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Assessing the role of dietary interventions on the gut microbiome: a potential therapeutic for IBD

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ASSESSING THE ROLE OF DIETARY INTERVENTIONS ON THE GUT MICROBIOME: A POTENTIAL THERAPEUTIC FOR IBD

by

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DEDICATION

I would like to dedicate this work to my good luck charm and best friend—Maverick. I would also like to dedicate it to my grandparents, who have been my greatest support system.

ACKNOWLEDGMENTS

I would like to thank my two readers, Dr. Garcia- Diaz and Dr. Tornheim, for their time and guidance throughout this thesis. I would also like to thank my advisor, Dr. Garcia-Diaz, for his constant support and advice.

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ABSTRACT

The gut microbiome consists of a wide diversity of bacteria that play a role in maintaining homeostasis and regulating inflammation. Therefore, in recent years, there has been an increased focus in sequencing the microbiome through new metagenomic advancements to better understand the functions of the bacteria. Through sequencing, it was discovered that the microbiome is composed of four main phyla, each with a different primary function. Many studies have been conducted investigating the impact of diets on the microbiome and they have shown its composition can change depending on the dietary patterns of a particular individual. For example, in a high animal-based protein diet there is an increase in microbiota processing of L-carnitine and phosphatidylcholine, which leads to increased levels of Trimethylamine N-oxide (TMAO). In a carbohydrate diet, there is an increase in microbiota accessible carbohydrates (MAC), which leads to an increase in bacteria that convert the MAC to short chain fatty acids (SCFA). In a high fat diet, more bacteria are needed to alter the bile acids in order to enhance their binding to receptors. When consuming a plant-based diet, it has been well demonstrated that there is an increase in *Prevotella* bacteria. Gluten free (GFD) and low Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols (FODMAP) diets also show a differing composition of the microbiome, when compared to a normal diet. While dietary changes allow for alteration of the microbiome without disrupting the symbiotic nature of the microbiota with the gastrointestinal system, dysbiosis of the microbiome can occur which can lead to the pathogenesis of many diseases, including Inflammatory Bowel Disease (IBD). IBD is an autoimmune condition characterized by persistent intestinal inflammation. While the development of IBD is largely multifactorial, there is strong evidence that alteration of the composition of the microbiome is a core component of the pathogenesis. Current treatments center around managing the inflammation through prolonged steroid use, Tumor Necrosis Factor (TNF) inhibitors, and other maintenance medications. However, emerging treatment options have explored utilizing dietary interventions to attempt to restore the symbiotic nature of the microbiome. Many of these dietary interventions, such as exclusive enteral nutrition (EEN) and Crohn's Disease exclusion diet (CDED) have been proven to lead to remission in Crohn's Disease (CD) patients. By understanding the complexity of the microbiome and its relationship to the digestive system, new advancements can be made in treating chronic diseases such as IBD through dietary interventions.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

AIEC .... Adherent Invasive E. Coli

BU .... Boston University

CD .... Crohn’s Disease

CDED .... Crohn’s Disease Exclusion Diet

IBD .... Inflammatory Bowel Disease

IBS .... Inflammatory Bowel Syndrome

EEN .... Exclusive Enteral Nutrition

FODMAP .... Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols

GFD .... Gluten Free Diet

HF/HS .... High Fat/High Sugar

LPS .... Lipopolysaccharide

LRD .... Low Residual Diet

LF .... Low FODMAP

MAC .... Microbiota Accessible Carbohydrates

PEN .... Partial Enteral Nutrition

SCD .... Specific Carbohydrate Diet

SCFA .... Short Chain Fatty Acids

TMA .... Trimethylamine

TMAO .... Trimethylamine N-oxide

TNF .... Tumor Necrosis Factor

UC .... Ulcerative Colitis

INTRODUCTION

There are many commensal bacteria that have formulated symbiotic relationships within specific organ systems in the human body. While this ranges from the respiratory tract to the skin, one prime example of this interaction is the human gut microbiota. Consisting of millions of different bacteria, the gut microbiome serves to enhance the immune system and provide essential vitamins and nutrients needed for proper growth (Ruan et al., 2020). The great diversity of this microbiome is important in preventing illness, as a lack of diversity has been implicated in many different gastrointestinal diseases, such as IBD (Holmes et al., 2011). Though each part of the digestive tract houses a specific subset of bacteria (**Figure 1)**, a healthy microbiota has been defined by a core set microbes consisting of *Bacteroides, Eubacterium, Faecalibacterium, Alistipes, Ruminococcus, Clostridium, Roseburia, and Blautia; with Faecalibacterium prausnitzii, Oscillospira guillermondii, and Ruminococcus obeum* (Ruan et al., 2020). It is also predominantly composed of four main phyla including Firmicutes, Proteobacteria, Actinobacteria and Bacteroidetes, with each having their own classes, species, and genus of bacterium within them (Ruan et al., 2020).

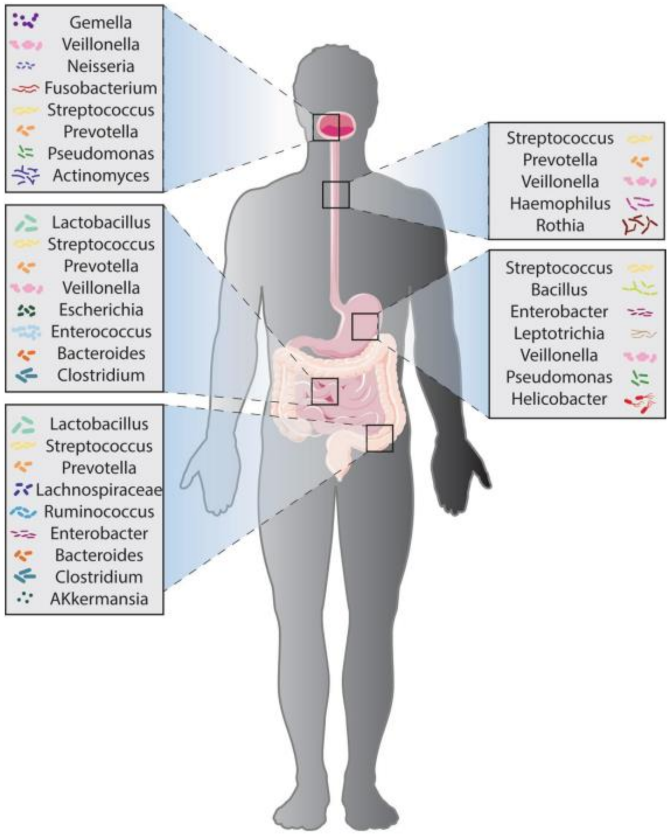

Figure 1. Diversity of the Gut Microbiome. Each part of the digestive tract has a different subset of bacteria that are most prominent to that area, which are shown in this figure. Taken from (Ruan et al., 2020).[1]

With the deep complexity of the microbiome, many of the bacterium have a specific functional role that contributes to health of the human. For example, two of the main phyla, Bacteroidetes and Firmicutes, play a major role in hydrolyzing carbohydrates that are not digestible and producing short chain fatty acids (SCFA) (Hills et al., 2019). These SCFA play a fundamental part in suppressing inflammation; therefore, a decrease

[1] Reprinted from Healthy Human Gastrointestinal Microbiome: Composition and Function After a Decade of Exploration, Vol 65, Ruan et al., Pages 695-705., Copyright (2020), with permission from Springer Nature

in these bacteria can lead to a reduction of SCFA and an increase in inflammation. Conversely, high levels of Proteobacteria can lead to an increase in inflammation even in the absence of disease; therefore, it is important to have minimal levels of Proteobacteria (Hiippala et al., 2018). It is clear through past studies that there is an abundance of bacteria within the gut that play important roles in preventing the pathogenesis of diseases. Therefore, this paper will further explore the composition of the gut microbiome as detailed by current literature and shed light on the major roles that each bacterium plays in maintaining the symbiotic relationship.

Impact of Nutrition on the Gut Microbiome

There are many factors that contribute to the diversity of the microbiome and often it is a complex interplay between environment, genetics, and diet, with dietary patterns being a prime influencer. Since nutrition significantly impacts the composition of the gut microbiome, numerous studies have investigated its role. Interestingly, a change in dietary patterns can lead to rapid changes in the gut microbiome composition (Singh et al., 2017), with long-term dietary patterns having a more significant influence. One study examining the effects of a meat-based diet compared to a vegetarian-based diet, showed that the animal diet led to decreased levels of *Firmicutes* and increased levels of *Bilophila Wasworthia*. Overall, a meat-based diet was shown to have more of an impact on the gut microbiome when compared to the plant-based diet (David et al., 2014).

The main source of protein is not the only factor that impacts the composition of the microbiota. In another study examining stool samples of people on a GFD to those on a regular diet, results indicated that those on the GFD diet had decreased *Veillonacea* and

Ruminococcus bacterium (Bonder et al., 2016), whereas a diet high in starch showed an increase in the *Ruminococcus* bacterium (Flint, 2012). In addition, a systemic literature review investigating the impact of different diets found that a protein-based diet increased microbial diversity, that a high fat diet showed increased levels of *Faecali* and *Pranusnitizii*, and that a diet high in non-digestible carbohydrates decreased overall bacterial abundance (Singh et al., 2017). Finally, as most people in the United States eat primarily a Western diet that tends to have a decrease in microbiota accessible carbohydrates (MAC), research on the effect of a decrease in MAC has shown that it leads to a decrease bacterial diversity in the gut microbiome and that this reduction in diversity can be inherited (Sonnenburg et al., 2016). As this decrease in diversity has been linked to numerous intestinal disorders, it has been suggested that incorporating more MAC in the diet may be important in decreasing the incidence rates of these diseases (Sonnenburg et al., 2016). This paper will provide an in-depth analysis of the effects of specific diets, including a high protein, high carbohydrate, high fat, vegan/vegetarian diet, gluten free diet, and Westernized diet, on the composition of the gut microbiome.

Dysbiosis of the Gut Microbiome

When functioning in a symbiotic manner, the gut microbiome is beneficial to enhancing an individual's health, and studies have shown that it can enhance host immunity (Ruan et al., 2020; Singh et al., 2017). However, the root cause of many intestinal disorders, and in particular IBD, stems from dysbiosis between the gut microbiome and the human body. IBD is an autoimmune condition that causes prolonged

GI inflammation and consists of both Crohn's Disease (CD) and Ulcerative Colitis (UC) (*CDC -What Is Inflammatory Bowel Disease (IBD)?*, 2020). CD can affect any part of the digestive tract, while UC only affects the colon. The incidence rates of IBD have been on a steady rise since the 1800s and are more prevalent in the Westernized world (Kaplan, 2015). While the cause behind the development of IBD remains multifactorial and largely poorly understood, it is likely that alterations in the gut microbiome play a significant role in the pathogenesis of the disease.

