A SMALL ANSWER TO A BIG PROBLEM: HOW RE-ESTABLISHING HOMEOSTASIS IN THE HUMAN GUT MICROBIOME AFTER SOLID ORGAN TRANSPLANTATION CAN IMPROVE SOLID ORGAN TRANSPLANT SUCCESS

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ABSTRACT

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This thesis examines and evaluates the manipulation of the microbiota as an immune-modulating therapy for improving the outcomes of solid organ transplants by helping prevent organ rejection, infection, and chronic disease development. Focusing on kidney, liver, and heart transplants, the factors contributing to gut dysbiosis before, during, and after solid organ transplant are analyzed and solutions to restore homeostasis to the gut microbiome, including fecal microbial transplant, synbiotic therapy, short chain fatty acid supplementation, diet and exercise interventions, and a sub-specialized transplant ICU are evaluated for their potential success in altering the human gut microbiome and preventing allograft rejection and the development of chronic diseases. These therapies are also evaluated in terms of cost, amount of care they require, and the challenges they face. This research was conducted through reviewing primary research papers and conducting interviews with faculty at Dell Medical School and the University of Texas School of Nursing. It concludes that without full understanding of the microbiome's role in health and disease it is too early to determine an approach for restoring homeostasis to the human microbiome. However, it is likely that there will not be just one solution to restoring a disrupted microbiome and that individual microbial differences will play a large role in determining the method and success of microbiome manipulation. Currently, diet and exercise programs are the most cost effective and safest therapeutic strategies and should be more strictly managed in transplant patients.

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TABLE OF CONTENTS

ABSTRACT..... 3

ACKNOWLEDGMENTS 4

INTRODUCTION 5

CHAPTER ONE: Solid Organ Transplants and the Microbiome 7
- **I. Solid Organ Transplant**
- **II. The Human Microbiome**

CHAPTER TWO: Effects of Solid Organ Transplants on the Microbiome 17
- **I. Pre-transplant**
- **II. During Transplant**
- **III. Post-transplant**

CHAPTER THREE: Methods to Aid in Restoring Homeostasis to the Microbiome.....33
- **I. Fecal Microbial Transplant**
- **II. Synbiotic Therapy**
- **III. Short Chain Fatty Acid Supplementation**
- **IV. Lifestyle: Diet and Exercise**
- **V. A Change in Perspective: A Sub-Specialized ICU**

CHAPTER FOUR: Conclusions and Final Recommendations.....53

BIBLIOGRAPHY 59

BIOGRAPHY.....67

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Introduction

Health and disease are profoundly influenced by diverse microbial communities known collectively as the microbiome. The human microbiome refers to the complex community of commensal and pathogenic microorganisms that exist in the nonsterile sites of the body. Half of the genes in an individual's intestinal microbiome have been found to be unique to the individual, demonstrating the innate variability of the gut microbiome. Vast individual microbial differences impede our understanding of how the intestinal microbiome regulates health, specifically in terms of immune system regulation and protection from pathogenic organisms. This lack of knowledge limits the ability of doctors to manipulate the intestinal microbiome for the patient's benefit.

Solid organ transplant patients experience a significant shift in the diversity and dominance of bacterial populations that constitute their intestinal microbiome, both before and after the transplant procedure. This shift often results in a microbial imbalance, referred to as dysbiosis. While it is not quite understood how, the gut microbiome is an important modulator of the immune system such that gut dysbiosis has been found to increase patients' risk of infection. This is a serious issue since patients must comply with strict immunosuppression medication after their transplant to prevent organ rejection. Furthermore, immunocompromised patients are at a heightened risk for developing chronic diseases such as type one and type two diabetes, cancer, and high blood pressure, all of which the gut microbiome plays a role in regulating. Developing any one of these chronic diseases increases health risks for the patient. The current risk for organ rejection, post-transplant infection, and chronic disease development in solid organ transplant patients makes the development of therapies to restore homeostasis to the intestinal microbiome critical to the future success of solid organ transplants.

This thesis advocates for the manipulation of the microbiota as an immune-modulating therapy to improve solid organ transplant outcomes through the prevention of organ rejection and chronic disease development by analyzing current methods for restoring homeostasis in the human gut microbiome and by making recommendations on how to reduce disruptions to the microbiome before, during, and after transplant. Kidney, liver, and heart transplants will be studied as they are the most commonly performed types of solid organ transplants. The effects of kidney, liver, and heart solid organ transplant on the human gut microbiome will be examined, as will the ways in which the preparation for solid organ transplant, the solid organ transplant procedure, and recovery protocols for solid organ transplants affect patients' gut flora. Current solutions for re-establishing homeostasis in the human gut microbiome including fecal microbiota transplant (FMT), short-chain fatty acid supplementation, synbiotic therapy, dietary and exercise changes, and the creation of a sub-specialized transplant ICU will be evaluated for their potential success in altering the human gut microbiome, reducing allograft rejection in solid organ transplant patients, and preventing the development of comorbidities. These therapies will be analyzed in terms of their predicted outcome, cost, the amount of care required, and the challenges they face.

Chapter 1- Solid Organ Transplants and the Microbiome

I. Solid Organ Transplantation

The field of solid organ transplants, although relatively new, has seen much progress in the 60 years since its origin. While it began as an experimental procedure, it has quickly evolved into a life-saving operation that saved 33,175 lives in 2019 (UNOS). A solid organ transplant is an operation in which an organ is surgically removed from its donor and transplanted into a recipient who suffers from irreversible and otherwise fatal organ failure. Transplants are necessary and lifesaving for the recipient. Today, there are nine types of organ transplants that are performed: kidney, lung, heart, liver, pancreas, skin, cornea, trachea, and vascular tissue.

To receive an organ transplant, potential transplant patients must be referred by a primary care physician and a specialist. The requirements to qualify for an organ transplant differ depending on the type of transplant needed. However, in all cases, the patient must meet strict medical criteria. The patient must not suffer from multiple-organ failure, have a persistent or pervasive infection, or have any other option as an alternative to organ transplant. Additionally, the patient's medical status and psycho-social factors are analyzed by a transplant center team to make sure the recipient patient will survive the procedure and benefit from a transplanted organ. This evaluation is difficult because a patient's medical status is constantly changing. Furthermore, the transplant team must evaluate the patient's mental health, how likely the patient would be to follow the strict lifelong regimen of anti-rejection drugs, and the likelihood of compliance with follow-up appointments and post-surgical interventions if necessary. The purpose of these evaluations is to ensure the organ will have the greatest chances for success in

the patient and to make sure the patient's quality of life is enhanced enough to compensate for the rigorous transplant surgery and post-transplant care regimen.

Once patients have been evaluated and placed on the waitlist for an organ transplant, they wait to be matched to an organ. When an organ becomes available, patients who match the medical and biological profile of the donor are ranked. This ranking takes into consideration how long the patient has been on the waiting list as well as their location. The transplant center is contacted when an organ becomes available and the transplant team considers the organ for the patient. The transplant team either accepts or declines it based on organ condition, patient condition, staff availability, and organ transportation. The transplant waiting list is necessary due to the lack of available organs and the high demand for them worldwide. Currently, 114,000 people are on the waiting list for an organ donation and 20 people die each day waiting for an organ to become available (UNOS).

The high demand for organs and lack of supply is still the biggest challenge in improving the success of organ transplants. As researchers continue looking for ways to increase organ supply, such as 3D printing organs from cultured human cells, the best way to improve the success of organ transplants is to maximize organ functionality after the time of surgery. However, long term survival rates have not improved and the chances of surviving 5 to 20 years after having a transplant procedure are still not significantly different for a 1980s-era patient than a present-day organ recipient (Rana, Abbas, & Godfrey, 2019). Infection and graft failure are still the main causes of death with death from malignancy increasing over time (Rana, Abbas, & Godfrey, 2019).

Researchers have focused on improving long term organ function through improvements in immunosuppressive therapies. Immunosuppressive drugs are essential for organ transplant

patients to ensure the recipient does not reject the allograft. An allograft is the transplant of an organ from one individual to another who is of the same species, but has a different genotype. Allograft rejection is a complex response that involves multiple cell types and cell mediators. The process of organ rejection is initiated by the presence of histocompatibility antigens. Antigens are foreign substances that induce an immune response in the body. Antibodies, protective proteins that neutralize and bind to antigens, are produced by B cells and operate to prevent antigens from penetrating body cells. They also mark antigens for removal from the body.

The major histocompatibility complex (MHC), found on chromosome six, is a group of genes that code for proteins located on the surface of cells that help the immune system identify foreign substances (Janeway et al., 2001). The MHC produces two classes of protein molecules, class I and class II proteins, which present antigenic peptides so T cells can recognize them. T cells play an important role in organ rejection in that they recognize antigens presented by MHC proteins. CD8 T cells recognize antigenic peptides from within the cell presented by MHC class I proteins while CD4 T cells recognize extracellular antigenic peptides presented by MHC class II proteins (Janeway et al., 2001). During organ rejection, CD4 and CD8 T cells recognize antigens expressed on the cells of the foreign graft.

Solid organ rejection occurs by two different pathways. In one pathway, called the direct pathway, T cells (CD4 and CD8) recognize foreign MHC molecules (class I and II proteins) on donor antigen-presenting cells. This is believed to be the main pathway in acute organ rejection. The other pathway, known as the indirect pathway, functions by T cells recognizing allopeptides on recipient MHC molecules (Bharat & Mohanakumar, 2007). Dendritic cells play a major role in the graft induced immune response and are involved in both rejection pathways. Dendritic

cells (DCs) specialize in the capture and processing of antigens and are abundant in organs and peripheral tissues so they can capture antigens (Roghanian). In the direct pathway, DCs migrate from the graft and into secondary lymphoid tissues where they activate T cells (Ingulli, 2010). In the indirect pathway, graft proteins are processed by the DCs which are then presented to T-cells. A study that examined the indirect and direct rejection pathways in mice concluded that the direct alloresponse was short-lived and the indirect alloresponse became the main pathway in long term graft rejection (Benichou et al., 2017). This implies that by targeting and controlling the indirect allorecognition pathway, graft rejection can be prevented.

