Mechanism of Fecal Bacteriotherapy in Treating Clostridium Difficile Infection and GI Tract Disorders

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Abstract

Clostridium difficile infection, an increasingly prevalent and virulent condition, is often resistant to treatment. Standard antibiotic therapy is rarely efficient when used to treat recurrent C. difficile infection. Fecal microbiota transplantation (FMT) is a safe, effective and inexpensive treatment that has a cure rate of about 90%, according to clinical trials and reports. This approach may also be applicable in treating ulcerative colitis, a type of inflammatory bowel disease, on the basis of the restoration of flora imbalances. Additionally, recent data suggests that a disproportion in composition of the gut microbiome may contribute to obesity. FMT, may restore a healthy balance. Using information from Touro College's database, this article discusses the reason behind the success of fecal microbiota transplantation when used to treat Clostridium difficile infection and ulcerative colitis. The effect of diet, environment and geography on the bacterial flora is also explored.

Introduction

The human gut microbiome consists of over 100 trillion microbial cells, exceeding the amount of human cells by a factor of 10. Bacteria are found in various parts of the body including nasal passages, oral cavity, skin, urogenital system and the gastrointestinal tract. The gut microbiota carries out a variety of valuable tasks for their human hosts; it assists in the development of innate and adaptive immunity, serves as an energy source, and kills foreign organisms. (Dave, et al. 2012). The quality and composition of the gut microbiome depends largely on diet, medication and weight of its host. The microbiota is capable of secreting or altering the production of molecules that affect both weight gain or loss and fat mass. (Rosenbaum, et al. 2015)

Colonization of the microbial microflora of the internal and external surfaces in a human begins at birth and is termed indigenous bacterial microflora. (Van der Waaij, 1989) The route of delivery when a baby is born determines which type of bacteria will develop. Infants delivered vaginally have microbiota comparable to their own mother's vagina, whereas infants born via Cesarean section develop microbes similar to the bacteria found on the skin surface. (Dominguez-Bello, et al. 2010) After birth, microbes develop and colonize, benefitting both themselves and their host, each contributing something vital to the other. (Van der Waaij 1989) For example, the anaerobic bacteria in our distal gut breaks down complex polysaccharides and ferments the resulting monosaccharides into short chain fatty acids. This is a mutually beneficial relationship; the microbes live in a sheltered anaerobic environment and receive a rich assortment of glycans while offering the host a source of carbon and energy. (Backhed, et al. 2005)

Of all the microbial communities being studied by human microbiome researchers, the gastrointestinal tract (GI) holds a promising future for the discovery of new concepts and therapies. The large colon holds the most abundant and diverse communities of microbes; it has both local and systemic effects on the body. (Dave et al. 2012) The gut microbiota can be thought of as a "microbial organ placed within a host organ," it plays vital roles in metabolism and immune system function. It is known that the microbial communities have a method of communication; they also can consume, store and redistribute energy. (Bakhed, et al. 2005)

Fecal Microbiota Transplantation (FMT) is an FDA experimental drug targeted to treat Clostridium difficile associated diseases (CDAD). It has been successfully used to treat patients with ulcerative colitis (UC) and different forms of inflammatory bowel diseases (IBD). Additionally, by comparing and analyzing the gut microbiome of lean and obese individuals, researchers have been able to hypothesize a cause for obesity and other weight issues. Using this knowledge, microbiologists can manipulate the microbiome and formulate a therapy to decrease obesity worldwide. This research can result in a new kind of pharmacopeia; implanting a more refined probiotic to combat infection, inflammation and dysbiosis.

Methods and Materials

To investigate the mechanism of fecal bacteriotherapy and how it can be utilized to treat Clostridium difficile associated diseases and other gastrointestinal disorders, many original research articles and reviews have been read. Touro College's online database was employed, particularly EBSCO, PubMed, ProQuest and Google scholar. Further research was done by perusing the footnotes and references in review papers to find original research.

Discussion
Fecal Microbiota Transplantation

The indigenous microbiota is vital to the human host, yet it can be altered by various factors of modern day life, most commonly repeated doses of antibiotics. (Van der Waaij, 1989). Dethlefsen et al. tested the fecal microbiota of adults before and after oral administration of ciprofloxacin for five days using pyrosequencing. A rapid reduction in richness of bacterial phyla was seen, resulting in decreased diversity, fullness and balance in the microbiome. Four weeks following the completion of the antibiotic, the microbiota returned to baseline. However,