As noted above, nutrition plays a large role in altering the diversity of the gut microbiome, and as a Westernized diet decreases the diversity of the microbiome, research has suggested that there is a possible link between this decreased diversity and the development of IBD (Albenberg et al., 2012; Sonnenburg et al., 2016). While there are numerous reasons for the development of IBD, there is clear alteration in the gut microbiome in these patients. Patients with IBD showed a decreased diversity in their microbiome, and an increase of the *Bacteroides fragilis* bacterium (Walters et al., 2014). Patients also showed a decrease in Firmicutes and an increase in Proteobacteria, which may increase inflammation and lead to the pathogenesis of IBD.

While alteration of the gut microbiome plays a clear role in the development of IBD, there are also environmental and genetic factors that may contribute. Certain genetic markers including NOD2, ATG16L1, IRGM, and LRRK2 have been well-documented in CD patients (Ananthakrishnan, 2015). Other factors such as lack of sleep, stress, exercise, appendectomies, and necrotizing enterocolitis have been shown to increase a person's risk for developing IBD (Ananthakrishnan, 2015). While it is challenging to pinpoint exactly what root cause may lead to the development of IBD, the dysbiosis of the gut microbiota is clearly involved.

Role of Nutrition in IBD Treatment

Current treatment for IBD consists of steroids and biologics aimed directly at decreasing the intestinal inflammation (*CDC -What Is Inflammatory Bowel Disease (IBD)?*, 2020). However, these treatments can cause many adverse effects, such as immunosuppression, leaving patients vulnerable to disease. Prolonged steroid use can also lead to the development of other diseases, such as Cushing's syndrome, which can not only affect the patient on a physical level but can be mentally challenging as well. As active IBD can lead to prolonged hospitalizations and life-threatening complications, there is an ever growing need to find a more sustainable and effective approach to treating this disease.

As indicated above, nutrition can play a large role in altering the gut microbiome and restoring the symbiotic nature of the bacterium, so recent studies have investigated how dietary treatment may assist in alleviating the symptoms of IBD. One study examining the effect of a specific carbohydrate diet (SCD) compared to low residual diet (LRD) found that SCD diet led to increased microbial diversity in IBD patients, while LRD diet decreased the diversity (Walters et al., 2014). Exclusive enteral nutrition (EEN) is a dietary invention that has be proven to lead to remission in CD patients, and studies investigating its effect on the gut microbiome found that it interestingly reduced microbial diversity and led to more dysbiosis, but that this enhanced dysbiosis actually promoted the re-development of a healthy gut microbiome (Albenberg et al., 2012;

MacLellan et al., 2017). Another study showed that a low FODMAP diet (LFD) assisted in alleviating many of the symptoms associated with IBD (Gibson & Shepherd, 2010). Finally, a recent study showed success of utilizing the CD Exclusion Diet (CDED) combined with partial enteral nutrition (PEN) in altering the microbiome in order to help patients attain remission (Levine et al., 2019). Although the relationship between nutrition, the gut microbiome, and IBD is not entirely understood, these studies show promising evidence that dietary inventions can assist in alleviating many of the symptoms associated with IBD and may be used in place of corticosteroids that could have adverse side effects.

Specific Aims

Specific aims of the following thesis include:

1. Comprehensive review of the composition and symbiotic relationship of the human gut microbiome.
2. Investigation of how different dietary patterns can alter the composition of the gut microbiome.
3. Exploration of the role of the gut microbiome and the role of nutrition in the pathogenesis of IBD.
4. Conclusion on how different nutritional inventions can alter the gut microbiome to reduce symptoms of IBD patients.

COMPOSITION OF THE MICRIOBIOME

The microbiome is composed of a wide array of archaea, viruses, eukaryotes and fungal species (Tomova et al., 2019). The composition and regulation of the gut microbiome is essential for promoting health and reducing the incidence of disease. Dysbiosis of the microbiome is implicated in the pathogenesis of many diseases including but not limited to IBD, Type II Diabetes, Cardiovascular Disease, and Colorectal cancer (Hills et al., 2019). In recent years, advancements in metagenomics and shotgun sequencing have led to progress in sequencing the highly complex human gut microbiome. A few main lab techniques have been applied in attempt to provide the most accurate description of the gut microbiome. For example, Next Generation Sequencing has been used in projects, such as the Human Genome Project, to work towards developing a comprehensive library of the multitude of microbes that exist in each part of the digestive tract. Shot gun sequencing, 16A rRNA and Metatranscriptomics have also all been used in order to provide the most comprehensive list of microbes and their function (D'Argenio & Salvatore, 2015). Therefore, this section will explore the composition of the gut microbiome as detailed by current literature.

The gut microbiome is highly diverse and its composition differs based on a multitude of factors, including specific sections of the GI tract (Ruan et al., 2020). Consistently, the two phyla Bacteroidetes and Firmicutes have been shown to compose the majority of the gut microbiome (Holmes et al., 2011; King et al., 2019; Tomova et al., 2019). The most common genera include *Bacteroides, Prevotella, Bifidobacterium, Eubacterium, Clostridium, Streptococcus, and Enterobacteria* (Tomova et al., 2019). A study conducted at George Washington University utilized their own metagenomic technology and previous results from the Human Microbiome Project to sequence the gut microbiota. Results from their study showed that four phyla had an abundance of over 1%, including Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria (King et al., 2019). However, congruent with past studies, the results showed that the two phyla Bacteroidetes and Firmicutes are clearly the most predominant (**Figure 2**). In every sample that they sequenced, they found the following: Bacteroidetes, Proteobacteria, Spirochetes, Actinobacteria and Firmicutes phylum. Each phylum also had a predominant genus. In Bacteroidetes, *Bacteroides* was the most common genus with *Bacteroides fragilis* present in almost all samples and most notably *Bifidobacterium* was present in almost all healthy human samples. For Actinobacteria, Proteobacteria, and Firmicutes, *Actinobacteria, Gammaproteobacteria* and *Clostridia* were the most predominant genus respectively (King et al., 2019).

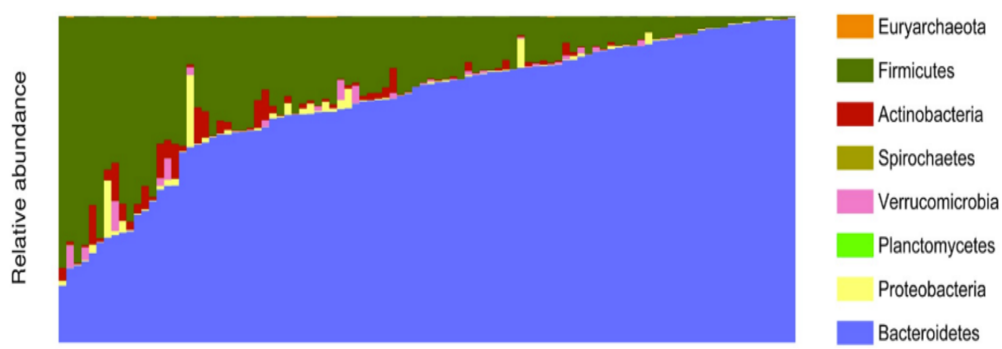

Figure 2. Bar chart of the phylogenic composition of all microbiome tax. The green and the blue indicate that Firmicutes (green) and Bacteroidetes (blue) have the highest abundance. Actinobacteria (red) and Proteobacteria (yellow) have the next highest abundance. This illustrates that the four dominant phyla consist of: Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria. Taken from (King et al., 2019).

While it is clear that the Bacteroidetes and Firmicutes are the major phyla in the microbiome, it has also been shown that the composition of the bacteria is dependent on the specific section of the digestive tract as many bacteria have a specialized function. In the oral cavity, the most predominant bacteria include Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, *Spirochaetes, and Fusobacteria*, (D'Argenio & Salvatore, 2015) compared to the colon where the most common bacteria include Bacteroidetes, Firmicutes, Verrucomicrobia, Proteobacteria and Actinobacteria (Ruan et al., 2020) (**Figure 1**). The differing compositions of the gut bacteria allow for the bacteria to preform specialized functions and survive in the vastly different environments of each part of the digestive tract. However, despite the fact that the microbiome varies throughout the digestive system, there is a stable core of bacterium that has been identified in a healthy human adult. These main microbes include *Faecalibacterium, Alistipes, Ruminococcus, Bacteroides, Eubacterium, Clostridium, Roseburia,* and *Blautia. Faecalibacterium prausnitzii, Oscillopsia guillermondii,* and *Ruminococcus obeum* were shown to be the three dominant taxa common amongst all adults (Ruan et al., 2020). The current hypothesis is that alterations in the subset of core microbiota can lead to the pathogenesis of many gastrointestinal disorders.

In addition to the specific set of microbes in the gut, according to Hiippala et al. (2018), there are also two distinct microbial ecosystems. These include the luminal ecosystem, composed of 90% Firmicutes and Bacteroidetes, and the mucosal layer, shown to have decreased diversity and increased prevalence of Firmicutes (Hiippala et al., 2018). It is the epithelium that allows for the symbiotic relationship between the host and the microbiome. Overall, through recent advancements in sequencing technology, it is apparent there is a wide diversity of gut microbes that aid in homeostasis and immune regulation. While there is still a large movement to continue to sequence and understand the complexity of microbiome composition, current literature and metagenomic technology offers a window into some of the most prevalent gut microbes.

Role of Each Bacterium

With such great diversity of the gut microbiome, many studies have investigated the role that specific bacterium may have in aiding the human digestive system. Gut bacteria are known to be involved in immune function, vitamin metabolism, processing of sugars, fat storage, angiogenesis regulation and behavior development (D'Argenio & Salvatore, 2015). A study conducted by Zou et al., examined 1,520 genomes from the human gut microbiota and attempted to determine the functions of 338 of the bacterial species. The results shown in **Figure 3** indicate that there is a wide range of processes that the gut microbiota regulate (Zou et al., 2019). They noted that virulence factors and genes associated with antibiotic resistance were increased in *Klebsiella, Escherichia, Citrobacter,* and *Enterobacter*. Other bacteria, such as Bacteroidetes, play a major role in sphingolipid and steroid hormone synthesis (Zou et al., 2019).

One of the other main roles of the commensal gut bacteria is to metabolize non-digestible carbohydrates and produce SCFA. The SCFA include butyrate, acetate, and propionate. The production of these SCFA serve as an energy source for the enterocytes and also aid in depressing intestinal inflammation (Hiippala et al., 2018). Therefore, a decrease in the diversity of microbes may lead to the reduction of the production of

SCFA, which can increase inflammation and lead to dysbiosis of the microbiome. This section will explore the roles of each of the four main phyla and what processes each one regulates.