Immunosuppressants act to prevent the immune system from recognizing foreign MHC molecules and allopolypeptides by suppressing the immune system, specifically targeting T cells, B cells, anti-donor antibodies, and other immune system molecules (Hartono, Muthukumar, Suthanthiran, 2013). They are classified as either induction or maintenance agents. Induction agents work to prevent acute rejection while maintenance agents prevent long term rejection. The introduction of new maintenance agents, calcineurin inhibitors (CNI), cyclosporine (CsA), and tacrolimus (Tac), in the 1980s resulted in improved graft outcome (Hartono, Muthukumar, Suthanthiran, 2013). Calcineurin inhibitors inhibit the action of calcineurin, an enzyme that stimulates T cells. Other immunosuppressive agents such as small molecules, biologics, Mycophenolate Mofetil (MMF), Sirolimus, Rabbit antithymocyte globulin (rATG), Alemtuzumab, and Belatacept have recently become available for clinical use (Hartono, Muthukumar, Suthanthiran, 2013). However, increased potency of newer immunosuppressive agents can increase the risk of polyomavirus infection and post-transplant EBV-associated lymphoma (Hartono, Muthukumar, Suthanthiran, 2013). The implementation of these

immunosuppressive drugs has helped improve short term outcomes in organ transplant patients, but have not improved the life span of the transplanted organ long term.

II. The Human Microbiome

The human microbiome refers to the community of microorganisms and their genes that exist in the nonsterile sites of the body, such as the skin, respiratory tract, and gastrointestinal tract (Hair & Sharpe). Everyone carries their own microbial communities, shaped by mode of birth, environment, genetics, diet, and other lifestyle and biological factors. Since the discovery of the microbiome in the late 1990s, scientists have predicted that there are over 100 trillion microbes living in humans, with the majority residing in the gut, specifically the large intestine (Hair & Sharpe). With this many microbes living in humans, the total number of genes in the microbiome is 200 times greater than the number of genes in the human genome. In addition, half of the genes in an individual's microbiome have been found to be unique to the individual (Ursell et al., 2012). This positions bacteria to be a powerful modulator of health and disease.

The microbiome acts as its own ecosystem separate from the human body and is considered an environmental factor that influences health and disease. Microbes live in symbiosis with their human host and are variable and unique to everyone. The intestinal microbiome is primarily made up of bacteria from the genus *Bacteroides* which includes *Peptostreptococcus*, *Eubacterium*, *Lactobacillus*, and *Clostridium* (Rowland et al., 2018). Most of the bacteria that make up the microbiome are not harmful, but beneficial. These microbial communities play essential roles in metabolism, food digestion, and immune system regulation.

Communication networks between intestinal microbes at mucosal membranes and the host have a large impact on the development of the immune system. This has been observed

through studies comparing germ-free and conventional animals. The results of these studies show that intestinal microbes are essential for the proper function and development of the mucosal immune system in early stages of life (Purchiaroni et al., 2013). This has a critical impact on lifelong immunity in adults. In addition, it has been found that the composition of the intestinal microbiome impacts gut-associated lymphoid tissue (GALT) which consists of isolated or aggregated lymphoid follicles which form Peyer's patches, groupings of lymphoid follicles that contain a variety of immune cells which provide immune surveillance in the digestive system (Seladi-Schulman). GALT contains around 70% of the body's immunocytes, cells that can induce an immune response by creating antibodies (Jung, Hugot, & Barreau, 2010). Defects in GALT development, a reduced cellular lamina propria, fewer plasma cells in the germinal center of the mesenteric lymph nodes (MLNs), and a reduction in antibody production and the number of Peyer's patches were all observed in germ-free animals compared to animals under normal conditions (Jung, Hugot, & Barreau, 2010). While the immune system is highly impacted by the microbial ecosystem in the gut, microbes themselves are affected by many factors. Altered gut microbiota can directly and indirectly affect immune cells in the gut.

Intestinal microbial composition is shaped by many external and genetic factors. The composition of the microbiome has been found to be more similar among blood relatives than unrelated individuals suggesting that the composition of the microbiome is influenced by genetic factors. However, external factors, such as diet, lifestyle, stress levels, and medications taken have been found to be more influential determinants of microbiome composition. Beginning as early as birth, an infant's method of delivery has been found to be an external factor that affects the early establishment of an infant's microbiome. Infants born by vaginal birth are exposed to their mothers' bacteria which affects the development of the infant's gut bacteria and stimulates

the immune system (Wen & Duffy., 2017). Infants delivered by cesarean section lack early exposure to bacteria which puts them at a higher risk for type two diabetes and obesity (Wen & Duffy., 2017). Breastfeeding too affects the development of an infant's microbiome. Breast milk contains commensal bacteria and probiotics that provide essential nutrients to the infant and influence the development of an infant's gut bacteria (Wen & Duffy., 2017). Breast milk from obese mothers has been found to lack diversity in bacteria compared to breast milk from normal-weight mothers. These findings show that the transfer of microbes from mothers to their infants is an external factor that affects infant growth, microbiota development, and health.

Another factor affecting the composition of the microbiome is the use of antibiotics and other medications. Antibiotics, used to treat bacterial infections, have an impact on the composition of the intestinal microbiome by reducing the diversity of bacteria in the microbiome and enabling resistant strains to overgrow and dominate (Wen & Duffy., 2017). Clindamycin, an antibiotic used to treat bacterial infections of the skin, internal organs, lungs, and blood, in young children has been found to have a negative long term effect on the composition of the gut microbiome by reducing the microbiome's resistance to colonization by pathogens and disturbing normal bowel function (Dudek-Wicher, Junka, & Bartoszewicz, 2018). Other antibiotic groups such as fluoroquinolones and β-lactams have the same effect of decreasing the diversity in the microbiota (Dudek-Wicher, Junka, & Bartoszewicz, 2018). However, the amount of variation in microbiota diversity depends on the initial bacterial profile of the individual's microbiome. Antibiotic treatment also affects the microbiome by liberating fucose and sialic acid, mucosal carbohydrates. An increased level of these substances presents pathogens such as *Salmonella typhimurium* and *C. difficile* with the opportunity to colonize the gut (Dudek-Wicher, Junka, & Bartoszewicz, 2018). Non-antibiotic drugs have also been associated with changes in

composition and function of gut microbiome composition. Proton pump inhibitors, metformin, antibiotics, and laxatives have been found to have the biggest impact on microbiota composition. Non-antibiotic drugs were found to increase upper GI tract bacteria, fatty acid production, *E. coli* levels, and antibiotic resistance mechanisms in the gut microbiome (Vich Vila et al., 2019).

Diet also has a major impact on the composition of the gut microbiome. High fat and sugar diets correlate to fewer bacterial species in the intestinal microbiome and cause dysbiosis, microbial imbalance, in the microbiome. This puts individuals who eat a high fat and sugar diet at a higher risk for disease such as inflammatory bowel disease. In contrast, diets rich in complex carbohydrates have shown an increase in gut microbiome diversity (Schnorr et al., 2016). Fiber rich diets help regulate the microbiome and have been found to restore levels of beneficial microbes and lower the number of harmful microbial metabolites. It also increases the number of short chain fatty acids (SCFAs) produced which help stabilize the microbiome and can protect against colorectal cancer and inflammation (Louis et al, 2014).

Exercise promotes diversity of microbes in the gut. Data from a study conducted on professional rugby players showed that exercise positively influences the diversity in the microbiome by increasing the amount of diversity among the Firmicutes phylum indicating a healthier intestinal environment. In addition, protein intake and creatine kinase levels also increased. Another study, which examined different levels of cardiorespiratory fitness, found that cardiorespiratory fitness was correlated to a more diverse microbiome (Monda et al., 2017). Cardiorespiratory fitness is defined as the ability of the circulatory, respiratory, and muscular systems to supply oxygen throughout the body during sustained physical activity (Lee et al., 2010). Higher levels of exercise and fitness correlated with enriched butyrate-producing taxa in the microbiome. Butyrate production is an indicator of good gut health.

While the microbiome is influenced by genetic factors and events at birth, its composition is variable and predominantly affected by many lifestyle choices and external factors. The variability of the microbiome makes it a good target to manipulate to improve an individual's overall health. However, the microbiome can just as easily be negatively affected by external factors as well, making it a tricky target.

After solid organ transplantation, the microbiota in the gut and other body sites changes. The change in the microbiome is different depending on the type of solid organ transplant and varies greatly among individuals. In liver transplant recipients, there is a post-transplant reduction in the amount of *Bifidobacterium*, *Lactobacillus*, and *Faecalibacterium prausnitzii* present in fecal samples, but an increased number of bacteria from the family Enterobacteriaceae (Wu et al., 2012). While in lung transplant recipients, there is an increase in the number of bacteria and fungi in the lower respiratory tract, but a decrease in the diversity of bacteria and fungi (Charlson, 2012). These changes have been associated with acute and chronic graft rejection which suggests that the microbiota has an impact on alloimmunity and transplant outcome. It has been found that different microbial community structures have different effects on transplant outcome such that some microbiota community structures increase alloimmunity and accelerate rejection, some community structures have neutral effects on graft rejection, and others dampen transplant rejection (Sepulveda et al., 2019).

The role that the human gut microbiome plays in transplant success has been underestimated. While gut microbes have been found to directly contribute to allograft rejection, they also indirectly regulate many other processes that contribute to the long-term health outcomes in transplant patients. Through short chain fatty acid production, they regulate blood pressure and cholesterol levels, two conditions that can lead to chronic disease such as type one

and type two diabetes, high blood pressure, and irritable bowel syndrome. The importance of restoring homeostasis in the gut microbiome is vastly important for long term health outcomes and utility of the organ donation.

Chapter 2- Effects of Solid Organ Transplants on the Microbiome

Introduction:

In looking for ways to improve transplant success, the gut microbiome is a good place to start. With emerging evidence that the gut microbiome is involved in many processes, such as promotion of angiogenesis and regulation of the immune system, it is reasonable to conclude that a patient's gut microbiome affects solid organ transplant outcome. Transplant success can be improved by preventing acute and chronic rejection, but also by preventing infection, chronic disease development, and improving mental health, all of which the microbiome influences. While the gut microbiome of the patient is primarily involved in determining patient transplant outcomes, the donor's gut microbiome may also have influence on the transplant outcome. This section analyzes the possible ways the donor and patient gut microbiomes are affected before surgery, how the transplant surgery itself affects the patient's gut microbiome, and how post-transplant recovery affects the gut microbiome. The impact of how changes in the microbiome before, during, and after transplant affect transplant success immediately and in the long term is also discussed. Recommendations on minimizing microbiome disruption are made when appropriate.

