2014

Introduction to the human gut microbiota and its effect in weight regulation

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https://hdl.handle.net/2144/15328
Boston University
INTRODUCTION TO THE HUMAN GUT MICROBIOTA
AND ITS EFFECT IN WEIGHT REGULATION

by

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B.S., University of California, Davis, 2009

Submitted in partial fulfillment of the
requirements for the degree of
Master of Science
2014
ACKNOWLEDGEMENTS

I would like to thank Dr. Stephanie Oberhaus and Dr. Theresa Davies for their guidance and help throughout this process.
INTRODUCTION TO THE HUMAN GUT MICROBIOTA AND ITS EFFECT IN WEIGHT REGULATION

ERIC M. GAVARRE

ABSTRACT

There has been a rapid increase in the number of overweight and obese individuals worldwide in the past 50 years. It has been assumed that an increased caloric intake and a more sedentary lifestyle are the main causes of this rise. However, recent evidence has shown that the microbes that live in the human gastrointestinal tract may play a role in the regulation of weight and obesity development. These microbes, termed the gut microbiota, are commensal and symbiotic microbes that are densely populated throughout an individual’s gastrointestinal tract. This paper presents the relevant research and possible mechanisms of how these microbes, mainly bacteria, are thought to play a role in weight regulation and obesity.
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<tr>
<td>%WMEN</td>
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<td>AMPK</td>
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<td>FIAF</td>
<td>Fasting Induced Adipose Factor</td>
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<td>FISH</td>
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<tr>
<td>rRNA</td>
<td>Ribosomal Ribonucleic Acid</td>
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<tr>
<td>SCFA</td>
<td>Short Chain Fatty Acid</td>
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<td>UN</td>
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<td>WMD</td>
<td>Weight Maintaining Diet</td>
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INTRODUCTION

There has been a rapid increase in the number of obese and overweight individuals worldwide in the past 50 years. It is estimated that obesity has doubled since 1980 ("WHO | Obesity and overweight," 2013). Due to this rapid increase, there has been an increase in studies on this topic recently. Some researchers hypothesize that the primary cause is due to two factors: increased caloric intake and an increased sedentary lifestyle causing an energy imbalance between calories consumed and calories expended (Cani & Delzenne, 2011; Zhao, 2013). However, recent research has shown that the multitudes of microorganisms that reside in the human gastrointestinal tract, the human microbiota, may have a role in weight regulation and the development of obesity (Shen, Obin, & Zhao, 2013; Turnbaugh et al., 2006). Although the mechanism of how the microbiota accomplishes this task is still being elucidated, there is strong evidence indicating that the human gut microbiota plays a role in weight regulation and development of obesity.

The World Health Organization (WHO) defines “overweight” and “obesity” as an unusual or excessive accretion of fat and triglycerides which may impair an individual’s health (“WHO | Obesity and overweight,” 2013). Obesity can include many different disease phenotypes such as fatty liver, hypertension, insulin resistance and the accumulation of abdominal adipose tissue that is associated with a low grade systemic and chronic inflammation (Cani & Delzenne, 2011; Shen et al., 2013). It raises an individual’s chance of having type II diabetes mellitus, cardiovascular disease, and
osteoarthritis as well as increasing the chances of certain cancers such as endometrial, breast and colon. The World Health Organization estimates that the number of overweight adults aged 20 years and older surpassed 1.4 billion in 2008. Of that number, 200 million men and 300 million women were obese. Childhood obesity is also increasing with approximately 40 million children aged 5 or younger who are overweight. This is especially harmful to children as it leads to complications such as breathing difficulties, hypertension, early markers of cardiovascular disease and psychological stress (“Obesity and Overweight for Professionals: Data and Statistics: Adult Obesity - DNPAO - CDC,” 2013).

The prevalence of obesity not only impacts an individual’s health, but it also has negative economic influences on an individual and the health care system. The Centers for Disease Control and Prevention (CDC) estimates that in the US, the estimated annual medical cost of obesity was $147 billion dollars in 2008. An obese person had average medical costs that were $1,429 more than a normal weight individual in previous years (“Obesity and Overweight for Professionals: Data and Statistics: Adult Obesity - DNPAO - CDC,” 2013). In the United States, obesity is a major health problem. In 2009, the CDC found that 35.7% of the US population aged 20 and over were obese (Figure 1) (CDC, 2012). In the recent past there has been a perception that obesity is a disease that is mainly prevalent in high income countries. However, the percentage of individuals who are overweight or obese in low and middle income countries is now rising (Zhao, 2013). Of the 40 million children who were overweight in 2011, 30 million lived in developing countries versus the estimated 10 million overweight children in developed countries.
Until recently, the United States had the highest percentage of obese individuals since researchers began studying this epidemic. However, in 2013 the Food and Agriculture Organization of the United Nations reported that Mexico surpassed the United States in having a higher percentage of obese individuals, 32.8%, as compared with the United States, 31.8% (Food and Agriculture Organization of the UN, 2013). This report further demonstrates that obesity has become a worldwide problem, and not just a problem that high income countries must try to combat. Being obese or overweight is now the 5th leading cause of death throughout the world. In fact, being overweight or obese is associated with more deaths throughout the world than being underweight. Sixty five percent of the world’s population live in a country where more people die from complications of being overweight or obese than from hunger or starvation (‘WHO | Obesity and Overweight,” 2013).
The Gut Microbiota