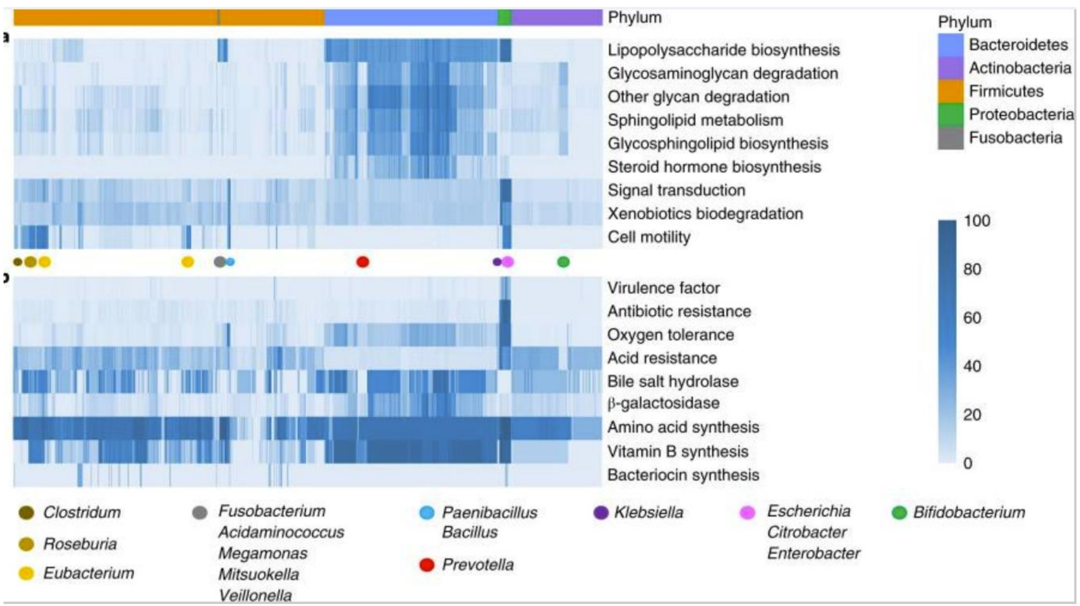

Figure 3. The roles of each bacterium in the gut microbiota. The different phyla are demarcated by a specific color as shown on the top and the species are listed on the bottom. As shown by the dark blue lines, that virulence factors and antibiotic resistance genes are increased in *Escherichia*, *Citrobacter*, *Klebsiella*, and *Enterobacter*. Bacteroidetes plays a major role in lipid biosynthesis. Taken from (Zou et al., 2019).

Bacteroidetes

As mentioned previously, Bacteroidetes and Firmicutes have consistently been shown to be the two most abundant microbes in the human gut microbiota. Both of these are obligate anaerobic bacteria that play a role in hydrolyzing non-digestible carbohydrates into SCFA (D'Argenio & Salvatore, 2015). Bacteroidetes in particular is a phylum of bacteria that has been shown to be important in the degradation of glycan, and seems to be involved in sphingolipid and steroid hormone synthesis (Gibiino et al., 2018; Zou et al., 2019). In certain diseases, such as IBD, the function of Bacteroidetes is reduced, leading to a decrease in the production of SCFA (Gibiino). Two important members of the phylum Bacteroidetes are *Bacteroides* and *Bacteroides fragilis*, both of which have been shown to have adverse and beneficial effects on the microbiome (Hills et al., 2019). Research also suggests that *Bacteroides fragilis* is important for communicating with the immune system through its production of a polysaccharide (PSA). The PSA that is produced is then involved in the immune responses that aid in the maintenance of gut homeostasis (Hiippala et al., 2018). This bacterium can also bind to IgA, which can be important for regulating the immune response (Hiippala et al., 2018).

Firmicutes

In a similar manner, Firmicutes plays a role in hydrolyzing indigestible carbohydrates. *Firmicutes prausnitzii*, a member of the Firmicutes phylum, is an oxygen-sensitive anaerobe that hydrolyzes both glucose and acetate to make formate, D-lactate, and butyrate (Hiippala et al., 2018). The production of butyrate in particular seems to play a major role in reducing intestinal inflammation through inhibiting both NK-kB and IFN-gamma. Through reducing inflammation, this bacterium may play an essential role in preventing colon cancer (Hiippala et al., 2018).

Actinobacteria

Another major phylum found in the gut microbiome is Actinobacteria. *Bifidobacterium*, a member of the phylum Actinobacteria, is thought to play an important role in maintaining a healthy human gut. In utero, it can assist in decreasing the risk of necrotizing enterocolitis and sepsis (Hiippala et al., 2018). This bacterium also plays a role in protection against *E. Coli* and seems to be reduced with age (Hiippala et al., 2018). It also is involved in the fermentation of oligosaccharides that leads to SCFA (Hills et al., 2019).

Proteobacteria

The fourth major phylum found in the human gut is Proteobacteria, a gram-negative bacterium. Since it is a gram-negative bacterium, it has an outer layer that is covered in lipopolysaccharide (LPS). This outer LPS increases the release of cytokines that promote inflammation, which can cause intestinal inflammation (Hiippala et al., 2018). Therefore, if this particular phylum increases, there can be more gut inflammation seen even in the absence of infection. This in part explains the increase in Proteobacteria seen in IBD. Proteobacteria remains one of the minor phyla since increased amounts of it is strongly linked to the development of disease (Hiippala et al., 2018).

One member of the Proteobacteria family is *Bilophila wadsworthia*. This bacterium most likely is involved in the inflammatory response. Feng et al. examined the role of *B. wadsworthia* in the pathogenesis of chronic diseases. Results from their study confirmed that this bacterium promoted systemic inflammation through the production of cytokines, such as serum amyloid A proteins and interleukin-6 (Feng et al., 2017).

EFFECTS OF DIET ON THE MICROBIOME

It has been well-documented that the composition of a person's microbiome is developed through an interplay of factors including diet, environmental influences and genetics. Diet in particular has been shown to be a major contributor to the overall microbiome composition. For example, *Prevotella,* a bacterium that has enzymes that degrade plant fiber, has been shown to be replaced with *Bacteroides* with increased time spent in the United States by the host (Hills et al., 2019). Although both are part of the Bacteroidetes phylum, as someone transitions to a more Westernized diet when living in the United States, which contains less vegetables, there is no longer a prominent need for *Prevotella* and therefore its presence decreases. Due to the strong influence of diet on the microbiome, three different enterotype profiles have been detailed to describe the composition of the microbiome according to a person's diet (Hills et al., 2019). Enterotype I is linked to a Western Diet and has decreased levels of *Prevotella* and increased levels of *Bacteroides*. Enterotype II prevails with a more plant-based diet and shows increased levels of *Prevotella* and decreased levels of *Bacteroides*. Finally, Enterotype III has a higher prevalence of *Ruminococcus*, a member of the Firmicutes phyla, which tends to be a pro-inflammatory bacterium that is linked to both Irritable Bowel Syndrome (IBS) and Inflammatory Bowel Disease (IBD) (Hills et al., 2019). As indicated, there is a clear link between diet and the composition of the microbiome.

Diet not only influences the composition of the gut microbiome, but it also impacts the metabolite that the microbiota produces. Upon ingestion of specific foods, the microbiome will process it and produce a specific metabolite. For example, the microbiota will produce short chain fatty acids (SCFA) from undigestible carbohydrates, also known as fiber. These SCFA are important for regulating homeostasis and decreasing inflammation. In a high animal-based protein diet, the microbiota creates Trimethylamine (TMA) from the choline and L-carnitine ingested which can then be converted to Trimethylamine N-oxide (TMAO) (Schoeler & Caesar, 2019). High levels of TMAO can be seen in the pathogenesis of cardiovascular disease and low levels can been seen in IBD (A. S. Wilson et al., 2020). Bile acids can also be altered and modified by the microbiota, allowing them to bind to their specific receptors and exert their effects (Schoeler & Caesar, 2019). **Figure 4** shows the interplay between the ingested product, the microbiota metabolites, and its transition into the body.

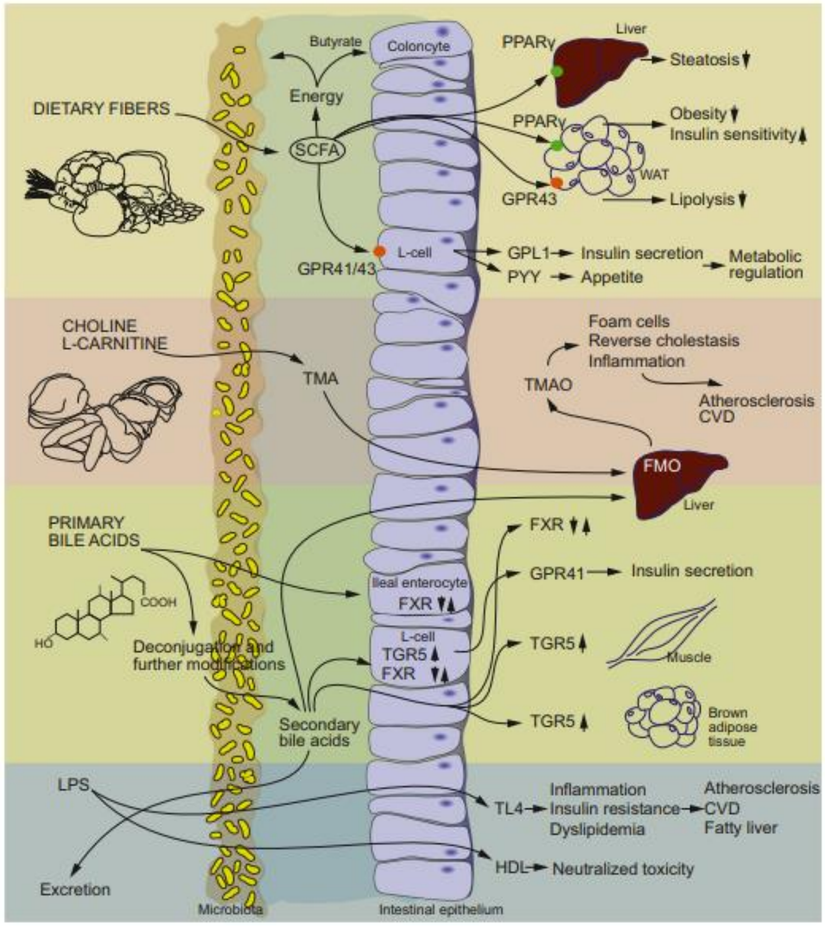

Figure 4. Microbiota metabolites and products from ingested food. On the left side of the image, it portrays what is being processed by the microbiota. The middle column shows the metabolite produced by the microbiota and the right shows what happens to the metabolite once it enters the body. Taken from (Schoeler & Caesar, 2019).

As it is clear that diet strongly impacts the gut microbiome and its metabolites, this section will explore the impact that different diets may have on altering the gut microbiome and will specifically highlight what occurs in a high protein animal-based, plant-based, high fat, high carbohydrate, gluten free, low FODMAP, and Western diets.

