A STUDY INTO THE HUMAN GUT MICROBIOME

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INTRODUCTION TO THE GUT MICROBIOME

When talking about the human microbiome, one refers to the trillions of microorganisms that reside on various tissues of the body. These microorganisms can be found to live inside and on humans, and are referred to as microbiota.[1] The human gut microbiome is so unique and diverse among individuals that it can be used as a sort of fingerprint comprised of microbial variability.[2] Human microbiota reside symbiotically through commensal and mutualistic (sometimes pathogenic) relationships.[3] Furthermore, there are several regions that make-up specific microbiome habitats, these regions include; the skin, the mouth and nose, the gut, and the vagina.[4] The human microbiome can be defined as the aggregate of those regions, which are comprised of the microbiota and the genetic material they possess.[3] More specifically, the gut microbiome is collectively comprised of the microorganisms that are found throughout the gastrointestinal tract of a human. This microbiome can span the stomach, duodenum, small intestine, cecum, large intestine, and the colon. Bacteria and other microbes that reside in the gastrointestinal tract are what make-up the gut microbiome. Much of the literature and research done on microbiomes is found to focus on the microbiota that reside specifically in the gut. This is due to this region, distal gut specifically, having the greatest abundance in bacteria as well as the most significant variation in genetic material when compared to all of the other human microbiomes.[2,3]

Depending on what one is researching and how it is being studied, the specific definition of the gut microbiome varies in the literature. For this paper, "gut microbiome" will signify a broad definition, covering microbiota and their genes from regions of both the upper and lower gastrointestinal tracts (i.e. stomach, small intestine, colon). This way a much broader understanding of what the gut microbiome actually is can be achieved. Exploring what the gut

microbiome entails can be confusing and convoluted, but here we will outline what the recent research defines as a healthy human gut. This will be explored in terms of the differences in diversity and the bacterial composition that has been found to make-up a normal human gut microbiome. Drawing attention to both the composition of microbiota and what it means to have "normal" human gut microbiome. The human gut microbiome is an important part of everyday health and its influence with our many physiological mechanisms is something we as humans could not live without. This research paper presents an explanation and overview of the gut microbiome and several of the many complex interactions it can have in human health and disease.

GUT MICROBIOME COMPOSITION AND DIVERSITY

So what is considered a healthy or normal gut microbiome? This all depends on what you are trying to ascertain when looking at the thousands of unique bacteria found in the gut. While we do know that some microbes provide beneficial symbiosis, full understanding of how diversity of those microbiota and their genes is still developing. Through research and initiatives such as the Human Microbiome Project[5] it has been found that even in individuals that lead healthy lifestyles, the variation of their internal gut microbiota can be staggering.[4] It is not as simple as stating "large amounts of diversity equals a healthy gut microbiome". Diversity is only a small portion of what constitutes as a healthy gut. To fully appreciate the complexity of the gut microbiota one must first find the common patterns present in individual to individual, defining what can be considered as "normal". Diversity in the gut can be defined with two factors in mind, the amount of distinctly unique microorganisms found in the gut and the abundance of those unique organisms in the gut.[4] This statement can be summarized as, large amounts of variation in taxa equates to a more diverse microbiome. In a human gut, it is commonly found

that the largest portion of biomass is comprised of prokaryotic bacteria while other microorganisms such as, eukaryotes, archaea and even viruses make-up a smaller portion of the gut microbiome.[3,6,7] Majority of gut microbial composition is made up of what are known as strict anaerobes, outnumbering both facultative anaerobes and aerobes by up to 100 times.[8] All these different types of organisms contribute to the diversity that can be seen in the gut of a human. As stated earlier bacteria hold the largest portion of the gut microbiome pie, it is only logical that they also display the most abundant diversity from species to species.

The shear abundance of this was demonstrated by Junjie Qin and the rest of the MetaHIT Consortium when they discovered and sequenced 3.3 million nonredundant microbial genes, 99% of which were bacterial.[9] For comparison, the near-complete human genome only holds 20,000-25,000 coding genes.[10] The study by Qin et al contained 124 fecal samples which were provided by healthy individuals as well as obese, overweight and even inflammatory disease patients.[9] Through metagenomics sequencing the MetaHIT Consortium was able to compare and contrast bacterial genes interpersonally. The research done by the MetaHIT Consortium provides a bedrock for other studies when looking at what may be considered a healthy composition of bacteria in the human gut. Actual population and species counts vary widely from study to study and individual to individual.[11] Nevertheless, an accepted estimate falls between 1000 and 1150 prevalent bacterial species which can be found in a normal human gut.[7,9,12] With at least 160 species being present in each individual, suggesting that most of those were shared across the cohort.[9] They thus concluded that this small shared bacterial could be identified as a common bacterial core, which might be commonly present in a human gut. Although a common bacterial core was identified in these samples, it did not mean this hypothesis was full-proof. Variability in abundance of those species was still relatively high, this

meant that even though those species could be commonly identified in this cohort, the abundance of each species was seen to differ greatly from individual to individual. Further complicating the idea of a common core, in a different study with similar sequencing methods, it was found that no single bacteria was ever present in the 154 sampled adult guts at a significant frequency.[13] This prompted a hypothesis that states, instead of having a common bacterial core that can be identified by abundance of certain bacteria, humans instead may share a common core microbiome in terms of metabolic function provided by the microbiota.[13] Proposing that as humans, we each hold a unique set of bacteria in our gut, but those unique bacteria provide common metabolic functions that can be seen in healthy adult humans. These two hypotheses each come from multiple data sets that can be commonly demonstrated in a range of studies. Further highlighting the complexity of the human gut, the microbiota that resides in each of us, and the functionality that they provide. Metabolic functionality provided by the gut microbiome will be further discussed later in the paper and will be tied back to the unique diversity found in each human gut.

As stated earlier it is widely accepted that the composition of the human gut microbiome is dominated by the presence of bacteria. Normal composition of bacterial biomass can be further characterized into phyla that seem to abundantly and commonly colonize the human gut. In a normal human gut the majority of the bacterial populations fall under two major phyla, the Bacteroidetes and the Firmicutes.[3,7,14,15] There two phyla have been found to provide the largest numbers in the population, each with a number of classes/genera that can also be commonly detected. The Actinobacteria and Verrucomicrobia phyla are right behind the two already mentioned phyla in terms of numbers.[14] Some common genera seen from these two phyla include; *Bacteriodes, Bifidobacterium, Prevotella, Streptococcus, Lactobacillus, Clostridium,*

Enterococcus and *Ruminococcus*.[11] Class and genera differences is what contributes to the large diversity and variety seen in individuals.[14] Certain types of bacteria are so abundant in the gut of humans that recently new classification was developed for our microbial ecosystems, called enterotypes. An enterotype is a classification of living organisms solely based on the bacteriological ecosystem present within their gut microbiome.[16] It was discovered that humans can be classified into three major enterotypes, each of which containing a specific genera of bacteria that was seen to dominate the microbiome composition. In the first classification, enterotype 1, individuals have a microbiome that contains high numbers of bacteria from the genus *Bacteroides* and their species variety is mainly made up from this genus.[16] In an enterotype 2 individual, *Prevotella*, also from the Bacteroidetes phylum, comprises the majority of their gut microbial diversity with a smaller portion originating from *Bacteroides* genus.[16] And lastly, enterotype 3 classification is given to those individuals that have high abundance of *Ruminococcus*, bacteria that fall under the other major phylum Firmicutes.[16] Even with the high interpersonal variability, the consistency of enterotypes can be used as a way to classify individuals through their bacterial make-up. This may be important because enterotypes may allow further insight to how an individual may react to certain diets and even drugs depending on what enterotype they fall under.[16] In 2011 when researching enterotyping in humans, Arumugam and colleagues found that determination of an enterotype was seen to be independent of the individual's age, body mass index, and gender and to an extent cultural origin.[16] While humans can always be classified into one of the three enterotypes listed, percentage make-up of dominating genera can vary greatly in individuals. Only providing a small part of what may be determined as a normal human gut microbiome.