in some individuals, it took 6 months for the bacterial phyla to completely return to normal. (Dethlefsen et al., 2008) Infectious diseases of the past were thought to be linked with bacteria capable of causing disease in a susceptible host. However, present day infectious diseases are linked to host interaction with potentially pathogenic bacteria that make up the microflora of the digestive tract (Van der Waaij, 1989). A particular infection that results from a disruption of the gut microbiota is Clostridium difficile infection. Clostridium difficile is an anaerobic, spore forming bacillus that produces 2 exotoxins that damage the intestinal mucosa. It is the most commonly recognized cause of anti-microbial associated diarrhea. Clostridium difficile associated disease mainly affects the large colon, causing diarrhea and pseudomembranous colitis. If progressed, the infection can lead to toxic megacolon, sepsis and death (McDonald et al., 2006). The microbial colonies that regularly control the Clostridium difficile communities are inhibited and blocked by antibiotics. Because C. difficile spores are generally resistant to antibiotics, this allows them to germinate back into their vegetative state after antibiotic treatment has ended. If the remaining microbes in the intestine cannot get rid of the infection, Clostridium difficile can multiply and release the toxins that return the disease symptoms. (Bakken et al., 2011)

There are different treatments that exist for recurrent Clostridium difficile infection, many of which involve the use of stronger antibiotics, such as vancomycin and metronidazole. However, as Dr. Johan Bakken says, "the use of antibiotics represents a double-edged sword by suppressing both the pathogen as well as the protective microbiota." In other words, repeated and extended uses of antibiotics have negative effects on the natural gut flora. It is a possible risk factor for further infection due to the continuously altered state of gut microbes. (Bakken et al. 2011)

Another approach to treat recurrent Clostridium difficile infection is to restore the damaged microbial communities using probiotics. Saccharomyces boulardii is a yeast that colonizes in the colon within 3 days of ingestion, survives gastric acids of the stomach and is cleared from the colon once treatment is discontinued. The effectiveness of the probiotic as an add-on to antibiotics was tested in two trials. The treatment did not decrease recurrence rates in those with their first episode of Clostridium difficile infection but it did decrease the frequency of relapses in those with the recurrent infection. The second trial showed effectiveness only in patients that were on a high dose of vancomycin. (McFarland et al., 1994) (Bakken et al., 2011) Thus, probiotics may have a role in treatment of Clostridium difficile infection, but their effectiveness is less than ideal. (Kelly, LaMont 2008)

Fecal Bacteriotherapy, or fecal microbiota transplantation (FMT), is an innovative and effective treatment for Clostridium difficile infection and other infections of the colon, including ulcerative colitis. It involves implanting stool from a healthy donor into a recipient's diseased colon, thereby allowing a newer and healthier microbiome to develop and flourish. From the trials done and the case studies reported, it seems that recurrent Clostridium difficile infection can be cured with a single treatment of FMT. The logic behind FMT is straightforward: strong antibiotics can cause an imbalance in the normal intestinal flora and reduce colonization resistance, which allows C. difficile to grow and cause symptoms of infection. With the introduction of healthy donor feces, the imbalance can be corrected, the cycle interrupted and the normal colon function returns. (Bakken, et al. 2011)

Although the first four patients to be treated with FMT in the 20th century was in 1958 for pseudomembranous enterocolitis, (Bakken, et al. 2011) the use of fecal material as a treatment for diseases dates back more than 2500 years ago in ancient China. During the Dong-jin dynasty in the 4th century China, Ge Hong, a well-known doctor described the use of human fecal suspension by mouth for people suffering from severe diarrhea or food poisoning. This treatment was considered a medical miracle that brought patients back from the brink of death. In the 16th century, during the Ming dynasty, Li Shizen prescribed a series of fecal material doses to treat abdominal diseases that caused severe diarrhea, fever, pain, vomiting, and constipation. This treatment was called "yellow soup" for aesthetic reasons. (Zhang, et al. 2012)

Nowadays however, the procedure is done in a more sterilized manner and reported to have been successful via colonoscopy (Persky, Brandt 2010), nasogastric tube, (Aas, et al. 2003) nasoduodenal tube, (van Nood, et al. 2013) and self-administered fecal enemas. (Silverman, et al. 2010) One method of implantation was not found to be superior over the other. (Rohlke, Stollman 2012)

Currently, the only FDA approved drug to treat C. difficile infection is the antibiotic vancomycin, which is inadequate for patients suffering from recurrent CDI. It is estimated that 80% of patients treated with the standard antibiotic therapy are cured, however, the remaining 20% are likely to recur 3-28 days after discontinuation of antibiotics. Disease caused by C. difficile has been shown to increase the length of stay of patients by a mean of 7 days, incurring an additional $10,500 in health care charges for every infected patient. (McFarland, et al. 1994) Alternative treatments, including probiotics, surgery and administration of different antibiotics, have been suggested, but none were effective, safe and inexpensive. Fecal microbiota transplant seems to

fit the criteria of being inexpensive, highly effective and safe. In fact, a study done to compare 4 different treatments for CDAD; metronidazole, vancomycin, fidaxomicin and FMT demonstrated that FMT was the "most cost-effective strategy." The incremental cost-effectiveness ratio (ICER) was $17, 016 relative to oral vancomycin. (Konijeti, et al. 2014) It is "the only therapy that restores the phylogenic richness of the recipient's intestinal microbiota without prolonging the perturbation of the normal microbiotic composition." (Bakken, et al. 2011)