High Protein Diet (Animal-Based)

The variety and prevalence of the gut microbiome can be altered based on the diet of an individual. In fact, altering ones diet can lead to changes in the gut microbiome within a single day (Singh et al., 2017). When studying the effects of an animal-based protein diet compared to a plant-based diet, it was shown that an animal-based diet had a greater effect on the composition of the gut microbiome than a plant (David et al., 2014), but increased protein consumption leads to greater diversity overall (Singh et al., 2017). In an animal-based protein diet, the bacteria *Bacteroides, Clostridia* and *B. wasworthia* seem to be increased, while the bacteria *Bifidobacterium adolescentis, Roseburia* and *Firmicutes* are decreased (David et al., 2014; Frame et al., 2020; Singh et al., 2017). As *Firmicutes* is involved in the metabolization of plant polysaccharides, it may be reduced in an animal-based diet as its function is not needed as much as it is in a plant-based diet. The increase in *B. wasworthia* and decrease in *Roseburia* could be implicated in the pathogenesis of IBD (David et al., 2014; Singh et al., 2017). As mentioned previously, in the Feng study, they concluded that *B. wasworthia* played a role in promoting systemic inflammation; therefore, this increase is why it is postulated to be implicated in IBD (Feng et al., 2017). Most importantly, these studies all show evidence that the profile of the gut microbiome can change depending on the main source of protein in a diet.

The shift in the composition of the microbiome to accommodate the increased protein intake, whether it be an increase in plant or animal protein, is important because one of the main roles of the microbiota when ingesting protein is to metabolize the amino acids (Gentile & Weir, 2018). While the metabolism of amino acids can lead to many different metabolites, one important product seen primarily in an animal-based protein diet as opposed to a plant protein diet is TMAO (Gentile & Weir, 2018). This is an important distinction because increased levels of TMAO, which occur in an animal protein diet, are often implicated in the development of cardiovascular disease (Gentile & Weir, 2018). Interestingly, although high TMAO levels lead to the pathogenesis of cardiovascular disease (Tang et al., 2013), in a study conducted by Wilson et al., they found that Crohn's Disease (CD) patients had decreased levels of TMAO (A. Wilson et al., 2015). In either case, the production of TMAO is highly dependent on the gut microbiome (Tang et al., 2013). Upon ingestion of L-carnitine, a compound that is seen primarily an animal protein diet, or phosphatidylcholine, a glycerophospholipid that is present in animal-based protein sources such as liver, eggs, beef and pork, aerobic anaerobes in the microbiome will convert it to TMA (Gentile & Weir, 2018; Tang et al., 2013; A. Wilson et al., 2015). These anaerobes include *Clostridia, Proteus, Shigella*, and *Aerobacter* (A. Wilson et al., 2015). Once it is converted to TMA, Proteobacteria can then convert it to TMAO (A. Wilson et al., 2015). As production of TMAO is decreased in CD, it is suggested that this could possibly be used as a biomarker for CD and since TMAO is often increased in cardiovascular disease it is suggested that patients at risk reduce their phosphatidylcholine intake through a plant-based, high fiber diet, as this would decrease a patients' ingestion of animal protein (Tang et al., 2013; A. Wilson et al., 2015).

Plant-Based Diet

A plant-based diet tends to be more prevalent in African and Indian cultures and has been shown to lead to a drastic shift in the microbiota. One major change seen in a plant-based diet is an increase in *Prevotella* and Bacteroidetes (Frame et al., 2020; Tomova et al., 2019). To enhance understanding of the changes in the composition, one study examined the fecal samples of vegetarians and vegans compared to individuals consuming a normal diet, and found there to be decrease in *Bacteroides, Bifidobacterium, Escherichia coli* and *Enterobacteriaceae*, indicating there is a shift in the composition of the gut microbiome when consuming a completely plant-based diet (Zimmer et al., 2012). From the Zimmer study, it was concluded there was a decrease in *Bacteroides* and it is postulated that this is due to the fact that *Bacteroides* mainly function in the alteration of bile, which is more relevant in a high animal protein diet (Tomova et al., 2019).

As stated previously, one important role of the gut microbiome is to utilize microbiota accessible carbohydrates (MAC) to create SCFA that promote homeostasis and anti-inflammatory processes. Since there is an increase in fiber in a plant-based diet, vegetarians tend to have more SCFA production, such as increased production of acetate, propionate, and butyrate, when compared to non-vegetarians (Tomova et al., 2019). In order to process the increased load of fiber, there is an increase in lactic acid bacteria, such as *Ruminococcus, E. rectale* and *Roseburia* and a decrease in *Clostridium* and *Enterococcus* (Tomova et al., 2019). *Ruminococcus* is important for the digestion of cellulose and other complex carbohydrates and will produce butyrate as a byproduct, which functions to decrease inflammation (Tomova et al., 2019). This bacterium may also be implicated in the conversion of phosphatidylcholine to TMA, which will then be converted to TMAO (Tomova et al., 2019). A plant-based diet also provides a source of phytochemicals that can be altered by the microbiome to form metabolites (Gentile & Weir, 2018). One such metabolite is polyphenols, which tend to increase *Bifidobacterium* and *Lactobacillus* (Tomova et al., 2019). The polyphenols play an important role in regulating inflammation. Polyphenols are most commonly converted to equol, urolithins, and enterolignans by *Bifidobacterium, Lactobacillus, Coriobacteriaceae, Clostridium, Bacteroides* and *Saccharamyces* (Tomova et al., 2019). Therefore, there is an increase in these bacteria with a plant-based diet as more polyphenols will need to be converted.

As mentioned, plant-based diets tend to predominate in certain countries. Therefore, numerous studies have compared the microbiomes of populations from differing countries. In a popular study, De Filippo and his team compared the fecal microbiota of Italian children, who tend to have a more Western based diet (n=15), to those children living in a rural African town, Burkina Faso (n=14), who tend to have a lower fat and high plant protein diet. As indicated in **Figure 5**, Burkina Faso children had increased amounts of Bacteroidetes and a decrease in Firmicutes. Of the Bacteroidetes, more than half of their microbiome was composed of *Prevotella*, which is known to be involved in the metabolism of plant protein. Due to the increase in MAC in a plant-based diet, the Burkina Faso children also had higher levels of SCFA (De Filippo et al., 2010). This is in comparison the Italian children who had increased levels of Firmicutes and decreased levels of Bacteroidetes.

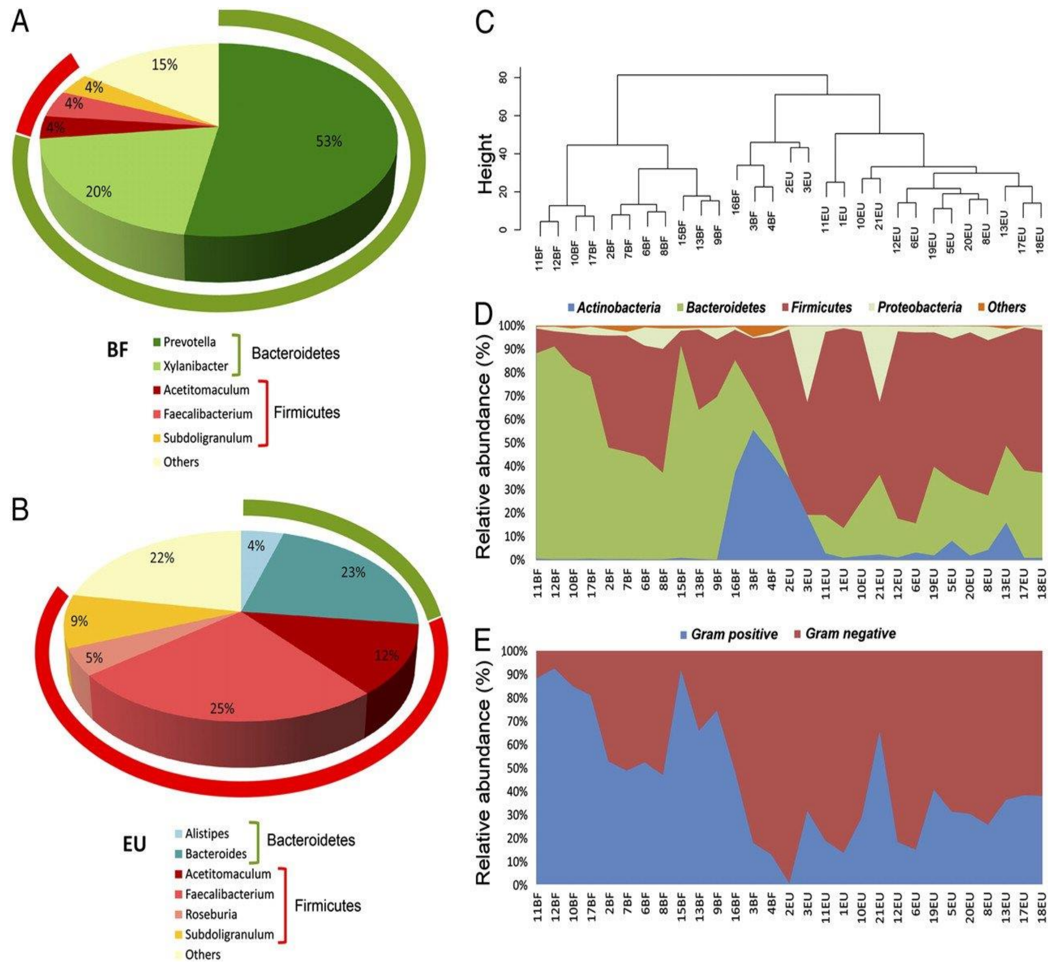

Figure 5. Comparison of Italian children microbiome to Burkina Faso children. A and B both highlight the major bacteria that composed the microbiome of two different groups of the children. C is hierarchical clustering of the samples. D shows the abundance of each bacterium in each child. E highlights the difference in gram positive vs gram negative bacterium. Taken from (De Filippo et al., 2010).

In another study, Lin et al. compared the fecal microbiota of children in Bangladesh, who tend to have a more carbohydrate rich, plant-based diet, to that of the United States, whose children tend to have a Western type diet (Lin et al., 2013). They found that the Bangladesh children had an increased diversity of their microbiome with an increase in *Prevotella, Butyribio*, and *Oscillospira*, and a decrease in *Bacteroides*. Notably, as seen in the Burkina Faso children, *Prevotella* was the dominant bacteria and they also had *Lactobacillus* in their microbiome. Interestingly, this study found that the US children had more Bacteroidetes, with an increase in *Bacteroides*, which is known to be implemented in a high-protein, high-fat diet (Lin et al., 2013). Overall, one major takeaway from these two studies is that a predominantly plant-based diet alters the microbiome and leads to increased levels of *Prevotella.*

High Fat

When examining a diet high in fat compared to one low in fat, studies have shown that in a low-fat diet there is an increase in *Bifidobacterium* (Singh et al., 2017). In a high-fat diet, there is an increase in *Faecalis, Pranisnitzii, Bacteroidetes, Actinobacteria,* and *Alistipes* and a decrease in *Firmicutes, Proteobacteria, and Bifidobacteria* (Frame et al., 2020; Singh et al., 2017). This leads to a decrease in SCFA production. However, conversely, in another study that examined mice fed a high-fat diet, they found that the mice had decreased levels of Bacteroidetes and increased Firmicutes and Proteobacteria (Gentile & Weir, 2018). While the alteration of the microbiome in a high fat diet may be more dependent on an individual's composition (Frame et al., 2020), one common theme is the microbes alteration of bile acids in a high fat diet.