As you move distally through the gastrointestinal tract, the type and number of bacteria present fluctuates depending on region.[11,14] The uniqueness of bacteria makes it so that in each gastrointestinal region, there are different types of bacteria present. This leads to bacterial numbers being distributed unevenly throughout the gut and a distinct difference in diversity and abundance can be observed throughout (FIG 1).[14] Populations range from 10^1 - 10^3 bacteria per gram starting in the stomach and duodenum and rising to 10^{11} - 10^{12} in the large intestine/colon.[17] There are also definite differences in the gut of a healthy human adult versus that of a patient with a gastrointestinal disorder. One example comes from Manichanh et al, and their study over microbiota and inflammatory bowel disease (IBD) which showed diminished numbers of Firmicutes in those affected by the disease.[15] These findings were further supported by Qin et al, where they compared IBD patient samples to those from healthy individuals only to find that previous hypotheses of differences in microbiota to be supported.[9] Since Bacteroidetes and Firmicutes are always found in high numbers in the human gut, they can be used to determine disease states with correlation to some pathogenic genera that fall within these two phyla as shown in the two studies discussed earlier.[14] Furthermore, certain genera like *Bacteroides, Prevoletta* and *Ruminococcus* have been associated with a healthy gut microbiome when their abundance is high.[14] The phyla Bacteroidetes and Firmicutes are sometimes seen to share an inverse ratio relationship when it comes to population numbers present in the human gut. In individuals that harbor an abundance of Firmicutes, the number of Bacteroidetes is seen to diminish, and vice versa.[14] These findings serve as broad descriptions of what can be seen in a normal human gut. Later we will discuss how bacterial composition can be influenced by various factors, contributing to the diversity of the human gut microbiome.

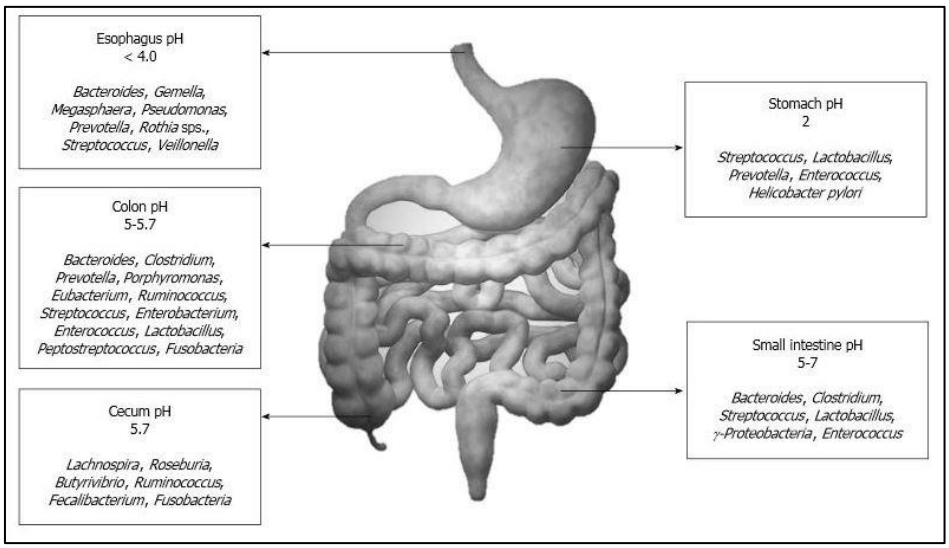

Figure 1.[14] The distribution of normal human microbiota throughout the gastrointestinal tract. Shows how several common genera reside in specific regions of the GI tract. (Figure sourced from Jandhyala, S.M., et al., 2015)[14]

ESTABLISHMENT OF HUMAN GUT MICROBIOME

Our understanding of how the human microbiome is established has increased dramatically in the recent years. And the importance of having healthy microbial exposure has only recently began to get wide acceptance. Establishment of the infant gut microbiome is determined by factors such as maternal microbiota, natal delivery method, and postnatal diet.[18] Unsurprisingly, as we develop as humans, our microbiome develops alongside us. Bacteria and other microbes begin to colonize our bodies as early as birth. As it is widely accepted that that an infant intestine is a sterile environment and introduction to commensal foreign microbes only occurs after birth.[11] On the other hand, it has also been shown that an infant intestine may never truly be sterile before birth, as very low levels of microbes have been found to be present in the meconium (earliest form of bowel movement) of prenatal humans, indicating that there might be small intestinal colonization even before birth.[19] Generally, this type of microbial detection prior

to parturition can be used as a marker for infections during term. However, Jimenez et al concluded that in healthy pregnancies this type of microbial presence may indicate minor mother-to-child exchange of commensal bacteria during pregnancy term and thus suggesting that prenatal infants may actually experience gut colonization before birth.[19] Further investigation into the implications of these findings still needs to occur as current research haven't yielded any significant results. So it is not until birth occurs in which the gastrointestinal tract of a newborn truly undergoes the dramatic colonization event of microbes.[11]

This rapid and extreme colonization occurs due to exposure to microbes that originate from the mother's own vaginal and fecal microbiome with a small portion coming from the environment during delivery.[20] Simple contact exposure leads to millions of microbes making their home in the new infant's gut/intestine, usually introduced through the oral cavity.[20] While this microbial colonization event might first seem erratic and chance driven, there is more structure to how microbes actually colonize a newborn gut than first thought. Interestingly, colonization of the intestines has been found to occur in a stepwise manner. Aerobes and facultative anaerobes such as coliforms and lactobacilli colonize the GI tract first, then strict anaerobes such as *Bifidobacterium, Clostridium,* and *Bacteroides* come in later to replace the first wave of microbiota present.[18,21,22] This form of colonization is quite beneficial for the longevity of the newly introduced microbiota, as this stepwise colonization ensures that proper stability is developed in the microbial community of the child's gut.[23] While it is still not fully understood how some types of microbiota succeed far better than others, colonization occurs quite quickly in the neonate child.[11] Immediately after birth, the same *E. coli* bacteria types can be found in both the mouth of the newborn and fecal matter originating from the mother. More impressively, even the gastric content of a 5 to 10 minute old baby shows similarities to that of

the bacteria found in the mother's cervix.[20] Showing that initial colonization occurs largely due to exposure to the mother's own microbiota. And that this exposure event may greatly shape the development of the infant's own gut microbiome even into adulthood.[11] This concept was also demonstrated by Ley et al, where their study found close relation in the gut microbiota of mice and their respective mothers, and low similarities in mice that were not related.[24] It only makes sense that the initial bacteria found in the infants is bacteria that can also be found in the mother, as exposure occurs through the mother's microbiomes extremely early. To reiterate, a child does not provide and "develop" their own microbiota after birth but instead the mother provides a starting point in which microbes can be introduced to the infant. The bacteria that migrate to the child will then develop alongside the infant, paving the way for future microbial generations. This first exposure and colonization is so critical to the development of gut microbiota that even the method of delivery affects how the gut microbiome of the infant develops.[20]