A study comparing the fecal material of a patient before and after FMT illustrates the effectiveness of the transplant. Before FMT, the patient's fecal material was composed of atypical bacterial populations including Veillonella, Clostrudium, Lactobacillus, and Streptococcus. Two weeks after FMT, Bacteroidetes began to appear and four weeks after the transplant, the stool studies were culture negative for C. difficile. The recipient's fecal bacterial composition greatly resembled the donor. Throughout the months following FMT the patient reported issues of loose stool, but those issues were resolved within two weeks without therapy. Six months later, the patient was completely cured from C. difficile infection. (Khoruts, et al. 2010)

Further data is needed to assess the effectiveness of FMT, yet given the promising data to date and anticipated research data on the intestinal microbiome, it seems to be the most reasonable treatment for recurrent C. difficile infection (CDI). Bakken et al. put together a guideline for the proper administration of FMT. Included in the guideline is a detailed description of primary indication, donor selection and directions for preparing and administering the stool. Many trials and studies that involving FMT used this guideline as instructions on how to proceed. In order to be eligible for FMT, a patient either has to have recurrent or relapsing CDI with at least three episodes of mild-moderate CDI and a failure of a 6-8 week taper with vancomycin. Patients on major immunosuppressive agents, decompensated liver cirrhosis or have had a recent bone marrow transplant cannot receive the fecal microbiota transplant as it may cause adverse effects.

Currently, little or no data is available to suggest that any reasons, other than the exclusion criteria based on medical history and laboratory testing, would recommend a specific donor over another. However, using intimate contacts as donors minimizes the risk of transmitting an infectious disease. Donors are interviewed thoroughly and must answer a detailed questionnaire regarding their past medical history. Donors are disqualified if they test positive for HIV, Hepatitis B or C infections or had exposure to HIV or viral hepatitis. Individuals that have high risk sexual behaviors, use illicit drugs, had tattoo or body piercing within 6 months or have a history of incarceration cannot

donate stool for FMT. Once a donor passes the medical and laboratory tests, their stool is screened for enteric pathogens such as C. difficile toxin A and B, fecal Giardia and Cryptosporidium antigens and Helicobacter pylori fecal antigen. (Bakken, et al. 2011)

The stool donor may or may not take an osmotic laxative the night before the procedure. The stool should be used as soon as possible, preferably within 6 hours. The choice of diluents may vary among practitioners, but either preservative-free normal saline or 4% milk is combined with the stool in a household blender. It should be mixed until it reaches a homogenous slurry consistency and filtered to remove particulate. The slurry stool mixture is to be used immediately. The optimal volume for transplantation has not been established yet; however if by nasogastric tube or via other routes from above, smaller volumes (25-50 mL) should be used and if by colonoscopy or other routes from below, larger volumes (250-500 mL) may be used. (Bakken et. al. 2011)

This is a basic outline on how a practitioner would administer fecal microbiota transplant. However, "practical and aesthetic barriers" have slowed down the widespread use of FMT, despite its success in dramatically resolving CDI after one treatment. Enrollment and screening of donors is a tedious process linked with substantial costs, thereby inhibiting the use of FMT in severe situations. Youngster et al. suggests that by establishing a repository of prescreened frozen donor stool, this treatment may be more easily obtainable from a broader population. He conducted a trial to evaluate the efficacy of FMT in treating relapsing CDI in a pilot cohort of 20 patients, comparing colonoscopic and nasogastric tube administration.

The donors for the study underwent extensive physical examinations and general laboratory screening tests to ensure that all results were within normal range for age and sex. Renal function and electrolytes, complete blood count with differential, complete liver function tests and fecal occult blood testing were some of the tests that fecal donors underwent. All fecal donations were set aside for four weeks to allow retesting of donors for HIV and hepatitis B and C before the feces was inoculated. To facilitate manipulation of the fecal material, donors were asked to take milk of magnesia the day before the donation. The feces was mixed in a commercial blender with normal saline and then were passed through 4 sieves to get rid of any particulate. The final product was concentrated 3-fold by centrifugation and then resuspended in sterile saline with 10% glycerol added as a cytoprotectant. The resulting mixture was frozen at -80°C. Each filtered sample was calculated to have come from 41g of fecal material. Inocula were stored frozen for up to 156 days and thawed in a 37°C water bath, then kept on ice until delivery.

