (SwissProt reviewed and/or annotation score ≥4/5) were compiled via keyword searches (LPS, lipopolysaccharide, short chain fatty acids, short chain fatty acid biosynthesis) on the UniProt database. Lists of associated Uniprot protein IDs were identified in the study population fecal microbiome data (109). The dataset containing the counts of identified UniProt protein IDs were used in subsequent analyses. Proteins IDs found in <5% of samples were excluded. Corncob was used for the targeted differential abundance analyses of the UniProt protein IDs utilizing the Wald test and correcting p-values with BH adjustment. Corncob is an individual regression model that assesses differential abundance irrespective of sequencing depth, excessive zeros, or dichotomous and continuous variables (111). Analysis of a subset of participants (n=21) was conducted on those with T2D taking metformin (n=14) compared to those with T2D not taking metformin(n=7) (112).
Results

**Microbial Composition according to T2D status**

The relative abundance of taxonomic groups at the family level per participant is shown on Figure 6. The microbial composition of two individuals was visibly different for the rest of the participants. However, those samples had a sequencing depth above the median of 1.9M. Given that there was no rationale for excluding these individuals based on the fecal microbiome data quality or their clinical profile, subsequent analyses were conducted both including and excluding these two outliers. Beta diversity was significantly different at the family level with both Bray-Curtis (p=0.019) and Aitchison (p=<0.001) (Table 8).

**Functional Richness and Diversity of Fecal Microbiomes according to T2D status**

The PALADIN analysis aligned 1,491,268 unique UniProt protein IDs including species information and 1,484,945 unique UniProt IDs agglomerating functions across multiple species. There was a trend for UniProt ID richness and Fisher diversity to be lower in T2D (p=0.087 and p=0.095) (Figure 7). After excluding the two outliers (n=48), richness and diversity was significantly lower in those with T2D.

UniProt ID richness and diversity measures were all inversely correlated with interleukin 6 (IL-6) (Inverse Simpson rho=-0.32, p=0.026; Shannon rho=-0.34, p=0.017; Observed Richness rho=-0.31, p=0.032; Fisher rho=-0.34, p=0.016) but did not remain significant after BH correction (Figure 8). Conversely, BMI and leptin were positively associated with Inverse Simpson (rho=0.35, p=0.015 and rho=0.358, p=0.011) and Shannon (rho=0.32, p=0.023 and rho=0.35, p=0.014), while total fiber was inversely correlated with Shannon diversity (rho= -0.28, p=0.049), but also all lost significance after BH correction. Although the relationship of
diversity and inflammation was apparent in this sample, no correlations were observed with BDNF, consistent with the lack associations with compositional richness and diversity explored previously.

Fecal Microbiome Functional Potential Profiles According to T2D status

77 UniProt protein IDs within LPS and SCFA biosynthesis pathways, and present in at least 5% of the samples, were identified for the targeted analysis of functional potential (Table 9). None of these UniProt protein IDs were significant predictors of T2D, HbA1c, or HOMA-IR after BH adjustments. Additionally, none of the UniProt protein IDs in SCFA production pathways were correlated with T2D status or any of the examined biomarker. Conversely, UniProt protein IDs annotated to LPS biosynthesis pathways were significantly correlated with FPG, insulin, CRP and LBP (Figure 9 and Table 10). For example, UniProt ID: KDSB_PHOV8, is a protein needed for the incorporation of LPS in bacteria was predictive of plasma insulin levels. Therefore, glycemic and inflammation markers were positively correlated with LPS biosynthesis pathways in the fecal microbiome.

Metformin Subset Analysis

A subset analysis included participants with T2D under metformin treatment (n=21). Although there was a trend for compositional and functional richness and diversity to be higher in the metformin subgroup, there were no statistically significant differences according to treatment (Table 11). Further, no UniProt protein IDs from the targeted list (Table 9) were significantly associated with metformin treatment (data not shown).
Discussion

In the present study, functional (UniProt) richness and diversity did not differ according to T2D status. Although compositional richness and diversity were lower in participants with T2D, this did not translate into functional differences, which was consistent with previous reports (105). Thingholm et al. noted that interindividual gut microbiome variability, including functional diversity, was associated with obesity, but not with T2D. Thus, it was suggested that instead of distinct microbial signatures in obesity and T2D, the progressive disruption of the gut microbiome in obesity may play a role in the development of T2D (95). In this study population, the lack of fecal microbiome differences according to T2D may be due to the high prevalence of overweight and obesity. However, richness and diversity are only one measure when characterizing the gut microbiome. When studying the microbiome, we should consider multiple metrics to better understand these complex ecosystems, including exploring the enrichment of proteins and participating gene pathways.

UniProt IDs related to LPS biosynthesis were positively associated with glycemic and inflammatory markers, but not T2D status. The development of low-grade chronic inflammation in obesity may be attributed to increased intestinal permeability and translocation of LPS across the intestinal barrier (2). Here we demonstrate that UniProt protein IDs within the LPS core biosynthesis, LPS biosynthesis, and lipid A biosynthesis pathways were positively associated with CRP and LBP levels. Consistently with our results, previous studies found significant enrichment of LPS biosynthesis enzymes in women with untreated T2D and positive correlations with HOMA-IR in non-T2D insulin resistance (113,114), and for serum LPS to be correlated with insulin resistance (115). A large proportion of the study participants without T2D had
prediabetes, and thus may be exhibiting signs of glycemic impairment that masked any differences according to T2D status. We did find associations with FPG and insulin, suggesting that even in this sample there still appears to be a relationship between the LPS producing potential of the gut microbiome, glycemic regulation, and insulin resistance. If LPS pathways underlie T2D, they could be a prime target for therapeutics or lifestyle interventions.