A big indicator of the importance of this initial exposure can easily be viewed in population differences of microbiota when sampling from infants that have undergone different birth delivery methods. Differences in microbial number have been consistently observed when comparing infants that have undergone a non-natural type of birth.[11] In a study that aimed to evaluate how different delivery methods could affect the establishment of the gut microbiome, it was found that infants that were delivered via a cesarean procedure harbored fewer microbiota counts than those that were delivered vaginally.[21] More precisely they found that the vaginally delivered infants had a larger population of *Bifidobacterium* than those that had been delivered via cesarean.[21] In a more recent study by Bäckhed et al, a clear difference was found between the two delivery methods in terms of the types of microbes gathered from the newborns.[25] They found that vaginally delivered babies harbored more microbes from the genera *Bacteroides,*

Bifidobacterium, Parabacteroides and *Escherichia*, which later were found to be the most abundant microbiota in the infant gut and commonly seen genera.[25] In contrast, infants who had been delivered via a C-section harbored greater microbe counts from the genera *Enterobacter, Haemophilus, Staphylococcus, Veillonella,* and *Streptococcus*, most of which are known skin and oral microbiota indicating that these types of bacteria colonize first in cesarean delivered infants instead.[25] Moreover, in the same study by Backhed et al, they calculated that in vaginally delivered infants 72% of early microbial colonizers matched with the maternal fecal microbiota, while in cesarean babies, only 41% matched.[25] This and other studies like it show how delivery method may compromise the early colonization of the gut microbiome and in turn the microbial make-up of the infant gut. This does not mean that C-section infants are exposed to less microbes, it instead shows that the types of microbes exposed differ and that early diversity and stability may be compromised because of it.

Differences in abundance counts and microbes present are high during the first month, however, the differences due to mode of delivery begin to diminish anywhere between the 4th and 12th month of age,[25] normally becoming almost undetectable past the 6th month.[11] This is likely due to the rapid nature of bacterial colonization and competition inside of the gut during this stage of establishment. In any case, there are still certain bacteria that remain less prevalent in C-section babies, specifically species in the *Bacteroides* genus.[25] So while the differences lower, one can still see the impact the initial exposure has in the composition of the gut microbiome of the infant. Infants that are born to cesarean births can still be exposed to a small portion of their mother's vaginal and fecal microbiota, as there is evidence that smaller numbers of *Bifidobacterium* are still transferred from mother to child.[26] However it is accepted that in

these cesarean infants the initial exposure can be attributed most to environmental bacteria that is present during the procedure.[20,25]

An infant's gut microbiome develops quickly during the first week after birth, during this time it increases in both diversity and stability.[18,23] The microbiome begins to grow a dominant phyla which depends on the prior exposure, whether it be from maternal or environmental microbes. Variation in the developing gut microbiome increases as the infant is further exposed to; mouth microbes from suckling, maternal microbes in breast-milk, and kissing from family members.[20] Soon after birth, the child continues to have constant exposure to new microbes, some of which may or may not make their way into the gut and become part of the microbiome. Though our understanding of this is still limited, external and internal host-related factors begin to shape the success of the gut microbiota and are one facet of how an infant's gut microbiome begins to develop differently from that of those introduced maternally.[11]

The critical time for gut microbiome development continues throughout the first year of a human's life. Factors such as dietary options play a huge role in the establishment of certain bacteria in the gut. As we will discuss later in the paper, diet can be a large influencer for the bacterial composition of the gut microbiome. This statement is even more accurate during this first year of critical human development. Bacterial population shifts can actually be observed to directly correlate with changes in the feeding method of the child, separated from breast/formula milk feeding to weaning and eventually the introduction to solid foods.[11,20] To fully understand how this process functions during the first year of development, Palmer et al conducted a time analysis during the first year of 14 healthy infants and the microbes found in their stool.[22] Looking at both composition and temporal patterns of their microbial communities during this time frame.[22] Here they found that the composition of several major taxonomic groups like

Firmicutes, Verrucomicrobia and *Bacteroides,* fluctuated as time went on, and more importantly, those fluctuations varied from baby to baby in terms of degree and temporal position during the first year.[22] They concluded that the variations in composition and temporal patterns, may lead to support a much broader definition of what a healthy infant gut microbiome actually looks like, as total consistency was not observed in their study.[22] As it has been observed that interpersonal variation of gut microbiota composition is greater between infants than between adults.[27] Leading to the belief that during our infancy, the gut microbiome undergoes more changes and therefore may be more pliable during early development.

The genera *Bifidobacterium, Collinsella, Veillonella* and *Lactobacillus* can be used as markers to identify the gut microbiome of a 4 month old baby, as it's during this time that one can begin to see differences between breast-fed and formula babies.[25] These differences were further studied and identified by Backhed and colleagues. Infants that had been exclusively fed breast milk displayed increased numbers of species under the *Lactobacillus, Bifidobacterium, Collinsella* and *Bacteroides* genera.[25] While in formula-fed infants there was in increase in *Clostridium difficile, Granulicatella adiacens, Enterobacter* and *Bilophila*.[25,28] These observed population fluctuations can also be affected by the delivery method. So any configuration of delivery method and milk diet whether it be, vaginal/breast-milk or C-section/breast-milk etc., have profound effects on the establishment of the infant gut microbiome. Even more interestingly, the stopping of breast-feeding can also have a dramatic impact on gut microbiome establishment of a 12 month old infant.[25] As it was seen that when breast-feeding is stopped, there is a shift in the microbiota of the gut to rapidly represent a more adult-like make-up.[25] With bacteria from *Bacteroides, Bilophila, Clostridium, Roseburia,* and *Anaerostipes* dominating the gut microbiome composition.[25] When compared to if the mother does not stop breast-feeing

around the 12th month, the infant gut microbiome is enriched instead with bacteria that can be found dominating during the 4th month of age.[25] Showing that a much "younger" microbiome may be established if the cessation of breast-feeding does not occur. Suggesting that breast-feeding both improves gut microbiome development early in first year but may hinder further development if kept after the first year.[25] Research into the complex human microbiome is still developing, so differences viewed by all the outline factors in this section have not yet been labeled good or bad. Mainly because even with differences in taxonomic make-up, functional similarities are present in individuals.

This early establishment is a magnified portrayal of how the microbiome shifts and changes through external and internal factors. These shifts display how dramatically the human gut microbiome can react to influence as it evolves. This however is only during this first developmental stage. In the next section we will discuss how the adult microbiome can be influence and how it reacts to several external and internal influences. And how it may or may not differ from what has been observed in an infant gut. As it is believed that during the first years of life the gut is adaptable but once it becomes stable and "adult" it may be much harder to change.