Microbes in the gut are necessary in order to properly digest and absorb fats. Upon eating a high-fat meal, the liver produces bile acids and secretes them into the small intestine. These bile acids are essential for lipid metabolism and are prime regulators of the gut. The microbiota help regulate the composition of the bile acids, their abundance, and their signaling (Gentile & Weir, 2018). The microbiota processing of bile acids aids their binding to specific receptors such as the Farnesoid X receptor and G-protein coupled receptors, which promotes their function (Schoeler & Caesar, 2019). In addition to the processing of bile acids, a high-fat diet can lead to an increase in the microbiota's production of lipopolysaccharide (LPS), a metabolite that is implicated in both cardiovascular disease and metabolic disease (Gentile & Weir, 2018). LPS may be implicated in these diseases because it is an endotoxin that promotes inflammation through activating toll like receptors, including TLR4 (Schoeler & Caesar, 2019).

Carbohydrates

There are two main types of carbohydrates—digestible and non-digestible. In a diet high in digestible carbohydrates, there is an increase in *Bifidobacterium* and a reduction in *Bacteroides* (Singh et al., 2017). The consumption of too much carbohydrates, which normally occurs in the Western world, can cause adverse effects on the microbiome. The indigestible carbohydrates that are accessible to the microbes, or MAC, are important because they are the primary source of energy for the enterocytes. In addition, the bacteria can produce SFCA from the MAC, which help with energy homeostasis, lipid and carbohydrate metabolism and decreased inflammation (Gentile & Weir, 2018). In a study exploring the impact of feeding mice a low MAC diet, results indicated a large reduction in the diversity of the microbiome and that decreases in MAC lead to decrease in SCFA, which in turn can promote inflammation (Gentile & Weir, 2018). Many pre-biotics contain MAC, which promotes the production of *Bifidobacterium* and *Lactobacillus* (Gentile & Weir, 2018).

One popular diet is the ketogenic diet, which stems from a low CHO consumption (Hills et al., 2019). This diet has often been used as a treatment for seizure disorders because it leads to an increase in *Akkermansia* and *Parabacteriodes* (Olson et al., 2018). In a study conducted by Olson et al., they showed in mouse models that an alteration of the microbiome in a ketogenic diet acts as a protection against seizures. Other studies all were congruent in the fact that a ketogenic diet decreases the diversity and richness of the microbiome, with an increase in Bacteroidetes and a decrease in Firmicutes and Actinobacteria (Newell et al., 2016; Zhang et al., 2018).

Gluten Free Diet

Multiple research studies have proven that changing the main protein source has the most impact on the gut microbiome's diversity as opposed to altering the carbohydrate source (Bonder et al., 2016). However, changing the carbohydrate source does seem to impact the composition of the microbiome. One diet that alters a person's carbohydrate intake is a gluten free diet (GFD). This diet is often recommended to patients who have celiac disease, which is an autoimmune condition that stems from the ingestion of gluten. Therefore, most patients must maintain a strict gluten free diet in order to prevent inflammation of their gut mucosa. In a study conducted by DiCagno et al., they followed 19 patients on a gluten free diet and 15 controls over the span of two years. During this time, they found a decrease in *Lactobacillus, Enterococcus,* and *Bifidobacterium* and an increase in Bacteroidetes, *Staphylococcus, Salmonella, Shigella*, and *Klebsiella* (Di Cagno, 2009). In another study that looked at fecal samples from 42 healthy subjects and 14 celiac patients, they found a decrease in the level of

Bifidobacteria and dysbiosis of the microbiome even when patients were on a GFD (Golfetto et al., 2014). Congruent with these findings, Starz et al. also found that a GFD led to a decrease in *Bifidobacterium* and an increase in *Enterobacteriaceae* and *Escherichia* (Starz et al., 2021).

In addition, one study looked at the stool samples of participants on a GFD over a 4-week timeframe and compared it to individuals not on a GFD. One major finding was a that a GFD led to a decreased in *Veillonacae* and *Ruminococcus* bacteria (Bonder et al., 2016). *Veillonacae* is thought to promote inflammation and thus it can be seen in the pathogenesis of many different gastrointestinal disorders and *Ruminococcus* is important for degrading indigestible starches (Bonder et al., 2016). Therefore, this diet is often recommended for individuals with gastrointestinal diseases in order to assist in alleviating some of the symptoms.

FODMAP Diet

A low FODMAP diet (LFD) is one common diet recommended to patients who experience a multitude of gastrointestinal issues, and it aims to reduce many of the symptoms associated with eating that a patient may experience. The LFD is a strict-elimination diet of short-chain fermentable carbohydrates including lactose, fructose, fructans, galactans, and polyalcohols. After a period of complete elimination, there is then a re-introductory period in which a person tests each food to see if it is tolerable (Reddel et al., 2019). An LFD diet is often recommended to alleviate symptoms associated with Irritable Bowel Syndrome (IBS). IBS is a disease that is characterized by abdominal pain and bloating; however, it is important to note that it is not autoimmune in origin and therefore is not classified as IBD. This diet is often recommended because when microbiota process FODMAPs, they produce colonic gases which can cause the bloating; therefore, LFD diets are supposed to decrease the production of gases (Reddel et al., 2019).

Numerous studies have proven that LFD alters the composition of the microbiome and that this alteration aids in the relief of gastrointestinal symptoms. Short-term implementation of a LFD reduces bacterial diversity and also decreases the level of *Bifidobacteria*, while increasing the ratio of Firmicutes to Bacteroidetes (Hills et al., 2019). In a study examining the microbiome of patients on a LFD compared to a high FODMAP diet (HFD), it was found that that the LFD had decreased levels of *Bifidobacteria* and increased *Actinobacteria*. On the other hand, the HFD had decreased diversity of the microbiome and decreased *Firmicutes* and *Clostridiales* (McIntosh et al., 2017). In another 4-week study examining the microbiota of patients on a LFD compared to a normal diet, they found a reduction of *Bifidobacteria,* which is congruent to past studies (Hills et al., 2019; McIntosh et al., 2017; Reddel et al., 2019). An LFD has also been shown to decrease butyrate-producing bacteria (Starz et al., 2021).

Western Diet

Finally, a Western diet that is high in fat and protein, but low in fiber has been linked to a lack of diversity of the microbiome (Frame et al., 2020). In addition, through examining the composition of the microbiome in a Western diet, studies have found it correlated with increased levels of *Bacteroides* and decreased levels of *Enterococci* and *E. Coli* (Frame et al., 2020). A Western diet has also been correlated with many different diseases including obesity, cardiovascular disease, and diabetes. In this type of diet there is a decrease in MAC. A study examined the gut microbiota of mice eating a diet low in MAC and found that it led to a decrease in the diversity of the gut microbiota of the mice and that this lack of diversity was transmissible to the next generation (Sonnenburg et al., 2016). In addition, after one generation it was still possible to alter this lack of diversity, but after multiple generations it was no longer a reversible change.

One study conducted by Agus et al., investigated the impact of a high fat, high sugar (HF/HS) diet, which is seen in a Western diet, on the microbiome. They found that a HF/HS diet led to an increase in Proteobacteria and *E. Coli* populations. With increased Proteobacteria, there was less of an abundance of anti-inflammatory bacteria and decreased production of SCFA, leading to a decrease in acetate, butyrate, and propionate concentrations. SCFA tends to bind to GPR43 receptors and with a decrease in SCFA, there was also a decrease in GPR43 receptors. Hypothesizing that the decrease in receptors may increase inflammation, investigators treated mice with GPR43 agonists and found that it gave them some protection against inflammation. The study suggested this as an effective treatment for Crohn's Disease (CD), as often there is uncontrolled inflammation in the pathogenesis of CD. In addition to decreases in GPR43, it was found that a HF/HS diet increases the *E. Coli* population that contributed to the overall dysbiosis by increasing the permeability of the gut mucosa (Agus et al., 2016).

It has been thought that the Western diet may lead to the pathogenesis of CD, as there is a high incidence rate of IBD in Western countries. The next section will explore the relationship between diet, IBD and the gut microbiome.

DIET, IBD, AND THE GUT MICROBIOME

What is IBD?

Inflammatory Bowel Disease, or IBD, consists of two different, incurable diseases that are classified based on the part of the GI tract that they affect. CD can lead to inflammation anywhere throughout the gastrointestinal tract, whereas ulcerative colitis is solely confined to the colon. Both diseases manifest with similar symptoms including abdominal pain, bloody stools, nausea, diarrhea, and weight loss. Patients can also experience extraintestinal manifestations of the disease including, erythema nodosum, pyoderma gangreaneosum, ophthalmologic manifestations, joint pain, and malabsorption that can lead to osteoporosis (Engel & Neurath, 2010). Due to the chronic inflammation caused by the disease, patients can face life-threatening complications and often need to have surgery. IBD also increases an individual's risk for colorectal cancer. It affects about 1.5 million Americans and 2.2 million in Europe, and is it often has both periods of remission and relapse (Ananthakrishnan, 2015). It tends to affect more females than males and the median age of diagnosis is 33-45 years old (Engel & Neurath, 2010). IBD is generally diagnosed through a colonoscopy, where the normal smooth surface of the intestines looks cobble stone in nature and it can be confirmed through biopsies (Engel & Neurath, 2010).

The prevalence and incidence rate of IBD has steadily increased since the 1800s when very few cases were diagnosed. The incidence rate has increased from about 0.6/100,000 to 8.3/100,000 and today the disease affects about 0.5% of the population and seems to be more prevalent in the Westernized world, possibly highlighting the role a

Westernized diet may play in promoting the development of the disease (Ananthakrishnan, 2015; Kaplan, 2015). As shown in **Figure 6**, there is a much higher incidence rate in the United States, Canada and Australia, compared to South America, Russia, and China. However, alarmingly, there has been an increase incidence rate in predominantly low-incidence areas, such as Asia (Engel & Neurath, 2010).