The gut microbiota is defined as the community of microbes that reside in an individual’s gastrointestinal tract (GI tract) (Gordon, 2012). This term incorporates microbes from all three domains: Archaea, Eukaryota and Bacteria. It also includes the viruses associated with each domain (Gordon, 2012). These are commensal and symbiotic microbes and the majority of them are bacteria (Shen et al., 2013). During the past 10 years, there has been an increased interest in the role that the human microbiota plays in human health due to advances in technology that have enabled researchers to investigate these intricate communities. The human microbiota includes microbes that live in the human gut as well as the microbes that exist elsewhere in the human body such as...
as in the oral cavity, nostrils and skin (Figure 2). The type of microbes in each of these sites depends primarily on where the microbes are located anatomically. Interestingly, there is marked variation, and some similarities, in composition of these communities between individuals. Researchers conservatively estimate that microbes have been living and performing metabolic and other functions within animals for the past 500 million years. During those years, these microbes have undergone a specific selection based on co-adaptation (Cho & Blaser, 2012).
Figure 2. Compositional differences in the Microbiome by Anatomical site. (Cho & Blaser, 2012)
In humans, the colon is the most densely colonized anatomical area and contains the most diverse populations of bacteria compared to other places in the body (Zhao, 2013). Some of the many functions of the gut microbiota include: nutrient and drug metabolism, epithelial cell proliferation, immune system and barrier function against enteric pathogens and the synthesis and bioavailability of several vitamins (Cani & Delzenne, 2009). Enzymes that are produced by these microbes enable the host to process compounds that would have otherwise passed through the host unaltered. It has been estimated that the short chain fatty acids that are produced after fermentation of dietary fibers account for 10% of the total dietary energy supply in humans (Duncan et al., 2008). In fact, most gut microbes are either harmless or are of benefit to the host (Lozupone, Stombaugh, Gordon, Jansson, & Knight, 2012).

One effect of having numerous bacteria reside within the colon is that some of their metabolites enter the blood stream. In fact, one third of all small molecules found in human blood can be traced to bacteria in the gut microbiota. Some of the metabolites have a beneficial effect to the human host. Effects of these metabolites include: anti-inflammatory properties, anti-oxidant properties, pain relief activity, a source of energy, a source of vitamins and gut barrier function control. For example, butyrate, a short chain fatty acid (SCFA), is produced by bacterial fermentation of dietary fibers. It has been shown that this SCFA could then act as a source of energy for human colonocytes as well as having many other effects such as: increasing satiety, alleviating inflammation, reducing carcinogenesis in the colon, mitigating oxidative stress and improving gut barrier function (Zhao, 2013).
However, there are bacterial-derived products that are harmful. Cytotoxins, genotoxins and immunotoxins are all examples of bacterial exotoxins or metabolites that have deleterious effects on the human host. Lipopolysaccharide (LPS) is one example of a damaging cell wall component released by Gram-negative bacteria either by release of cell wall “bleb” or by cell lysis (Zhao, 2013). It has been shown that lipopolysaccharide, when injected subcutaneously, can provoke an inflammatory response as well as aggravate chronic inflammation that is seen in conditions such as obesity and insulin resistance (Cani et al., 2007).

There are approximately 1.5 kilograms of bacteria that dwell in the human colon. Those trillions of cells help to breakdown otherwise indigestible food (Ley, Turnbaugh, Klein, & Gordon, 2006). New technologies for studying the microbiota, such as 16s rRNA sequencing and shotgun high throughput sequencing, have led to findings that alter how researchers and scientists consider bacteria that live inside humans. Human beings are now being considered supraorganisms that are composed of both human and microbial cells (Zhao, 2013). It is estimated that human beings are composed of 10% human cells and 90% prokaryotic cells (Lederberg, 2000). These prokaryotic cells not only add a wide array of different cells to a host, but also add a tremendous amount of genetic material to that host. The human genome contains approximately 23,000 genes, but the genes that are associated with the gut microbiota, termed the gut microbiome, exceed 3 million, i.e. more than 130-fold more bacterial genes than human genes (Shanahan, Dinan, Ross, & Hill, 2012; Zhao, 2013). The aggregate of the microbiome and the human genome is termed the metabolome (Wall et al., 2009). These recent
findings are relevant to medicine because all genes present in the body have the potential to affect human health, either positively or negatively (McNulty et al., 2013). Therefore, because the metabolome consists of mainly genes from these microbes, it can be said that human beings only genetically inherit 1% of the genes that reside in cells in their body. The rest are acquired from the immediate environment breast milk, contact with other people, as well as other sources (Zhao, 2013).

Figure 3. Human beings as supraorganisms. Humans consist of both human cells and microbial cells. The gut microbiota interacts with host genetics and the environment to influence health of the host (Zhao, 2013)

There are more than 1000 phylotypes of bacteria in the gut microbiota. A phylotype can be described as collections of 16S rRNA sequences which have 97-99% similar identities organized into the same operational taxonomic unit symbolizing one
species level sequence or “phylotype”. These phylotypes are usually identified by using shotgun high-throughput sequencing or 16s rRNA sequencing (Shen et al., 2013). There are 6 divisions of bacteria present in the gut microbiota: Proteobacteria, Actinobacteria, Fusobacteria, Verrucomicrobia, *Firmicutes* and *Bacteroidetes*. However, about 90% of the bacteria belong to either the *Firmicutes* or *Bacteroidetes* (Cho & Blaser, 2012; McNulty et al., 2013). Other microbes that are present are Methanogenic Archaea, eukaryotes (mainly yeasts) and viruses (mainly phages). In the investigation to find bacterial species which represent a “a core microbiota”, a few species have been identified as notable members in the human colon: *Faecalibacterium prausnitzii*, *Roseburia intestinalis* and *Bacteroides uniformis* (Lozupone et al., 2012).

Although these six divisions are consistently the main components of the gut microbiota, the proportions of species and phyla as well as the specific species that are present, vary across individuals (Song et al., 2013). Cultured-based studies have indicated that healthy individuals share many of the same bacteria in their colon, termed the “core microbiota”. However, culture-independent sequencing studies contradict this concept. Other studies have shown that species within the gut microbiota vary over time and across geographic populations (Lozupone et al., 2012). Interestingly, although these populations differ in the composition of the gut microbiota, the functional gene profiles remain quite similar (Turnbaugh et al., 2009). Jumpertz et al conducted studies that showed individual variability of bacterial composition in fecal matter. Researchers collected stool samples of subjects at various time points after being placed on certain diets. Samples taken from the same subject in their study were more similar, irrespective
of current diet, than when compared to samples taken from different subjects. This reinforces the notion of individual variability in the gut microbiota (Jumpertz et al., 2011).