UniProt proteins IDs within SCFA biosynthesis pathways were associated with T2D status or inflammatory, glycemic, and satiety markers. SCFAs, primarily propionate, butyrate, and acetate, are the byproduct of microbial fermentation of fiber, and have been suggested to have an anorexigenic effect through the stimulation of satiety hormones (2). Alterations in SCFA production, including butyrate, in T2D have been inconsistently suggested in previous studies (95). The high prevalence of obesity and T2D in this study population which could have attenuated differences in SCFA production (116). Other studies have not seen differences in pathways or gene families according to T2D in samples with obesity (95). Interestingly, the group with T2D has significantly higher fiber intake, but the lack of functional differences in SCFA metabolism pathways may be indicative of a low capacity to ferment fiber in T2D (105).

Metformin, a commonly used T2D treatment can alter the composition of the gut microbiome as well as alter its functional potential. Specifically metformin has been shown to increase the abundance of genes coding for the production of butyrate and propionate, which is thought to potentially provide some of the beneficial impacts observed in the use of this treatment (1,94,117,118). Therefore we conducted a subset analysis of the microbiome according to metformin treatment among participants with T2D (94). The composition or functional
potential of the gut microbiome did not differ according to metformin treatment. Specifically, UniProt ID richness and diversity appeared slightly higher in participants taking metformin but did not reach statistical significance. Additionally, no SCFA or LPS related UniProt protein IDs were associated with metformin use among those with T2D. However, our analysis included a small sample size and did not account for other medications.

**Strengths & Limitations**

A strength of this study was the focus on an underrepresented population in microbiome research, but also one that disproportionately experiences a higher risk and incidence of chronic diseases like T2D. Including diverse populations in studies of the gut microbiome is essential in understanding its role in health in a populations-specific manner. However, this convenience sample did not include metabolically healthy participants, as most had overweight or obesity and high rates on pre-diabetes in the group without T2D. Even though some associations may have been attenuated due to the underrepresentation of healthy participants and small sample size, these findings are pivotal in characterizing the relationship between the gut microbiome and cardiometabolic health in Bhutanese refugee adults in NH.

Exploring the functional potential expands upon the compositional characterization in an attempt to better understand the important features of the microbiome that influence health and chronic disease. Common limitations in microbiome studies include geographic and environmental factors, medication use, and day-to-day variability (95). With such limitations, obesity has been highlighted in its relationship to the gut microbiome, but historically associations can only be seen with community level metrics, like alpha diversity. Findings on other features of the gut microbiome in obesity and T2D have been inconsistent (95,119). This
may be partly due to limited consideration given to potential confounders and population characteristics. Population-specific studies that consider these confounders are therefore warranted. Additionally, it is important to note that analytical approaches to study functional potential are fairly new and there has been limited standardization of protocols. However, as more research is expanded to different populations concurrently with standardization of analytical pipelines, a clearer understanding of the role of the microbiome in human health will emerge.

Conclusion

This study was an unprecedented characterization of the functional potential of the gut microbiome of Bhutanese refugee adults in NH with a high prevalence of obesity and T2D. The findings highlight the interconnectedness of the gut microbiome in chronic diseases through inflammation and glycemic regulation. Although T2D was not a predictor of functional features of the gut microbiome, LPS biosynthesis potentials were related to glycemic markers and inflammation. The findings highlight the interconnectedness of the gut microbiome in chronic diseases through inflammation and glycemic regulation. The underlying pathways and mechanisms linking increased functional potentials with observed metabolic responses should be further explored. Similar investigations of the functional characterization of the gut microbiome should be carried out in other underrepresented populations, particularly those with higher risks of chronic diseases.
Thesis Conclusion

The gut microbiome plays a key role in human metabolic health and is implicated in chronic diseases such as obesity and T2D. Although exact mechanisms have yet to be elucidated, gut microbes are thought to influence glycemic regulation and insulin resistance through inducing inflammation and mediating signaling along the gut-brain axis. The purpose of this project was to characterize composition and functional potential of the gut microbiome in Bhutanese refugee adults in NH and expand upon the understanding of the inflammatory and gut-brain axis pathways underlying the microbiome’s relationship to T2D.

This study identified a substantial chronic disease burden in this population, with high prevalence of overweight and obesity as well as prediabetes and type 2 diabetes. Composition of the gut microbiome was related to glycemic status, indicating lower species richness and diversity as well as differences in beta diversity according to T2D. Findings suggest an association between gut microbial communities and community structure according to T2D in this population. Although the functional potential was not related to T2D status, UniProt protein IDs from LPS biosynthesis pathways were positively associated with glycemic and inflammatory markers. Low-grade chronic inflammation tied to endotoxemia has been of significant interest regarding insulin resistance and this finding highlights the relationships between the potential for LPS production in the gut microbiome and systemic inflammation. LBP levels were also related to various glycemic markers, indicating a connection between LPS, inflammation, and glycemic regulation. However, no associations were found in SCFA producing potentials according to T2D status or other explored markers including fiber, inflammation, or glycemic markers. Furthermore, while fiber remains at the core of SCFA production pathways, no notable associations were found with fiber intake in this sample.
South Asian adults in the United States experience a higher prevalence of prediabetes and T2D, which is corroborated by the findings in this study on Bhutanese refugee adults (66). Thus far, there have been very limited investigations on obesity and metabolic health in Bhutanese refugee in the United States (79). Additionally, among microbiome research, southern Asia is the most underrepresented (120). Greater inclusion of diverse populations in microbiome research is important to better understand relationships to health and to expand intervention applications to underrepresented groups. This sample lived primarily as a cultural enclave with relatively homogenous lifestyle factors. Nevertheless, environmental, lifestyle, and genetic factors still play large roles in metabolic diseases and the gut microbiome, that make studying these relationship complex.