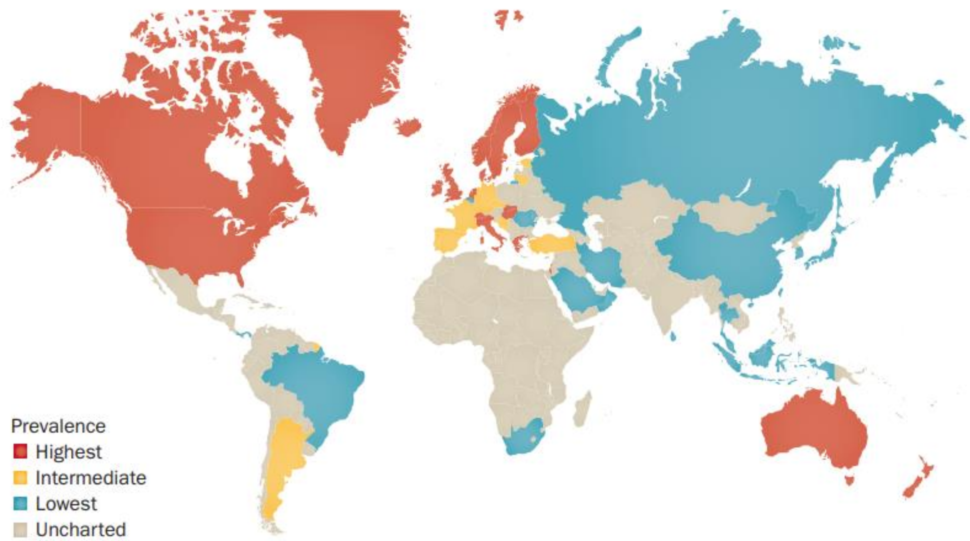

Figure 6. Prevalence of IBD in 2015. Highest prevalence seen in United States, Canada, and Australia. Lowest prevalence seen in Russia, Brazil, China, and South Africa. Taken from (Kaplan, 2015).[2]

Although both CD and UC currently lack a cure, there are many different treatment options for patients. One treatment consists of aminosalicylates and corticosteroids. Salicylates, such as mesalamine, are predominantly used as maintenance medication once remission is attained. In order to achieve remission, patients can take

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[2] Reprinted from The global burden of IBD: from 2015 to 2025, Vol 12, Kaplan et al., Pages 720-727., Copyright (2020), with permission from Springer Nature

corticosteroids such as budesonide or prednisone (Engel & Neurath, 2010). Another popular treatment option is Tumor Necrosis Factor (TNF) inhibitors. These are often given as injections and are usually tapered off once remission is attained. The downside of these treatments is that patients can experience a multitude of side effects, including depressed immune function which can lead them vulnerable to many illnesses. TNF inhibitors in particular can also lead to the development of certain blood cancers. Other treatments include, methotrexate, antibiotics and infusions (Engel & Neurath, 2010). Unfortunately, none of these treatment options can cure IBD and are highly variable in effectiveness from person to person. Therefore, it is imperative to find new and targeted treatment options for patients, and to decrease the amount of adverse side effects seen with treatment.

There are a multitude of reasons why one may develop IBD and it is normally due to an interplay between diet, lifestyle, behavior, and dysbiosis of the microbiome (Ananthakrishnan, 2015). Kaplan et al., describe four main reasons for the difference in the prevalence of IBD worldwide. These include: the genetic makeup of an individual, environmental exposures, health care capacities and the fact that many countries may have differences in their surveillance of IBD (Kaplan, 2015). In terms of genetics, there seems to be some genetic risk with an 8% chance of developing CD if your parent also has the disease. There are certain genetic loci such as NOD2, ATG16L1, IRGM, and LRRK2 that have been shown to be correlated with the development of IBD (Ananthakrishnan, 2015). NOD2 in particular seems to have a predominant role in the pathogenesis of IBD because in the presence of peptidoglycan from bacterial cell walls, this mutation can activate NFκB and the mitogen-activated protein kinase pathways (Engel & Neurath, 2010).

In addition to genetic factors, there are environmental factors that are thought to play a role in the development of IBD. These include smoking, appendectomy, the hygiene hypothesis, infectious pathogens and antibiotics, medications such as aspirin, non-steroidal anti-inflammatory drugs, oral contraceptives, and postmenopausal hormonal therapy (Ananthakrishnan, 2015). One of the most important of these influencers is diet, as detailed above. CD seems to be associated with lower intake of fruits and vegetables. In addition, persons with a high fiber diet tend to have a decreased risk of developing IBD, because the increased fiber leads to increased production of SCFA, which inhibits inflammatory mediators (Ananthakrishnan, 2015). A high fat diet leads to the production of *Bilophila wadsworthia*, which promotes an inflammatory response and may be implicated in the pathogenesis of IBD. Other important environmental factors include stress, lack of sleep, and exercise (Ananthakrishnan, 2015).

With the specific genetic and environmental factors present, the gut microbiome can cause inflammation through numerous mechanisms. One way is that the bacteria can attach to the walls of the gut and begin entering the cells. This promotes the monocytes and macrophages to produce TNF-alpha, which is often the target of IBD treatment (Stojanov et al., 2020). Microbes can also cause inflammation through activating toll like receptors (TLR), which are normally important for controlling the immune response and maintaining gut homeostasis (Stojanov et al., 2020). For example, *Clostridium* is implicated in T cell differentiation and other microbes production of SCFA produces an anti-inflammatory effect (Stojanov et al., 2020). While the exact mechanism of dysregulation and inflammation is not clear, it is apparent that alteration of the microbes and their function is a main driver of the excessive and uncontrolled inflammation seen in IBD. Therefore, this section will detail the dysbiosis seen in the gut microbiome of patients with IBD and how specific treatments can target the gut microbiome in order to help patients achieve long-lasting remission.

Dysbiosis of the Gut Microbiome in IBD

Clearly, a healthy gut microbiome plays a crucial role in preventing the development of IBD. However, dysbiosis of the gut bacteria has been linked to the pathogenesis of many gastrointestinal disorders, including IBD. Normally, in the intestinal microbiota of healthy adults, the increased Firmicutes, Bacteroidetes, and Actinobacteria and decreased Proteobacteria play an anti-inflammatory role and assist in preventing dysbiosis. However, there are many genetic mutations that have been discovered to be implicated in IBD. Investigation of these mutations have indicated that they are linked to alterations in the gut microbiome and down-regulation of the healthy microbiota (Glassner et al., 2020). Common mutations in IBD that enhance a person's genetic risk include NOD2, ATG16L1, CARD9 and CLEC7A. NOD2 is part of an intracellular pathway that leads to the development of a specific Pattern Recognition Receptor capable of interacting with both gram positive and negative bacteria (Glassner et al., 2020). Patients with this mutation have been shown to have a decrease in *Faecalinbacterium* and increased *Escherichia*. Another common mutation is a mutation in CARD9, which increases a person's susceptibility *Candida albicans, Aspergillus frumigatus and Cryptococcus neoformans* (Glassner et al., 2020).

While genetic mutations can lead to changes in the microbiome, it is thought that environmental factors may also be implicated in altering the gut microbiota. Factors such as antibiotics, infections, breast feeding, diet and cigarette smoking are all implicated in promoting the inflammatory nature of the disease (Ananthakrishnan, 2015; Glassner et al., 2020). In fact, having infectious gastroenteritis has been shown to increase the development of IBD by 40%. Another hypothesis, called the hygiene hypothesis, postulates that a child's immune system may be negatively impacted if they are not exposed to enough microbes during early childhood which could also lead to the pathogenesis of IBD (Glassner et al., 2020). In addition, when developing the microbiome, environmental factors play a large role. A child's microbiome will rapidly grow during their first two years of life, with the first colonizers usually being *Enterobacteriaceae, Streptococci,* and *Staphylococci*. Following that, *Bifidobacteria, Clostridium* and *Bacteroidetes* will begin populating the microbiome (Marques et al., 2010). The type of birthing delivery and feeding seems to play a prominent role in the development of the microbiome, with C-section babies having lower numbers of *Bifidobacteria* (Marques et al., 2010). While this paper will not go into an in-depth description of the development of the microbiome, it is important to recognize that a variety of factors contribute to its development all of which can be instigating factors in the dysbiosis of the microbiome seen in gastrointestinal disorders.

The combination of genetic changes, environmental factors, and microbiota changes can all lead pro-inflammatory pathways that can cause dysfunction of rich-mucous barrier and damage to the underlying epithelium. The complex interplay of all these factors and their resulting damage is shown in **Figure 7.**

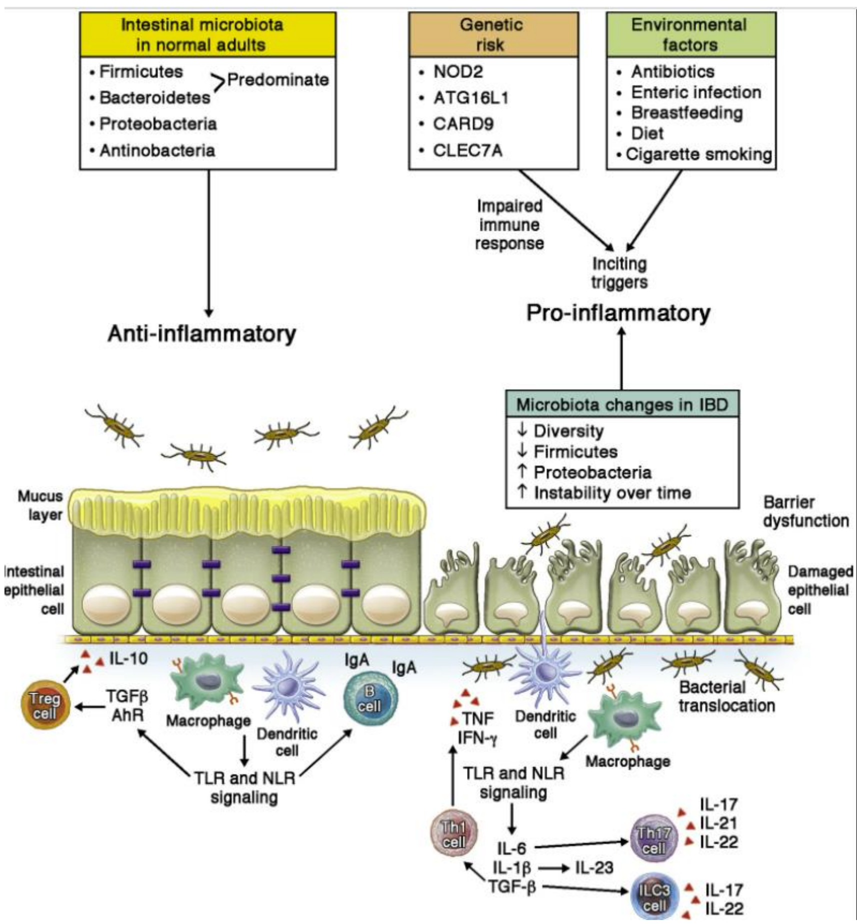

Figure 7. The effects of genetic risk, environmental factors and microbiota changes in the pathogenesis of IBD. This compares the alterations seen in IBD to a normal health microbiome. Take from (Glassner et al., 2020).[3]

[3] Reprinted from The Microbiome and Inflammatory Bowel Disease, Vol 145 /Issue 1, Glassner et al., Pages 16-27., Copyright (2020), with permission from Elsevier

While there are both genetic and environmental factors that play a role, the root cause of developing IBD is still poorly understood and it is classified as a disease of unknown etiology. However, one aspect of the disease that is well-documented is the alteration of the gut microbiome in patients with IBD. There is a clear decrease of diversity and decrease of anti-inflammatory bacteria. There tends to be more of a decrease in diversity in CD when compared to UC (Ananthakrishnan, 2015). One study found that those bacteria that are increased include *Proteobacteria, Pasteurellacea, Veillonellaceae, Fusobacterium and Ruminococcus*, while those that are decreased include *Clostridium, Bacteroides, Suterlla, Roseburia, Bifidobacterium and Faecalibacterium prausnitzii.* For fungi, *Candida albicans, Candida tropicalis, Clavispora Iusitaniae, Cyberlindnera jadinii, and Kluyveomyces marxianus* are increased, while *Saccgarmyces cerevisiae* is decreased. The *Caudivirales* virus also seems to be increased in IBD (Glassner et al., 2020). A large decrease in the Firmicutes/ Bacteroidetes ratio has also been seen in IBD patients (Stojanov et al., 2020). In the Firmicutes phylum, *Clostridia* and *Faecalibacterium prausnitzii* were specifically decreased and Firmicutes was observed to more decreased in CD patients (Stojanov et al., 2020). When comparing CD to UC, it seems that CD has a more unstable and altered gut microbiome than seen in UC (Glassner et al., 2020).