Variation in the Gut Microbiota

After the initial realization that the gut microbiota may have a role in weight regulation and the development of obesity, researchers sought out to learn more about the details of these many microbes inhabiting not only the human colon but also throughout the body. It was found that the composition of the microbiota varied by anatomical site. They also found that interpersonal variation is extensive, and is surprisingly more variable than temporal variation seen in individuals (Cho & Blaser, 2012) (Figure 4). Researchers are currently investing what factors drive this variation.
Figure 4. Diversity of the human microbiota at different phylogenetic scales. Variation in the gut microbiota between 4 individuals: A, B, C and D. The graph depicts variation seen at the phylum level. One difference is that individual A has a high proportion of Bacteroidetes where as individual D has a high proportion of Firmicutes. The tree depicts the phylogenetic relationships between species level phylotypes in the Firmicutes in individuals B and C. Red branches are specific to individual B, blue branches are specific to individual C and purple branches are shared. This tree demonstrates that although it may seem like there is less variation between individuals B and C at the phylum level, there is great variation seen at lower taxonomic levels. (Lozupone et al., 2012)
One major factor that drives changes in the composition of the human gut microbiota is diet. As will be mentioned later, it has been shown that the composition of bacteria in obese individuals can be modified by consuming a fat-restricted diet or a carbohydrate restricted-diet (Ley et al., 2006).

The environment is also an important factor that has been shown to affect the constitution of the gut microbiota. This includes the environment in which a baby was born as well as the environment to which the human child is later exposed. For instance, there is increasingly more evidence for the transfer of the microbiota from the mother to the infant during the first months of the infant’s life. Prior to the rupture of the amniotic sac, the fetus is essentially sterile. However, during vaginal delivery, the first bacteria colonies in the infant resemble those from the mother’s vaginal canal as well as those present in the mother’s breast milk. Lactobacilli predominate in these colonies. Consistently, it was observed that this genus of bacteria flourishes in the infant’s gastrointestinal track at first. Lactobacilli is the pioneer population of bacteria in the colon and could possibly be responsible for preparing the colon for subsequent populations of colonizing microbes (Cho & Blaser, 2012). This preparation leads to many changes in the microbiota that occur throughout a person’s life. The microbiota in infants is very unstable and there are marked changes that occur early in life until a more established community is formed (Lozupone et al., 2012).

A genetic component is also being investigated as a possible factor that helps to drive the variation seen in the microbiota. Two separate experiments showed that the
microbiota is more similar in twins and between a mother and a daughter than unrelated individuals (Dicksved et al., 2008; Turnbaugh et al., 2009). These findings suggest that there is a genetic factor that partially determines the composition of the gut microbiota. Possible examples of a genetic component that is responsible for influencing composition of the gut microbiota include: host digestive physiology, pH of the gut, presence of bile acids as well as components of the innate immune system (Sylvia H. Duncan & Flint, 2013; McNulty et al., 2013; Vijay-Kumar et al., 2010).

Another example of how the environment and genetics both could affect the composition of the microbiota is seen when studying the microbiota from populations across the world. The microbiota from Italian children was seen to be vastly different from the microbiota present in children from rural Africa (Filippo et al., 2010). Furthermore, the gut microbiota found in children and adults in the US drastically varied from the gut microbiota found in populations from Malawi and Venezuela (Yatsunenko et al., 2012). It is difficult to determine what caused the variations in these examples for many different reasons. For example, not only are there genetic differences between the populations, these populations also differ in environmental exposure, sanitation levels, diet and antibiotic use (Lozupone et al., 2012). However, these two studies indicate that the environment, diet and genetics may all play a factor in determining the composition of the gut microbiota.

Although the composition of the gut microbiota undergoes temporary and minor perturbations, in response to things such as a change in diet, it can also undergo a major
perturbation due to an enteric infection or the use of antibiotics. The effects of either of these could lead to a new steady state composition of the gut (Cho & Blaser, 2012).

**Why Study the Gut Microbiota?**

It is important to study the composition of the gut microbiota and to identify the factors that drive change within it because many disorders in human health such as obesity, malnutrition, inflammatory bowel disease and certain neurological disorders have been associated with disturbances in the normal state of the gut microbiota. However, it is also important to note that identifying the composition of the gut microbiota alone will not guide researchers to an understanding of how the gut microbiota is established and maintained or the mechanisms of its effects on health and disease. This in combination with sequencing community DNA, studying cultured isolates that have well characterized genome content and *ex-vivo* phenotypes together will provide the most information about the functions of the gut microbiota (Lozupone et al., 2012).

The gut microbiota has gained attention in the past 10 years due to a possible link to weight regulation and the development of obesity in mice as well as humans. This was first observed when researchers found a difference in the ratio between two phyla in obese and non-obese individuals. This dysbiosis, whether at the level of the phylum or more specific taxon level, was hypothesized to aid in the development of obesity through weight regulation. The research presented in this thesis describes findings related to this hypothesis. This research, along with future research, is critical to human health as it
attempts to expand on an area of the human system that has not been previously studied. It will enable future clinicians and the general public the ability to monitor and possibly manipulate the gut microbiota in order to achieve better health.
PRESENTATION OF PUBLISHED DATA

Animals that are reared under sterile or germ-free conditions are classified as gnotobiotic (Gordon, 2012). They have been valuable to the study of the gut microbiota because they are able to provide an in-vivo model of the human gut microbiota when they are colonized with a defined population of sequenced human gut microbes. This provides researchers the opportunity to study microbes in ways that are not possible during human studies by tightly controlling experimental variables (McNulty et al., 2013). Germ free (GF) mice are defined as mice that do not have microorganisms living in or on them. These mice have been valuable to the gut microbiota investigations because they allow for the gut microbiota to be transplanted from a conventional mouse to a GF mouse. This process is termed conventionalization (Zhao, 2013). Researchers have relied on studying the microbiota that is located in the caecum because it is an anatomically distinct structure found at the distal small intestine and the proximal portion of the colon. There are a sufficient amount of microbes residing in the caecum which allow researchers to perform a metagenomic analyses on these microbes (Turnbaugh et al., 2006).