I. PRE-TRANSPLANT

Donor:

To understand the different factors affecting patient outcomes pre-transplant it is important to consider both living and non-living donors. Living donors can donate a kidney and parts of their liver, lungs, pancreas, and intestines while non-living donors can donate those

organs in addition to their heart. The role the gut microbiome of a living or non-livings donor plays in transplant success is unclear. However, the gut microbiota is known to interact with distant organs and play a role in the regulation of many bodily systems. Therefore, disruption to the gut microbiome could have a negative effect on procured organs for transplant. In addition, deceased donors experience a variety of preoperative interventions that can result in poorer transplant outcomes, while the organ itself also experiences preoperative procedures that affect graft function post-transplant.

In solid organ transplantation, the quality of care the donor receives pre-transplant affects the recipient's outcome post-transplant. For instance, if the non-living donor is not properly attended to, hormonal and inflammatory changes, hyperglycemia, and increased plasma levels of interleukin-6 due to brain death can result in poorer graft utilization, graft dysfunction, and increased chances of rejection (Kumar, 2016). Established protocols for donor management require that the non-living donor's temperature, hormone levels, fluids, ventilation, and cardiovascular system must all be properly controlled to improve graft function and surgical outcomes (Kumar, 2016). In addition, organs procured for transplant from non-living donors are in a state of distress due to bodily changes that occur after death. These organs spend more time in preservation solution, compared to organs from living donors, before being transferred to the recipient. Organ distress and a greater amount of time spent in preservation fluid may reduce organ function temporarily and delay the organ from being fully functional after its transplant. This has been observed in kidney transplants where the transplanted kidney can take weeks to become fully functional after surgery.

The role the donor's gut microbiome plays in affecting transplant outcomes is still not well understood, but it seems that it may be of more concern in deceased donors than living

donors. Considering living donors, it appears unlikely that the donor's gut microbiome affects the organ transplant. This conclusion was reached by the fact that living donors undergo an intense screening process and must be in very good health to become an organ donor. This makes it highly unlikely that the living donor is experiencing gut dysbiosis which could negatively affect transplant outcomes. However, the microbes on the procured organ itself may cause infection, which is true for deceased donors as well.

Currently, it is not medical practice to treat whole organs with antiseptic solutions to eliminate bacterial, viral, or fungal contaminants due to the time constraints of organ viability (Block). Hypothermic preservation and the treatment of organs for donation with organ preservation solutions can keep microorganisms alive on the donor organs and facilitate their growth (Oriol et al., 2018). This is potentially dangerous, especially for lung and skin transplants, because the microbes from the donor may be transferred to the recipient and result in infection or poorer graft outcome. Despite this possibility, all organ donors undergo meticulous preoperative screenings to ensure the absence of infectious agents before organ removal. Also, while there is a high incidence of culture-positive preservation fluid, very few preservation fluids test positive for pathogenic microorganisms. In addition, it has been found that transmission of bacteria from the donor to the recipient is infrequent in deceased donor liver transplantation (Chan et al., 2019). Overall, it is rare for a patient to contract infections from the donor organ, especially in heart, liver, and kidney transplants as they are sterile organs, but not impossible.

The deceased donor's microbiome may be more influential in affecting transplant outcomes than the living donor's microbiome. Most deceased donors are brain dead and when brain death is caused by severe brain injury, there is a disruption in the brain-gut axis resulting in chronic dysfunction of the gastrointestinal system (Zhu et al., 2019). This was observed in an

experiment in which changes were found in the gut microbiota of mice after experimental stroke and traumatic brain injury (Zhu et al., 2019). The experiment demonstrated that the brain and the gut microbiome have a direct relationship and disrupting it causes stress to intestinal microbe communities. Significant changes in the diversity to the microbiota have been observed within two hours of traumatic brain injury (Zhu et al., 2019). Depending on the type of injury, brain death may occur in minutes or over a period of days. Since changes to the microbiome occur at a relatively accelerated rate, it is possible that dysbiosis in the donor's intestinal microbiome may affect the microbes present on organs being removed for organ donation which may impact organ function in the recipient after transplant.

Living donor organ transplants have better outcomes and lower rates of rejection than deceased donor transplants. This is partly because living donors are better genetic matches to the recipient due to the ability to test potential donors ahead of time and find the most compatible patient to match with the donor. Rejection kinetics of transplanted organs are greatly dependent on the extent of genetic disparities between the donor and recipient. However, environmental factors may affect rejection kinetics too (McIntosh et al., 2018).

The main environmental factor affecting transplant outcomes is the process of cooling organs after removal. This process is essential for keeping the organ viable for transplant. Typically, a heart or lung has a six-hour window in which it can be kept viable for transplant while a liver will keep for 8-12 hours and a kidney for 24-46 hours (HRSA). New super cooling methods have been reported to be able to keep livers stored at sub-zero temperatures without freezing which allows the liver to be stored outside of a body for a day and a half and still can recover and function properly. While this technology can hopefully be applied to other organs, the larger the organ the more difficult the super cooling process is (de Vires et al., 2019).

Currently, the method of organ preservation is different for different organs. The basic concept behind the method of preservation is the same. All organs are preserved by hypothermic preservation using a type of solution specific for the organ. For the liver and kidney, a cold storage solution developed at the University of Wisconsin at Madison has proven to be the preferred solution while a donated heart uses crystalloid cardioplegia solution (Southard & Belzar, 1995). Other solutions such as Celsior solution, Kyoto solution, and Bretschneider's HTK solution can also be used, each having slight advantages and disadvantages over the others.

The cooling process itself is important because good organ preservation is a major determinant of graft outcome after transplant and re-vascularization. Even if the organ is a good genetic match for the recipient, the care the organ receives upon removal from a donor is critical for maximizing organ function in the recipient.

Recipient:

The microbiome of a patient awaiting an organ donation is affected by many individual and environmental factors. Organ transplant recipients are already suffering from dysbiosis because of their need for an organ transplant. The extent of dysbiosis depends on the severity of their condition, but can be made worse by other factors such as the hospital environment, pre-surgery drugs, a lack of exercise, and restricted diet.

To qualify for an organ transplant, organ recipients must suffer from irreversible and otherwise fatal organ failure. The waiting list for an organ transplant can range from a couple of months to years, with the longest wait time being around two to five years. The median waiting time for high priority candidates is around 2.6 months, while median waiting time for intermediate priority candidates is around 8.5 months (Goldstein et al., 2016). Waiting times

vary due to blood type, ethnicity, UNOS (United Network of Organ Sharing) status at listing, and age. Due to long wait times, patients may be hospitalized for a prolonged amount of time. Patients typically wait at home, but are usually in and out of the hospital because of complications associated with their condition. In severe cases, patients are kept in the hospital to monitor their health status. Extended hospital stays and trips in and out of the hospital can cause changes in the gut microbiome as hospitals themselves have been found to be microbial ecosystems that facilitate microbe exchange between patients, staff, and visitors who occupy the hospital. In fact, the hospital may serve as a vector for infection transmission in extended stay patients.

The Hospital Microbiome Project, one of the largest microbiome analyses undertaken, examined how microbial exchange works in the hospital setting. The results of the Hospital Microbiome Project showed that on the first day a patient is admitted to the hospital, microbes spread from the surroundings to the patient. However, this is quickly reversed by the second day when the patient's microbiome colonizes the room, increasing the diversity of the microbes in the room. Long term stays have shown that potentially harmful bacteria, including *Staphylococcus aureus* and *Staphylococcus epidermidis*, acquired antibiotic resistant genes and promoted hospital acquired host infections (Wang & Raun., 2017).

According to Dr. Michelle Wright at the University of Texas School of Nursing, the environmental switch from home and hospital is very disruptive to the microbiome. Many aspects of the hospital environment affect a patient’s gut microbiome, including, but not limited to, encounters with different healthcare providers, the hospital diet, and exposure to antibiotic resistant bacteria, known as “superbugs”.

In transplant patients, with extended hospitalization prior to transplant surgery, this is potentially harmful as they are exposed to these factors for longer periods of time. This increases the risk of disruption in the microbiome and the chances of contracting a hospital acquired infection. In serious cases, this can put transplant patients at risk for not being able to receive an organ if one becomes available. In addition, extended hospitalization affects the patients diet by limiting what they can eat to what is available in the hospital. High fiber diets and plant based diets have been found to be associated with healthier microbiomes. However, hospitals have been criticized for not serving quality nutrient rich food. Recently, the New York state Legislature's Senate Health Committee passed a landmark bill to guarantee hospital patients plant-based options (NY State Senate). While the quality of hospital food has been increasing over the past decade, dietary switches may induce shifts in the microbiome composition, function, secreted metabolites and proliferative and inflammatory markers (Pietzner et al., 2017). All food must be prepared and handled properly by all hospital staff to prevent infection before surgery.

Pre-surgery preparation is another factor that disrupts the gut microbiome. Fasting, mechanical bowel cleansing and antibiotics are all factors that have only just started being examined for their role in gut flora disruption. Medications may affect the composition of the microbiome while the microbiome is known to affect pharmacokinetic properties of medications and contribute to the effectiveness or side effects of these medications. Prior to surgery, patients take antibiotics 30 minutes before their transplant and immunosuppression medications are taken a couple of hours before. While antibiotics are known for killing bacteria or preventing them from multiplying, immunosuppressants also affect the microbiome. These changes have a greater effect after the transplant surgery when immunosuppressants begin to be routinely taken.

Immunosuppressants primarily alter the microbiome by reducing many taxa commonly found in a healthy microbiome.

The microbiome is also affected by exercise, patients awaiting transplants may have difficulty working out due to their medical conditions, but regular exercise has been found to play an important role pre- and post-transplant to increase quality and life and prevent development of chronic diseases such as diabetes, de novo tumors, and high blood pressure. A low intensity routine preoperative training program for patients awaiting organ transplant has great effects on reducing the length of hospital stay and increasing quality of life and general robustness (Beekman, Berzigotti, & Banz 2018).

The patient's condition and their age are two other factors that influence the intestinal microbiome prior to transplant. Relevant to pediatric transplant patients, teenagers going through adolescence experience microbiome changes. It is unknown how needing an organ transplant affects typical microbiome development, but it is a factor to be considered. Overall, even before the transplant surgery, multiple factors are contributing to the disruption of the microbiome and putting the new organ at risk.