Future research is needed to identify effective interventions for this population to address the prevalence and incidence of chronic diseases like T2D. Based on the findings of this study, both compositional diversity and inflammatory pathways appear as the strongest associations with glycemic regulation and insulin resistance. Addressing intestinal permeability and LPS producing pathways may provide a mechanism in which to target glycemic regulation in this population. However, any interventions developed should center the population’s cultural preferences and utilize the community’s feedback for the development of successful future study designs and intervention implementation. Next steps include developing a community report of this study’s findings to provide the participating community with an understanding of what research was done and how the findings can be interpreted.
Tables

Table 1. Regression models predicting T2D and glycemic impairment

<table>
<thead>
<tr>
<th>Logistic Regression Models</th>
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<tbody>
<tr>
<td>Model 1  T2D = CRP + IL6 + TNF-α + LBP + Age + Sex</td>
</tr>
<tr>
<td>Model 2  T2D = Total Fiber + Insoluble Fiber + Soluble Fiber + Whole Grains + Age + Sex</td>
</tr>
<tr>
<td>Model 3  T2D = Observed Species Richness + Age + Sex</td>
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<table>
<thead>
<tr>
<th>Linear Regression Models</th>
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<tbody>
<tr>
<td>Model 4 Log transformed HOMAIR = LBP + Observed Richness + Age + Sex</td>
</tr>
<tr>
<td>Model 5 Log transformed HbA1c = LBP + Observed Richness + Age + Sex</td>
</tr>
<tr>
<td>Demographic</td>
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<td>-----</td>
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<tr>
<td>n</td>
</tr>
<tr>
<td>Clinical</td>
</tr>
<tr>
<td>CVD (% With CVD)†</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
</tr>
<tr>
<td>BMI Category (% Overweight/Obese)</td>
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<td>Smoking Status (% Smoker)</td>
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<td>Physical Activity Score</td>
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<td>Metformin (% Use)</td>
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<tr>
<td>Statin (% Use)</td>
</tr>
<tr>
<td>NSAID (% Use)</td>
</tr>
<tr>
<td>PPI (% Use)</td>
</tr>
<tr>
<td>Demographic</td>
</tr>
<tr>
<td>Age (y)</td>
</tr>
<tr>
<td>Sex (% Female)</td>
</tr>
<tr>
<td>Years in US (y)</td>
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<tr>
<td>Household Size</td>
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<tr>
<td>Food Security (% Food Secure)</td>
</tr>
<tr>
<td>High School Completion (% Completed)</td>
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<tr>
<td>Dietary Intake</td>
</tr>
<tr>
<td>Total Dietary Fiber (g)</td>
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<tr>
<td>Insoluble Fiber (g)</td>
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<tr>
<td>Soluble Fiber (g)</td>
</tr>
<tr>
<td>Whole Grains (oz equivalents)</td>
</tr>
<tr>
<td>Daily Caloric Intake (kcal)</td>
</tr>
</tbody>
</table>

*Statistically significant at alpha level 0.05
†CVD excluding hypertension
††Wilcoxon used to generate p-value
†††Fisher’s exact test used; assumptions were not met for chi-squared
Table 3. Clinical Factors, Dietary Intake, and Inflammatory Markers

<table>
<thead>
<tr>
<th></th>
<th>All Participants</th>
<th>Non-T2D</th>
<th>T2D</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glycemic Status Markers</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>50</td>
<td>4.3</td>
<td>0.2</td>
<td>28.3</td>
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<tr>
<td>HbA1c (%)</td>
<td>50</td>
<td>5.9</td>
<td>4.9</td>
<td>10.4</td>
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<tr>
<td>FPG (mg/dL)</td>
<td>50</td>
<td>120.0</td>
<td>88.0</td>
<td>274.0</td>
</tr>
<tr>
<td>Insulin (μIU/mL)</td>
<td>50</td>
<td>14.4</td>
<td>0.9</td>
<td>81.4</td>
</tr>
<tr>
<td><strong>Inflammatory Markers</strong></td>
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<td></td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>50</td>
<td>2.8</td>
<td>0.1</td>
<td>29.0</td>
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<tr>
<td>IL6 (pg/mL)</td>
<td>49</td>
<td>2.0</td>
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<tr>
<td>TNFα (pg/mL)</td>
<td>49</td>
<td>8.1</td>
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<td>51.1</td>
</tr>
<tr>
<td>LBP (μg/mL)</td>
<td>50</td>
<td>4.2</td>
<td>1.3</td>
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</tr>
<tr>
<td>Leptin (pg/mL)</td>
<td>50</td>
<td>8638.9</td>
<td>360.9</td>
<td>93520.6</td>
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<tr>
<td><strong>Lipid Profile</strong></td>
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<tr>
<td>Total Cholesterol (mg/dL)</td>
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<td>179.5</td>
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<td>LDL Cholesterol (mg/dL)</td>
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<td>80.0</td>
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<tr>
<td>HDL Cholesterol (mg/dL)</td>
<td>50</td>
<td>41.5</td>
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<td>64.0</td>
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<tr>
<td>Triglycerides (mg/dL)</td>
<td>50</td>
<td>134.0</td>
<td>35.0</td>
<td>454.0</td>
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</table>

*Statistically significant at alpha level 0.05
Table 4. Partial spearman correlation analysis of diabetes markers, inflammatory markers, and dietary intake

<table>
<thead>
<tr>
<th></th>
<th>HOMA-IR</th>
<th>HbA1c</th>
<th>FPG</th>
<th>Insulin</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>Rho</td>
<td>P-value</td>
<td>n</td>
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<tr>
<td>Inflammatory Markers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-Reactive Protein (mg/L)</td>
<td>50</td>
<td>0.23</td>
<td>0.111</td>
<td>50</td>
</tr>
<tr>
<td>Interleukin-6 (pg/mL)</td>
<td>49</td>
<td>0.22</td>
<td>0.132</td>
<td>49</td>
</tr>
<tr>
<td>Tumor Necrosis Factor Alpha (pg/mL)</td>
<td>49</td>
<td>0.22</td>
<td>0.142</td>
<td>49</td>
</tr>
<tr>
<td>Lipoprotein-Binding Protein (ug/mL)</td>
<td>50</td>
<td>0.35</td>
<td>0.016*</td>
<td>50</td>
</tr>
<tr>
<td>Leptin (pg/mL)</td>
<td>50</td>
<td>0.27</td>
<td>0.068</td>
<td>50</td>
</tr>
<tr>
<td>Dietary Intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Dietary Fiber (g)</td>
<td>50</td>
<td>0.06</td>
<td>0.708</td>
<td>50</td>
</tr>
<tr>
<td>Insoluble Fiber (g)</td>
<td>50</td>
<td>0.04</td>
<td>0.798</td>
<td>50</td>
</tr>
<tr>
<td>Soluble Fiber (g)</td>
<td>50</td>
<td>0.08</td>
<td>0.587</td>
<td>50</td>
</tr>
<tr>
<td>Whole Grains (oz equivalents)</td>
<td>50</td>
<td>0.12</td>
<td>0.427</td>
<td>50</td>
</tr>
<tr>
<td>Daily Caloric Intake (kcal)</td>
<td>50</td>
<td>-0.06</td>
<td>0.664</td>
<td>50</td>
</tr>
</tbody>
</table>