The seminal study about the role that the gut microbiota plays within host metabolism was first performed by Bäckhed and colleagues in 2004. They made the observation that GF C57BL/6 mice contained 42% less body fat than conventionally raised mice (CONV-R), which harbored a microbiota beginning at birth. Additionally, researchers observed that the epididymal fat pad weights were 47% greater in the CONV-R animals. These characteristics were apparent even though the CONV-R mice consumed
29% less standard rodent chow diet than the GF mice. The GF mice were maintained under gnotobiotic isolators, kept on a strict 12 hour light schedule, and fed an autoclaved diet. They were purchased from a separate laboratory. Researchers determined the amount of body fat using dual energy x-ray absorptiometry. They then transplanted the gut microbiota from the cecum of CONV-R mouse into GF mice. CONV-R mice were fed a low-fat, polysaccharide diet and were maintained in a specified pathogen-free state prior to the conventionalization. The conventionalized mice were then placed in gnotobiotic isolator for 10-28 days under the same conditions as the GF mice. Total body fat content was measured five minutes after the mice were anesthetized with an intraperitoneal injection of ketamine and xylazine. The amount of normal standard rodent chow was measured in grams day. Three different conventionalizations were performed: a 10 day conventionalization, 14 day conventionalization and a 28 day conventionalization. They found that after a 14 day conventionalization, mice had a 57% total body fat content increase and a 61% increase in epididymal fat weight. This increase in total body fat was associated with a 7% decrease in lean body mass. A similar increase in total body fat was seen after a 10 day conventionalization and the 28 day conventionalization did not produce further increases in total body fat content nor epididymal fat pad weight. The increases in fat content were seen in the 14 day conventionalization despite the conventionalized mice consuming 27% less standard rodent chow than GF mice (Figure 5). It is important to note that these effects were not unique to one sex or the other. Also, the increases in fat were also seen in the NMRI inbred strain of GF mouse (90% increase in total body fat content with a 31% decrease in
rodent chow consumption) (Bäckhed et al., 2004). These findings led to many studies attempting to explain how the gut microbiota was able to affect the weight of mice and the possible mechanisms behind this process.

**Figure 5. Changes seen between GF, conventionalized and conventionally raised mice.** Graph A depicts differences in total body fat percentage between the 3 groups. Graph B depicts the differences in Epididymal fat weight (g). (Bäckhed et al., 2004)
In 2005, Ley and colleagues wanted to expand on the idea that the gut microbiota has an effect on obesity and the development of obesity. They sequenced 5,088 bacterial 16s rRNA sequences from the microbiota located in the caecum of genetically obese mice (ob/ob), lean mice (ob/+) wild type siblings and their (ob/+) mothers. These mice were all fed the same polysaccharide-rich diet. Their results indicated that when compared to lean mice and their siblings, regardless of kinship, ob/ob mice had a 50% decrease in the amount of *Bacteroidetes* as well as a proportional increase in the amount of *Firmicutes*. These differences were seen throughout the phylum and not due to differences in food consumption (Ley et al., 2005).

Turnbaugh and colleagues sought to examine if obesity is associated with changes in these two phyla, the *Firmicutes* and *Bacteroidetes*. They investigated the distal gut microbiomes of ob/ob, ob/+ and +/- littermates through random shotgun sequencing of their caecal microbial DNA. The ob/ob mouse was used in this experiment because the researchers knew that these mice were obese due to increased food intake because of a leptin deficiency. The lean mice (ob/+ and +/-) mice were used as a comparison to the ob/ob mice. The researchers quantified the amount of short-chain fatty acids (SCFA’s) in the caecal microbiota of 4 lean and 5 obese conventionally raised C57BL/6J mice via gas chromatography mass-spectrometry quantification. The results showed that their caecum had an increased concentration of the products of bacterial fermentation, butyrate and acetate (Figure 6a). They hypothesized that this may occur because many of the members of the *Firmicutes’* domain are butyrate producers, and that the microbiota found in obese mice contained a higher percentage of *Firmicutes* than did their lean littermates.
They hypothesized that the obese gut microbiota was more efficient at extracting energy from the diet than the microbiota from a lean individual. They verified this hypothesis by using bomb calorimetry of the fecal gross energy content (kcal g\(^{-1}\)) of 9 lean and 13 obese conventionally raised C57BL/6J mice. The results indicated that the fecal matter of the obese mice contained less energy than that of the lean mice (Figure 6b). The researchers also tested this by attempting to pass this trait on to lean individuals. The colonization of 10 GF C57BL/6J mice with an obese (ob/ob) microbiota from 9 obese mice showed an increase of 47% body fat. In contrast, a GF C57BL/6J mouse colonized with microbiota from a lean +/+ donor showed an increase in 27% body fat over a period of 2 weeks. The body fat content was measured before and after a 14 day colonization using dual energy x-ray absorptiometry (Figure 6c). There was no change in caloric intake or food consumption (Turnbaugh et al., 2006).
Figure 6. Analysis of the ob/ob microbiome. (A) Gas-chromatography mass-spectrometry quantification of short-chain fatty acids in the caeca of 4 lean and 5 obese conventionally raised C57BL/6J mice. (B) Results from bomb calorimetry of fecal gross energy content in 9 lean (+/+, ob/+), and 13 obese (ob/ob) conventionally raised C57BL/6J mice. This evidence suggests that the obese microbiome may be more efficient at extracting energy than the lean microbiome. (C) 10 GF mice wild type C57BL/6J mice were colonized with the caecal microbiota from 9 obese (ob/ob) donors resulting in greater total body fat content (Turnbaugh et al., 2006).

There have only been a few studies examining the gut microbiota in human subjects. However, one of the first was performed by Ley and his colleagues in 2006. They sought to determine if the composition of the gut microbiota in obese individuals differed from that of lean individuals. They studied the microbiota in 12 obese men and women aged 21 to 65 years old over the course of one year. Their body mass index (BMI) ranged from 30 to 43 kg/m². They assigned each subject to either a fat restricted diet (Fat-R) or a low calorie diet (Carb-R) with the goal to monitor how these diets affected the composition of their gut microbiota. The recommended caloric intake for
men was 1500-1800 kcal/day and 1200-1500 kcal/day for women. Four fecal samples were taken: one prior to the start of the study and three more at 12, 26 and 52 weeks after the onset of the diet therapy. Fecal samples were compared with samples from two healthy men, aged 32 and 36 who both had a BMI of 23 kg/m$^2$. The microbiota was monitored by sequencing the 16s rRNA sequences from the volunteers’ stool samples. They found that 70% of the phylotypes were distinctive to each volunteer regardless of to which diet restricted group they were assigned. However, despite this interpersonal variation among the phylotypes, they found that 92.6% of all 16s rRNA sequences belonged to either the Firmicutes or Bacteroidetes phylum. Additionally, they found that obese individuals at the beginning of the study had more Firmicutes (P=0.002) and fewer Bacteroidetes (P<0.001) than did lean controls. Throughout the course of the study, the relative abundance of Firmicutes decreased (P=0.002) and the relative abundance of Bacteroidetes increased (P<0.001). These changes were seen irrespective of which diet the subject was placed on. These changes were seen throughout all species detected within the phyla and not due to blooms or extinctions of specific bacterial species. Another interesting finding was that the increased abundance of Bacteroidetes was correlated with the percentage of body weight lost and not with changes of dietary calorie content overtime (Figure 7) (Ley et al., 2006). However, this study is limited due to the small sample size of volunteers used.
Figure 7. Relative abundance of Firmicutes and Bacteroidetes seen throughout the experiment. (Ley et al., 2006).

