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Gut Bacteria and their Influence on Metabolic Disorders

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Abstract

The human gut microbial genome encodes for several metabolic processes that are not encoded for in the human genome. Through the study of metagenomics, mice, and human models, researchers have shown that changes in the gut bacterial composition can generate oxidative stress, release endotoxins, and induce lipogenesis. These pathways can disrupt normal metabolic function, resulting in obesity and other related metabolic disorders such as diabetes. Most of the health implications associated with obesity originate from the biological reactions carried out by the gut bacteria, which are strongly impacted by environmental factors. Probiotics, prebiotics and fecal transplantation are methods that can be used to replace destroyed microbes due to environmental impacts. Several other diseases can originate from disruptions in the gut bacterial community, thus future research must be conducted.

Introduction

The human body houses several different microbial communities within the oral cavity, stomach, intestines, female vagina, and on the surface of human skin. Each anatomical site contains different microbial species that differ in their abundance (Cho and Blaser 2012). Microbes can either increase or decrease in numbers depending upon the timing in which an individual is exposed to certain microbes, genetics, and environmental factors such as diet and antibiotics (Califf et al 2014). For this reason, the human microbiome is very specific and "personalized" to each individual (Califf et al 2014).

The gut micro-biome represents a complete ecosystem located within the large and small intestines. Although the microbiome composition strongly differs between individuals, research has noted that most healthy humans contain microbial species in the gut belonging to the

Firmicutes, Bacteroidetes, and Actinobacteria phyla (Califf et al 2014; Cho and Blaser 2012). Together, the microbial species found within the gut aid in energy absorption, along with the breakdown of carbohydrates and proteins, which directly help regulate metabolic pathways (B□ckhed 2012).

The diversity and population of different microbial species found within the gut can be altered through several different factors, including diet, antibiotics, and exposure to different microbes (Califf et al 2014; Cho and Blaser 2012). Microbial diversity influences metabolic pathways through the production of endotoxins, oxidative stress, and lipogenesis, which can ultimately result in obesity and other related metabolic disorders (Brown et al 2011; Nadal et al 2008).

Metagenomics

The human gut micro-biome is composed of 10^{14} bacterial cells, proving it to be 100 times larger than the Human Genome (Cani and Delzenne 2009). Several metabolic pathways carried out in the human body are regulated by the genetic material encoded in the microbial genome, not in the human genome. For this reason, the gut microbiome can be considered a "virtual organ" and a "meta-genome," as it is an extension of the human genome (Harley and Karp 2012; Qiao et al 2013).

Shortly after the human genome was sequenced, researchers were interested in sequencing the genomes of different microbes that live within the human body. This led to the establishment of the Human Microbiome Project (Gevers et al 2012). Traditionally, microbial species are cultured in a laboratory under environmental conditions suitable for growth, allowing for researchers to study individual microbes. However, many microbes found within the human

body require specific environmental conditions that cannot be duplicated in a laboratory setting, thus leading to the development of metagenomics (Gevers et al 2012). The field of metagenomics uses advanced DNA sequencing technology to study microbial communities without cultivating them in the laboratory (Human Microbiome Project). Researchers sequence the 16S ribosomal RNA (rRNA) gene through the processes of Polymerase Chain Reaction (PCR) and amplification to properly identify a particular bacterial strain and classify it into a certain phylogeny (Gevers et al 2012). This particular genomic region is sequenced because it contains several conserved genes along with variable regions, providing great insight to evolution and its taxonomy (Microbial Reference Genomes). This technique proves to be very beneficial because it allows researchers to sequence the genes from the whole microbial community as opposed to studying individual genomes through methods involving bacterial cultures (Gevers et al 2012).

The purpose of metagenomics is to use the findings based upon 16S rRNA sequencing to help understand the role of microbial communities in human health and disease (Human Microbiome Analysis). However, this type of research poses some minor ethical controversies because researchers are dealing with non-human genomes (Gevers et al 2012). Due to the microbial genome being separate from the human genome, some questions arise as to whether or not experimentally manipulating the microbial community is essentially altering biological identity (Gevers et al 2012). Since it is difficult to determine who exactly owns a microbial genome, some researchers propose that regulations, similar to those of gene therapy, may apply to metagenomics, in future research (Gevers et al 2012).