Recommendations:

Pre-surgery efforts to improve organ transplant outcomes should focus on the deceased donors and patients. To improve transplant outcomes, patients should be kept at home and on their regular routine as much as possible as environmental changes are known for disrupting the microbiome. While this cannot always be done, especially in serious situations, hospital level care should be brought to the patient's home whenever possible to prevent long term stays in the hospital.

To reduce the length of time solid organ transplant patients spend in the hospital, pre-transplant exercise can also be a method for keeping patients healthy and at home. Pre-surgery exercise is important even though exercise capacity is reduced in solid organ transplant candidates. Studies have found that exercise training pre-transplant is safe and should consist of aerobic training or a combination of aerobic training and resistance training (Janaudis-Ferreira et al., 2019). Pre-transplant exercise increases the exercise capacity of the patient, may positively affect gut microbiome composition, and can improve post-transplant outcomes as well.

Regarding deceased donors, as discussed earlier, the quality of pre-transplant care the donor receives affects the recipient's outcome post-transplant. The time after brain death and before organ procurement is a critical period in which organ quality can be affected. While there are standard clinical guidelines for maintaining cardiovascular and hemodynamic stabilization, there is currently no optimal approach to hormonal replacement in brain-dead donors (Ingelfinger, 2018). While thyroid hormones, vasopressin, insulin, and glucocorticoids are commonly used, optimizing hormonal replacement therapy in deceased donors is important for not only improving transplant outcomes, but also for helping address the major issue of organ shortage by increasing the number of organs available for donation.

Methylprednisolone is a corticosteroid medicine that prevents the release of substances in the body that cause inflammation. Studies have found that when methylprednisolone, vasopressin, and thyroid hormone are used together to treat deceased donors less graft dysfunction and improved early survival were observed in heart transplant patients. Methylprednisolone therapy also was found to improve liver function after liver transplant (Kotsch et al., 2008). Continuing to understand and improve hormonal replacement in deceased donors is important for creating an optimal approach to hormonal replacement therapy that can

be used to help solve the issue of organ shortage and improving organ functionality in the patient.

II. DURING TRANSPLANT

Microbes alter their phenotypic expression to optimize fitness by assessing their local environment (Rowley et al., 2006). One of the most disruptive events to the intestinal microbiome is surgery as it is highly stressful on the body. Surgery alters the gut microbiota composition, microbial abundance, and function (Guyton & Alverdy 2017). In a study examining the effect of abdominal surgery on piglets, data showed that two weeks after the surgery was performed, there was a reduction in microbes of the families *Enterobacteriaceae*, *Bacteroidaceae*, and *Rhodospirillacea*e when compared to the control group (Stavrou & Kotzampassi, 2016). For the most part, gut flora can adapt to surgical stress. This is evident by the fact that most surgical patients do not experience infectious complications post-surgery. However, in specific patients, bacteria can sense host stress, humoral alterations, and specific environmental cues that result in gut flora becoming virulent and pathogenic (Stavrou & Kotzampassi, 2016). This results in post-surgical infections that can be threatening to solid organ transplant patients.

The anesthetic used during the transplant procedure has also been found to alter the recipient's microbiome. In a mouse model, 16s ribosomal RNA sequencing was used to analyze the effects of volatile anesthetics on the composition and diversity in the intestinal microbiome (Serbanescu et al., 2019). The results showed that after exposure to isoflurane, a general anesthetic, the diversity in the gut microbiome decreased. In addition, other good gut bacteria were found to be depleted suggesting that volatile anesthetics may contribute to gut microbial

dysbiosis in the patient post-surgery (Serbanescu et al., 2019). The relationship between the microbiome and use of anesthetics is complicated as the gut microbiome influences pharmacokinetic properties which determine the effectiveness and proper dosage of the drugs. The gut microbiome must be taken into consideration before an anesthesiology treatment plan is created, but at the same time the anesthetics alter the composition of the microbiome. This dual relationship complicates the treatment plan for patients and living donors.

Recommendations

To reduce gut microbiome disruption, it is critical to reduce surgically induced stress. Surgical stress impairs metabolism, immune function, and wound healing and can result in infection and increased mortality (Finnerty et al., 2013). These are all processes in which the microbiome is involved in. Reducing operative stress and host inflammatory responses by developing minimally invasive surgical techniques may help reduce post-transplant infections and ensure the patient's best outcomes. In addition, the type of anesthesia used during surgery may also reduce stress.

Minimally invasive surgery has been found to improve surgical outcomes and help reduce postoperative morbidity. Small incision open surgery, laparoscopic surgery, and robotic surgery are all minimally invasive surgical techniques that have been employed in kidney transplantation. Recently, the first purely laparoscopic living donor surgery for liver transplant was performed (Uwechue et al., 2017). While minimally invasive surgical techniques reduce risk of infection and hospital recovery time, they are greatly limited by medical imaging technology. Research to develop new imaging technologies that improve image quality and enable 3D color visualization as well as real-time tracking devices should be a priority as it has great ability to

improve minimally invasive surgery technology and have a positive impact on patient outcomes (Goel, 2019).

Anesthesia also has an effect on reducing stress on the body due to major surgery. Total intravenous anesthesia, a general anesthesia technique that provides anesthesia through an exclusively intravenous route and does not use inhalation agents, has been found to have a positive effect on preventing postoperative inflammation and other stress response mechanisms. Epidural anesthesia and general anesthesia are commonly used during kidney transplantation (SarinKapoor et al., 2007). However, in kidney transplantation, total intravenous anesthesia can be used instead of balanced anesthesia, a general anesthetic technique that uses smaller doses of two or more agents (Modesti., 2006). Total intravenous anesthesia has many advantages such as prevention of excessive postoperative inflammation and faster recovery times (Chen, Jiang, & Wu, 2016).

III. POST-TRANSPLANT

After a successful organ transplant surgery, new threats to the gut microbiome arise while pre-transplant threats persist. In the days following surgery, patients are still exposed to the hospital environment and are limited in their diet and exercise routines. However, this changes once patients are discharged and overtime regain dietary freedom and increased ability to exercise. However, post-surgery the patient must begin to regularly take immunosuppressant drugs to prevent organ rejection. Broad-spectrum antibiotic prophylaxis is also frequently prescribed to prevent perioperative complications immediately following surgery.

Transplant recipients are required to stay in the hospital following surgery to ensure proper recovery. Kidney transplant patients typically remain in the hospital for three to five days

while liver and heart transplant patients stay in the hospital for about a week before being discharged. As discussed previously, the hospital environment, hospital and post-surgery diet, and limited physical activity can negatively affect the microbiome of the patient.

New to the patient after a successful organ transplant surgery is the routine administration of immunosuppressive drugs required to prevent acute and chronic rejection. Combinations of medications and high doses are prescribed at first and are lowered overtime. The combination of drugs varies for the type of organ transplanted and for the individual. Typically, immunosuppressive drugs such as Prednisone, Tacrolimus, Cyclosporine, and Mycophenolate are prescribed. Immunosuppressive drugs are known to lower the body's resistance to infection, so anti-infection medications are also prescribed as infections can have severe consequences for transplant patients. Commonly taken anti-infection drugs include Nystatin, Acyclovir, Clotrimazole, and Ganciclovir. Anti-infection medications are usually only taken for one to three months after transplant (Umich). Anti-ulcer medications and anti-hypertensive medications may also be prescribed and are to be determined by the physician.

Immunosuppressive drugs, required for transplant recipients, have been found to alter microbial community structures. Research shows that bacterial diversity in the gut microbiome significantly decreased when treated with immunosuppressive drugs (Baht et. al, 2017). Using 16s RNA sequencing, the phylogenetic diversity in the intestinal microbiome was found to be reduced by Sirolimus, an immunosuppressant taken by kidney transplant patients. Tacrolimus, another immunosuppressant prescribed to kidney, liver, and heart transplant patients, and Sirolimus were found to alter gut flora by increasing the amount of *Lactobacillus* and *Akkermansia muciniphila* making the microbiome resemble the microbiome of diabetic patients (Zhang et al., 2018).

Other taxa that were found to be reduced in immunosuppressed patients were *Roseburia*, *Oscillospira*, *Rothia*, *Micrococcaceae*, and *Staphylococcus* (Bhat et al., 2017). These taxa are butyrate-producing bacteria that are important for maintaining intestinal health and promoting insulin sensitivity. In addition to the alterations to the intestinal bacterial profile, rats treated with immunosuppressive drugs were found to develop hyperglycemia and increased cholesterol levels (Bhat et al. 2017). These results demonstrate the consequences of immunosuppressive drugs in transplant patients.

Like immunosuppressant drugs, antibiotic prophylaxis is prescribed post-transplant, but only for 24-48 hours in most cases. Broad-spectrum antibiotic prophylaxis is routinely utilized after almost all surgical procedures to prevent postoperative surgical site infections (SSIs) from occurring. They decrease the amount of bacteria in the surgical wound to allow for proper healing. Surgical site infections are one of the most common healthcare-associated infections and, depending on the type of transplant, occur in 3-53% of transplant patients (Anesi et al., 2018). SSIs are associated with increased graft rejection and mortality of solid organ transplant patients.

Antibiotics eliminate pathogenic bacteria, but also eliminate beneficial bacteria as well. The effects on the composition of the gut microbiome varies depending on the type and duration of antibiotics taken. Altering the microbiome with antibiotics results in decreasing the metabolic potential of the microbiome and makes it easier for pathogenic bacteria to colonize (Rahim, Taylor, & Hirota., 2017). Emerging data has shown that antibiotic prophylaxis may increase the risk of *Clostridium difficile* infection and graft-versus-host-disease (Horton, Haste, & Taplitz, 2018). Ironically, antibiotic prophylaxis which is used to prevent SSIs may increase chances for infections due to its effect on the microbiome.

Recommendations

While prophylactic antibiotics decrease the risk of surgical site infections and improve morbidity and mortality outcomes, they can cause significant alterations to an already disrupted microbiome. Developing other ways to reduce the incidence of surgical site infections that don't cause antibiotic resistance, *Clostridium difficile* infection, and alterations of the gut microbiome should be considered. Using non-absorbable antimicrobials has been found to be successful in performing the role of prophylactic antibiotics and do not disrupt the gut microbiome. In addition, insulin therapy has been found to decrease rates of post-transplant infection and sepsis.