Duncan et al. did a study to determine the relationship between BMI, weight loss and major bacterial groups found in human subjects’ fecal samples during a 4 week diet (S H Duncan et al., 2008). Using 16s rRNA-based quantitative fluorescent in situ hybridization (FISH), the researchers monitored major bacterial groups in 18 obese male subjects. This data was later extended to include another 15 obese male subjects and 14 unspecified, non-obese subjects. A subject was determined to be obese if their BMI was greater than or equal to 30kg/m². A certain bacterial group was expressed relative to the total number of bacteria that was present in a subject’s fecal sample. This total number of bacteria was estimated using a broad probe (Eub338) which can detect nearly 73% of bacteria present by direct staining using 4,6-diamido-2-phenylindole. There were similar amounts of total number of bacteria between obese and non-obese subjects. They found that obese subjects had a total bacteria amount of $5.52 \times 10^{10}$ cells/g feces and non-obese
subjects had $5.59 \times 10^{10}$ cells/g of feces. The researchers measured the amount of *Bacteroidetes* using the Bac303 probe. There was no difference in the total amount of *Bacteroidetes* between obese and non-obese subjects consuming a weight maintaining diet (27.2 vs 21.9%, SED=2.96, P=0.084). Additionally, they did not find a significant relationship between BMI and the proportion of *Bacteroidetes* (correlation 0.13, P=0.40) (Figure 8). 23 obese subjects were then placed on two weight loss diets for 8 weeks: a high-protein low carbohydrate, ketogenic diet or a high-protein moderate-carbohydrate, non-ketogenic diet. Each subject spent 4 weeks on one diet. The researchers did not identify a difference in the ratio of *Firmicutes* and *Bacteroidetes* between the non-obese and obese individuals at the end of the study. Additionally using the Bac303 probe, they did not find an increase in members of the *Bacteroidetes* phylum throughout the weight loss (R2: 0.08, P=0.11). However, they did find that the abundance of 2 specific groups of bacteria within the *Firmicutes*’ phyla, *Roseburia spp.* and *Eubacterium rectale*, both decreased with decreased carbohydrate intake. Interestingly, they found that fecal butyrate concentration decreased, a finding consistent with Turnbaugh et al. However, conclusions from this study may be limited because the study was performed only for 4 weeks and had a relatively small sample size.
In 2007, researchers sought to further clarify why GF mice, fed the same diet as conventional mice, failed to develop obesity. Conventional mice and GF C57BL/6J mice were fed a high-fat, sugar-rich “western” diet. They found prior to conventionalization, that there is a change in the composition of the gut microbiota in genetically obese (ob/ob) mice such that there was a 50% decrease in Bacteroidetes and a corresponding increase in the percentage of Firmicutes when compared to the microbiota of their lean (+/+ and ob+) littermates. Additionally, metagenomic analyses of the microbes that resided in the distal gut microbiota of obese mice, fed a standard low-fat rodent chow,
revealed that the microbiota was enriched with genes that were able to extract calories from complex plant-derived polysaccharides. Additionally, GF +/+ mice that were fed a standard low-fat, high polysaccharide rodent chow were conventionalized with the microbiota from either obese ob/ob mice or from their lean littermates (+/+ and ob/+). They found that the GF mice that were conventionalized with the microbiota from obese mice had a higher incidence of adiposity over a 2 week period than the GF mice that were conventionalized with the gut microbiota from their lean littermates (Backhed, Manchester, Semenkovich, & Gordon, 2007).

Figure 9. Change in body weight seen in Conventionalized Western and GF Western mice over the course of 8 weeks (Backhed et al., 2007).

In 2008, Turnbaugh and colleagues continued to investigate the relationship that the gut microbiota may have in the development of obesity. They explored the relationship between diet, gut microbial ecology and energy balance in an obese mouse
model consuming a prototypical “western” diet. Ten GF C57BL/6J mice were fed a low-fat, carbohydrate rich rodent chow. At 12 weeks of age, these mice were gavaged with the caecal microbiota from a conventionally raised mouse. Four weeks after the conventionalization, 5 of the GF mice were then switched to a diet that a ‘Western’ diet that was high in both saturated and unsaturated fats. 41% of calories in this diet came from these fats. The other 5 mice remained on the carbohydrate rich diet. Twelve weeks after the conventionalization, the researchers noted that the mice on the ‘Western’ diet gained considerably more weight (5.3±0.8g versus 1.5±0.2g; p<0.05) than did the mice consuming the carbohydrate chow. The caecal contents were then examined. 16s rRNA gene sequence based surveys and UniFrac-based analysis were performed on the caecal contents of these ten mice along with the caecal contents of the conventionally raised donor mouse. They found that diet induced obesity (DIO) produced a bloom of bacteria within a specific class of Firmicutes, the Mollicutes. This class is normally present at a smaller percentage of the gut microbiota in the distal intestine, however, during diet induced obesity, it became a dominant member (Figure 10). They stated that this bloom was associated with an increase in fitness for this particular class of bacteria compared to other bacteria in the Firmicutes phyla as well as members of the Bacteroidetes phyla. Interestingly, when the diet was manipulated to limit weight gain and reduce adiposity, this bloom was diminished. When they studied this class of bacteria in further detail, they found that the Mollicutes class had evolved the ability to import and metabolize certain types of carbohydrates that were commonly present in Western diets, such as glucose, fructose and sucrose. The products of this bacterial metabolism were SCFA’s that the
host was able to utilize. They confirmed results from previous experiments when they showed that when the microbiota from diet induced obese mice was transplanted into GF mice, it produced a greater increase in adiposity than when the microbiota from lean donors into GF mice. Additionally, there was an increased adiposity in GF mice seen when transplantation of microbiota from mice who were consuming a “western” style diet than from mice that were consuming a carbohydrate rich diet. The GF mice were fed a low-fat polysaccharide-rich carbohydrate diet (Turnbaugh, Backhed, Fulton, & Gordon, 2008).