Partial spearman correlation adjusted for age and sex

*Statistically significant at alpha level 0.05
Table 5. Logistic regression models 1-3 predicting T2D status

<table>
<thead>
<tr>
<th>Model</th>
<th>Variable</th>
<th>Coefficient</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>CRP</td>
<td>0.00</td>
<td>1.00</td>
<td>(0.86, 1.13)</td>
<td>0.985</td>
</tr>
<tr>
<td></td>
<td>LBP</td>
<td>-0.17</td>
<td>0.84</td>
<td>(0.52, 1.34)</td>
<td>0.462</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>-0.06</td>
<td>0.95</td>
<td>(0.90, 0.99)</td>
<td>0.019*</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td>0.44</td>
<td>1.55</td>
<td>(0.33, 7.36)</td>
<td>0.585</td>
</tr>
<tr>
<td>Model 2</td>
<td>Soluble Fiber</td>
<td>-0.30</td>
<td>0.74</td>
<td>(0.46, 1.20)</td>
<td>0.226</td>
</tr>
<tr>
<td></td>
<td>Whole Grains</td>
<td>-0.45</td>
<td>0.64</td>
<td>(0.29, 1.42)</td>
<td>0.272</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>-0.04</td>
<td>0.96</td>
<td>(0.92, 1.01)</td>
<td>0.104</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td>0.23</td>
<td>1.26</td>
<td>(0.24, 6.69)</td>
<td>0.785</td>
</tr>
<tr>
<td>Model 3</td>
<td>Observed Species Richness</td>
<td>0.01</td>
<td>1.01</td>
<td>(1.00, 1.02)</td>
<td>0.174</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>-0.05</td>
<td>0.95</td>
<td>(0.91, 1.00)</td>
<td>0.022*</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td>0.39</td>
<td>1.48</td>
<td>(0.32, 6.92)</td>
<td>0.619</td>
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</tbody>
</table>

*Statistically significant at alpha level 0.05
Table 6. Linear regression models 4-5 predicting T2D status

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>SE</th>
<th>P-Value</th>
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<tr>
<td>Model 4 HOMAIR</td>
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<td></td>
</tr>
<tr>
<td>LBP</td>
<td>1.27</td>
<td>0.08</td>
<td>0.004*</td>
</tr>
<tr>
<td>Observed Species Richness</td>
<td>1.00</td>
<td>0.00</td>
<td>0.587</td>
</tr>
<tr>
<td>Age</td>
<td>1.00</td>
<td>0.01</td>
<td>0.620</td>
</tr>
<tr>
<td>Sex</td>
<td>1.13</td>
<td>0.30</td>
<td>0.691</td>
</tr>
<tr>
<td>Model 5 HbA1c</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>LBP</td>
<td>1.04</td>
<td>0.02</td>
<td>0.010*</td>
</tr>
<tr>
<td>Observed Species Richness</td>
<td>1.00</td>
<td>0.00</td>
<td>0.795</td>
</tr>
<tr>
<td>Age</td>
<td>1.00</td>
<td>0.00</td>
<td>0.144</td>
</tr>
<tr>
<td>Sex</td>
<td>0.99</td>
<td>0.06</td>
<td>0.875</td>
</tr>
</tbody>
</table>

*Statistically significant at alpha level 0.05

Table 7. Wilcoxon Rank Sum Test of Alpha Diversity Measures by T2D status

<table>
<thead>
<tr>
<th>Measure</th>
<th>W</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed Richness</td>
<td>407</td>
<td>0.045*</td>
</tr>
<tr>
<td>Shannon</td>
<td>405</td>
<td>0.049*</td>
</tr>
<tr>
<td>Simpson</td>
<td>400</td>
<td>0.061</td>
</tr>
<tr>
<td>Inverse Simpson</td>
<td>400</td>
<td>0.061</td>
</tr>
<tr>
<td>Fisher</td>
<td>409</td>
<td>0.040*</td>
</tr>
</tbody>
</table>

*Statistically significant at alpha level 0.05
Table 8. Differences in beta diversity identified according to T2D via PERMANOVA