Non-absorbable antimicrobials, such as Rifaximin, an antibiotic with broad spectrum activity and limited intestinal absorption, have been found to have a negligible impact on the intestinal microbiome and decrease gut inflammation while also decreasing transplant mortality and improving overall survival rates (Horton, Haste, & Taplitz, 2018). Rifaximin is safe, has minimal drug interactions, and is thought to help prevent and act as a therapeutic for other gastrointestinal diseases as well (Koo & DuPont, 2010).

The best way to maintain gut microbiome structure in transplant patients is through microbiome profiling and avoiding the use of broad-spectrum antibiotic in individuals with low gut flora diversity. Stool samples from patients could be used to gain insight into the diversity of their individual gut microbiome and inform physicians on whether broad-spectrum antibiotics or narrower-spectrum antibiotics should be prescribed. In addition to examining the gut microbiome composition of the patient, the patient's individual susceptibility to infections due to genetic polymorphisms should also be considered. Recent evidence shows that genetic polymorphisms in innate immunity are associated with increased risk of bacterial infections after liver transplant

(van Hoek, de Rooij, & Verspaget, 2012). This has been found to also be true in heart and kidney transplant recipients. The ability to identify these genetic markers through individual genomic sequencing can help tailor non-absorbable antimicrobials to the individual patient and improve transplant outcomes.

Hyperglycemia, an abnormally high blood glucose level, is common after extreme physiological stress and can be a result of transplant surgery. Optimal insulin therapy could be a potential therapy for transplant patients as it has been found to significantly decrease rates of infection and sepsis while also improving organ function and helping reverse the post-traumatic catabolic state due to surgery (Finnerty et al., 2013). However, the complex conditions of transplant patients make it difficult to understand the physiological effects that insulin therapy may have on transplant patients (Iestyn et al., 2019). More research must be conducted to understand the potential benefits of insulin in the realm of solid organ transplants.

Chapter 3: Analyzing Potential Methods of Restoring Homeostasis to the Microbiome

I. Fecal Microbial Transplant

Recently, altering the composition of the microbiome has been found to be possible through the unconventional method of fecal microbial transplant. Fecal microbial transplant (FMT) is a therapy that has been successful in treating recurrent *Clostridium difficile* (*C. diff*) infection, an infection that leads to severe colon inflammation. FMT works by infusing bacteria from a healthy donor's stool into a patient's disrupted microbiome. The transplanted bacteria repopulate the gut of the recipient with diverse microbes that outcompete the detrimental *C. diff* bacteria. This reverses dysbiosis and restores normal gut function by establishing a new gut microbiota in the recipient.

Fecal microbial transplant has been 90% successful in treating recurrent *Clostridium difficile* infection (Gupta, Allen-Vercoe, Petrof, 2016). The success seen with treating recurrent *Clostridium difficile* infection suggests that FMT could also reset an organ transplant recipient's disrupted microbiome to a healthy homeostatic state. If FMT were to be successful in solid organ transplant patients, the rates of post-transplant infection, organ rejection, and development of comorbidities would likely decrease. Many researchers have had similar ideas and FMT is currently being explored in a variety of clinical settings. Research has seen preliminary success in using FMT to treat functional constipation, immunotherapy-induced colitis, neurodegenerative disease, and prevention of cancer-related disorders like graft versus host disease (Dupont et al., 2020).

Recently, FMT was tested to determine if it could be a successful therapy for treating obesity. In a randomized placebo controlled pilot trial, experimental group participants received 30 FMT capsules followed by 2 doses of 12 capsules over a 12-week period and the control group received placebos instead of the FMT capsules (Allegretti et al. 2019). The results showed three important things: that FMT was a safe therapy, donor community engraftment occurred, and that the stool samples of the participants became more like the stool samples from the FMT donor. The study was a mild success, but concluded that a longer follow up needed to be conducted to make final conclusions.

Currently, FMT is not FDA-approved and is considered investigational (Allegretti). FMT may be used in clinical trials, but it can only be performed for clinical care to treat recurrent *Clostridium difficile* infection. It is important to note that FMT has been approved for use in immunocompromised patients making FMT a possible therapy for restoring the gut microbiome in solid organ transplant patients. However, immunocompromised patients are still at high risk for infection making donor screening even more critical.

Even if FMT cannot be used to reverse gut dysbiosis, it can still indirectly improve patient outcomes by preventing the development of comorbidities such as obesity and diabetes. This reduces the burden of disease and amount of stress put on the transplant recipient resulting in a longer lifespan, lower healthcare costs, and less care required to deal with chronic disease.

The cost of FMT depends on the hospital and the method of administration which can be by colonoscopy, enema, nasogastric tube, or capsules. Health insurance does cover components of FMT administration. OpenBiome, a nonprofit stool bank, sells FMT capsules for $1950 per dose and provides FMT upper and lower delivery microbiota preparations for $1595 per treatment. The costs can be up to $3500 if one chooses to use and screen their own donor. In

certain situations, FMT must be performed twice to see results, doubling these costs. Most insurance companies do cover FMT which helps to reduce the cost burden on the patient.

FMT also has no known side effects and is a quick outpatient procedure. The entire procedure takes around two hours with the transplant portion only taking around 10 minutes and patients with *C. diff* usually recover in 3-4 days. Besides the transplant itself, FMT does not require any extra care. From what is currently known about dysbiosis in the human gut microbiome in kidney, liver, and heart transplant patients, FMT should be able to be used as a potential therapy for all three transplant types.

A major challenge in fecal microbial transplantation includes strict donor screening as to prevent the transmission of multidrug resistance organisms through FMT. Screening protocols to detect multidrug resistant organisms and potentially disease causing organisms have been implemented in all major stool banks globally (Allegretti). Standardized screening protocols are essential for patient safety and increasingly important in immunosuppressed patients (Allegretti). In addition to stool screening, uniform protocols for stool preparation need to be established and the best method for FMT administration must be determined. Since FMT is a recently discovered therapy, these challenges need to be addressed before FMT can be used.

It is important to note that while recurrent *C. diff* infection can be treated with one or two FMT treatments, chronic disorders may require more frequent FMT administration over a longer period and may not be improved by FMT at all. In addition, recurrent *C. diff* infection is considered a “relatively pure” form of dysbiosis in which FMT from one unrelated donor is acceptable (Dupont et al., 2020). For transplant patients with a long history of critical illness FMT may not work as well or have a significant impact on the disrupted microbiome. The best results of FMT may come from its ability to stabilize short chain fatty acid production, increase

Tregs and IL-10 production, stimulate IgA production, and restore the bacteriophage population (Dupont et al., 2020). These results could indirectly help improve health outcomes for FMT recipients.

II. Synbiotic Therapy

Gut dysfunction caused by dysbiosis in the intestinal microbiome is a contributing factor to disease progression. Under conditions of critical illness such as organ failure it is difficult for the microbiome to maintain homeostasis, especially after invasive procedures like transplant surgery. Due to their susceptibility to infection transplant patients are at risk of developing several complications including ventilator-associated pneumonia, enteritis, systemic inflammatory response syndrome (SIRS), and sepsis. Synbiotic therapy has seen some success as a potential method for restoring homeostasis in gut microbiota and the overall gut environment.

Synbiotics are combinations of prebiotics and probiotics that have been found to successfully alter the structure of the intestinal microbiome. Prebiotics are high-fiber foods that promote the growth of certain colonic bacteria while probiotics are live, non-pathogenic microorganisms which may protect the gut barrier, reduce pathogen overgrowth, decrease bacterial translocation, and prevent infection (Pickard et al. 2017). When prebiotics and probiotics are administered together they work to enhance the survival of probiotic bacterial strains and stimulate the gastrointestinal bacteria resulting in a healthier gut (Anderson et al. 2004). Synbiotics are preferred over probiotics alone because they improve survival of probiotic bacteria as it passes through the upper intestinal tract and implant in the colon (Peña, 2007).

Recent research suggests that synbiotics can improve gut microbial composition and increase antioxidant capacity, making synbiotic therapy a promising option to reestablish

homeostasis in the gut microbiome (Morshedi, Saghafi-Asl, & Hosseinifard, 2020). A study found that synbiotics altered the gut microbiome composition by reducing the amount of *Clostridium* and *Bacteroides* and increasing the amount of *Lactobacillus* in the microbiome (Morshedi, Saghafi-Asl, & Hosseinifard, 2020). In addition, synbiotic therapy has been found to reduce bacterial infection in transplant patients.

In examining the clinical efficacy of synbiotics in abdominal surgery patients, multiple studies have found that in randomized controlled trials, groups treated with probiotics and synbiotics saw a reduction in the number of bacterial infections they developed compared to patients in groups that did not receive synbiotics and probiotics. These results were observed when liver transplant patients in the experimental group were treated with *L. plantarum 299v*, a probiotic (Rayes et al., 2002). The results showed that 13% of those who received the treatment had infectious complications. This was a significant difference when compared to the control group in which 48% had infectious complications (Rayes et al., 2002). Another study showed that in liver transplant patients treated with the synbiotic Synbiotic2000, 3% of the experimental group had infectious complications while 48% of the control group had infectious complications (Rayes et al., 2005). A study in living liver transplantation patients showed that patients in the experimental group who received BLO, a synbiotic, had 4% infectious complications while the control group had 24% infectious complications (Eguchi et al., 2011). These results conclude that preoperative administration of probiotics and synbiotics results in a decrease in infectious complications and can improve the health of transplant patients (Shimizu et al., 2013).

Synbiotic treatment has also seen positive outcomes in preventing complications in patients with sepsis, a life-threatening condition caused by the body's extreme response to infection (Zeratsky). Solid organ transplant recipients are at a higher risk for sepsis than the

general population due to their immunocompromised state (Donnelly). Sepsis can be a complication of ventilator-associated pneumonia, which has been found to be the main cause of infection following heart transplant and major heart surgery (Hortal et al., 2009). Methods of reducing the incidence of infection and sepsis are crucial and would have a major impact on the mortality rate due to sepsis.