Fetal inheritance of Gut Microbes

The effect of environmental exposure to certain microbes is manifested in the process of child birth. Before birth, the fetal gut is determined to be sterile and lack any microbes; however, the microbial community begins to develop once the baby is exposed to the outside environment. The external environment includes the vaginal canal during vaginal delivery, or adult human skin in the case of a cesarean section (Califf et al 2014). Research has shown that the particular mode of delivery impacts the initial inheritance of an individual's microbiome. For instance, babies who experience a vaginal birth express a microbiome similar to the bacteria found in the vaginal canal, whereas babies delivered via cesarean section, initially express a bacterial composition similar to the bacteria found on adult human skin (Califf et al 2014; Cho and Blaser 2012).

Furthermore, studies have also observed a correlation between diet and fetal inheritance of a microbiome. In the early infant years, babies primarily consume the mother's milk, which is rich in lactobacilli (Cho and Blaser 2012). Interestingly enough, researchers have observed lactobacilli to be the most abundant microbe present in the fetal gut, indicating that microbial development is also initiated by the consumed diet (Cho and Blaser 2012). As the baby continues to grow and develop, research proposes that the intestinal maturity continues to be regulated by environmental and genetic factors (Cho and Blaser 2012). For instance, the oral cavity presents a different microbial community before teeth emerge compared to after they emerge, which can be influenced by genetic tendencies and gut composition.

Throughout the course of human life, microbial composition does not remain static. For example, diets high in carbohydrates alter levels of *Rosenburia spp*., the bacteria responsible for producing butyrate, an essential energy source for cells located in the colon (Caricilli and Saad

2014). If the production of butyrate is hindered, that may result in an unhealthy colon, because these bacteria aid in the processes regulating toxin and waste elimination.

Also, early treatments with antibiotics can strongly shift microbial diversity and contribute negatively to the overall development of the child, because antibiotics can kill off many bacteria species important in nutrient absorption and metabolic processes (Cho and Blaser 2012). If a child lacks the microbes necessary to absorb nutrients, this can present problems of malnutrition and other health implications in the future (Cho and Blaser 2012). Therefore, the gut microbial community plays a major role in human health and constantly changes throughout one's life.

Obesity and Microbial Diversity

Following the emergence of metagenomics, researchers have gained great insight into the relationship between gut bacteria and metabolic disorders. For example, obesity is a disease characterized by a multitude of metabolic disorders that result in excessive amounts of body fat (Cani and Delzenne 2009). Generally, obesity tends to be regarded as a disease resulting from poor diet intake, genetics, and lack of physical activity (Cani and Delzenne 2009). Though these are contributing factors, the excess of body fat is mediated by the role of the microbial species present in the human gut (Cani and Delzenne 2009).

Recent studies have shown that an altered gut flora is strongly correlated with the accumulation of body fat and weight gain. For example, researchers involved in one particular study examined many 16S rRNA gene sequences from both genetically obese mice and lean mice. They observed that the obese mice expressed an increased level of Firmicutes and a decrease in the number of Bacteroidetes compared to the lean mice (Cani and Delzenne 2009).

Firmicutes are bacterial microbes that are involved in energy absorption, and Bacteroidetes are involved in the breakdown of carbohydrates and proteins (Cecchini et al 2013). The energy extracted from the Firmicutes is then invested into further metabolic pathways by the Bacteroidetes (Cecchini et al 2013). In effect, the obese mice were likely experiencing higher rates of energy absorption, and possible disrupted metabolic pathways due to the failure of the energy to be invested into metabolic pathways (Cani and Delzenne 2009; Cecchini et al 2013). Ultimately, the high extraction of energy, along with the failure to input that energy into metabolic processes causes that energy to be stored as fat in the body, which can accumulate over time and lead to obesity. These results indicate that the composition of the gut microbial community influence metabolic pathways involving energy absorption, ultimately contributing to the amount of body fat accumulated and metabolic phenotype (Cani and Delzenne 2009).