Patients were ordered to stop taking antibiotics for 2 days before the procedure. Recipients designated for a colonoscopic administration underwent a standard bowel preparation with 4 liters of polyethylene glycol electrolyte solution. Then, 90 cc of the thawed inoculum was placed in the right colon via endoscopy. The fecal material was further diluted to 250 cc for adults and 160 cc for pediatric recipients. Patients were given a single dose of loperamide at the time of the procedure in order to retain the material for as long as possible. The patients assigned to nasogastric tube administration were given omeprazole orally for 2 days before the procedure, to ensure that the fecal bacteria will not be affected by the acids in the stomach. After inserting the nasogastric tube, 90 cc of inoculum was administered. In order to minimize vomiting and aspiration, the inoculum was not diluted. (Youngster, et al. 2014)

The goal of the trial was the clinical resolution of diarrhea off antibiotics for C. difficile infection, without a recurrent episode within 8 weeks. Patients who showed no improvement were offered a second FMT by their preferred route of administration, using the same donor inoculum to minimize exposure to infectious agents.

Of the 20 patients in both study arms, 14 were cured after the first FMT; 8 of them were in the colonoscopy group and 6 in the nasogastric tube group. One patient refused a second transplant but the remaining 5 patients were given a second infusion via NGT using feces from their original donors. Four patients were cured after the second FMT, resulting in an overall cure rate of 90%. No patient relapsed within the 8 week period following the initial cure.

There were some serious adverse effects that were deemed unrelated to the trial by investigators and the Institutional review board, which may have been caused by the poor health of the patients with recurrent CDI. 2 patients died; one from an obstructive pulmonary disease and another from metastatic laryngeal cancer. A third patient was diagnosed with adenocarcinoma of the esophagus and a fourth (treated via NGT) was hospitalized for Fourier gangrene.

This trial demonstrated that the transplantation of unrelated frozen donor stool is effective in treating recurrent CDI with an overall cure rate of 90%. Additionally, NGT appears to be a feasible route of administration for elderly patients with the infection. One 89 year old female patient had 16 documented cases of CDI in the previous 15 months. She was hospitalized 4 times and placed on the intensive care unit twice due to the infection. After two infusions, she was cured and was free from any symptoms of the disease off treatment for 12 months. (Youngster, et. al. 2014)

Seeing the success with frozen inocula, Youngster et al. directed another study evaluating the effectiveness of oral capsulized frozen fecal microbiota transplantation in treating relapsing C. difficile infection. The donors were thoroughly screened, as described in the previous study (and the guideline by Bakken, et al. 2011) and similar processing of the fecal material took place. To ensure that the acidic environment of the stomach will not destroy the capsule, commercially available acid-resistant hypromellose capsules were used. The stability of the capsules was tested by mimicking the acidic environment of the stomach. Trypan blue filled capsules were used, at a temperature of 37°C and a pH of 3 or less, and the capsules were stable for 115 minutes before the dye was released.

From July 2013 through January 2014, a total of 20 patients were treated using stool obtained from four donors. The recipients followed up six months later in July 2014. The goal of the study was the clinical resolution of diarrhea, while not receiving antibiotic for C. difficile, without relapse within 8 weeks.

The procedure went as follows: each patient was given 15 capsules, directly from an investigator on 2 consecutive days. 2 days prior to FMT, patients were ordered to stop all antibiotics. They were asked to fast 4 hours prior to and 1 hour post taking the capsule for both days. If there was no improvement after 72 hours, patients were retested and offered another treatment for positive test results.

From the 20 patients treated, 14 had clinical resolution of diarrhea after the first FMT. The remaining 6 patients were retreated 7 days after the first procedure. 5 of the 6 had resolution of diarrhea after the second FMT, however one patient relapsed within the 8 week follow up time period. Thus the overall rate of diarrheal resolution was 90%. (Youngster et. al. 2014)

Fecal microbiota transplantation used to treat C. difficile infection is a highly effective, safe and inexpensive method as described studies and research. Very few, if not any, complications were caused by FMT. The fecal material can be manipulated and can be transplanted as a liquid via NGT or colonoscopy and can also be ingested as a capsule. FMT is currently an FDA experimental drug and therefore the trials and studies are with small samples and lack of placebo or other trial to compare. Granted, the trials and studies done are encouraging, however this may be due to the small sample size. Furthermore, even with careful screening, there is a potential risk of transmission of infections via microbiota transplantation. Larger studies would be needed to confirm these outcomes and to evaluate long-term safety and effectiveness. (Youngster et. al 2014)

Fecal Microbiota Transplant for Ulcerative Colitis

Encouraged by the success for fecal microbiota transplant in the treatment of C. difficile infection, researchers looked to other conditions related to GI microbial imbalance, such as inflammatory bowel disease (IBD), obesity and diabetes mellitus. (Borody, et al. 2103) Inflammatory bowel diseases are thought to be caused by an overall community dysbiosis rather than a single underlying pathogen. In patients with IBD, an increase in Actinobacteria and Proteobacteria and a decrease of Lachnospiraceae, Bacteroidetes, Clostridium leptum, and Bifidobacteria are observed. (Clemente, et. al. 2012)