Administering synbiotics to septic patients as a drug therapy showed positive results in a study that aimed to evaluate how effective symbiotics are at modulating the gut microbiome and in reducing complications in patients with sepsis. Incidences of enteritis and ventilator-associated pneumonia were significantly lower in the cohort that received synbiotic therapy than in the control group (Shimizu et al., 2013). Higher rates of *Bifidobacterium* and *Lactobacillus*, the most common probiotics, and higher levels of organic acid concentration were seen in the group who received synbiotic treatment when compared to the control group (Shimizu et al., 2013). The study concluded that synbiotics were effective at modulating the gut microbiota and could have preventative effects on the rates of ventilator-associated pneumonia and enteritis (Shimizu et al., 2013). Symbiotics also showed a decrease in septic complications in patients with systemic inflammatory response syndrome (SIRS) proving that through intestinal microbiota maintenance, septic complications in patients with SIRS can be significantly reduced (Shimizu et al., 2013).

Due to solid organ transplant recipients' high risk for infection, implementing synbiotic therapy before and after the transplant surgery could be beneficial to prevent enteritis, ventilator-associated pneumonia, SIRS, and other outcomes of sepsis. Synbiotics also provide a way to reduce the use of antibiotics to treat these infections which can reduce multidrug resistance and further damage to gut flora. In addition, synbiotic therapy may help reduce rates of organ

rejection because of its ability to modulate the composition of the intestinal microbiome and reduce septic complications in SIRS patients who are at increased risk for organ failure.

There is a lack of evidence for using probiotics and synbiotics to treat most health conditions. This has resulted in the FDA not yet approving symbiotic therapies to treat health problems (Wu, Chen, & Huang, 2017). However, 74 clinical studies found that synbiotic administration was safe in children as well as adults (van den Nieuwboer et al., 2015).

In terms of cost, there have not been any studies that have analyzed the cost of synbiotic therapy as it is relatively new. Studies to test the cost-utility of synbiotic therapy need to be conducted, but can only be conducted after synbiotic therapy has been approved and used widely to treat dysbiosis in the gut microbiome. An analytical study that analyzes quality-adjusted life-years, costs (US dollars), and cost per infection avoided would be necessary to analyze the cost-effectiveness of synbiotic therapy. The amount of care required in implementing the use of synbiotics pre- and post-transplant is relatively low. Patient's would have to monitor when their synbiotics were taken, if the correct dosage was taken, and that the prescription gets refilled.

Symbiotics appear to be beneficial to kidney, liver, and heart transplant patients. In kidney patients, synbiotics have an extra benefit of being effective at lowering concentrations of p-Cresol, a uremic toxin that is typically elevated due to the disrupted microbiome (Guida et al., 2017). In liver transplant patients, probiotics containing *Lactobacillus* with the use of prebiotics were found to be effective in reducing infectious complications post-transplant (Jorgensen et al., 2018). Heart transplant patients would also benefit as they frequently contract infections, one of the main causes of mortality in the heart transplant patient population. Overall, synbiotics have been found to be effective in reducing the amount of potentially pathogenic microorganisms in

the gut microbiome, eliminating potential toxins, providing antioxidants and nutrients through fermentation, and stimulating the immune system (Bengmark, 2004).

Although the results of these experiments support the use of synbiotic therapy and highlight its benefits, there are still challenges facing the implementation of synbiotic therapy. The biggest of these challenges is that the mechanism of how synbiotics act in the gastrointestinal tract remains unknown. It is hypothesized that synbiotics work by increasing the abundance of beneficial bacteria, such as *Bifidobacterium* and *Lactobacillus*, resulting in an increase in the production of short chain fatty acids in the gut. Short chain fatty acids play an important role in maintaining intestinal homeostasis and gut flora maintenance and may also signal through cell surface G-protein coupled receptors to activate signaling cascades that control immune function, therefore enhancing the immune system (Venegas et al. 2019). However, further clinical research must be performed to understand the mechanisms of immune responses involved in synbiotics' therapeutic effect on the microbiota. This information is critical in determining the effectiveness of synbiotic therapy, when it should be prescribed, and how often synbiotics need to be taken by the patient.

III. Short Chain Fatty Acid Supplementation

A disrupted gut microbiome is characterized by a decreased abundance of beneficial taxa. *Lachnospiraceae*, *Ruminococcaceae*, and *Clostridium* are all bacteria that through bacterial fermentation of indigestible fiber produce short chain fatty acids (Needell et al., 2017). Short chain fatty acids are important metabolites that work to maintain intestinal homeostasis. Acetate, propionate, and butyrate are short chain fatty acids that are key contributors in regulating the gut microbiota-epithelium interaction (den Besten et al., 2013). They help maintain gut homeostasis

by interacting with metabolite sensing G protein-coupled receptors on endothelial cells (den Besten et al., 2013). Decreased production of short chain fatty acids can lead to a rise in gut pH further causing damage to the gut flora and infectious complications (Shimizu et al., 2013).

A lack of microbiota-generated short chain fatty acids is linked to comorbidities such as obesity, type one and type two diabetes, cancer, and inflammatory bowel disease (Li, 2020). This is dangerous as transplant patients are at higher risk for developing these comorbidities. Short chain fatty acid supplements can help compensate for the reduction of short chain fatty acid production due to gut flora disruption. By restoring proper levels of short chain fatty acids through supplements, metabolic balance can be restored to the gut microbiome. Short chain fatty acids have been found to be successful in reshaping the intestinal microbiome by reducing the abundance of *Clostridium* and *Bifidobacterium* bacteria (Needell et al., 2017)

While short chain fatty acids have seen some success in directly altering the gut microbiome composition, they have also had success in affecting enteroendocrine hormones, glucose homeostasis, and colon inflammation (Koh et al., 2016). The short chain fatty acid propionate has been found to play an important role in regulating appetite. Long term supplementation with colonic propionate has been found to prevent weight gain by releasing peptide YY (PYY) and glucagon like peptide 1 (GLP-1) from colonic cells (Chambers et al., 2016). Supplementation with propionate over a seven-week period resulted in reduced fasting glucose levels and increased insulin release in women (Venter et al., 1990). In addition, it has been proposed that short chain fatty acids play a role in preventing chronic inflammation, a risk factor for colorectal cancer (Koh et al., 2016). However, in considering the effects short chain fatty acids have on cancer, other factors such as genetic background, cellular energetics, and environmental context must be considered.

Short chain fatty acids supplements such as sodium butyrate and inulin-propionate ester are FDA approved and are commercially available. Costs around $30-50 dollars for a bottle of supplement pills. Kidney, liver, and heart transplant patients would benefit from short chain fatty acid supplements because of their anti-inflammatory properties and immune system regulation.

An issue with short chain fatty acid supplements is that they may be absorbed before they reach the colon, thus preventing the colon from benefiting from the supplement. Butyrate has been found to best reach the colon when it is fermented from fiber, so increasing the amount of high fiber foods in a transplant patient's diet post-transplant may provide a better result and be more cost effective than short chain fatty acid supplements. Butyrate production can be stimulated by supplementing one's diet with carbohydrates that can only be degraded by bacteria in the colon. However, fiber degradation does not always lead to butyrate production, so to improve the results of dietary supplements they may need to be personalized to one's individual gut microbiota. In addition, the route of delivery could play a role in the effectiveness of the supplement. Proper route of administration is important because exogenously administered short chain fatty acids may act slightly differently than microbial produced metabolites which target the colon.

IV. Lifestyle (Diet & Fitness)

Diet:

Diet has been found to have a significant impact on microbial composition and gut health. Many patients waiting for an organ transplant suffer from malnutrition which increases their risk of contracting infections. Despite a lack of studies investigating the gut microbiota in

malnourished adults, malnutrition and the gut microbiome have been well studied in children. In regards to severe acute malnourished children and their gut microbiome, a malnourished microbiome has decreased diversity, is depleted of anaerobic species, and is enriched with aerobic species (Tidjani Alou et al., 2017). Restoring the microbiome may not be done through diet alone, but it is an important factor nonetheless.

Up to a fifth of kidney transplant patients suffer from malnutrition (Nolte Fong & Moore, 2018). The prevalence of malnutrition in cirrhosis, the end stage of many liver diseases, is as high as 65%–90% (O'Brien & Williams, 2008). The role that gut microbes play in severe malnutrition is not yet understood. However, malnourished patients have higher rates of morbidity which decreases organ transplant utility. Nutrient repletion in patients before transplant surgery can improve transplant outcomes.

Malnourished patients are more susceptible to infection which is dangerous pre- and post-transplant. Severely malnourished liver transplant patients have been found to be at higher risk during surgery, required more blood products during the transplant, and had longer postoperative hospital stays (Stephenson et al., 2001). Data from this study showed that nutrient repletion in patients with end stage liver disease before transplant surgery could help improve transplant outcomes. However, nutrient repletion in end-stage liver disease patients can be complicated especially since liver disease and kidney disease patients are limited in what they can and cannot eat. Attempting to replete protein losses may result in aggravating hepatic encephalopathy and increasing carbohydrates can lead to hyperglycemia (Hammad et al., 2017). While there are a multitude of factors controlling nutritional status in malnourished liver disease patients, it has been suggested that aggressive nutritional support consisting of sufficient intake of vitamins, dietary supplements, and trace minerals should be taken by all liver transplant patients pre-

transplant (DiCecco et. al., 1989). Establishing a healthy diet pre-transplant helps prevent malnutrition progression post-transplant.

A solid organ transplant recipient's post-transplant diet needs to focus on addressing malnutrition and micronutrient deficiencies and take precautions to prevent infection. The post-transplant diet is a short-term diet that must be re-evaluated after lost energy stores have been replenished. The post-transplant diet is replaced by a long-term diet that still focuses on preventing infection, but now aims to prevent chronic disease such as diabetes and obesity.

Weight gain can be an issue for many patients post-transplant due to increased freedom with their food and increased appetites due to new medications. This is dangerous for the patient because they are at an increased risk for metabolic abnormalities such as obesity, dyslipidemia and diabetes. Transplant medications may increase blood pressure and fluid retention, so salt should be limited to prevent high blood pressure. Fat should also be controlled to maintain a healthy weight and not put stress on the heart. All organ transplant recipients must consult with a dietitian to make sure they are eating properly to maintain a healthy weight and not put stress on their new organ. Eating a healthy, well balanced diet that includes carbohydrates, protein, and fats, and ensuring that foods are prepared properly with no raw or undercooked meat or fish will decrease the chances of complications and infections arising after transplant, promoting a healthy microbiome.

In terms of challenges and cost, tailoring a diet to one's individual needs requires a dietitian who is usually provided by hospitals as part of the transplant care team. Overall, altering one's diet is a low-cost method for altering the gut microbiome and can be done by the transplant patient themselves with a dietician's help. Consistently keeping a well-balanced diet is likely to

encourage a successful transplant and many health benefits like increased short chain fatty acid production as previously discussed.