Additionally, these same researchers carried out another study using human subjects. They examined the gut flora of 12 obese subjects, and compared them to 12 lean subjects. Similar to the results obtained using the mice models, the researchers also observed a lower number of Bacteroidetes and a higher number of Firmicutes, in the obese individuals, as compared to the lean individuals (Cani and Delzenne 2009). Next, the researchers placed the obese individuals on a very strict diet, which limited the amount of fat, carbohydrate, and caloric intake (Cani and Delzenne 2009). After intervening with the diet of the obese individuals for 52 weeks, the researchers examined the gut flora of the obese individuals again (Cani and Delzenne 2009). They noticed that the gut flora was much more comparable to that of the lean individuals, because the numbers of Bacteroidetes and Firmicutes in both groups were similar (Cani and Delzenne 2009). Based upon the results, the researchers were able to conclude that diet does

impact the composition and diversity of the microbial community located within the human gut; however, the mechanism by which this happens is still unknown.

Furthermore, another study using both antibiotics and a high fat diet, displayed the impacts of such environmental agents. In this particular study, the researchers analyzed six different groups of mice, all experiencing different treatment protocols. The groups are as follows: mice fed a normal diet, versus mice fed a high-fat diet; mice treated with antibiotics along with a normal diet, versus mice treated with antibiotics and a high fat diet; obese mice without antibiotic treatment, versus obese mice treated with antibiotics (Cani et al 2008). Overall, the results indicated that after a four week antibiotic treatment, all mice treated with antibiotics expressed alterations in the diversity of the gut microbial community, regardless of the consumed diet (Cani et al 2008). Also, mice fed just a high-fat diet, compared to mice fed both a high fat diet and antibiotics expressed very dissimilar gut floras. They displayed only a 22% similarity between each gut flora (Cani et al 2008). Although genetics can contribute to the gut composition, the results indicate that antibiotics and high fat diets are clearly strong factors that play a role in altering the composition of the gut microbiome.

Mechanisms by which Gut Bacteria Initiate Metabolic Disorders

Additional research was conducted to better understand exactly how the gut microbial community influences the development of obesity by affecting many different metabolic pathways. Recent studies have shown a correlation of the gut microbial community to human metabolism by directly interrupting energy homeostasis along with alterations in the redox state. Normally, when humans ingest food, metabolic processes are able to compensate for the amount of energy that was expended throughout each day, ultimately controlling how much energy is

stored as fat (Cani and Delzenne 2009). Those who ingest a higher percentage of calories compared to the amount of calories expended disrupt energy homeostasis and, as a result, develop more body fat.

Recent research using mouse models suggests that the microorganisms present in the gut strongly influence the regulation of energy homeostasis. In one particular research study, germ free mice lacking gut microorganisms were compared to conventionally raised mice possessing a normal gut flora. The conventionally raised mice consumed about 30% less food in their diet compared to the germ-free mice; however, the germ-free mice had 40% less body fat compared to the normal mice (Cani and Delzenne 2009). These findings were further expanded upon as the researchers conventionalized the germ free mice by transplanting a gut microbiota obtained from a normal, conventionally raised mouse, into the germ free mice and observed the results over a two week time period (Cani and Delzenne 2009). After transplanting a gut microbiota into the germ free mice, the mice were fed a lesser amount of food, but expressed a 60% increase in body fat (Cani and Delzenne 2009).

Researchers concluded that the observed weight gain was due to an increase in glucose absorption in the intestines, energy extracted from non-digestible consumed food, and a higher level of glucose and insulin in the blood. Glucose and insulin stimulate the activity of certain enzymes that aid in lipogenesis, the process by which food is converted into energy and then stored as fat in the body (Cani and Delzenne 2009). In comparison to the germ-free mice lacking any microbes in the gut, there was less weight gain, despite being fed a larger diet compared to the conventionally raised mice. Each of the processes observed in the conventionalized mice are carried out by the microbial species present in the gut. This provides insight into the importance

of microbes in extracting the energy from ingested foods and initiating fat production in the body, which is crucial to health when maintained at healthy levels (Cani and Delzenne 2009).