Ulcerative colitis (UC), a type of IBD, is a chronic devastating inflammatory bowel disease that affects the large intestine. Unfortunately, the current medications used to treat UC have significant adverse effects; they mainly target the inflammation without focusing on the dysbiosis. (Kunde, et al. 2013)   The colonic microbiome in UC patients lacks diversity and is dysfunctional. The cause of inflammation of the colon in UC is hypothesized to be caused by inappropriate stimulation of the innate mucosal immune system because of dysbiosis in a genetically susceptible individual. Borody et al. asks the following question: C. difficile infection causes reduced microbial diversity which results in symptoms of colitis; if FMT can reverse this, perhaps a similar treatment can be used to treat ulcerative colitis? (Borody, et al. 2013)

Borody et. al. conducted a trial assessing the clinical, histological and colonoscopic effects of "human probiotic infusion" (HPI), or fecal material transplant in six selected patients with ulcerative colitis. All patients had suffered from severe recurrent symptoms of UC and each, respectively, were on series of moderate to high dose steroids and anti-inflammatory medications. The donors were chosen by the recipient patients and screened thoroughly to prevent any transmission of parasites or bacterial pathogens. The feces (200–300 g) was diluted in 200 to 300 mL normal saline and administered to the patient via retention enema within 10 minutes of its preparation. This process was repeated daily for 5 consecutive days. One week following HPI, some symptoms of ulcerative colitis had resolved in several patients. A complete reversal of symptoms was achieved in all patients 4 months post HPI, by which all other UC medications had been discontinued. From 1 to 13 years later, patients remained asymptomatic with a healthy colonoscopic appearance and normal histology.

However, the pathogenesis of UC is unknown since it is characterized by persistent inflammation. It is suggested that the disease is driven by a chronic infection caused by the absence of vital luminal anaerobes. If the ability of the normal flora to resist colonization by pathogens is hindered, for example, by previous antibiotic use, the numbers of protective anaerobes in the colon, such as Bacteroides, decrease. This would allow the establishment of other pathogenic anaerobes or spore bearing bacteria and a form of recurrent, chronic colitis. The persistence of UC may be due to the implantation of spores in the mucosal surface, analogous to Clostridium difficile infection. Antibiotics may help briefly but are unlikely to be curative because the spores are not eliminated. (Borody et. al. 2003)

Kunde et. al. conducted a pilot study to evaluate the efficiency and safety of fecal microbiota transplantation in children with ulcerative colitis. Ten children, ages 7 to 21 with mild to moderate UC according to the pediatric ulcerative colitis activity index (PUCAI) were enrolled in the study. Clinical response and improvement was defined as decrease in PUCAI by more than 15 points on the index. A decrease in PUCAI to less than 10 points was considered clinical remission. Each donor produced a 90 gram stool sample every day which was blended with 250 mL normal saline and filtered through 2 gauze pieces to remove sediments. It was then separated into 4 aliquots of 60 mL each and kept in a warm water bath (37°) until administration. Each patient received FMT as retention enema for 1 hour (60-mL enema every 15 minutes) daily for 5 days. One subject however, was not able to tolerate the retention enema for 3 consecutive days and was therefore not included in the results. Within one week following the procedure, 7 of the 9 subjects (78%) showed clinical response. Four weeks after FMT, six of the nine patients (67%) maintained the clinical response. However, 3 of the nine subjects (33%) went into clinical remission one week following FMT. All of the adverse events related to FMT, besides fever, were self-limiting, and did not require intervention from health care providers. The majority of them were similar to subjects' baseline symptoms of UC such as bloating and diarrhea.

The dramatic response to FMT after one week was not achieved in all subjects, perhaps due to the severity or duration of the disease, or the ability to retain larger FMT volumes for longer periods. Using FMT via fecal enema to treat UC in children is feasible, as proved in the trial. However, the sample size was small and therefore limits future indication. In order to improve FMT a standardization procedure would need to be arranged for donor selection and ideal routes of administration. (Kunde, et al. 2013)

The Gut Microbiota and Obesity

The World Health Organization (WHO) reports that in 2014, more than 1.9 billion adults, 18 years and older were overweight and of these, over 600 million were obese. Obesity is a growing, worldwide epidemic that has been accompanied by increases in obesity related disorders, including type II diabetes, hypertension and nonalcoholic fatty liver disease. Much emphasis has been placed on the dietary excess or host genes in obese individuals.