Exercise:

The effect of exercise on the gut microbiota has been observed in human longitudinal studies. These studies show that exercise has an independent impact on microbiome function and composition. Consistent aerobic exercise was also seen to have a beneficial impact on the gut microbiome. However, long term high intensity aerobic training is likely required to see significant taxonomic and metagenomic changes in gut microbiota (Mailing et al., 2019). Exercise programs that are of the right intensity, frequency, and target the specific needs of an individual have been found to be beneficial for solid organ transplant patients.

Exercise is important post-transplant because it has been proven to uniquely change the composition of the gut microbiome independent of diet. In a twelve-week experiment, mice in the exercise group were found to have a unique microbiome where *Faecalibacterium*, *Clostridium*, and *Allobaculum* were all present (Yeager, 2019). *Faecalibacterium*, *Clostridium*, and *Allobaculum* were absent in the fecal samples collected from the mice who did not exercise. The study also found that despite eating a high fat diet, the mice who exercised did not experience inflammation in their intestines compared to the mice who did not exercise and did experience inflammation.

When the mice stopped exercising, the gut microbiome changed and reverted to the baseline composition. Similar results were seen in a human study in which lean, sedentary people enrolled in an exercise program for six weeks developed high levels of *Clostridiales*, *Lachnospira*, *Roseburia*, and *Faecalibacterium* in their gut increasing gut diversity and making

their microbiome unique (Yeager, 2019). These two studies corroborate the theory that exercise results in distinct gut microbiome changes.

Much remains unknown about the gut-exercise connection, but there are a couple hypotheses on the influence of exercise on the gut microbiome. One hypothesis is that during exercise, bile acid and lactate circulation increases resulting in a change in pH of the gut therefore shifting the composition of the gut microbiome (Yeager, 2019). Another idea is that exercise increases the number of microbes that produce butyrate, a short chain fatty acid that promotes homeostasis in gut microbiota. Exercise has also been thought to alter gene expression of immune cells in gut tissue resulting in the production of more anti-inflammatory cell-signaling proteins and antioxidant enzymes (Allen et al., 2018). The immune cells may promote growth of butyrate-producing bacteria and produce more antimicrobial compounds that regulate gut microbe composition. While these mechanisms are only theories as to how exercise impacts the gut, the many proposed mechanisms imply that there are multiple pathways that link exercise and gut health.

Many patients suffering from end-stage kidney disease, end-stage liver disease, and heart failure before organ transplant can participate in exercise training that positively impacts transplant outcomes in other ways besides diversifying the microbiome. One of the major impacts exercise has on patients pre-transplant, is that it makes patients stronger for surgery. End-stage kidney disease patients benefit from a variety of training programs including aerobic training, resistance training, and a combination of both. The American Society of Nephrology reported that, when compared to standard care, a 12-month program of exercise-based rehabilitation significantly slowed the rate of kidney function decline and improved cardio-respiratory fitness, making patients stronger for a transplant operation (ASN). Patients with

cirrhosis, the end stage of many liver diseases, have benefitted from exercise programs that target their specific needs and are of the right intensity and frequency as to not put stress on the body and liver. Exercise training in patients with stable heart failure have also resulted in positive outcomes such as fewer cardiac events and lower rates of hospital readmission due to heart failure (Piña et al., 2003).

Post-transplant, exercise plays an important role in maintaining a healthy lifestyle for transplant patients. In kidney transplant recipients, uremic sickness also known as hemolytic-uremic syndrome (HUS) is increasingly common post-transplant due to several risk factors that stem from kidney disease (Remuzzi & Ruggenenti, 1995). HUS can occur in patients who have never suffered from uremic syndrome before (de novo post-transplant HUS) or in patients whose end stage kidney failure was due to uremic syndrome (recurrent post-transplant HUS). De novo HUS is usually caused by either infections or immunosuppressive drugs such as calcineurin inhibitors and infections, while recurrent post-transplant HUS occurs in patients who usually have a genetic predisposition for the disease. Patients with uremic sickness are at an increased risk for cardiovascular events due to accelerated atherosclerosis, a disease in which fatty material is deposited on the inner walls of arteries (Rafieian-Kopaei et al., 2014). Accelerated atherosclerosis can occur in native arteries of a transplant recipient as well as in the arteries of a transplanted heart or kidney (Fellström et al., 1998). Cardiovascular events are a main cause of death in pre- and post-transplant patients (Romano, Lorenzon, & Montanaro, 2014).

Exercise has been found to be a key factor in reducing cardiovascular risk and increasing graft function. Multiple studies have found that physical training improves graft function, quality of life, and multiple factors related to increased cardiovascular risk such as glomerular filtration rate, arterial pressure, and homocysteine levels (Romano, Lorenzon, & Montanaro, 2014).

Furthermore, exercise increases cardiovascular function and oxygen delivery to the transplanted organ, thus improving graft function. Hypertension in kidney patients is reduced because of exercise decreasing arterial pressure augmentation (Romano, Lorenzon, & Montanaro, 2014). Levels of homocysteine, an amino acid related to higher prevalence of cardiovascular disease seen at elevated levels in kidney organ transplant recipients, are also significantly decreased with routine exercise (Romano, Lorenzon, & Montanaro, 2014). Anxiety and depression are reduced because of exercise which improves the patient's mental state and overall health.

The intensity and duration of exercise has been found to have an impact on health outcomes. High intensity interval training has been found to have better results in terms of maximum oxygen uptake and systolic blood pressure compared to moderate exercise. Stable heart transplant recipients benefited more from high intensity interval training than moderate training. The high intensity interval training group had a 17% increase in maximum oxygen uptake compared to the moderate exercise group which saw a 10% increase in maximum oxygen uptake (Dall et al., 2015). In addition, the systolic blood pressure of the high intensity interval training group decreased considerably compared to the moderate exercise group (Dall et al., 2015).

Consistent exercise post-transplant appears to be an important low cost, easy to implement, and effective therapy for stabilizing the microbiome and promoting whole-body health in all solid organ transplant patients. All exercise routines must be approved by a patient's transplant team and transplant patients need to be extra cautious about exercising outside, due to increased chances of skin cancer caused by immunosuppressive drugs.

Limitations on physical activity resulting from organ failure may prevent patients from using exercise to alter the composition of their gut microbiome pre-transplant. However, pre-

transplant exercise can still improve patient outcomes by affecting the mental state of the patient, improving cardio-respiratory fitness, and making the patient stronger for surgery, therefore increasing transplant success. Besides improving gut microbiome diversity, post-transplant exercise has many benefits for patients, including preventing chronic disease development such as inflammatory bowel disease, obesity and metabolic disease, and mental illness. Tailored exercise programs are necessary for all types of transplant patients to foster post-transplant health. Physicians must emphasize the importance of consistent exercise to transplant patients, empower the patient to exercise consistently by helping them to design a workout program, and inform the patient on how to exercise safely.

V. A Change in Perspective: A Sub-Specialized Transplant ICU

Caring for solid organ transplant patients pre- and post-transplant is demanding, complex, and requires a multidisciplinary approach. The rigorous management of solid organ transplant patients presents challenges to the healthcare team at every stage of the transplant process. Establishing a sub-specialized ICU with a collaborative leadership model to tend to the critical needs of solid organ transplant patients would allow for more collaborative multidisciplinary care and provide an environment to better understand the dynamics of the microbiota in the transplant population (Sakpal et al., 2018).

The need for a sub-specialized transplant critical care unit is evidenced by the increasing number of patients requiring ICU care before and after transplant surgery. In the pre-transplant process, numerous complications can arise that jeopardize the patient's eligibility to receive a lifesaving organ. While on the transplant waitlist, end stage kidney disease patients can experience renal failure that must be diagnosed and treated appropriately (Lata et al., 2016).

Patients suffering from end stage renal disease and who are on the renal transplant waitlist are at high risk of contracting catheter-related bloodstream infections, a common infection among patients receiving hemodialysis (Lata et al., 2016). There are many other complications that can occur in patients waiting to receive an organ donation that can result in them becoming ineligible for transplant. Thus, it is imperative to the patients to receive personalized care by multiple specialists who understand the patient's pathophysiology and can curate aggressive therapies to ensure their eligibility for solid organ transplant.

In addition to complications that can occur pre-transplant, the complications that get the most attention are the ones that arise post-transplant. Acute and chronic post-transplant complications arise more often than expected. Bacterial, viral, and fungal infections may be acute or chronic complications while malignancy, cardiovascular disease, chronic rejection, and post-transplant diabetes mellitus are common chronic complications. The incidence and severity of post-transplant complications requires a multidisciplinary approach to successfully attack these issues head on.

The sub-specialized transplant critical care units would have experienced specialists and an intensive care team to provide complex and interdisciplinary care. While many hospitals have surgical intensive care units, few have specific transplant surgical intensive care units that cater to only transplant patients. This is inadequate because, after transplant surgery, many patients' conditions require intense care and resources available in the ICU. In liver transplants, 45.7% of patients require immediate ICU stay after transplant surgery (Sakpal et al., 2018) and up at 35% return due to infection, graft failure, septic complications, and cardiopulmonary dysfunction (Sakpal et al., 2018). After kidney transplants, between 6.6% and up to 20% of patients needed to stay in the ICU after surgery. It's important to note that these statistics underestimate the use

of the ICU by solid organ transplant patients because they fail to account for patients who get admitted to the ICU with an acute illness and recover, those who pass away in the ICU without their status being upgraded, and those who are admitted to the ICU for a condition that causes them to be removed from the transplant wait-list (Sakpal et al., 2018).

The sub-specialized ICU would utilize a transplant and intensive care physician collaborative leadership model to provide multidisciplinary practices and processes for organ transplant patients. The sub-specialized ICU would operate on a closed ICU model as closed ICUs have been reported to have better evidence based management, more effective utilization of resources, fewer complications, lower mortality rates, and typically shorter ICU stays (Hanson et al., 1999). In a closed ICU model the intensivist, a physician who specializes in the pathophysiology of the critically ill and acts as the primary care physician of the ICU, is primarily responsible for the treatment and care the patient receives in the ICU.