INFLUENCING THE HUMAN GUT MICROBIOME

In the previous section we discussed how the gut microbiome is initially established, what factors play a role and how it evolves into what is believed to be a stable community. Now we will begin to look at how the gut microbiome may be influenced after the critical one year of development. Most importantly we will begin to discuss several factors such as age, diet, and medicine and how they aid or disrupt the gut microbiome in humans. Importance of age was

already demonstrated earlier but now the focus will fall on how a stable adult gut may evolve or be changed as human age. As with a lot of things having to do with the gut microbiome, its adaptability during adulthood is still not yet fully understood. And there are several studies that conflict with each other when it comes to this field of study. This section will provide a more in depth look at how diet plays a part in the gut microbiome and some prominent theories pertaining to diet. Looking at differences between what is known as a "Western diet" and several different diets. It has also been seen that several lifestyle differences may in fact also influence the gut microbiome. For example, antibiotic use or nonuse during early childhood has been found to play an important role in the destruction/survival of several microbiota that were established during birth.[12,29] It seems like age however may be the largest factor on just how easy it is to change the gut microbiome.

After the critical one year of development, the microbial gut populations become more established and flexibility is seen to diminish.[11] Despite this there is still a relatively large increase in diversity during the next two years of a child in order to set up what will become the stable adult gut microbiome.[22,27,30] In a comprehensive study by Yatsunenko et al, a large sample size was gathered that included individuals varying in age and population (326 individuals aged 0-17 and 202 adults aged 18-70, from 3 different populations USA resident, Malawian, and Amerindian).[30] They discovered several interesting findings that have helped with the understanding of how the gut microbiome may be influenced. They saw that in all populations the gut microbiota of children adapted to phylogenetically resemble one closer to that of an adult within the first three years after birth, backing up previous studies in the field.[22,30] Secondly, their study also supported past conclusions of interpersonal variation being significantly greater in children than in adults;[31] this was true for both taxonomic and gene composition.[30] As

discussed in the previous section, this finding coincided with many other studies that focused on infant microbial populations. Showing just how turbulent the gut microbiome is when it is first being established. These two age-specific findings support the idea that age really does matter when talking about the gut microbiome as adaptability and flexibility of the gut microbiome is greater during the early years of childhood.[12] Thirdly, data gathered showed that the fecal microbial composition differed in individuals, and the differences were found to be dependent on what population group the individual fell into. Specifically, there was a large difference in the microbes found in the sampled USA residents when compared to those in the Amerindian and Malawian populations, unsurprisingly, this was true from all age groups in the cohort.[30] As we have already discussed how maternal microbes are passed onto the child. This could be an example of how the effects of maternal microbial transferring may be seen even in larger populations, as well as, the importance of kinship in interpopulation differences. While kinship and maternal microbial transferring may have a small role in population-specific differences, diet may actually be the one factor that greatly contributes to interpopulation differences.

When you think about what the gut is and its primary purpose in digestion, it is easy to come to the conclusion that what we put into our bodies food-wise could have an effect on the composition of the microbiota present there. While this would seem like an easy hypothesis to prove and it would only make sense that gut microbiota is influenced by the diet humans partake in. The literature is not fully expanded, and our understanding of just how much the adult gut microbiome can be influenced by diet is simply not completely developed yet. However it is still important to show several instances where connections between diet and microbial composition do occur and the complex relationships between the two.

In relation to the gut microbiota, the most commonly studied and documented diet is the "Western diet". Many studies have observed the microbiota that are found in adult humans that follow this diet and search for abundance and diversity characteristics in these individuals. As the name suggests, a Western diet is one that broadly represents the diets of the Western world and is characterized mainly by high-fat, high-sugar, low fiber content, and meat consumption.[32-34] The high-fat/high-sugar components of this diet can be used to represent a broad spectrum of "typical" human diets. Individuals that fall under the Western diet have been shown to be characterized by the type 1 *Bacteroides* enterotype.[33] As discussed earlier, this means that these individuals predominantly display abundant numbers of bacteria from the genus *Bacteroides*, which lies within one of the two major phyla, Bacteroidetes.[16] Consequently, bacteria in this enterotype have been linked with high animal proteins, amino acids and saturated fats, which are all prevalent in a Western diet.[33] On the other hand, the type 2 enterotype which is characterized by higher numbers of *Prevotella*, has been observed to correlate with a different diet type, one that emphasizes high carbohydrates and simple sugars.[33] High quantities of these two biomolecules is seen to characterize diets found in more agricultural societies with less animal protein intake and more greens. Findings and correlations like these indicate that people with different diets provide a different environment for the microbiota in their gut. Suggesting that gut microbiota composition can indeed be affected by the diet one partakes in.[33]

Large cohorts on strictly vegetarian individuals have not yet taken place, however, throughout several gut microbiome studies the microbial composition of vegetarian guts has been sampled before. Allowing us to see the difference between a vegetarian diet and a Western diet. The gut microbiota in vegetarians actually show high numbers of *Prevotella* bacteria as well as higher *Clostridium* composition, a genus under the other major phyla Firmicutes.[33,34] Indicating

that the enterotype 2 classifcation may be prevalent in humans that partake in less meat intake. Furthermore, differences in microbial composition can also be seen in vegan fecal samples. As *Bacteroides, Bifidobacterium* and *Enterobacteriaceae* counts were seen to have greatly diminished values when compared to samples gathered from adults that participated in traditional omnivore diets.[35] They also found that vegans had higher counts of genera that fell under Firmicutes, such as *Clostriudium* and *Lactobacillus*.[35] Once again showing similarities in diets with less meat intake and difference to those with high animal protein. More importantly however, they found that in all three subject types (vegetarian, omnivore, and vegan) there was no differences in the overall bacterial counts, suggesting that while diet may influence what type of bacteria is present, it does not influence the overall abundance of the microbiota of the gut.[35] There are some studies that confusingly enough may contradict our characterization of diets when looking at phylum level composition. Further enforcing the idea that the gut microbiome is a complex entity and many different factors influence its composition. In one study, children of the rural African village, Burkina Faso, that have traditionally partaken in low-fat/high-fiber diets and showed significant increase in the abundance of their Bacteroidetes when compared to European children who had instead higher Firmicutes representation.[36] The diet of the African children mainly focused on high fiber and high plant-polysaccharides similar to that of a vegetarian, so their microbial composition should have fallen closer to an enterotype 2 classification.[36] This study however focused on children gut microbiota and results may be attributed to how children's gut microbiome is more impressionable than that of its gut counterpart. On the other hand, findings such as the ones discussed here illustrate that differences in gut microbiota can be seen across culture and may actually be influence greatly by the diets of people.

Most of these examples, of course, were from an adult gut and the diet most likely had been established throughout several years of living. Therefore, the contrast between enterotype 1 and 2 and their corresponding diets, suggests that exposure to a diet long-term may indeed influence the microbial make-up of an adult gut.[33] This does not mean that an adult gut cannot see quick changes due to diet. In fact it has also been observed that microbiota can adapt quickly when someone changes their diet abruptly even in adulthood.[37,57] Rapid shifts in the gut microbiome are seen to occur when a newly introduced diet imposes different metabolic requirements, the bacteria then adapt to fit those metabolic functions needed (i.e. plant or protein breakdown).[57] Detecting specific diet influence can be difficult because of how different microbiota can adapt and shift. But still illustrates the point that the adult gut microbiome is also affected by diet intake. The gut microbiota may shift due to certain diets providing more benefits to a certain type of bacteria, as seen with the enterotype classifications.[33] Even more likely, shifts occur when a diet is changed and the preferred nutrition of the established microbiota is not met, resulting in diminished numbers of certain bacteria and increase of other types.[33] These shifts may encompass changes in specific genera and if significant enough can manifest into shifts in class, family and even as large as phylum shifts.