<table>
<thead>
<tr>
<th></th>
<th>R²</th>
<th>F</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td><strong>Bray-Curtis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family</td>
<td>0.05</td>
<td>2.71</td>
<td>0.019</td>
</tr>
<tr>
<td>Genus</td>
<td>0.04</td>
<td>1.78</td>
<td>0.063</td>
</tr>
<tr>
<td><strong>Aitchison</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family</td>
<td>0.06</td>
<td>3.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Genus</td>
<td>0.04</td>
<td>1.97</td>
<td>0.003</td>
</tr>
<tr>
<td>UniProt ID</td>
<td>Protein Name</td>
<td>Pathway</td>
<td>Function</td>
</tr>
<tr>
<td>-----------------</td>
<td>--------------------------------------------------</td>
<td>----------------------------------------------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>BCACT_ROSHA</td>
<td>Butyryl-CoA:acetate CoA-transferase</td>
<td>Lipid metabolism; butanoate metabolism.</td>
<td>Coenzyme A-transferase that converts butyryl-CoA to butyrate. Can also use propionyl-CoA as substrate in vitro.</td>
</tr>
<tr>
<td>MAM_FAEPA</td>
<td>Microbial anti-inflammatory molecule</td>
<td>N/A</td>
<td>Plays an anti-inflammatory role in the mammalian intestine. The supernatant of this bacteria, or 2 purified fractions containing peptides derived from this protein, exerts inhibitory effects on IL1-beta-induced IL-8 secretion in intestinal epithelial CaCO-2 cells (PubMed:18936492, PubMed:26045134). It may play a role in maintaining the intestinal barrier, which is important in diabetes mellitus.</td>
</tr>
<tr>
<td>ACDS_MEGEL</td>
<td>Acyl-CoA dehydrogenase, short-chain specific</td>
<td>SCFA (butyrate)</td>
<td>Has an optimum specificity for 4-carbon length fatty acyl-CoAs.</td>
</tr>
<tr>
<td>ATOA_ECOLI</td>
<td>Acetate CoA-transferase subunit beta</td>
<td>Lipid metabolism; short-chain fatty acid metabolism.</td>
<td>Coenzyme A transferase which is involved in short-chain fatty acid degradation and catalyzes the activation of short-chain fatty acids to their respective CoA thiolesters (PubMed:1103739, PubMed:3025185). During acetocacetate degradation, catalyzes the transfer of CoA from acetyl-CoA to acetoacetate by a mechanism involving a covalent enzyme-CoA compound as a reaction intermediate (PubMed:1103741). Utilizes a variety of short chain acyl-CoA and carboxylic acid substrates but exhibits maximal activity with normal and 3-keto substrates (PubMed:1103739).</td>
</tr>
<tr>
<td>UniProt ID</td>
<td>Protein Name</td>
<td>Pathway</td>
<td>Function</td>
</tr>
<tr>
<td>---------------</td>
<td>-------------------------------</td>
<td>----------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>KDSB_BACTN</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>KDSB_PARD8</td>
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<tr>
<td>KDSB_PHOV8</td>
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</tr>
<tr>
<td>KPSU5_ECOLX</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3-deoxy-manno-octulosonate</td>
<td>Activates KDO (a required 8-carbon sugar) for incorporation into</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cytidylyltransferase</td>
<td>bacterial lipopolysaccharide in Gram-negative bacteria.</td>
</tr>
<tr>
<td>A0AG5VDT2_9FIRM</td>
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<td>A0A3D5PT93_9FIRM</td>
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<td>C9LH4_9FIRM</td>
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<tr>
<td>A0A316ESJ7_MEGEL</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bifunctional protein HldE</td>
<td>Catalyzes the ADP transfer from ATP to D-glycero-beta-D-manno-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>heptose 1-phosphate, yielding ADP-D-glycero-beta-D-manno-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>heptose.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Catalyzes the phosphorylation of D-glycero-D-manno-heptose 7-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>phosphate at the C-1 position to selectively form D-glycero-beta-D-</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>manno-heptose-1,7-bisphosphate.</td>
</tr>
<tr>
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<td>F0R60S_PHOSB</td>
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<td>D4IP0_9BACT</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Multifunctional fusion protein</td>
<td>Catalyzes the hydrolysis of UDP-3-O-myristoyl-N-acetylglucosamine to</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>form UDP-3-O-myristoylglucosamine and acetate, the committed step in</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>lipid A biosynthesis.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Involved in unsaturated fatty acids biosynthesis. Catalyzes the</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>dehydration of short chain beta-hydroxyacyl-ACPs and long chain</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>saturated and unsaturated beta-hydroxyacyl-ACPs.</td>
</tr>
</tbody>
</table>
Table 9B. UniProt protein IDs related to lipopolysaccharide metabolism from the UniProt database [CONTINUED]

<table>
<thead>
<tr>
<th>Protein ID</th>
<th>UniProt entry</th>
<th>Function/Activity</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>KDSC_ECOBD</td>
<td>3-deoxy-D-manno-octulosonate 8-phosphate phosphatase KdsC</td>
<td>Bacterial outer membrane biogenesis; lipopolysaccharide biosynthesis.</td>
<td>Catalyzes the hydrolysis of 3-deoxy-D-manno-octulosonate 8-phosphate (KDO 8-P) to 3-deoxy-D-manno-octulosonate (KDO) and inorganic phosphate.</td>
</tr>
<tr>
<td>LPXZ_BACTN</td>
<td>Bifunctional enzyme LpxC/FabZ</td>
<td>Glycolipid biosynthesis; lipid IV(A) biosynthesis; lipid IV(A) from (3R)-3-hydroxytetradecanoyl-[acyl-carrier-protein] and UDP-N-acetyl-alpha-D-glucosamine: step 2/6.</td>
<td>Catalyzes the hydrolysis of UDP-3-O-myristoyl-N-acetylglucosamine to form UDP-3-O-myristoylglucosamine and acetate, the committed step in lipid A biosynthesis. Involved in unsaturated fatty acids biosynthesis. Catalyzes the dehydration of short chain beta-hydroxyacyl-ACPs and long chain saturated and unsaturated beta-hydroxyacyl-ACPs.</td>
</tr>
<tr>
<td>GLMU_BIFAA</td>
<td>Bifunctional protein GlmU</td>
<td>Bacterial outer membrane biogenesis; LPS lipid A biosynthesis.</td>
<td>Catalyzes the last two sequential reactions in the de novo biosynthetic pathway for UDP-N-acetylglucosamine (UDP-GlcNAc). The C-terminal domain catalyzes the transfer of acetyl group from acetyl coenzyme A to glucosamine-1-phosphate (GlcN-1-P) to produce N-acetylglucosamine-1-phosphate (GlcNAc-1-P), which is converted into UDP-GlcNAc by the transfer of uridine 5-monophosphate (from uridine 5-triphosphate), a reaction catalyzed by the N-terminal domain.</td>
</tr>
<tr>
<td>LPXD_BACFN</td>
<td>UDP-3-O-acylglycerol N-acetyltransferase</td>
<td>Bacterial outer membrane biogenesis; LPS lipid A biosynthesis.</td>
<td>Catalyzes the N-acylation of UDP-3-O-acylglycerol using 3-hydroxyacyl-ACP as the acyl donor. Is involved in the biosynthesis of lipid A, a phosphorylated glycolipid that anchors the lipopolysaccharide to the outer membrane of the cell.</td>
</tr>
<tr>
<td>RFAP_ECOLX</td>
<td>Lipopolysaccharide core heptose(I) kinase RfaP</td>
<td>Bacterial outer membrane biogenesis; LPS core biosynthesis.</td>
<td>Catalyzes the phosphorylation of heptose(I) of the outer membrane lipopolysaccharide core.</td>
</tr>
<tr>
<td>FABI_ENTFA</td>
<td>Enoyl-[acyl-carrier-protein] reductase [NADH] FabI</td>
<td>Lipid metabolism; fatty acid biosynthesis.</td>
<td>Catalyzes the reduction of a carbon-carbon double bond in an enoyl moiety that is covalently linked to an acyl carrier protein (ACP). Involved in the elongation cycle of fatty acid which are used in the lipid metabolism (By similarity).</td>
</tr>
</tbody>
</table>
## Table 9B. UniProt protein IDs related to lipopolysaccharide metabolism from the UniProt database [CONTINUED]