Figure 10. Changes seen in the gut microbiota with diet induced obesity. Black boxes indicate nodes that were reproduced in >70% of all jackknife replications Pie charts reveal the average relative abundance of bacterial lineages in the two different diets. (Turnbaugh et al., 2008)
In 2010 Fleissner and colleagues’ results conflicted with previous findings that GF mice were resistant to diet induced obesity. GF mice were fed with three different diets: a low fat diet, a high fat diet and a “western diet”. They found that when GF mice were fed a low-sucrose, lard-based high fat diet, the GF mice became obese. Intriguingly however, when these mice consumed a high sucrose, high palm oil “western” diet, the GF mice were resistant to obesity. The authors also found that with the mice on the high fat diet and western diet, the relative amount of Firmicutes increased and the amount of Bacteroidetes decreased. This study did use a different strain of GF mice, the C3H strain (Fleissner et al., 2010).

In 2011, Jumpertz and colleagues set out to find how the gut microbiota affected nutrient metabolism in humans. Bäckhed and colleagues in 2004 postulated that the gut microbiota had been able to alter nutrient absorption by modulating the expression of lipoprotein lipase inhibitor in mice. However, it had not been studied whether the gut microbiota had this same effect in humans. Jumpertz and colleagues set out to monitor the variations of the gut microbiota during caloric consumption that ranged from 2400kcal/day to 3400kcal/day. These changes were observed by pyrosequencing bacterial 16S rRNA genes present in the stool samples provided by twelve lean and nine obese individuals. Ingested calories were measured through bomb calorimetry. They found that when their subject ingested more calories, there was a swift change in the subject’s gut microbiota. It was seen that in lean individuals, a twenty percent increase in the Firmicutes’ population and a corresponding decrease in Bacteroidetes was associated with increased energy loss in the subject’s stool of about 150kcal. However, there was no
change seen in the weight maintaining diet that researchers initially placed the subjects on (Jumpertz et al., 2011). As was the case with the other human trials, this study did have a small sample size.

Figure 11. Associations between the Bacteroidetes and the Firmicutes in the distal gut. Graphs A and B represent the 2400kcal/d diet. Graphs C and D represent the 3400 kcal/d diet. These graphs show the associations between the changes in relative number of Firmicutes and Bacteroidetes in the weight maintaining diet (WMD) and as a percentage of weight maintaining needs (%WMEN) (Jumpertz et al., 2011)
Zhang and colleagues set out to solve the discrepancy between previous results of papers that either did find a difference in the ratio of *Firmicutes* and *Bacteroidetes* or did not. A group of adult C57BL/6J mice consumed a high fat diet for 12 weeks. Throughout the experiment, the microbiota from the diet induced obese (DIO) mice was compared to lean mice that were fed a diet rich in carbohydrates. These mice displayed significant levels of newly acquired obesity and insulin resistance. The researchers subsequently placed the mice on a normal fat diet for 10 weeks. Researchers observed a relative increase in the amount of *Firmicutes* and a decrease in the amount of *Bacteroidetes* in the DIO mice that were fed a high fat diet for 12 weeks. Additionally, they found that the relative abundance of a third phylum, Proteobacteria, increased while the mice were consuming the high fat diet. The researchers then sought to see if these changes were reversible if the mice reverted to being fed a normal fat diet. They placed the DIO mice on a normal chow diet for 10 weeks. After 4 weeks on this diet, the previous changes that were seen in the microbiota when the mice consumed a high fat diet, reverted to a composition similar to that of the mice consuming the diet rich in carbohydrates. The researchers performed a redundancy analysis on the fecal matter of the DIO mice and isolated 77 key phylotypes that responded to the alterations in the diets (Zhang et al., 2012).

Recently, Fei and Zhao sought to see what the ramifications of a long term high fat diet on GF mice were. They fed mice a non-western high fat diet. After the first 8 weeks, they noted that the GF mice gained a substantial amount of body weight (Figure 12). However, after this initial period the mice began to lose weight. After 17 weeks, the
body weight of the mice was no different than the mice that they were being compared to being fed normal rodent chow (Fei & Zhao, 2013).

Figure 12. Initial weight changes in body weight and fat pad weight between 4 strains of mice (Fei & Zhao, 2013).
DISCUSSION

The results presented here indicate that the gut microbiota has a role in weight regulation and that it can be altered through diet or microbiota transplantation in GF mice. They also indicate the ratio of *Firmicutes* to *Bacteroidetes* may vary in obese individuals and mice, and that this ratio may be associated with obesity. However, the results from different studies vary regarding whether *Bacteroidetes* or an increased ratio of *Firmicutes* vs. *Bacteroidetes*, or vice versa, correlates with the development of obesity.

The first studies to examine the gut microbiota identified a difference in the ratio of *Firmicutes* to *Bacteroidetes* in obese and non-obese mice. They hypothesized that these differences in the composition of the gut microbiota may have contributed to the development of obesity (Bäckhed et al., 2004; Ley et al., 2005; Turnbaugh et al., 2006). As mentioned above, Ley et al. re-examined these findings and again identified a difference in the ratio of *Firmicutes* to *Bacteroidetes* between obese and non-obese subjects. They concluded that obesity has an effect on the composition of the gut microbiota and suggested that their results may lead to findings where the manipulation of the composition of the gut microbiota may be useful in helping combat obesity in individuals. That same year, Turnbaugh and colleagues found that the caecum of obese mice contained an increased concentration of butyrate and acetate as well as noting that the microbiota of obese mice had had a higher concentration of *Firmicutes*. They determined that this was consistent with the fact that many of the members of the *Firmicutes*’ domain were butyrate producers. They suggested that their findings
reinforced the perception that the gut microbiota should be viewed as a possible contributor, along with host genotype and lifestyle, to the development of obesity and its associated disorders. These papers were the first to indicate that the gut microbiota may have a role in weight regulation in mice. They were also the first to identify the difference in ratio of *Firmicutes* to *Bacteroidetes*, which led to future studies examining this concept.