Further examples of diet influencing the gut microbiome have been demonstrated in a lab environment with the use of mice. Lab raised mice make excellent test subjects in studies about the gut microbiota because they contain many of the same gut microbiota that can be found across mammals, including humans.[32] More so, gnotobiotic mice, animals raised in a germ-free environment without microbial exposure, can be used to simulate a controllable environment and replicate the human gut microbiome via humanization.[32] Humanization is a process in which adult human fecal microbiota is transplanted into gnotobiotic mice to begin colonization event by

that microbiota. This is done to more accurately represent an applicable human gut model via mice in a controllable environment.[32] One example of this mechanism was conducted by Turnbaugh et al, as they fed humanized mice a Western diet and studied the results of their gut microbiota in a span of 4 weeks.[32] This was done to that observe how the colonized bacteria behave as a diet is introduced under a span of time. It was found that humanized mice on a Western diet acquired a different composition than humanized mice that were fed a low-fat/plant polysaccharide rich diet (LF/PP). Specifically, mice on the Western diet displayed an increase in Erysipelotrichia, a class under the Firmicutes phylum, which was not seen in the LF/PP mice.[32] Interestingly, the Western diet was seen so correlate with an increase in several Firmicutes classes which led to a proportional underrepresentation of bacteria in the Bacteroidetes phylum.[32] As discussed earlier, Bacteroidetes is the phylum in which *Bacteroides* resides and is an enterotype associated with the Western diet in humans. While these finding may conflict with the idea of certain Western diet gut compositions, it is another example that diet does indeed play a role in influencing gut microbiota and that bacterial associations are not as simple as first thought to be. Highlighting that many factors play a role in how gut microbial composition develops specially in humans.

Medically, antibiotics are used to destroy bacteria that may be causing an infection or disease in our bodies. Their use however, can also demonstrably influence our microbial composition and lead to reduction of microbial diversity in our guts.[7] Displaying that they have the ability to produce both short-term and long-term changes in the composition of our microbiota.[38] An example of how our gut microbiome can be altered by antibiotics and how they interfere with the protection provided by our gut microbiome, can be seen in cases of *Clostridium difficile* infections (CDI). This is an infection that is caused when the pathogenic *C.*

difficile is able to make its way into our large intestine, reproduce, and release several exotoxins into our internal environment.[39] The disruption and reduction of our gut microbiota through the use of antibiotics allows the pathogenic *C. difficile* to be introduced and survive in the "empty" tissues of the large intestine.[39] Without our full commensal microbiota the resistance in our bodies to pathogens can be reduced significantly. Allowing pathogenic or even opportunistic indigenous bacteria to thrive and cause complications in human health. This concept was also seen in rat studies done by Manichanh et al, where they treated lab rats with an antibiotic cocktail of vancomycin and imipenem and transplanted exogenous bacteria into their intestines.[38] Their results showed that even with a short antibiotic treatment, long-term effects on the gut microbiota can be observed.[38] Diversity was significantly reduced right after antibiotic treatment, and a reshaping of the two major gut phyla (Bacteriodetes and Firmicutes) occurred.[38] They found that 3 days after the antibiotic cocktail treatment there was a 10-fold decrease in bacterial count and phylogenic diversity in the intestinal microbiome (FIG 2).[38] Specifically, they saw a near-extermination of Bacteroidetes and large decrease of the Firmicutes microbiota. As the two major phyla decreased, two minor gut phyla (Proteobacteria and Tenericutes) saw a 1% to 31% increase in all of the samples tested.[38] This type of increase was attributed to what was mentioned earlier in the *C. difficile* example, opportunistic niche filling. They analyzed the shifts further after a month of antibiotic nonuse and found that phylum ratios returned to their original proportions (FIG 2), with the two minor phyla (Proteobacteria and Tenericutes) decreasing and the two major phyla (Bacteroidetes and Firmicutes) recovering their dominance.[38] Interestingly however, it was found that phylogenic diversity did not fully recover like the bacterial counts. The loss in diversity was mainly due to lowered Bacteroidetes phylotypes and this lead to a reshaping of the gut microbiome after 3 months.[38] With Firmicutes being higher

and Bacteroidetes being lower than in the control proportions before the antibiotics.[38] This type of shift in phylum proportions shows the detrimental nature of antibiotic on commensal bacteria. While this study was not conducted on humans, the strong long-term effects of antibiotic found here show that our gut microbiota may be more shapeable than anticipated.

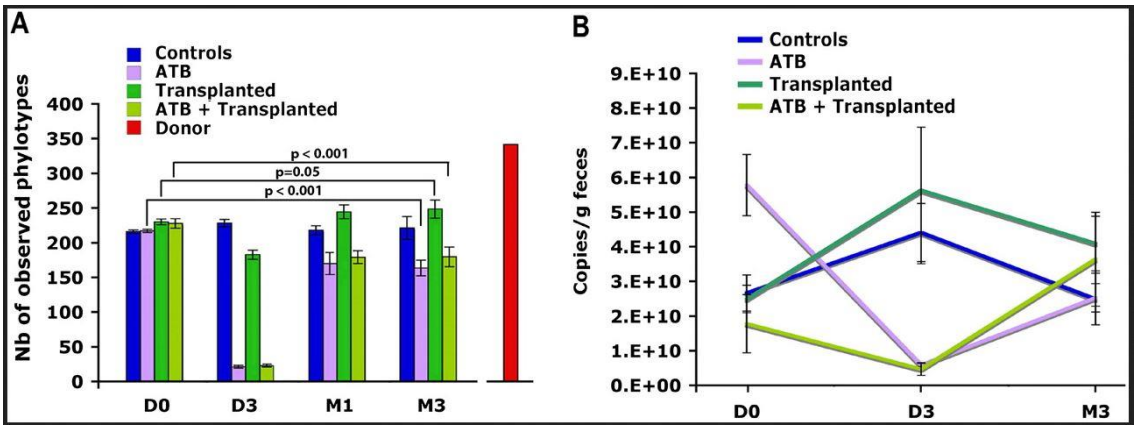

Figure 2.[38] Shifts in bacterial count and diversity over a three month span. Showing counts for antibiotic use, transplanted samples, antibiotic and transplant sample and donor. **A.** Displays the number of observed phylotypes in each sample group from day 0, day 3, month 1 and month 3. **B.** Feces sample counts for all mentioned samples. Displaying shift in bacterial counts after three days and three months. (Figure sourced from Manichanh, C., et al., 2010)[38]

Knowing how to shape and influence the gut microbiome can be a very important discovery. As it may lead to possibilities on how to combat several gastrointestinal diseases and other complications relating the human gut. The adult gut microbiome has displayed that it can be both adaptable and stable, two properties that can be beneficial to human health. Further understanding of this may provide insight to what can be done to maximize the beneficial functions provided by gut microbiota. As we will discuss in the next section, the gut microbiome provides many different interactions with the human body. These interactions create a dynamic that is both beneficial and detrimental to several metabolic functions.