Likewise, another proposed mechanism by which metabolic disorders such as obesity arise is due to alterations in the redox state. For instance, researchers imposed a long term high fat diet on experimental mice models and compared them to the control group, consisting of mice fed a normal diet. Specifically in the study, the researchers observed the bacterial strains of *E. coli*, *Lactobacilli*, and *Enterococcus* in both the mice fed a high fat diet, and those fed a normal diet (Qiao et al 2012). The results indicate that the experimental group fed a high fat diet experienced higher levels of oxidative stress as compared to the control group (Qiao et al 2012). In other words, the high fat diet resulted in the production of many free radicals; however, there were very few antioxidants present to rid the body of them. Additionally, they noticed an increase in the number of *E.coli* and *Enterococcus* bacteria along with a decrease in the number of *Lactobacilli,* in the gut of the experimental group compared to the control group (Qiao et al 2012). Through several different analyses, the researchers concluded that the diversity seen among the microbial species present in both the experimental and control group is significantly correlated to the amount of oxidative stress present with the organism (Qiao et al 2012).

Overall, the researchers found that high fat diets change the gut microbiota, which in turn, increases oxidative stress due to the resulting microorganisms. Though this study allows researchers to successfully make associations between bacterial species and biological processes, unfortunately it fails to provide the specific mechanism by which some microbes directly alter the process. Regardless, the studies have shown that diet alters the composition of the gut flora, which in turns generates an altered biological response due to different levels of particular microbial species.

Finally, a third mechanism by which gut bacteria alters metabolic pathways is through the production of endotoxins such as lipopolysaccharides (LPS). LPS is an endotoxin that originates in the membrane of Gram-negative bacteria residing in the intestinal gut (Boroni Moreira et al 2012). Normally, the present LPS is absorbed by enterocytes in the gut, however when this process fails, the LPS can reach the circulatory system, generating metabolic endotoxemia. High fat diets tend to prevent LPS degradation by the golgi, and instead, aid in the transportation of LPS across the intestinal barrier and into the plasma, where it becomes part of the circulatory system (Boroni Moreira et al 2012). Once LPS reaches the circulatory system, this toxin becomes present in the bloodstream and can hinder insulin signaling, ultimately causing an increase in weight gain (Boroni Moreira et al 2012). LPS activates toll-like receptor 4 (TLR4), which is a protein that aids in pathogen recognition to initiate an immune response. As a result, the body experiences acute inflammation due to a series of activated pathways occurring in the adipose, muscle and liver tissues (Caricilli and Saad 2014; Ding et al 2010). These activated pathways counteract insulin signaling, which results in insulin resistance. The adipose, muscle and liver tissues fail to respond to normal levels of insulin, thus cannot extract glucose from the bloodstream. As a result, the pancreas continues to produce an excessive amount of insulin, putting the individual at a high risk for developing diabetes (Caricilli and Saad 2014; Ding et al 2010).

A primary factor responsible for the harsh effects of endotoxins on metabolic pathways is due to a weakened intestinal membrane. In the previously mentioned study, there was an observed decrease in expression of ZO-1 and Occludin, which are proteins responsible for the tight junctions of the intestinal membrane (Cani et al 2008). Due to low expression of these proteins, there were fewer tight junctions, which weakened the stability of the membrane. In

turn, this caused an increase in intestinal permeability (Cani et al 2008). Due to a more unstable membrane, the ingested fats can more easily transport LPS across the membrane and relocate it into the plasma. Once this occurs, an inflammatory response is initiated, which was observed in the study by an increased expression of IL-1 and other genes involved in the immune system's inflammatory response (Boroni Moreira et al 2012;Cani et al 2008). The activation of pathways regulating inflammation hinders insulin signaling, which in turn, disrupts energy extraction from food and its proper expenditure, leading to insulin resistance. In response, cells fail to respond to normal levels of insulin, and the pancreas continues to produce more insulin, which leads to an excess of glucose in the blood (Boroni Moreira et al 2012). Insulin resistance plays a role in the development of obesity because energy extraction from ingested food and expenditure is not properly regulated, therefore leading to increased fat stores, weight gain, and possible type II diabetes (Boroni Moreira et al 2012). Thus, this complex cascade of pathways all stem from the response of the microbes present in the intestinal gut. The type of diet consumed influences the composition of the gut microbiome, which can weaken the intestinal barrier permeability, allowing for endotoxins to invade the plasma, initiate an immune response, and contribute to malfunctioning metabolic processes.