Ley’s findings in human subjects in 2006 indicated that, like in the gut microbiota of mice, a change in the composition of the gut microbiota in humans through dietary alterations is possible. They also found that obese individuals had a higher ratio of *Firmicutes* to *Bacteroidetes*, which was consistent with their previous findings in obese and non-obese mice. This study was one of the first to indicate that the gut microbiota may have a role in weight regulation in humans. Although Duncan and colleagues’ results in humans did not show a change in the ratio of *Firmicutes* to *Bacteroidetes*, they did find that two SCFA producing members, *Roseburia spp.* and *Eubacterium rectale*, did decrease with decreased carbohydrate intake. They suggested that their findings indicated that the composition of the gut microbiota is capable of being altered through diet. Secondly, they argued that their results illustrate that microbes living in the colon may have specific niches that vary depending on the host’s diet and conditions of the human gut. Their findings demonstrate the importance that future research focus on examining the gut microbiota of both mice and humans at a more specific level than at the phyla level. The conflicting findings regarding the changes seen in the ratio between
*Firmicutes* and *Bacteroidetes* may be due to differences in the bacterial species that were detected in the various studies.

In 2007, Bäckhed and colleagues found an increase in the *Firmicutes* to *Bacteroidetes* ratio in obese mice versus their lean counterparts. They also found that the obese microbiota was better able to extract calories from plant saccharides than their lean counterparts’ microbiota. They proposed that the gut microbiota has the ability to affect both the way that the host is able to extract calories from the diet as well as affect gene expression of genes that are involved in host energy expenditure and storage. Turnbaugh (2008) also wanted to study the relationship between the gut microbiota and energy balance. They found a bloom in a class of bacteria within the *Firmicutes*’ phylum, the Mollicute class, which was associated with diet induced obesity. They stated that their findings suggested that the Mollicute-enriched community enable the host to utilize calories in their diet as well as having an effect on the host metabolism of the absorbed calories.

Fleissner’s study (2010) was also interesting because it was one of the first to show that GF mice are not resistant to diet-induced obesity (DIO) with all diets. Although this study used a different strain of GF mice than previous studies, the C3H strain, the researchers used their data to assert that the absence of a microbiota does not protect against obesity. They found that GF mice that were fed a low-sucrose, lard based high fat diet, became obese. However, in accordance with previous results, they also observed that GF mice were resistant to a high sucrose, high palm oil ‘western’ diet. Therefore,
they postulated that diet affects the composition of the microbiota more than previously thought (Fleissner et al., 2010). Although these results are not consistent with some of the previous findings, they indicate that the gut microbiota is an essential component to the development of DIO in mice and that it could possibly have a causative role in the development of obesity in humans (Zhao, 2013).

Also presented in this paper are opposing results seen in recent studies regarding possible differences in the ratio between the *Firmicutes* and the *Bacteroidetes* in obese and non-obese individuals. While some studies have shown that there is a difference in the ratio of *Firmicutes* to *Bacteroidetes* between obese and non-obese individuals (Ley et al., 2006; Turnbaugh et al., 2006), other studies performed have shown that there is no difference in this ratio (S H Duncan et al., 2008; Schwieritz et al., 2010). Researchers hypothesized that the reason for the conflicting results may be because the changes seen in the microbiota between these individuals did not occur at the division level, but occurred at the phylotype level (Filippo et al., 2010; Zhang et al., 2010). However, this may be a limitation of all of the studies presented here. Due to the vast number of bacterial species living within the human gut microbiota, the variation in the gut microbiota between individuals and the changes seen in the microbiota throughout an individual’s life, it is not possible to identify and monitor all of the species of the human gut microbiota with the current technology (Lozupone et al., 2012).

Zhang and colleagues (2012) sought to resolve the controversy behind the *Firmicutes/Bacteroidetes* (F/B) ratio. They found that there was an increase in the
amount of *Firmicutes* after the mice were placed on a high fat diet, and that this change was quickly reversible. They concluded that when mice consume a diet high in fat, it caused increases or decreases in the number of specific phylotypes that were present in the gut microbiota. Secondly, their results showed that the gut microbiota in mice is adaptable and capable of responding to the perturbations of the diet.

Jumpertz et al found that when obese and lean human subjects were placed on an initial weight maintaining diet, an increased F/B ratio was not seen in obese versus lean individuals. However, they did find that when subjects ingested more calories, there were swift corresponding changes within the gut microbiota. They stated that when lean individuals overfed on either the 2400kcal/day diet or the 3400kcal/day diet, there was a decrease in the amount of energy lost in a subject’s stool. The researchers suggested that this study produced two important ideas regarding the gut microbiota. First, the composition of the microbiota can be quickly altered based on how many calories a person ingests. Second, the gut microbiota could play a valuable role in the nutrient harvest. These results again indicate that the gut microbiota has a role in weight regulation in humans, even though the mechanism has yet to be clarified.

Researchers have proposed many different mechanisms for how this may be possible. One hypothesis is that a bloom of endotoxin producers in the colon contributes to additional weight gain in the host. Lipopolysaccharide (LPS) is a molecule made up of both a lipid component and a polysaccharide component that is found in the outer membrane of Gram-negative bacteria. It has been shown that when injected into a host, it
provokes an immune response. Researchers have used LPS as a model endotoxin to study the effects of bacteria in the colon. In 2007, P. Cani and colleagues performed a low-rate infusion of LPS into mice. They induced metabolic endotoxemia through the subcutaneous injection of LPS (300μg kg⁻¹ day⁻¹) in 12 week old C57BL/6J mice using an implanted osmotic mini-pump for 4 weeks. The results were compared to mice that had the same implanted osmotic mini-pump which was injecting a 0.9% sodium chloride solution as well as to mice that were fed a 72% high fat diet without an implanted LPS infusion mini-pump. The mice with the LPS and saline infusion mini-pumps were fed a normal fat diet. The infusion of LPS provoked a systemic inflammation, similar to the chronic low grade inflammation that is seen in obesity, in the mice. The mice subsequently displayed many characteristics of metabolic syndrome such as obesity and insulin resistance. They hypothesized that the infusion of LPS was able to increase gut permeability leading to increased bacterial endotoxemia. This subsequent endotoxemia lead to low grade inflammation, like what is seen in obesity, and eventually to characteristics of metabolic syndrome (Cani et al., 2007).
CONCLUSION