<table>
<thead>
<tr>
<th>UniProt ID</th>
<th>Function</th>
<th>Enzyme Activity</th>
<th>Location</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>UPPS_LACPL</td>
<td>Ditran,polycis-undecaprenyl-diphosphate synthase ((2E,6E)-farnesyl-diphosphate specific)</td>
<td>N/A</td>
<td>Bacterial outer membrane biogenesis; enteroacterial common antigen biosynthesis</td>
<td>Catalyzes the sequential condensation of isopentenyl diphosphate (IPP) with (2E,6E)-farnesyl diphosphate (E,E-FPP) to yield (2Z,6Z,10Z,14Z,18Z,22Z,26Z,30Z,34E,38E)-undecaprenyl diphosphate (di-trans,octa-cis-UPP). UPP is the precursor of glycosyl carrier lipid in the biosynthesis of bacterial cell wall polysaccharide components such as peptidoglycan and lipopolysaccharide.</td>
</tr>
<tr>
<td>WECG_CITK8</td>
<td>UDP-N-acetyl-D-mannosaminuronic acid transferase</td>
<td>Bacterial outer membrane biogenesis; enteroacterial common antigen biosynthesis</td>
<td>Catalyzes the synthesis of Und-PP-GlcNAc-ManNAcA (Lipid II), the second lipid-linked intermediate involved in enterobacterial common antigen (ECA) synthesis.</td>
<td></td>
</tr>
<tr>
<td>KDGGP_BACTN</td>
<td>2-keto-3-deoxy-D-glycero-D-galacto-9-phosphonononic acid phosphatase</td>
<td>N/A</td>
<td>Involved in the biosynthesis of 2-keto-3-deoxy-D-glycero-D-galacto-9-phosphonononic acid used in cell-wall polysaccharides (PubMed:18804026).</td>
<td></td>
</tr>
<tr>
<td>LPXA_ECO57 LPXA_AKKM8</td>
<td>Acyl-[acyl-carrier-protein]--UDP-N-acetylglucosamine O-acyltransferase</td>
<td>Glycolipid biosynthesis; lipid IV(A) biosynthesis; lipid IV(A) from (3R)-3-hydroxytetradecanoyl-[acyl-carrier-protein] and UDP-N-acetyl-alpha-D-glucosamine: step 1/6</td>
<td>Involved in the biosynthesis of lipid A, a phosphorylated glycolipid that anchors the lipopolysaccharide to the outer membrane of the cell</td>
<td></td>
</tr>
<tr>
<td>FABZ1_LACLA FABZ2_LACLA FABZ_LACPL FABZ_STRE4 FABZ_STRMU</td>
<td>3-hydroxyacyl-[acyl-carrier-protein] dehydratase FabZ</td>
<td>Lipid A biosynthesis</td>
<td>Involved in unsaturated fatty acids biosynthesis. Catalyzes the dehydration of short chain beta-hydroxyacyl-ACPs and long chain saturated and unsaturated beta-hydroxyacyl-ACPs (By similarity).</td>
<td></td>
</tr>
<tr>
<td>A0A0F5INZ5_9BACT A0A133Z6B7_9BACT</td>
<td>Multifunctional fusion protein</td>
<td>Glycolipid biosynthesis; lipid IV(A) biosynthesis; lipid IV(A) from (3R)-3-hydroxytetradecanoyl-[acyl-carrier-protein] and UDP-N-acetyl-alpha-D-glucosamine: step 2/6</td>
<td>Involved in unsaturated fatty acids biosynthesis. Catalyzes the dehydration of short chain beta-hydroxyacyl-ACPs and long chain saturated and unsaturated beta-hydroxyacyl-ACPs. Catalyzes the hydrolysis of UDP-3-O-myristoyl-N-acetylglucosamine to form UDP-3-O-myristoylglucosamine and acetate, the committed step in lipid A biosynthesis.</td>
<td></td>
</tr>
</tbody>
</table>
Table 9B. UniProt protein IDs related to lipopolysaccharide metabolism from the UniProt database [CONTINUED]