Methods to Maintaining a Health Gut

Evidently, several research studies have shown the significance of environmental factors such as high-fat diets and antibiotics in altering the composition of the microbial community. Unfortunately, it is extremely difficult to completely avoid using antibiotics, as they provide relief for many bacterial infections. Additionally, while high fat diets can be reduced, it is difficult to completely avoid them. Therefore, alterations in the microbial population are

inevitable, but can still be regulated. One way is through the use of probiotics, which are dietary supplements that contain live bacteria to replace those lost in the intestinal gut due to environmental factors (Cani et al 2008). Bacterial species belonging to the genus of *bifidobacteria* have been shown to reduce the concentration of LPS in the plasma; however, this species tends to decrease in numbers as a result of antibiotic usage (Cani et al 2008; Nadal et al 2008). Therefore, by consuming probiotics containing *bifidobacteria*, individuals may be able to restore the intestinal gut composition and maintain a strong intestinal barrier to prevent an excessive amount of LPS leakage into the plasma (Cani et al 2008; Nadal et al 2008). Ultimately, they can reduce the risk of obesity, insulin resistance and other metabolic disorders.

Likewise, prebiotics are another way to restore a damaged microbiome within the gut. Unlike probiotics, prebiotics are not pills containing live bacteria; rather, they are dietary fibers found in foods such as onions, garlic, bananas, and the skin of apples. They nourish certain bacteria in the colon, which allow for those bacterial species to properly function and maintain a healthy gut, as opposed to directly ingesting certain microbes (Cani and Delzenne 2009).

Furthermore, intervention methods such as fecal transplantation can be used to restore a healthy gut. During this process, fecal matter is collected from a donor, mixed with a certain solution, and then placed into the gut of a patient suffering from an unbalanced microbiome via a colonoscopy or endoscopy (Harley and Karp 2012). The purpose of transferring microbes from the stool of a donor into the gut of a patient is to replace good bacteria that have been killed off due to environmental factors. This method also prevents the overpopulation of *Clostridium difficile*, which is a pathogenic bacteria specie that can cause many problems including fatal diarrhea (Califf et al 2014; Harley and Karp 2012).

Much more research regarding probiotics, prebiotics and gut bacteria must be conducted since there are several microbial organisms located in the gut that are also impacted by diet and antibiotics, which need to be investigated (Cecchini et al 2013). Also, the amount of fat and carbohydrates ingested must also be monitored, as high-fat diets clearly impact the composition of gut bacteria.

Conclusion

Overall, metagenomics has provided scientists with a great deal of evidence that gut bacteria does in fact contribute to the stimulation of certain pathways that direct metabolic phenotypes. Those who maintain a gut with very little bacterial diversity are characterized as having higher body fat, insulin resistance, and increased inflammation as compared to those possessing a very diverse gut microbiome (Caricilli and Saad 2014). Each of the metabolic pathways contributing to the development of obesity stem from the reaction of gut bacteria towards consumed foods. Without gut bacteria, the body would fail to extract energy from foods and invest it in necessary metabolic processes and aid in nutrient absorption. Gut bacteria can regulate weight gain, providing insight into how certain disorders, such as obesity, are biologically regulated. This can lead to advances in therapeutic strategies to treat obesity, because caloric intake is not solely responsible for the weight gain, but also the reaction of the gut bacteria to certain foods (Chen et al 2014).

Further research needs to be conducted because gut microbial diversity shows possible correlation to other diseases such as irritable bowel syndrome and Crohn's disease (Brown et al 2011). These diseases show similar characteristics of autoimmune disorders seen in rheumatoid arthritis and psoriasis, thus gut bacteria may indirectly impact auto-immune diseases as well.

(Brown et al 2011). Since the microbial community directs several pathways linked to digestion and immunity, it is likely that there are several other diseases that are non metabolic that may be strongly impacted by the gut bacteria. Much more research must be conducted to determine further correlations between gut composition and human health. This research can generate great insight into several other diseases and possible cures simply by maintaining a healthy gut.

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