Another study that examined the microbiome in both CD and non-IBD patients using Next Generation Sequencing, found congruent results to Glassner et al. Through their investigation, they also concluded that IBD patients tend to have a decrease in abundance of bacteria and showed large alterations in the composition of their microbiome when compared to non-IBD patients. They found an increased abundance in bacteria which include *Enterobacteriaceae, Pasteurellacea, Veillonellaceae, and Fusobacteriaceae,* and decreased abundance in *Erysipelotrichales, Bacteroides, and Clostridiales.* When examining contributing factors to this dysbiosis, they discovered that antibiotic use tended to exacerbate the dysbiosis found in CD patients (Gevers et al., 2014).

In another study, Lewis et al., used shot gun metagenomic sequencing to analyze fecal samples from CD patients starting EEN or anti-TNF antibiotics. They discovered that in all patients with active disease there was an increase in *Escherichia* and decreased *Preveotella* (Lewis et al., 2015). As mentioned in the plant-based diet section, *Preveotella* is essential for enhancing the production of SCFA that are needed to decrease inflammation. Therefore, this decrease may play a role in the pathogenesis of the disease. In addition, there is an increase of lactose-fermenting bacteria including *Streptococcus, Lactobacillus,* and *Klebsiella* (Lewis et al., 2015).

It was also found that 22% of CD patients had the pathogen *Adherent invasive E. Coli* (AIEC) in the microbiome, compared to 6.2% in healthy patients and there was a reduction of *Faecalibacterium prausnitzii*, which functions to produce pyruvate (Ananthakrishnan, 2015). This increase of AIEC seen in the intestinal mucosa of patients with CD was further proven in a study conducted by Michaud et al. In this study, they compared the intestinal ileal and colonic mucosa of patients with CD to those with UC to healthy controls. In the ileal group, they found AIEC in 21.7% of CD patients and 6.2% of controls. This is similar to the colonic specimens in which they found the majority of

AIEC strains in CD patients, when compared to UC patients and controls. These results indicated that the increased prevalence of AIEC is specific to the ileal region in CD (Darfeuille-Michaud et al., 2004).

Successful Dietary Interventions

Current treatment for IBD tends to be more of a shot-gun approach with little personalization of treatment. Most patients are given strong corticosteroid treatments aimed at reducing the inflammation in the gut. These treatments can have lasting adverse consequences on the patient's health and may leave them susceptible to many opportunistic infections. One common treatment that is administered to IBD patients includes anti-TNF therapy. The purpose of this therapy is to directly target the inflammation seen in IBD through shutting off the inflammatory pathway. Even though this approach targets the exact same pathway in each patient, the gut microbiome seems to be important in the effectiveness of the treatment. When examining the gut microbiome of those patients who had success with this treatment, patients with an abundance of the *Bifidobacterium species, Collinsella species, Lachnospira species, Lachnospiraceae, Roseburia species, and Eggerthella taxa* showed more successful results, suggesting that the gut microbiome may play a role in the effectiveness of therapy (Glassner et al., 2020). As the alteration in gut microbiota is at the core of IBD, there has been new research into investigating microbiota-based strategies for targeting treatment for IBD. The goal of this strategy would be to diagnosis IBD based on a "microbial signature." This signature could be used as a prognostic criterion in order to predict the potential complications and response to therapy. In terms of treatment, the goal would be to replace the inflammatory bacteria with anti-inflammatory bacteria such as *Faecalibacterium prausnitzii*. Utilizing this approach, the gut microbiome could then be reset via a fecal microbiota transplant and redesigned with the administration of specific medications (Glassner et al., 2020). Other bacteria that may have therapeutic benefits in IBD and could be administered with this approach include *Lactobacillus* and *Bifidobacterium* (Stojanov et al., 2020).

A new and emerging treatment option for patients with IBD is centered around different dietary interventions that are targeted at altering the gut microbiome. While many of these dietary interventions will not cure IBD, they can help in alleviating symptoms and attaining remission. Results from nutritional therapy have been encouraging. In a study conducted by Agrenio et al., results indicated that when compared to the control, a patient before nutritional therapy had increased levels of Proteobacteria, decreased levels of Bacteroidetes and Firmicutes, and an overall decrease in diversity of the microbiome, which is congruent with the previous studies discussed. Interestingly, as shown in **Figure 8**, they found that after being treated with nutritional therapy, patients had comparable levels of Bacteroidetes, Firmicutes and Proteobacteria to the control and had an overall increase in bacterial diversity (D'Argenio & Salvatore, 2015).

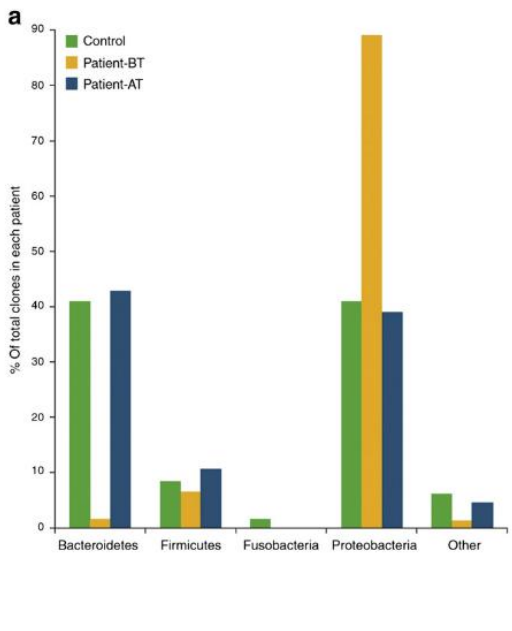

Figure 8. IBD Patients before and after nutritional therapy compared to healthy controls. Results show that post-therapy patients microbiome closely resembles that of a healthy control. Taken from (D'Argenio & Salvatore, 2015).

As apparent from the study conducted by Argenio et al., nutritional therapy may be useful at restoring the dysbiosis of the microbiome in patients with IBD back to a symbiotic relationship. Therefore, there have been multiple different dietary interventions that have been tested and proven successful in helping IBD patients achieve remission. These include, exclusive enteral nutrition (EEN), the Crohn's Disease exclusion diet (CDED), and the specific carbohydrate diet (SCD). Other dietary interventions mentioned previously including the low FODMAP diet and a Gluten Free diet have been shown to help alleviate symptoms associated with the disease, but not lead to remission. Therefore, these next sections will highlight the three dietary treatments that have been proven to lead to remission in IBD patients.

Exclusive Enteral Nutrition (EEN) Treatment

One treatment option for patients with IBD is EEN. As many studies have indicated, there is a clear reduction of diversity of the gut microbiome seen in patients with CD, with a primary loss of anti-inflammatory bacteria. EEN has been shown to lead to remission in CD patients through alteration of the gut microbiome. A study found that EEN works by further decreasing the abundance of the bacteria and causing enough dysbiosis that the gut eventually resets and restores to a normal microbiome (MacLellan et al., 2017). In another study, Gatti et al., looked at the effects of EEN in patients with CD. Their results also showed that EEN reduced the abundance of microbial diversity; however, upon ceasing EEN, patients' microbiome returned to their baseline normal. This means that this specific treatment may not be very effective at achieving long term remission for patients. Overall, they found a decrease in Firmicutes post-EEN treatment and an increase in Proteobacteria. They also found a reduction in *F. prausnitzii*, which is a butyrate producing bacteria that is thought to be productive against IBD (Gatti et al., 2017). Although it seems counterintuitive to see an increase in Proteobacteria since it is often a main contributor to dysbiosis, they believe, similar to the conclusion drawn by MacLellan et al., that causing too much dysbiosis will force the gut microbiome to reset.

Kaakoush et al. were also interested in the effect of EEN on the gut microbiome of children with CD. They used multiple techniques, including 16s rRNA gene and whole-genome high throughout sequencing, in order to examine differences seen in the microbiota of children throughout EEN treatment. Congruent with many past studies, before starting EEN, CD patients had reduced diversity of their gut microbiota and a loss of the symbiotic nature of the bacterium. Similar to Gatti et al.'s results, they found that during treatment there was an even further decrease in bacteria, with 80% of patients going into remission. However, after stopping the therapy many patients relapsed again and there was an increase in bacteria (Kaakoush et al., 2015). Again, this study highlights that while EEN may be an effective therapy at helping patients achieve remission through creating further dysbiosis within the microbiome, it is not effective at maintaining a lasting remission after therapy.

Crohn's Disease Exclusion Diet (CDED)

There is recent evidence that CD exclusion diet may lead to remission in patients. The CD exclusion diet is aimed at reducing the exposure of specific foods that may adversely affect the gut microbiome and uses a combination of both enteral nutrition and whole food restriction (Starz et al., 2021). The goal of this diet is to reduce the exposure of patients to animal fat, dairy, gluten and emulsifiers, which are typical staples of a Western diet (Sigall-Boneh et al., 2014). Through decreasing these factors, the goal is to decrease the levels of AIEC, which would lead to a decrease in intestinal permeability. A study conducted by Levine et al., compared the effectiveness of a CDED to EEN. It was a 12-week study that placed the participants in two groups—one group receiving EEN and another receiving CDED and partial enteral nutrition (PEN). Results showed that patients on the CDED and PEN diet had as sustained increase in *Clostridia*, a bacterium that tends to be decreased in CD patients, and had a decrease in Actinobacteria and Proteobacteria, with the Proteobacteria decrease being sustained. This is compared to the EEN group that initially showed the same results, an increase in *Clostridia* and a decrease in both

Actinobacteria and Proteobacteria; however, both the Actinobacteria and Proteobacteria began to return to normal levels seen in CD patients by 12 weeks. Overall, as shown in **Figure 9**, the CDED plus PEN group, showed higher rates of steroid free remission and a sustained decreased in the Proteobacteria (Levine et al., 2019). As an increase in Proteobacteria has major implications in CD, this sustained decrease is an encouraging result for this new dietary therapy.

In another study conducted by Sigall-Boneh et al., they followed 47 patients who were on the CDED. The results indicated that of the patients who followed the CDED, 70% of children and 60% of adults achieved remission. While slightly more effective in children, this study yields promising results about the effectiveness of utilizing the CDED in achieving lasting remission for CD patients (Sigall-Boneh et al., 2014).