Yet, in addition to increased calories or shifts in host metabolism, the composition of the intestinal microbiota can contribute to the progression of obesity. Both diet induced obesity and gene induced obesity have microbe independent and microbe dependent pathways. (Baackhed et al. 2004, Cox, Blaser 2013)

A study was done to investigate the effect that diet has on shaping the gut microbiome. The fecal material of 15 European children and that of 14 children from a rural African village, Bukina Faso were compared and tested with 16S rRNA sequencing, and biochemical analysis. The goal of the study was to explore three questions: the split of bacterial diversity between the two populations, the connection between the gut microbiota and diet, and the distribution of well-known bacterial pathogens within the two populations given the different hygienic and geographic conditions.

The diet of Bukina Faso children is mostly vegetarian; rich in plant polysaccharides, starch and fiber and low in fat and animal protein. All the food was grown and harvested nearby the village by women. The European children were eating a typical Western diet high in animal protein, sugar, starch and fat and low in fiber. A dataset consisting of 438, 219 of filtered, high quality, classifiable 16S rRNA was generated. Of all of the sequences in all the Bukina Faso and European samples 92.4% were found to belong to the most populated bacterial phyla: Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria. However, it was the proportions that stood out: Actinobacteria and Bacteroidetes were more abundant in the Bukina Faso children than the European children 10.1% versus 6.7% and 57.7% versus 22.4%, whereas Firmicutes and Proteobacteria were represented more in EUROPEAN than BUKINA FASO children, 63.7% versus 27.3% and 6.7% versus 0.8%. Since Prevotella, Xylanibaeter (Bacteroidetes) and Treponema (Spirochaetes) were exclusively found in the Bukina Faso children. De Filippo et. al. hypothesized that these 3 genera could be a result of high fiber intake due to the environmental factors distinguishing the two populations (diet, hygiene, climate and geography). Perhaps these three groups contribute to the extraction of metabolic energy from ingested plant polysaccharides. It is reasonable to deduce that the increase in the Firmicutes to Bacteroidetes (F/B) ratio in European children possibly triggered by their high-calorie diet might predispose them to future obesity. This Firmicutes/Bacteroidetes ratio may also be considered a useful obesity biomarker. (De Filippo, et al. 2010)

A study done by Turnbaugh et al. further demonstrates the effect of diet on the gut microbiota. Mice raised in a germ free environment without any exposure to microbes, and then colonized at specific life stages with different microbial communities are referred to as gnotobiotic animals. Adult human fecal microbiota was transplanted into the recipient mice to establish a stable gut community similar their donors. An aliquot of the fecal dilution was introduced by gavage to the 15 recipient mice. These mice were maintained on a low fat plant polysaccharide (LF/PP) rich diet and fecal samples were collected 1 day, 1 week and 1 month after colonization. Half of the mice were then switched to a high-sugar, high-fat Western diet. The LF/PP and Western diet were maintained for 2 additional months, after which the mice were killed. Results showed that when the diet of the mice were switched from a LF/PP regimen to a high-fat, high sugar "Western" diet the structure of the microbiota was shifted within a single day. The representation of metabolic pathways in the microbiome were changed and the microbiome gene expression was altered. The mice fed a Western diet had an increased representation of the Erysipelotrichi class of bacteria within the Firmicutes phylum relative to mice fed the LF/PP diet. In mice that consumed a Western diet, the Bacilli, another class of the Firmicutes was abundant and a significant decrease in member of Bacteroidetes was found. These mice increased adiposity, demonstrating that this trait is transmissible via microbiota transplantation. Using germ free mice, researchers were able to see a clear correlation between the foods the mice were fed and its effect on the composition of their gut microbiota. Gnotobiotic technology is a promising tool for discovering new classes of probiotics that affect nutrient harvest in a given diet. (Turnbaugh, et al.2008)

A study comparing the fecal material of 31 adult female monozygotic and 23 dizygotic twin pairs and their mothers, concordant for obesity or leanness, was performed, to address how host genotype, environmental exposures and host adiposity influence the gut microbiome. All twins were 25-32 years old of European or African ancestry. Analysis of 16S rRNA datasets produced by the three PCR-based methods, plus shotgun sequencing of community DNA, revealed a lower proportion of Bacteroidetes and a higher proportion of Actinobacteria in obese versus lean Europeanropean and African individuals. Overall, across many methods, obesity was associated with a decrease in diversity of the gut microbiota. "This reduced diversity suggests an analogy: the obese gut microbiota is not like a rainforest or reef, which are adapted to high energy flux and are highly diverse, but rather may be more like a fertilizer runoff where a reduced diversity microbial community blooms with abnormal energy input." (Turnbaugh, et al. 2009)

There is an interesting case study regarding the transmission of "obese bacteria." A 32 year old female patient with recurrent CDI elected to undergo FMT after many failed antibiotic therapies. Around the same time, she tested positive for Helicobacter pylori.