<table>
<thead>
<tr>
<th>UniProt ID</th>
<th>Enzyme Name</th>
<th>Enzyme Function</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPXF_BACTN</td>
<td>Lipid A 4’-phosphatase</td>
<td>Bacterial outer membrane biogenesis; LPS lipid A biosynthesis.</td>
<td>Probably removes the 4’-phosphate group from lipid A. Removal of this phosphate group confers resistance to cationic antimicrobial peptides (CAMPs), inflammation-associated peptides produced by the human host. This LPS modification helps maintain the stability of this commensal bacterium in gut microbiota.</td>
</tr>
<tr>
<td>RPOE_HAEIN SIRB2_ECOLI</td>
<td>ECF RNA polymerase sigma-E factor</td>
<td>N/A</td>
<td>Sigma factors are initiation factors that promote the attachment of RNA polymerase (RNAP) to specific initiation sites and are then released. Extracytoplasmic function (ECF) sigma-E controls the envelope stress response, responding to periplasmic protein stress, increased levels of periplasmic lipopolysaccharide (LPS) as well as heat shock and oxidative stress; it controls protein processing in the extracytoplasmic compartment (By similarity).</td>
</tr>
<tr>
<td>LPXK_BACFN LPXK_BACTN LPXK_PARD8 LPXK_PHOV8</td>
<td>Tetraacyldisaccharide 4’-kinase</td>
<td>Glycolipid biosynthesis; lipid IV(A) biosynthesis; lipid IV(A) from (3R)-3-hydroxytetradecanoyl-[acyl-carrier-protein] and UDP-N-acetyl-alpha-D-glucosamine: step 6/6.</td>
<td>Transfers the gamma-phosphate of ATP to the 4’-position of a tetraacyldisaccharide 1-phosphate intermediate (termed DS-1-P) to form tetraacyldisaccharide 1,4’-bis-phosphate (lipid IVA).</td>
</tr>
<tr>
<td>ARNE_ECO57</td>
<td>Probable 4-amino-4-deoxy-L-arabinose-phosphoundecaprenol flippase subunit ArnE</td>
<td>Bacterial outer membrane biogenesis; lipopolysaccharide biosynthesis.</td>
<td>Translocates 4-amino-4-deoxy-L-arabinose-phosphoundecaprenol (alpha-L-Ara4N-phosphoundecaprenol) from the cytoplasmic to the periplasmic side of the inner membrane.</td>
</tr>
</tbody>
</table>
Table 10. Significant UniProt protein IDs from targeted analysis associated with fasting plasma glucose (FPG), insulin, c-reactive protein (CRP), and lipopolysaccharide binding protein (LBP) via Wald test from Corncob.

<table>
<thead>
<tr>
<th>UniProt Protein</th>
<th>Gene</th>
<th>Protein Name</th>
<th>Pathway</th>
<th>Biomarker Associated</th>
<th>Coefficient</th>
<th>Std. Error</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A0A3D5PT93_9FIRM</td>
<td>rfaE2</td>
<td>Bifunctional protein HldE</td>
<td>LPS core biosynthesis</td>
<td>FPG</td>
<td>0.039</td>
<td>0.010</td>
<td>0.002</td>
</tr>
<tr>
<td>C9LLH4_9FIRM</td>
<td>hldE</td>
<td>Bifunctional protein HldE</td>
<td>LPS core biosynthesis</td>
<td>Insulin</td>
<td>0.043</td>
<td>0.014</td>
<td>0.010</td>
</tr>
<tr>
<td>D4IIP0_9BACT</td>
<td>lpxC</td>
<td>Multifunctional fusion protein</td>
<td>Lipid IV(A) biosynthesis</td>
<td>Insulin</td>
<td>0.057</td>
<td>0.011</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>KDSB_PHOV8</td>
<td>kdsB</td>
<td>3-deoxy-manno-octulosonate cytidylyltransferase</td>
<td>Lipopolysaccharide biosynthesis</td>
<td>Insulin</td>
<td>0.024</td>
<td>0.008</td>
<td>0.010</td>
</tr>
<tr>
<td>LPXD_BACTN</td>
<td>lpxD</td>
<td>UDP-3-O-acylglucosamine N-acyltransferase</td>
<td>LPS lipid A biosynthesis</td>
<td>Insulin</td>
<td>0.029</td>
<td>0.009</td>
<td>0.010</td>
</tr>
<tr>
<td>FABI_ENTFA</td>
<td>fabI</td>
<td>Enoyl-[acyl-carrier-protein] reductase [NADH] FabI</td>
<td>Lipid metabolism; fatty acid biosynthesis</td>
<td>CRP</td>
<td>0.194</td>
<td>0.038</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FABZ1_LACLA</td>
<td>fabZ1</td>
<td>3-hydroxyacyl-[acyl-carrier-protein] dehydratase FabZ</td>
<td>Lipid A biosynthesis</td>
<td>CRP</td>
<td>0.256</td>
<td>0.035</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FABZ2_LACLA</td>
<td>fabZ2</td>
<td>3-hydroxyacyl-[acyl-carrier-protein] dehydratase FabZ</td>
<td>Lipid A biosynthesis</td>
<td>CRP</td>
<td>0.178</td>
<td>0.047</td>
<td>0.001</td>
</tr>
<tr>
<td>GLMU_ENTFA</td>
<td>glmU</td>
<td>Bifunctional protein GlmU</td>
<td>Lipid A biosynthesis</td>
<td>CRP</td>
<td>0.204</td>
<td>0.047</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ARNE_ECO57</td>
<td>arnE</td>
<td>Probable 4-amino-4-deoxy-L-arabinose-phosphoundecaprenol flippase subunit ArnE</td>
<td>Lipopolysaccharide biosynthesis</td>
<td>LBP</td>
<td>1.411 x 10^-6</td>
<td>0.202 x 10^-6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LPXA_ECO57</td>
<td>lpxA</td>
<td>Acyl-[acyl-carrier-protein]--UDP-N-acetylglucosamine O-acyltransferase</td>
<td>Lipid IV(A) biosynthesis</td>
<td>LBP</td>
<td>1.523 x 10^-6</td>
<td>0.407 x 10^-6</td>
<td>0.003</td>
</tr>
</tbody>
</table>
Table 11: Functional diversity and richness measures according to metformin use among those with T2D status via Wilcoxon test. No differences in functional richness or diversity observed according to metformin use among those with T2D