FUNCTION PROVIDED BY THE HUMAN GUT MICROBIOME

We have already talked extensively on how the human gut microbiome is established and how there are many variables at play when dealing with its evolving composition. Now we will begin to discuss why it is so important to know and understand our gut microbiome. The extensive volume of bacteria present in our gut has so much potential in impacting our health and wellness. This section will focus on the many functions the gut microbiome can provide and enhance for the human body. There is staggering evidence that the gut microbiota and its make-up can indirectly or directly play a role in immunity, metabolism and other physiological functions that eventually affect most of our health.[7] Through their natural drive to survive, gut microbiota provide mechanisms that interact with our bodies in a beneficial way. These interactions can be seen as a form of immunomodulation, as several mechanisms function to regulate the immune response in the gut in a healthy and beneficial manner. Some microbiota have been found to be beneficial through probiotic mechanisms in aiding with digestion and the integrity of our gastrointestinal tract.[7] As well through their microbial byproducts. All of which will highlight the importance of maintaining a healthy gut microbiome and understanding how its composition and complexity relates to its beneficially functional contributions.

When one thinks of bacteria, it is usually not in a positive connotation. In our gut however, bacteria do several things that are very beneficial to us as humans. In fact, a good amount of diversity in gut microbiota can be seen as a healthy component for any living organism. This is due to the greater the diversity, the greater the likelihood that the bacteria will provide more mechanisms our bodies can benefit from. The bacteria in the gut interacts greatly with the host's own immune system, and these interactions can be both beneficial and necessary. Researchers have found that there are numerous complex interactions between the immune

system and the abundant gut microbiota in humans.[7] To fully reap the benefits of our gut microbiota, however, our immune system must first be able to recognize and distinguish which bacteria can be helpful and which can be harmful. Incredibly, through years of coexistence, the gut microbiome has been established to be more beneficial than we ever though.

An immune response occurs when the immune system detects any foreign material that has entered the human body. T regulatory cells are a very important component of a healthy immune response. These adaptive immune cells are the ones responsible for keeping peripheral tolerance, suppressing and limiting inflammation and preventing autoimmune diseases.[40] Incredibly, it has been discovered that certain microbiota in the human gut may actually play a role in promoting the differentiation of these important T regulatory cells and thus promoting the maintenance of healthy homeostatic immunity.[7] Several studies have shown that this ability to promote differentiation can be seen in the certain *Clostridium* species that importantly are readily found in human intestines as well as mice.[41,42] The mechanism in which this is possible through involves the production of butyrate, a short-chain fatty acid, which is a byproduct of certain *Clostridium* species.[7] Butyrate consequently is a control factor in expression of the Foxp3 promoter, which under normal physiological and immune conditions controls Treg development.[42] Microbial colonization also impacts the presence of T helper cells in mammalian gastrointestinal tracts. In mice that are germ free, the number of T-helper (Th), Th1 and Th17, is lowered, and as such their intestinal immune response is governed by T-helper 2 cells.[43] Importantly, this type of T-helper cell imbalance can actually be reversed when the mice are introduced to normal gut microbiota.[43,44]

Establishment of commensal microbiota has been linked with healthy development of even our Gut-associated lymphoid tissues (GALTs). In mice that were raised as germ-free,

researchers could see that the GALT and Peyer's patches in these mice had impaired development as opposed to mice that had been colonized by human commensal microbiota.[44] The consequences of the impairment were further highlighted when researchers looked at which antibodies were being produced in the two mice groups. In the germ-free mice, higher levels of IgE+ B cells were present, which produce IgE antibodies that have been attributed to allergic responses.[44,45] In the commensally colonized mice however, IgA+ B cells had a larger presence, these are cells that produce the IgA antibody that provide an essential function neutralizing pathogens at the mucosal membranes of the intestines.[44,46] It is clear when looking at the differences found, that the gut microbiota play an important part in keeping our immune system up and running. This is an example can be seen as both an immune mechanisms and how gut microbiota play a role in even the structural development and integrity of the gastrointestinal tract. These types of findings support the idea that certain microbiota of the gut have positive contributions to the development of our internal immunity, and they also illustrate how complex each interaction can actually be. There are far more examples of how microbiota contribute to several immune mechanisms ranging from T-helper cells, innate lymphoid cells regulation, B cell development and more effecter T cells mechanisms.[44] These however will not be fully discussed in this paper as the examples are numerous. And evidence shows how interactions with our gut microbiota can have effects in other immune functions ranging from innate response to more complex adaptive responses such as the ones mentioned above.[44]

Astonishingly, relatively new discoveries suggest that the gut microbiota may in fact even influence a variety of brain functions in humans.[47] Several mechanism have been identified that show how these interactions may occur. While most of these studies have been done in mice, the humanization techniques allow us to make conclusions on how human gut microbiota

may affect some our brain functions as well. The vagus nerve is a crucial component in normal gut maintenance. This nerve functions through the parasympathetic division of our ANS, and is normally stimulated through cytokine and endotoxin detection which leads it to transmit anti-inflammatory signals from the gut to the brain.[47] Keeping inflammation at a minimum in the gut is a critical function of our healthy immune system, being that we do not want inflammation damaging gut cells and microbiota present.[48] Its close and intimate connection to the gut, makes the vagus nerve a susceptible mechanism for bacteria to influence the brain.[47] Depending on gut microbiota composition and abundance of certain taxa, several things may occur. Induced anxiety is one way in which bacteria can interact with our brains. In mice that were colonized with *Citrobacter rodentium,* anxiety-like symptoms suddenly manifested even when no innate response took place.[49] The vagal sensory neurons of these particular mice, showed increase cFos protein expression when compared to control mice.[49] The expression in cFos can be used as a marker for the activation of brain neurons in mammals.[47] What makes this finding significant is that there was no inflammation stimulus/response in either of the mice groups, thus the anxiety symptoms were attributed to the introduction of *C. rodentium* and that this bacteria somehow was able to activate a functional neuronal response in the gut and have a signal sent to the brain via a vagus nerve.[49] In another study, hypothalamic-pituitary-adrenal (HPA) reaction to stress was compared in germ free mice and gnotobiotic mice. This study found that the germ free mice showed higher levels of HPA stress which could be reduced with the reintroduction of *Bifidobactetriun infantis*.[50] Studies such as these with mice are useful because they indicate that commensal microbiota can affect the brain through neuronal interactions.[50]

A much simpler way in which human benefit from the abundance of commensal gut microbiota, is the concept of competition. Although large, the GI tract can only accommodate so

much surface area to microbiota. Meaning that the more space commensal and useful bacteria take in our gastrointestinal tract, the less room there is for pathogenic bacteria to thrive.[12] It needs to be stated again that gut microbiota do not work for humans, simply they strive for survival independent of host benefits. So the competition is not simply commensal versus pathogenic, but instead all phylogenic taxa compete against each other. The benefits are only seen if pathogenic bacteria happens to be overregulated by established gut microbiota. The competition amongst human microbiota is a healthy way in which this regulation can occur. The bacteria has to compete for both space and nutrients in the gut. This means that regulation occurs naturally among the microbial community through complex feedback loops that are up-kept spontaneously.[12] These feedback loops are mainly seen to be kept in control by the interactions with host mechanisms and the metabolic mechanisms microbiota provide.[12] It is then where the conclusion can be made that if regulation occurs naturally in commensal microbiota, prevention of "harmful" bacteria may also occur. One might think that competition among commensal gut microbiota may hinder the functional roles provided. But in the contrary, metabolic functions can be shared by several different types of bacteria, contributing to the diversity in individuals. Meaning while some of us may have completely different microbiota composition, our functional benefits derived from said microbiota are similar. This is due to the large number of phylotypes found in bacteria. Several bacteria may provide the same functionality but come from completely different phylum or even class. It has also been found that related bacteria do over-compete with one another and instead provide functional overlap as a result.[12] This is extremely beneficial in keeping a healthy gut healthy, because if an internal or external influence hinders one of these beneficial bacteria, another phylogenetically related

bacteria can still provide the same function. This outlines both the resilience in our gut microbiome and the importance of functional overlap.