Obesity has had a dramatic increase in the past fifty years. This could be due to many factors, but diet and lifestyle choices are thought to be the most likely causes. An increased caloric intake combined with a decreased caloric expenditure, as has become more common in developed countries, has caused many people to become overweight. Although this may be the primary cause of weight gain, recent research presented here indicates that the gut microbiota has a role in weight regulation in the human host. The mechanism by which the gut microbiota is able to accomplish this is still being elucidated. However, these studies show that a dysbiosis of the gut microbiota negatively affects how the host regulates energy balance and expenditure. They indicate that the gut microbiota has role in weight regulation that was not previously known.

There have been contradictory results concerning a possible change in the ratio of \textit{Firmicutes} to \textit{Bacteroidetes} in obese and non-obese mice and individuals. Many studies found a difference in the ratio between \textit{Firmicutes} and \textit{Bacteroidetes} between obese and non-obese individuals. However, other studies found no difference in this ratio. In 2008, the results from Gordon et al showed that there is an increase in a specific taxon of \textit{Firmicutes}, the Mollicutes class. This finding indicates that research solely focusing on changes at the phyla level may not be specific enough to show what is occurring at lower taxon levels. For instance, their results show that changes in diet was associated with changes in one class of \textit{Firmicutes}. The studies performed by Duncan et al and Ley et al also show the contradiction in findings regarding the F/B ratio in humans. These studies
suggest that the gut microbiota may have to be monitored at a more specific level than the phylum if researchers seek to determine how the composition of the microbiota affects health and energy expenditure. However, the findings suggest that a dysbiosis in the composition of the gut microbiota is associated with weight gain and obesity development.

Another interesting aspect of these studies is that they demonstrate that the gut microbiota is capable of being altered either through diet or conventionalization. This is an important finding because it could lead to interventions in the future to combat obesity and aspects of metabolic syndrome.
FUTURE DIRECTIONS

The study of the intestinal microbiota is an important endeavor due to its importance in human health. It has been shown that the dysbiosis of these microbes may lead to obesity and other health complications. The understanding of how exactly the gut microbiota affects human health will require further studies. With this information, medical professionals and the general public will be able to use this knowledge to achieve better health and possibly alleviate one of the causes of weight gain.

The core features of the gut microbiota should be a topic of study that is first clarified. Examples of these features could be specific species of bacteria and the compositions of those bacteria that provide the host with the most benefits. If features of the gut microbiota are known, it will provide the opportunity to manipulate the gut microbiota from a state of dysbiosis to a state that resembles a more ‘healthy’ gut microbiota. It will also provide the opportunity to learn what promotes the health of desired species and what can be done to exclude the undesirable species. Although researchers have found certain similarities between individuals, identifying the core features will be difficult at first due to the complex nature of the gut microbiota and the variation between individuals (Lozupone et al., 2012). Additionally, without more detailed information regarding the core features of the microbiota, it is difficult to assess how different dietary substrates influence individual taxa, how taxa cooperate or compete within the intestine for nutrients and how the collection of these taxa lead to host phenotypes (McNulty et al., 2013). Currently, there are a few projects that are attempting
to decipher the core features of the human gut microbiota. They include the European Metagenomics of the Human Intestinal Tract and the US Human Microbiome Project (National Institutes of Health, 2014).

A greater understanding of the gut microbiota could also lead to advances in medicine and treatments and well as personalized diets. For instance in 2012, Vrieze et al found that the insulin sensitivity of male human subjects with a history of metabolic syndrome increased after they were given small intestinal infusions of allogenic or autologous microbiota from lean healthy donors. These findings lead the researchers to suggest that the gut microbiota may be a therapeutic target for insulin resistance in the future (Vrieze et al., 2012). Additionally, as shown throughout this paper, the gut microbiota may be a target for weight regulation.

Another topic of study would be the effects, benefits and disadvantages of probiotics and prebiotics. It has been shown that a decreased diversity in the gut microbiota can lead to many gastrointestinal and extraintestinal disorders. One such disorder, irritable bowel syndrome, has been associated with a reduction of specific organisms that leads to a higher risk of developing this disorder (Shanahan et al., 2012). Therefore, probiotics and prebiotics, either used separately or in combination, could help people achieve a more healthy gut microbiota by providing the opportunity to replenish certain beneficial bacterial species or possibly alter the composition of the gut microbiota back to a more ‘healthy state’.
Lastly, it will be worthwhile to study the effectiveness of bacteriotherapy in helping the gut microbiota revert into a healthy state. Fecal bacteriotherapy, or fecal transplant, has been shown to be effective at altering the gut microbiota. Additionally, it has been shown recently to be an effective treatment in treating recurrent *Clostridium difficile*-associated diarrhea (CDAD) in humans. Mortality rates due to *Clostridium difficile* infections have increased in the United States recently (Redelings, Sorvillo, & Mascola, 2007) (Figure 13). Fecal transplant introduces a fecal preparation from a healthy donor as a homogenate by injection into the caecum using a colonoscope. In a recent study involving 317 patients suffering from CDAD across 27 case series, fecal transplant had a 92% success rate at disease resolution (Gough, Shaikh, & Manges, 2011). In fact, 89% of the cases of CDAD were resolved after the first fecal transplant. In one particular case, researchers found that one month after the procedure, *C. difficile* was not present in a patient’s stool sample after previously being affected with CDAD. Additionally, species from the donor were present in the stool sample which suggests that donor’s microbiota possessed the capability to persist in the recipient’s caecum during this interval (Lozupone et al., 2012). Fecal transplant could have many possible roles in the future. It could be used to promote or establish a healthy colony of microbes in the colon, treat intestinal infections without the use of antibiotics and could be used as a possible treatment for one of the causes of obesity.
Figure 13. Yearly rates of *Clostridium difficile*-related mortality rates per million population in the United States between 1999 and 2004 (Redelings et al., 2007).
REFERENCES


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