<table>
<thead>
<tr>
<th></th>
<th>No Metformin N=7</th>
<th>Metformin N=14</th>
<th>W</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>IQR</td>
<td>Median</td>
<td>IQR</td>
</tr>
<tr>
<td>Richness</td>
<td>121295</td>
<td>48586</td>
<td>141486</td>
<td>45846</td>
</tr>
<tr>
<td>Shannon</td>
<td>10.6</td>
<td>0.338</td>
<td>10.9</td>
<td>0.698</td>
</tr>
<tr>
<td>InvSimpson</td>
<td>15762</td>
<td>5886</td>
<td>22729</td>
<td>21651</td>
</tr>
<tr>
<td>Fisher</td>
<td>36537</td>
<td>18398</td>
<td>44683</td>
<td>19589</td>
</tr>
</tbody>
</table>
Figure 1. The Microbiota-Gut-Brain Axis. The microbiota-gut-brain axis is a bidirectional relationship between the gut microbiota, digestive system, and the brain. The neuronal, endocrine, and immune pathways are three intertwined components of the microbiota-gut-brain axis and are mediated by gut bacteria and bacteria-derived metabolites, impacting hunger and satiety, inflammation, and eating behavior.
Figure 2. Impact of Obesity Interventions on the Microbiota-Gut-Brain Axis. Upper panel: Changes in the microbiota-gut brain axis that may result from obesity interventions. Lower Panel: Obesity interventions that are being explored as mediating the microbiota-gut-brain axis. Further research is needed to identify successful interventions in these key areas for future clinical applications to address obesity and metabolic diseases.
Figure 3. Participant recruitment and data collection in coordination with Building Community in New Hampshire (BCNH).
Figure 4. Diversity measures according to T2D status. *Statistically significant at alpha level 0.05. Observed species richness, Shannon, and Fisher diversity were significantly higher in the non-T2D group compared to the T2D group. Other alpha diversity measures, Simpson and inverse Simpson were higher in the non-T2D group but did not reach statistical significance.
Figure 5. Spearman correlation matrix heatmap of inflammatory associated taxonomic groups with clinical biomarkers and dietary data. (A) Spearman correlation matrix of inflammatory associated genera and observed species richness with clinical biomarkers and dietary data. (B) Spearman correlation matrix of inflammatory associated species with clinical biomarkers and dietary data. *Statistically significant at alpha 0.05. P-values were adjusted using the Benjamin–Hochberg method. All significance was lost after BH correction.
Figure 6. Iris plot of individual sample family level relative abundance and T2D status
Figure 7. Boxplot of UniProt functional richness and diversity measures according to T2D status. No significant differences in functional richness or diversity via Wilcoxon rank sum test according to T2D status.
Figure 8. Heatmap of Spearman correlations of UniProt richness and diversity measures, all significance lost after BH corrections. Markers measured include dietary fiber measures, age, interleukin-6 (IL6), C-reactive protein (CRP), lipopolysaccharide binding protein (LBP), brain derived neurotrophic factor (BDNF), fasting plasma glucose (FPG), hemoglobin A1c (HbA1c), Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), tumor necrosis factor α (TNFα), body mass index (BMI), and leptin.

Figure 9. Significant UniProt proteins related to LPS biosynthesis pathways associated with glycemic and inflammatory markers. Results of the targeted UniProt analysis identified...
11 UniProt proteins to have significant positive associations with fasting plasma glucose (FPG), insulin, c-reactive protein (CRP), or lipopolysaccharide binding protein (LBP) via Wald test from Corncob.
Supplementary Figure 1. Age-adjusted Spearman correlation matrix heatmap of inflammatory associated species with clinical biomarkers and dietary data stratified. (A) Age-adjusted spearman correlation matrix of inflammatory associated genera and observed species richness with clinical biomarkers and dietary data. (B) Age-adjusted Spearman correlation matrix of inflammatory associated species with clinical biomarkers and dietary data. *Statistically significant at alpha 0.05. P-values were adjusted using the Benjamini–Hochberg method. All significance was lost after BH correction.
References


120. Abdill RJ, Adamowicz EM, Blekhman R. Public human microbiome data are dominated by highly developed countries. PLOS Biology. 2022 Feb 15;20(2):e3001536.
21-Mar-2019

Bigornia, Sherman J
ANFS, Kendall Hall
Durham, NH 03824

IRB #: 8042
Study: The Impact of SNAP-Ed on Dietary Quality, Food Safety Handling Behaviors, and Insulin Resistance among Bhutanese Adults Residing in New Hampshire
Approval Date: 21-Mar-2019

The Institutional Review Board for the Protection of Human Subjects in Research (IRB) has reviewed and approved the protocol for your study as Expedited as described in Title 45, Code of Federal Regulations (CFR), Part 46, Subsection 1101(b). Approval is granted to conduct your study as described in your protocol.

Researchers who conduct studies involving human subjects have responsibilities as outlined in the attached document, Responsibilities of Directors of Research Studies Involving Human Subjects. (This document is also available at http://unh.edu/research/irb-application-resources.) Please read this document carefully before commencing your work involving human subjects.

Note: IRB approval is separate from UNH Purchasing approval of any proposed methods of paying study participants. Before making any payments to study participants, researchers should consult with their BSC or UNH Purchasing to ensure they are complying with institutional requirements. If such institutional requirements are not consistent with the confidentiality or anonymity assurances in the IRB-approved protocol and consent documents, the researcher may need to request a modification from the IRB.

Upon completion of your study, please complete the enclosed Study Final Report form and return it to this office along with a report of your findings.

If you have questions or concerns about your study or this approval, please feel free to contact Melissa McGee at 603-862-2005 or melissa.mcgee@unh.edu. Please refer to the IRB # above in all correspondence related to this study. The IRB wishes you success with your research.

For the IRB,

[Signature]

Julie F. Simpson
Director

cc: File
Nov 10, 2021 9:24:07 AM EST

Sherman Bigornia
Agriculture, Nutrition,& Food Systm

Study Title: The Impact of SNAP-Ed on Dietary Quality, Food Safety Handling Behaviors, and Insulin Resistance among Bhutanese Adults Residing in New Hampshire
IRB #: IRB-FY2022-38
Modification: Addition of CIBBR Pilot Study
Modification Approval Date: November 10, 2021

The Institutional Review Board for the Protection of Human Subjects in Research (IRB) has reviewed and approved your modification to this study, as indicated above. Further changes in your study must be submitted to the IRB via Cayuse IRB/Human Ethics for review and approval prior to implementation.

Researchers who conduct studies involving human subjects have responsibilities as outlined in the document, Responsibilities of Directors of Research Studies Involving Human Subjects.

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For the IRB,

Julie F. Simpson
Director