The arrangement of our gut microbiome can also be a critical component of how we as humans digest our sustenance. Thus displaying another way in which humans benefit from having an established gut microbiome, as it has a huge impact on several metabolic mechanisms. One way our bacteria help digestion is through the breaking down of normally indigestible substrates found in certain foods.[7] The common and already mentioned bacterial genus, *Bacteroides*, provides several bacterial species (such as *Bacteroides ovatus*) that have the rare capacity to digest important substrates known as xyloglucans, which can be found in the vegetables consumed in our omnivore diets.[7,51] As humans we do not have a coding gene that provides us with proper xyloglucan degradation proteins. The only way we can gather energy from this complex polysaccharide is through the certain commensal *Bacteroides* species and its unique mechanism. The importance of these types of digestive mechanisms can be observed in the fact that 92 % of individuals in a public metagenomics database contained at least one of the *Bacteroides* strains that was capable of this function.[7] Several microbiota are also capable of creating short chain fatty acids whilst inside the human gut by breaking down indigestible fibers that we humans might digest.[7] The genus *Bacteroides* display the ability to create short chain fatty acids such as butyrate, propionate and acetate, which can be crucially important energy sources for both human cells and microbiota living in the GI tract.[14] Butyrate, especially, can be a great source of nourishment for the cells that line the human colon, allowing them to grow and divide as well as preventing D-lactate buildup, which can be a harmful metabolite when in excess.[14,52] Furthermore, butyrate has been seen to inhibit incidence of tumorigenesis in colon cells as well.[14] Mechanisms such as these have been observed throughout many different types

of bacteria found in the gut with varying degrees of importance and usefulness. Nevertheless, these showcase how dependent humans are to the microbiota found living inside the gut. They can both enhance our abilities to digest nutrients and provide substrates that are essential for healthy metabolic functions.

GUT MICROBIOME CONTRIBUTIONS TO DISEASE AND HEALTH

Earlier we outlined several mechanisms in which the gut microbiome provided healthy and positive outcomes. The shear abundance and diversity of the human gut microbiome can be a double-edge sword, as not all interactions with it are going to be beneficial to us as humans. It is no surprise then that certain bacteria in the gastrointestinal tract can cause harmful effects in our health and contribute to various disease states. Just as how some benefits were easily detectable and some difficult, the contributions to disease in humans by gut microbiota can differ in complexity as well. Disease states can occur via several situations, such as, the introduction of pathogenic bacteria, microbiota not being where they are supposed to be, and in some cases breakout of infections in gut tissues.[2] Here we will look at several examples of how gut microbiota play a role in the pathogenesis in humans.

First we will discuss how inflammation can be induced and extended through gut microbiota. Controlling inflammation in the gut is very important for proper immune response, and if not up-kept can lead to chronic inflammation and other complications. Inflammatory bowel disease (IBD) are conditions that occurs in the small intestine and colon in which several inflammatory states are observed and can be further distinguished as Crohn's disease (CD) and ulcerative colitis (UC).[11,53] Variation away from the normal gut microbiota as discussed earlier and harmful imbalance has been linked to the occurrence of IBD all throughout the

gastrointestinal tract.[11] Susceptibility to inflammation in these diseases has been found to occur due to either abundance of certain bacteria or the decrease of others. It was found that in patients with IBD, the abundance of *Bacteroides* was higher than that of healthy patient samples.[54] And significant reductions in *Clostridium* species were present in patients with ulcerative and infectious colitis not seen in healthy subjects.[54] The effectiveness of antibiotics in reducing and preventing inflammation in IBD patients is also another way in which researches concluded that microbiota play a role in IBD pathogenesis since the antibiotics destroy large number of microbial gut communities.[2] Interestingly, patients that have IBD also show reduced counts in the Firmicutes species *Faecalibactrerium prausnitzii*, a bacterial species that has anti-inflammatory capabilities and is consistently linked with lower protection of gut mucosal tissues if not present or in low numbers.[55] In IBD patients, reduced numbers of other Firmicutes is also observe. This is important because Firmicutes holds several taxa that have the ability to produce short-chain fatty acids (butyrate, acetate) which also have anti-inflammatory properties.[11,56] While evidence may be limited, it displays the elaborate relationship of diversity and abundance of gut microbiota in relation to IBD. Probiotic bacteria have also been found to be a successful way to treat variations of IBD. Introduction of certain probiotic microbiota can actually be seen to lower inflammatory remission and even treat small flare-ups in individuals affected.[2] Emphasizing that some bacteria can have positive effects on pathogenesis while other do not.

Correlations between gut microbiota and irritable bowel syndrome (IBS) have also been studied and it is widely accepted that gut microbiota play a role in its occurrence in humans.[11] As discussed earlier, microbiota have the ability to interact with our metabolic and immune functions. As such, inflammation associated with IBS it is believed to be because of variations in our gut bacteria just like in IBD.[58] Individuals with IBS show different abundance counts when

compared to healthy gut microbiota compositions. Distinctively, they showed increase in Firmicutes and reduction of Bacteroidetes taxa consequently resulting in reduced microbial diversity.[2] Reduced numbers of *Bifidobacterium,* genus under the phylum Actinobacteria, are also observed in individuals with IBS.[59] And just like in IBD, reduced numbers means that these individuals are missing critical species that aid in our metabolic and immune mechanisms. Without these bacteria present, the immunity of an individual may predispose them to this type of chronic inflammation in the gut.

Most intriguingly, the unique relationship between gut microbiota and obesity can be used to examine the dynamic interactions humans have with microbiota. And considering how we have discussed the complex relationship between diet and gut microbiota, it shouldn't be a surprise that our microbial companions also contribute to obesity in humans. Research in this field shows that not only can the gut microbiome be influenced by obesity but it can reciprocally influence the development of obesity in an organism.[17] Like most gut microbiome studies, mice were first mammalian test subjects in obesity/microbiome research. In 2006, Turnbaugh et al, made the interesting discovery that obesity could indicate different types of microbial ratios in mice.[60] They discovered that mice who were characterized as obese showed a significantly larger ratio of Firmicutes when compared to Bacteroidetes.[60] The lean mice in the study displayed the opposite, with their Firmicutes to Bacteroidetes ratios being shifted towards Bacteroidetes dominance.[24,60] Obese mice showed that their abundance of Bacteroidetes was lowered by 50% with their Firmicutes increasing to make-up that reduction.[24,60] Further analysis revealed that there was also a difference in the amount of energy being extracted from the diets. It was found that obese mice actually harbored higher amounts of bacteria that promote the expression of enzymes responsible for polysaccharides breakdown found in their diets.[60] It was suggested that

obese mice were actually extracting more energy than necessary, and as a consequence, stored more adipose, further promoting obesity.[60] In the same study germ free mice were transplanted with microbiota that had come from obese mice, and notably, the previously germ free mice showed an increase in body fat compared to germ free mice that had been transplanted with "lean microbiota".[60] Indicating that weight gain and predisposition to dietary differences may be attributed to the type of bacteria found in the gut as it can influence our bodies through specific metabolic interactions.