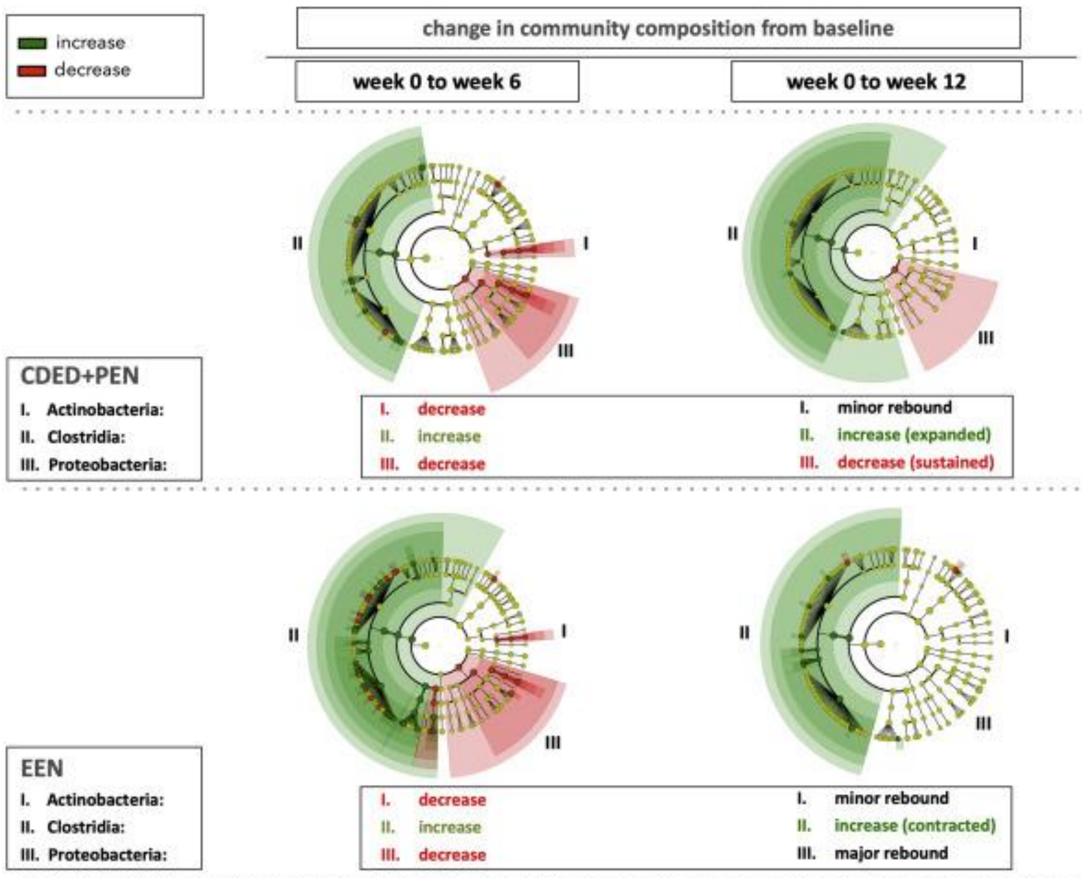

Figure 9. Comparison of the microbiome in CDED+PEN to EEN. As indicated, both start with the same microbiome (low Actinobacteria and Proteobacteria with an increase in Clostridia). However, CDED+PEN leads to more lasting changes in the microbiome, as opposed to EEN which mostly rebounds back to the normal levels. Taken from (Levine et al., 2019).[4]

Specific Carbohydrate Diet (SCD)

The SCD allows for a patient to eat carbohydrate food consisting of monosaccharides only and excludes any disaccharides and most polysaccharides. The goal of this diet is to decrease bacterial species that can metabolize carbohydrates to

[4] Reprinted from Crohn's Disease Exclusion Diet Plus Partial Enteral Nutrition Induces Sustained Remission in a Randomized Controlled Trial, Vol 157/Issue 2, Levine et al., Pages 440-450., Copyright (2019), with permission from Elsevier

create metabolites that promote inflammation (Dubrovsky & Kitts, 2018). Patients with IBD are frequently recommended to try this diet because it is thought that due to dysbiosis of the microbiome in IBD, patients have difficulty breaking down disaccharides due to enzyme deficiencies and therefore consuming only monosaccharides can optimize absorption (Kakodkar et al., 2015). In a case study that looked at 50 patients with IBD, results showed that a SCD may assist in helping patients achieve remission. Though this is a recent discovery, it is postulated that there is a change in the microbiome of patients once they begin SCD that alters it in a manner that allows it to reduce symptoms associated with IBD (Kakodkar et al., 2015).

In another case study that followed the fecal changes of a patient on a SCD diet for two weeks, they found that before the implementation of a SCD, *Fusobacteria* was most predominant, followed by *Veillonellaceae*. After the diet, there was a decrease in *Fusobacteria* and an increase in *Enterobacteria* (Dubrovsky & Kitts, 2018). As there is evidence that increased *Fusobacteria* may play a role in the pathogenesis of IBD, this case study provides compelling evidence that SCD may be able to reduce *Fusobacteria* levels, offering a therapeutic benefit to IBD patients.

This diet has been tried and proven to be successful in IBD patients. Suskind and his team examined the effect of the SCD on patients who were classified as having active CD. They found that upon implementing the diet, Bacteroides, Firmicutes and *Parabacteroides* were decreased, while *Eubacterium, Ruminococcus* and *Subdoligranulum* increased the most. In addition, Proteobacteria levels decreased in nearly every patient. Results suggested that this form of therapy may work to improve the dysbiosis seen in IBD patients, as opposed to EEN which increases the dysbiosis (Suskind et al., 2018). The fact that EEN, which increases dysbiosis, and SCD, which decreases dysbiosis, both have been proven diets to enhance remission in IBD patients adds to the complexity behind the pathogenesis of IBD. Though the root cause is poorly understood, it is clear that microbial communities play a large role in development and progression of IBD. This is evidenced in the fact that changes to the microbiome have been continuously shown in to lead to remission in patients.

CONCLUSIONS AND FUTURE DIRECTIONS

As highlighted throughout this paper, the gut microbiome is highly complex and alterations of the microbiome can lead to the pathogenesis of many different diseases including IBD. While many different bacteria compose the microbiome, the four main phyla include Bacteroidetes, which is important in the degradation of glycan and the production of SCFA, Firmicutes, which plays a role in digesting indigestible carbohydrates, Actinobacteria, which is important for gut homeostasis and Proteobacteria, which is pro-inflammatory in nature. Dysbiosis in these main phyla is at the root of many pathological conditions.

Although a wide array of factors can influence the composition of a person's microbiome, one primary influencer is diet. In a high animal-based protein diet, there is an increase in *Bacteroides, Clostridia* and *Bilophilia wasworthia* and a decrease in *Roseburia* and *Firmicutes*. As *B. wasworthia* leads to systemic inflammation, an increase in this many be implicated in the pathogenesis of IBD. In addition, when metabolizing a primarily animal protein, the microbiota converts the increased levels L-carnitine and phosphatidylcholine into TMA, which is then converted into TMAO. Interestingly, high levels of TMAO are seen in cardiovascular disease, while low levels of TMAO are seen in IBD. In a high fat diet, there is an increase in *Faecalis, Pranisnitzii, Bacteroidetes, Actinobacteria* and *Alistipes*, and a decrease in *Firmicutes, Proteobacteria* and *Bifidobacteria*. In addition, the microbiota also alters the bile acids in a manner that aids their binding to receptors, allowing the bile acids to exert their function. A carbohydrate diet leads to the increase of *Bifidobacterium* and a decrease in *Bacteroides.* Ingestion of carbohydrates produces MAC which can then be used as a source of energy for the enterocytes and the microbiota can also process the MAC to produce SCFA.

In a plant-based diet, there is an increase in *Prevotella* and decreased levels of *Bacteroides, Bifidobacterium, Escherichia coli* and *Enterobacteriaceae*. This diet is usually high in fiber, which leads to increased production of SCFA, such as acetate, propionate, and butyrate. Multiple studies comparing the microbiome of children on a pre-dominantly Western diet compared to a plant-based diet have proven there is a significant difference in the composition between the two and that there is a large increase in *Prevotella* in a plant-based diet. In the gluten free diet, which is the standard diet for a person with Celiac Disease, there is a decrease in *Lactobacillus, Enterococcus, Veillonacae, Ruminococcus* and *Bifidobacterium* and an increase in *Bacteroidetes, Staphylococcus, Salmonella, Shigella* and *Klebsiella*. A low FODMAP diet, which is a strict elimination diet often recommended for patients with IBS, leads to decreased levels of *Bifidobacteria, Firmicutes*, and *Clostridiales* and increased Actinobacteria. There is also a decrease in overall diversity of the microbiome and a reduction butyrate-producing bacteria, which reduces the production of colonic gases. Finally, a Western diet, characterized by a high fat, high protein, low fiber diet, has higher counts of *Bacteroides* and lower counts of *Enterococci* and *E. Coli*.

The alterations of the gut microbiome caused by a Westernized diet has been implicated in the pathogenesis of IBD, an autoimmune disease of the gastrointestinal tract characterized by uncontrolled intestinal inflammation. IBD has been increasing in prevalence and current treatment lacks consistent efficacy. While there are a multitude of factors that can lead to the pathogenesis of IBD including genetics, environmental, and diet, one clear fact is that there is dysbiosis of the microbiome. Overall, IBD patients have a decrease in diversity of their microbiome, a decrease in Firmicutes, and an increase in Proteobacteria. CD patients also have an increase in AIEC and reduction of *Faecalibacterium prausnitizii*.

Recent treatment options have explored targeting the microbiome through dietary interventions in order to improve patient's symptoms and aid them in achieving lasting remission. EEN treatment has been one option that has been shown to lead to remission in IBD patients. The premise of EEN is that creating so much dysbiosis causes the gut microbiome to reset. Though effective at achieving remission, it is not effective at achieving lasting remission. A more promising treatment option is the CDED. Compared to EEN this has been shown to lead to lasting remission within CD patients. It leads to an increase in *Clostridia,* and a decrease in Actinobacteria and Proteobacteria. Finally, a SCD, another dietary intervention, has also been proven to be effective. It works in the opposite manner of EEN, and decreases dysbiosis. It leads to a decrease in *Bacteroides, Firmicutes, Fusobacteria* and an increase in *Eubacteria, Ruminococcus* and *Subdoligranulum.*

While much is still unknown about the gut microbiome, there have been great advancements in sequencing the microbiome to determine its composition. Changes within the microbiome that deviate from normal are implicated in the pathogenesis of many diseases, including IBD. Current treatment for IBD is centered on more of a shot gun approach aimed at controlling inflammation and reducing symptoms. However, recent developments in dietary interventions aimed at altering the microbiome have shown promising results, with many patients attaining long lasting remission. While these treatments are still in development, there is great hope that with increased understanding of the microbiome it will be possible to treat a wide array of chronic diseases through its alteration, which will decrease patient exposure to the harmful side effects of current medications. As researchers slowly begin to unravel the mystery of the microbiome, it may become feasible to restore the symbiotic relationship.

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