The collaborative leadership model encourages collaborative efforts among physicians in different specialties and ICU physicians to curate therapies for transplant ICU patients. Collaborative efforts between specialists, including but not limited to, critical care, pulmonology, anesthesiology, nephrology, neurology, hematology, and gastroenterologists can improve the care of solid organ transplant patients. With our incomplete understanding of what is “normal” for the intestinal microbiome and how communities of microbes interact and play together, having a diverse care team, including gastroenterologists and neurologists, would likely improve patient outcomes. Social workers and palliative care specialists are also essential to help patients deal with the burdens of solid organ transplant and those who have exhausted treatment options.

Overall, the sub-specialized transplant critical care unit would work to ensure that patients remain eligible to receive organ transplants and care for the patient before and after their

surgery. Having an interdisciplinary team of specialists to coordinate therapies are treatment plans for patients would result in increased welfare of individual patients and understanding of modern surgical critical care for complex solid organ transplant patients (Wolffe, Hugenholtz, Wiersinga, 2018).

As the dynamics of the microbiota have yet to be fully understood, developing and determining what targeted therapies, such as synbiotic therapy, fecal microbial transplant, and short chain fatty acid supplementation are best to restore the microbiome is difficult. Even once the mechanism of how the gut microbiome modulates health in conditions of critical illness, individual differences must also be considered. A sub-specialized transplant ICUs in select hospitals provides collaborative multidisciplinary care that can focus on individual needs and treatments.

While this is the most expensive and demanding in terms of care potential solution for altering the gut microbiome and reducing allograft rejection in solid organ transplant patients, it may provide the most benefit in the long term if it is used as a space in which information on how to best restore the microbiota and understand the exact mechanism in which the gut microbiome effects transplant health can be studied. To do this, transplant patients in the sub-specialized ICU should be monitored over the course of their ICU stay and transplant procedure as well as in the years following. Due to the individual variations in gut flora, recording and mapping longitudinal changes in the microbiota for a specific patient may be more informative than making inter-individual comparisons (Rahim, Taylor, & Hirota., 2017). This information will help physicians understand which targeted approaches, such as the use of synbiotics, FMT, etc. are best for restoring the gut microbiota and promoting successful long term outcomes in transplant recipients.

Chapter 4: Conclusions and Final Recommendations

The human gut microbiome profoundly influences health and disease. This has been observed in solid organ transplant patients who experience gut dysbiosis due to their critical illness and many factors involved in the solid organ transplant process. Dysbiosis of the gut microbiome has been found to be a variable involved in influencing organ rejection, infection, and chronic disease development. In a world in which organs for transplant are limited and 3D bioprinted organs are still in early stages of development, increasing transplant patient outcomes is essential for maximizing the utility of the donated organ.

Throughout this thesis, factors that may negatively affect a patient's gut microbiome, from the time they are put on the organ waitlist to post-transplant recovery, were identified and recommendations were made on how to reduce their impact. In the pre-transplant phase, disruptions to the gut microbiome have mainly been associated with environmental changes and medications taken by patients. Environmental changes affect patients who require extended hospitalization prior to their transplant surgery. Many aspects of the hospital environment can have a role in microbiome disruption including encounters with multiple healthcare providers, limited exercise and diet, and exposure to antibiotic resistance bacteria. Reducing the amount of time patients spend in the hospital and bringing hospital level care to the patient's home whenever possible appears to be the best solution to this problem along with ensuring that the patient is engaging in exercise training which is known to promote microbiome health, decrease length of hospitalization, and improve surgical outcomes.

While most transplant candidates are not hospitalized for extended periods of time, all transplant patients are required to take medications for their critical illness. The composition of the gut microbiome has been found to be altered by many types of drugs such as the phosphate

binder lanthanum carbonate, taken by patients suffering from kidney failure, which decreases gut microbial diversity. The quality of care the organ donor receives is also influential in the recipient's outcome post-transplant and optimizing hormone replacement therapy in brain-dead organ donors has potential for improving transplant outcomes.

During the transplant surgery, the largest contributor to gut microbiome disruption identified was surgical stress. In many patients, gut flora can adapt to surgical stress. However, sometimes bacteria can sense host stress and environmental cues that results in gut bacteria becoming virulent and pathogenic. Developing minimally invasive surgical techniques through research focused on improving image quality, enabling 3D color visualization, and the invention of real-time tracking devices would improve minimally invasive surgery technology. Reduction of surgically induced stress can also be affected by the type of anesthetic used during the surgery.

In the post-transplant phase, immunosuppressant drugs and antibiotics are new factors introduced to the transplant patient that are known to alter gut microbiome composition. The use of non-absorbable antimicrobials has been found to have a negligible impact on the gut microbiome composition. In addition, microbiome profiling is a technique that can inform physicians on what type of antibiotics should be prescribed to an individual post-transplant based on individual genetic differences.

By identifying potential factors that affect the gut microbiome before, during, and after solid organ transplant, and making recommendations on how to reduce gut microbiome disruptions during these stages of solid organ transplant, efforts to improve transplant outcomes by decreasing gut microbiome disruption may focus on these suggested areas. Other potential pharmacological and nonpharmacological methods for resolving gut dysbiosis including fecal

microbiota transplant, short-chain fatty acid supplementation, synbiotic therapy, dietary and exercise changes, and the creation of a sub-specialized transplant ICU were analyzed for their potential success in altering the human gut microbiome, preventing organ rejection, and chronic disease development in transplant patients.

The analysis of five potential methods for restoring homeostasis to the microbiome suggests that as of now, there is not enough information to determine which of the proposed methods would be the most successful for liver, kidney, and heart transplant patients. In all the studies conducted using fecal microbial transplant, synbiotic therapy, and short chain fatty acid supplementation, researchers found that further study is required to determine if these treatments would be useful as a method of helping restore the microbiome to homeostasis and aid in preventing graft rejection and the development of chronic disease in transplant patients. In addition, more research must be conducted to determine which individuals would benefit from these potential therapies as it is likely that the effectiveness of these methods depends in part on the individual variation of the gut microbiome. The creation of a sub-specialized transplant ICU, a nonpharmacological intervention that was examined, is likely to help with this effort by providing an environment to better understand the dynamics of the microbiota in the transplant population while also providing collaborative care for patients.

As mentioned multiple times, one of the biggest obstacles to better understanding how the gut microbiome interacts with therapies is the large interpersonal variation of the gut microbiota. The unique differences in an individual's gut flora mean that a universal cure for restoring gut homeostasis in all individuals is unlikely. Diet and exercise adjustment is a low cost and safe nonpharmacological strategy for all patients, but patients may require other therapies, or a combination of therapies, depending on individual differences in microbial structure and the

various types of gut dysbiosis. While synbiotic therapy, fecal microbial transplant, and short chain fatty acid supplementation may each show success in altering gut microbe composition by themselves, the outcomes of these therapies would likely be aided by adherence to a well-balanced diet and routine exercise.

Both diet and exercise are known to have important roles in maintaining healthy gut microbiome compositions and are important for good gut health. Diet is heavily monitored throughout the entire transplant process and there are strict dietary guidelines instructing patients on what they should be eating before and after their transplant surgery. Kidney transplant patients on dialysis are restricted in their diets, liver transplant patients need adequate nutritional support as liver failure can cause malnutrition, and heart transplant patients must maintain a low sodium diet. After solid organ transplantation, the importance of maintaining a well-balanced diet is emphasized by physicians when they converse with patients about life post-transplant. However, exercise training guidelines are not well defined for transplant patients throughout the transplant process because of insufficient evidence to define specific exercise training guidelines for pre-transplant candidates.

While research shows that exercise is beneficial and safe for transplant patients before and after their transplant surgery, the inability to define specific guidelines on the frequency, intensity, and length of exercise programs negatively impacts patients who would benefit from understanding how they should be exercising and participating in an exercising training program pre-transplant. Physical therapists, who play an important role as part of the transplant team, are most involved after transplant as exercise training is emphasized in the post-transplant phase. Many people find it surprising to learn that kidney, liver, and heart transplant candidates can exercise before their transplant surgery and patients may be scared to exercise pre-transplant due

to fear that it will exacerbate illness and make their condition worse. Lack of information about pre-transplant exercise creates a need for pre-transplant exercise training guidelines that would communicate to patients that exercise pre-transplant is safe and allow patients to improve their transplant outcome by improving their exercise capacity, strength, mental health, and quality of life. More research on pre-transplant exercise is needed to create well defined guidelines for patients, but doing so could have great benefits for all patients.

As more evidence emerges demonstrating that the microbiome regulates the intestinal immune system and is involved in transplant success by playing a role in preventing acute and chronic rejection, infection, chronic disease development, and improving mental health, it is critical that gut microbiome health is prioritized throughout the transplant process. Thinking critically about how the microbiome is affected during the transplant process and taking steps to eliminate and minimize factors that disrupt gut flora as well as increasing research into how interpersonal gut microbiome variation affects therapies such as fecal microbial transplant, short-chain fatty acid supplementation, and synbiotic therapy is important for understanding how to resolve gut dysbiosis and potentially improve outcomes for transplant patients.

Other nonpharmacological interventions such as diet and exercise changes and the creation of a sub-specialized transplant ICU may have important roles in improving health and gut microbiome composition and increasing our understanding of the role the gut microbiome plays in transplant success respectively. Focusing research on exercise training pre-transplant will be beneficial in helping create guidelines for exercise training which communicates to patients that exercise pre-transplant is safe and may improve their surgical outcome by improving their exercise capacity, strength, mental health, and quality of life. Physical therapists who are already included in the transplant team can help take on this role and work with patients

before and after surgery to develop a manageable exercise training program for each individual patient. This is likely to help improve gut microbiome composition during the pre- and post-transplant phase for all transplant patients while fecal microbial transplant, synbiotic therapy, and short chain fatty acid supplementation are therapies that may only benefit specific individuals based on gut microbiome variation.

While there is a long way to go in understanding the gut microbiome's role in solid organ transplantation and how to manipulate it for long term transplant success and improved patient outcomes, the manipulation of the gut microbiome as an immune-modulating therapy should continue to be a focus in the field of solid organ transplantation.

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Biography

Krista Bangs is graduating from The University of Texas with degrees in Plan II Honors and Biochemistry. During her time in college, she was a part of the Texas 4000 for Cancer cycling team, a peer mentor and researcher in the Scott Stevens Vertebrate Interactome Mapping biochemistry research lab, a member of Texas Sweethearts, a Plan II Peer Mentor, and had the privilege of studying engineering global health in Barcelona, Spain. Krista plans on attending medical school after graduation.