In a recent anecdotal case report done on a patient who was treated for CDI (*Clostridium difficile* infection) via fecal microbiota transplantation (FMT), it was observed that she experience significant unintentional weight gain after the treatment occurred.[61] The patient was treated via FMT from samples provided by her daughter, who at the time had a body mass index of 26.4 at 140 pounds. Before the FMT procedure, the patient, who had no prior history of obesity, weighed in at a stable 136 pounds and BMI of 26.[61] The treatment against the CDI was successful and the patient suffered no relapse. Sixteen months after the FMT, the patient's body weight had shifted to 170 pounds with a BMI of 33, officially becoming obese.[61] Interestingly the donor daughter's weight also increased to 170 pounds sometime after the FMT procedure. And at 36 months post transplantation, the patient weighed in at 177 pounds (BMI of 34.5) despite diet and exercise efforts to control weight gain. While there are many reasons a person experiences weight-gain, this case study provides evidence that the FMT may have played a part in the onset of obesity as supported by other animal studies.[61] The occurrence of significant weight gain without prior history before the FMT and the mirror weight gain of both the daughter and mother (patient) suggests that transplantation may have influence on obesity and its relationship with the gut microbiome.[61] This is just one instance of how microbiota may

influence weight gain in humans. And although many large scale human studies have not yet been as successful at replicating the same findings presented here and by many animal models there is still a strong indication that the gut microbiome plays a role in human obesity as well.

Nonetheless, in humans some of these findings and microbial comparisons can still definitely be applied. This was observed through studies involving obese individuals and lean healthy individuals and analyses of their corresponding gut microbiota. In obese individuals that underwent a diet change to mimic that of a lean person, their Bacteroidetes to Firmicutes ratios actually shifted to match that more of a lean individual.[60] The microbial content and composition in obese individuals was also seen to shift as the person's weight changed.[62] More research into the connections between obesity and gut microbiota needs to be conducted. As current research has yet to find a significant correlation between specific bacteria, at either a phylum and species level, despite the fact that differences in interpersonal compositions have been observed. The understanding is limited when it comes to knowing exactly what bacteria causes what and how. For example, recent research has found that a risk of developing type 2 diabetes mellitus may also be the make-up of our gut microbiome.[11] More specifically, it may be the lack of butyrate producing bacteria that enhances the risk of this condition, as this was observed to be true in individuals with Type 2 diabetes, even though their overall gut microbiome was not overtly compromised.[63] Although many of the specific mechanisms to how our gut microbiota can contribute to disease need further study, it is still widely accepted that it does indeed influence several disease states. Further studies need to take place for us to fully understand the correlations between phylogenic variety and human diseases.

CONCLUSION

As discussed throughout the paper, research on the gut microbiome and its many effects on human health have increased dramatically in the recent years. Interest was elevated as researchers discovered how abundant and rich the internal colonization of our guts actually was. These studies showcase how the gut microbiome can be both resilient and influenceable and how these characteristics contribute to the large interpersonal variability found throughout humans. Interpersonal variety gives some insight into how microbiota can be useful and good for human health. Because although humans may be unique interpersonally, common functional benefits can be observed. Another good example of the importance of the gut microbiome to human development can be seen in the fact that its establishment occurs right at birth. The dependency on a healthy microbial community begins early in development and further evolves when diet is introduced into the body. Bacteria both help and adapt during these early interactions. And those interactions only become more complex as one grows into adulthood. The importance of commensal bacteria can now more than ever be seen. As humans have evolved to allow bacteria to thrive in the gut and create commensal relationships in the process. Things like, providing enzymes for complex digestion and nutrient extraction or maintaining proper gut homeostasis through abundance and population ratios, all showcase just why the gut microbiome is important for our bodies. Interactions such as these are numerous, and they all begin to adapt the moment a human is birthed. Our understanding of such interactions may still limited, but the fact they occur and have been observed to provide benefits to humans is momentous.

Bacteria present in our GI tract provides beneficial mechanisms that interplay with numerous human physiological processes. Proper digestion, strong immunity and good mental health can all be influenced by the composition of the human gut microbiome. Most of the problems that occur in relation to the gut microbiome manifest themselves as differences in

composition of the microbiota, be it by reduced or increased numbers, decreased diversity and/or overabundance of certain bacteria. All of which may be traced back to how the microbiome was established in the first place in those individuals and how it was up kept through their life. The bacterial diversity and abundance of someone's gut microbiome can be compromised right at the get-go depending on the type of exposure during birth. This type of disruption may contribute to the many differences seen in microbiota ratios discussed in human diseases. Gut microbial disruption contributes to not only interpersonal differences but maybe also to predisposition to certain types of disease like obesity, IBS, or diabetes. Like discussed earlier, our gut microbiome is extremely sensitive during the first three years of life. Thus, greater importance should be placed on having proper exposure during this critical time period to allow our gut to become a rich environment in which diverse microbiota can thrive in.

Although diversity does not necessarily equate to healthiness, it does mean that we can have a greater pool of commensal bacteria to interact with and thus increasing the chances of functionally viable microbiota presence. Healthy microbial composition may be further enhanced by what we eat, as seen through differences in enterotype classification. Since diet has been seen to shape both infant and adult gut microbiomes, more emphasis and studies on what types of diets benefit our microbiota may be needed. This way one can discern what food types provide long-term benefits for both the body and the microbiota within. Providing proper nutrition to the gut microbiota may allow us to combat the predisposition to several diseases and complications in humans. This can only be done by understanding what and how diets shape the internal environment of the human gut. Even limiting antibiotic use may provide more benefits than previously believed. As it has been shown that antibiotics can be detrimental to functional diversity through long-term effects. And a healthy gut microbiome encompasses both phylogenic

variability and functional diversity. Abundance of specific bacteria does not indicate healthy composition, instead it is seen that functional overlap and variety may be more important to our overall health. By being able to identify functional contributions in the gut provided by microbiota, researchers may be able to come up with solutions to the many problems seen in human health. Prevention of obesity or IBS may in the future be a possibility through these new findings and increased understanding of our gut microbiome.

With interpersonal variability being high even in healthy individuals, further large scale studies still need to take place in order to gather more data on the development, establishment and variability of the human gut microbiome. Understanding how gut microbial make-up works may lead to breakthroughs to several problems in our health. The gut microbiome is intertwined with many of our bodily functions so grasping its complex relationships can only be beneficial. The recent increase in research pertaining to the human gut microbiome has led to increased appreciation for the microbial organisms that reside inside of us. Through the many examples discussed, the gut microbiome has shown that it provides a variety of metabolic and immune functions that highlight its contributions to both human health and disease and its importance cannot be understated any longer.

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