The patient chose her 16 year old daughter, who was approximately 140 pounds to be her stool donor, and, subsequently, her weight increased to 170 pounds. The patient herself was 136 pounds at the time of the transplant. The patient was retreated for H. pylori with metronidazole, tetracycline, bismuth and proton pump inhibitor, and two weeks later she had the FMT. Six hundred cc. of the suspension of the donor stool in sterile water was infused through a colonoscope. The patient dramatically improved after the procedure and CDI did not recur. However, 16 months later, the patient presented again, and reported an unintentional weight gain of 34 pounds. Despite a medically supervised liquid protein diet and exercise program she had been unable to lose weight. Her weight continued to climb despite the diet and exercise and at 36 months post FMT she weighed 177 pounds.

Alang and Kelly offer a several of hypotheses to explain the rapid weight gain following FMT. There is a known link between H. pylori treatment and weight gain, resulting from the increase in appetite after the suppression of the bacteria. Yet, the patient was never obese before FMT and the fact that the stool donor also gained weight leads to the possibility that her obesity resulted from the transplant (Alang, Kelly 2014).

This hypothesis is supported in a study testing the fecal material of twins discordant for obesity. This experiment offers an opportunity to examine connections between obesity and its associated metabolic disorders, diet, and the gut microbiota. Ridaura et al. conducted a study by transplanting the fecal material of twins discordant for obesity into mice in order to replicate the donors' microbiota community in the colon. The impact of these differences on the body composition and metabolic phenotypes were able to be detected, and the effects of diet-by-microbiota interactions analyzed. The mice harboring the transplanted microbiomes from the obese twins exhibited higher expression of microbial genes involved in detoxification and stress responses, in biosynthesis of cobalamin and metabolism of essential amino acids. In contrast, the transplanted microbiomes from lean co-twins exhibited higher expression of genes involved in digestion of plant-derived polysaccharides, fermentation to butyrate and propionate. The results suggest that the transplanted microbiota of the lean co-twins had greater capacity to breakdown and ferment polysaccharides than the microbiota of their obese co-twins. (Ridaura, et al. 2013)

From these studies, it is evident that the gut microbiota reflects the health, diet and metabolism of its host. It seems that the composition of the phyla that make up the gut microbiome in healthy individuals is properly balanced. However, a disrupted microbiome can contribute to obesity by altering energy extraction from food.

Conclusion

By transplanting stool from a healthy donor into a recipient's diseased colon via fecal bacteriotherapy, a newer and healthier microbiome can flourish and the function of the large intestine can be restored. Fecal microbiota transplant has been proven by many clinical trials to be a safe, effective and inexpensive procedure. FMT has been successful in treating many cases C. difficile infection, as well as ulcerative colitis and has a promising future in the field of diet and obesity. The stool material can be readily administered in different ways, as a slurry consistency administered via colonoscopy or nasogastric tube, or packaged in a pill and taken orally; this therapy is easily accessible to almost all populations.

However, one potential drawback of FMT is the small sample size in many of the clinical trials done. Additionally, fecal bacteriotherapy may not be the ideal treatment for every individual suffering with GI tract disorders, for example, it may not be ideal in immunocompromised patients. However, FMT is an FDA approved drug and will undergo further standardization regarding donors and the preparations and administration of the stool.

The future of the gut microbiota related treatment is an active topic of research; much is anticipated from this exploration into the microbiome.

References

Aas J, Gessert C, Bakken J. Recurrent Clostridium difficile Colitis: Case Series Involving 18 Patients Treated with Donor Stool Administered via a Nasogastric Tube. Clinical Infectious Diseases. 2003;36(5):580-585

Alang N, Kelly C. Weight Gain After Fecal Microbiota Transplantation. Open Forum Infectious Diseases. 2014;2(1)

Backhed F, Ding H, Wang T et al. The gut microbiota as an environmental factor that regulates fat storage. Proceedings of the National Academy of Sciences. 2004;101(44):15718-15723

Backhed F, Ley R, Sonnenburg J, Peterson D, Gordon J. Host-Bacterial Mutualism in the Human Intestine. Science. 2005;307(5717):1915-1920. doi:10.1126/science.1104816.

Bakken J, Borody T, Brandt L et al. Treating Clostridium difficile Infection With Fecal Microbiota Transplantation. Clinical Gastroenterology and Hepatology. 2011;9(12):1044-1049

Borody T, Paramsothy S, Agrawal G. Fecal Microbiota Transplantation: Indications, Methods, Evidence, and Future Directions. Current Gastroenterology Reports. 2013;15(8)

Borody T, Warren E, Leis S, Surace R, Ashman O. Treatment of Ulcerative Colitis Using Fecal Bacteriotherapy. Journal of Clinical Gastroenterology. 2003;37(1):42-47.

Rivka Steinberg

Clemente J, Ursell L, Parfrey L, Knight R. The Impact of the Gut Microbiota on Human Health: An Integrative View. Cell. 2012;148(6):1258-1270.

Cox L, Blaser M. Pathways in Microbe-Induced Obesity. Cell Metabolism. 2013;17(6):883-894. doi:10.1016/j.cmet.2013.05.004.

Dave M, Higgins P, Middha S, Rioux K. The human gut microbiome: current knowledge, challenges, and future directions. Translational Research. 2012;160(4):246-257.

De Filippo C, Cavalieri D, Di Paola M et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europeanrope and rural Africa. Proceedings of the National Academy of Sciences. 2010;107(33):14691-14696.

Dethlefsen L, Huse S, Sogin M, Relman D. The Pervasive Effects of an Antibiotic on the Human Gut Microbiota, as Revealed by Deep 16S rRNA Sequencing. PLoS Biology. 2008;6(11):e280

Dominguez-Bello M, Costello E, Contreras M et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proceedings of the National Academy of Sciences. 2010;107(26):11971-11975.

Kelly C, LaMont J. Clostridium difficile — More Difficult Than Ever. New England Journal of Medicine. 2008;359(18):1932-1940.

Khoruts A, Dicksved J, Jansson J, Sadowsky M. Changes in the composition of the human fecal microbiome following bacteriotherapy for recurrent Clostridium difficile-associated diarrhea. Lawrence Berkeley National Laboratory. 2010:354-360.

Konijeti G, Sauk J, Shrime M, Gupta M, Ananthakrishnan A. Cost-effectiveness of Competing Strategies for Management of Recurrent Clostridium difficile Infection: A Decision Analysis. Clinical Infectious Diseases. 2014;58(11)

Kunde S, Pham A, Bonczyk S et al. Safety, Tolerability, and Clinical Response After Fecal Transplantation in Children and Young Adults With Ulcerative Colitis. Journal of Pediatric Gastroenterology and Nutrition. 2013;56(6):597-601.

McFarland L, Surawicz C. Greenberg R. A Randomized Placebo-Controlled Trial of Saccharomyces boulardii in Combination with Standard Antibiotics for Clostridium difficile Disease. JAMA. 1994;271(24):1913.

Persky S, Brandt L. Treatment of recurrent Clostridium difficile-associated diarrhea by administration of donated stool directly through a colonoscope. The American Journal of Gastroenterology. 2000;95(11):3283-3285.

Ridaura V, Faith J, Rey F et al. Gut Microbiota from Twins Discordant for Obesity Modulate Metabolism in Mice. Science. 2013;341(6150):1241214-1241214. doi:10.1126/science.1241214.

Rosenbaum M, Knight R, Leibel R. The gut microbiota in human energy homeostasis and obesity. Trends in Endocrinology & Metabolism. 2015;26(9):493-501. doi:10.1016/j.tem.2015.07.002.

Silverman M, Davis I, Pillai D. Success of Self-Administered Home Fecal Transplantation for Chronic Clostridium difficile Infection. Clinical Gastroenterology and Hepatology. 2010;8(5):471-473.

Turnbaugh P, Hamady M, Yatsunenko T et al. A core gut microbiome in obese and lean twins. Nature. 2008;457(7228):480-484.

Turnbaugh P, Ridaura V, Faith J, Rey F, Knight R, Gordon J. The Effect of Diet on the Human Gut Microbiome: A Metagenomic Analysis in Humanized Gnotobiotic Mice. Science Translational Medicine. 2009;1(6)

Van der Waaij D, Van der Waaij BD. The colonization resistance of the digestive tract in different animal species and in man; a comparative study. Epidemiology and Infection. 1990;105(2):237-243.

Van der Waaij D. The Ecology of the Human Intestine and its Consequences for Overgrowth by Pathogens such as Clostridium Difficile. Annual Review of Microbiology. 1989;43(1):69-87.

van Nood E, Vrieze A, NiEuropeanwdorp M et al. Duodenal Infusion of Donor Feces for Recurrent Clostridium difficile. New England Journal of Medicine. 2013;368(5):407-415.

Youngster I, Russell G, Pindar C, Ziv-Baran T, Sauk J, Hohmann E. Oral, Capsulized, Frozen Fecal Microbiota Transplantation for Relapsing Clostridium difficile Infection. JAMA. 2014;312(17):1772.

Youngster I, Sauk J, Pindar C et al. Fecal Microbiota Transplant for Relapsing Clostridium difficile Infection Using a Frozen Inoculum from Unrelated Donors: A Randomized, Open-Label, Controlled Pilot Study. Clinical Infectious Diseases. 2014;58(11):1515-1522.

Zhang F, Luo W, Shi Y, Fan Z, Ji G. Should We Standardize the 1,700-Year-Old Fecal Microbiota Transplantation? Am J Gastroenterol. 2012;107(11):1755-1755.

Rohlke F, Stollman N. Fecal microbiota transplantation in relapsing Clostridium difficile infection. TherapEuropeantic Advances in Gastroenterology. 2012;5(6):403-420.