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FECAL MICROBIOTA TRANSPLANTATION

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ACADEMIC DISSERTATION

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To my family

ABSTRACT

BACKGROUND

The impact of gut microbiota on human health has captivated lay people as well as researchers. The development of modern sequencing methods and their applications in microbiota research have promoted the discovery of complex microbiota communities in the human host. Fecal microbiota transplantation (FMT) is an effective treatment for recurrent *Clostridioides difficile* enteritis, and it has also been investigated for many other gut dysbiosis-related conditions.

AIMS

The aim of this dissertation is to investigate the efficacy of FMT in three different diseases and explore the potential of FMT in different clinical settings.

PATIENTS AND METHODS

This dissertation is built on four studies. The first (I) is a retrospective case series presenting patients treated with FMT and having significant comorbidities or patients who have been treated for conditions other than *Clostridioides difficile*.

The three subsequent studies (II–IV) are randomized controlled trials, in which FMT or placebo was given once by colonoscopy (II–III) or by gastroscopy (IV) for the treatment of irritable bowel syndrome (IBS) (II), for the maintenance of remission in patients with ulcerative colitis (III) or for obesity (IV). Additionally, obesity surgery was performed for patients with obesity half a year after FMT. Microbiota analysis was conducted on the subjects participating in the IBS trial before the FMT treatment and in different time points during the 18-month follow-up.

RESULTS

Fecal microbiota transplantation cured recurrent *Clostridioides difficile* enteritis in 11/13 patients with significant comorbidities. Other antibiotic-resistant bacteria, ESBL *Escherichia coli* and antibiotic-resistant *Salmonella*, were successfully eradicated from three patients. FMT slightly and transiently reduced irritable bowel symptoms 12 weeks after the treatment compared to the baseline, but there was no statistically significant difference between the treatment group and the placebo at the 12-week time point. However, the microbiota was changed to resemble that of the fecal donor. No advantage of FMT was found in ulcerative colitis or obesity patients compared to the placebo.

CONCLUSIONS

FMT is effective and safe for the treatment of recurrent *Clostridioides difficile* enteritis for patients with significant comorbidities. The FMT treatment regimens that were used in the clinical trials for IBS, quiescent ulcerative colitis and obesity were safe but ineffective for the treatment of these conditions. Nevertheless, FMT studies for indications beyond *Clostridioides difficile* enteritis should be continued. New research settings and developing methods for analyzing the microbiota continue to expand our knowledge on microbiota-host interactions and will likely lead toward improvements to FMT protocols and donor selection criteria, which may eventually facilitate the use of FMT or other bacteriotherapies in the treatment of new indications.

TIIVISTELMÄ

TAUSTA

Suoliston mikrobiston moninaiset vaikutukset ihmisen terveydelle ovat suuren kiinnostuksen ja lisääntyvän tutkimuksen kohde. Geenien sekvensointiin perustuvien tutkimusmenetelmien kehittyminen ja käyttö mikrobiston tutkimuksessa ovat edistäneet suoliston mikrobiston ja ihmisen välisten vuorovaikutusten selvittämistä. Ulosteensiirto on tehokkaaksi todettu ja yleisesti hyväksytty hoitomuoto toistuvan *Clostridioides difficile* infektion hoitoon. Ulosteensiirtoa tutkitaan monien muiden suoliston mikrobiston epätasapainoon liitettyjen sairauksien hoitoon.

TAVOITTEET

Väitöskirjan tavoitteena on selvittää ulosteensiirron tehoa kolmen eri sairauden hoidossa ja tutkia ulosteensiirron potentiaalia erilaisissa kliinisissä tilanteissa.

POTILAAT JA MENETELMÄT

Väitöskirja perustuu neljään osatyöhön, joista ensimmäisessä (I) kerättiin ulosteensiirrolla hoidettuja potilaita, joilla oli jokin immunitettä alentava perussairaus sekä sellaisia potilaita, joita oli hoidettu jonkin muun syyn kuin toistuvan *Clostridioides difficile* infektion vuoksi. Kolme jälkimmäistä osatyötä (II-IV) olivat satunnaistettuja lumekontrolloituja tutkimuksia, joissa potilaille annettiin tähystimen kautta ulosteensiirre tai omasta ulosteesta valmistettu lumesiirre joko paksusuolen tähystyksessä (II-III) tai mahalaukun tähystyksessä (IV) joko ärtyvän suolen oireyhtymän hoitoon (II), haavaisen paksusuolitulehduksen rauhallisen vaiheen, remission, ylläpitoon (III) tai lihavuuden hoitoon (IV). Lihavuudesta kärsiville potilaille tehtiin lisäksi lihavuusleikkaus puoli vuotta ulosteensiirron jälkeen. Ärtyvän suolen oireyhtymästä kärsiville potilaille tehtiin mikrobistoanalyysi ennen hoitoa ja sen jälkeen.

TULOKSET

Ulosteensiirto paransi toistuvan *Clostridioides difficile* suolitulehduksen 11/13 potilaalta, joilla oli vaikea liitännäissairaus. Kolmella potilaalla antibiooteille vastustuskykyisten bakteerien, ESBL *Escherichia colin* ja antibioottiresistentin *Salmonellan*, häätäminen suolistosta onnistui ulosteensiirrolla. Ärtävän suolen oireyhtymässä todettiin lievää ohimenevää oireiden vähenemistä ulosteensiirron jälkeen, muttei tilastollisesti merkitsevää eroa lumeryhmään verrattuna. Suoliston mikrobisto kuitenkin muuttui samankaltaiseksi kuin ulosteenuovuttajalla. Haavaista paksusuolitulehdusta sairastavilla potilailla ulosteensiirrosta ei ollut apua remission ylläpidossa lumeseen verrattuna, eikä myöskään lihavuuden hoidossa ulosteensiirto ollut annettua lumehoitoa tehokkaampi.

PÄÄTELMÄT

Ulosteensiirto on tehokas ja turvallinen hoitomuoto toistuvan *Clostridioides difficile* infektion hoitoon myös potilailla, joilla on muu vaikea sairaus. Ulosteensiirto kontrolloiduissa tutkimuksissa käyttämällämme menetelmällä ei ollut tehokas ärtävän suolen oireyhtymän, haavaisen koliitin remission ylläpidon tai lihavuuden hoidossa. Näissä ja monissa muissa tautiryhmissä ulosteensiirtoa kannattaa kuitenkin edelleen tutkia. Kehittyvät tutkimusasetelmat ja mikrobiston tutkimusmenetelmät tuovat lisätietoa mikrobiston ja terveyden yhteyksistä ja edesauttavat ulosteensiirtohoitoon kehittämistä esimerkiksi luovuttajien valinnan osalta, ja todennäköisesti johtavat lopulta ulosteensiirron tai siitä edelleen kehitettyjen mikrobiterapioiden käyttöönottoon myös uusissa tautiryhmissä.

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ORIGINAL PUBLICATIONS

This thesis is based on the four following studies, and in this text, they are referred to by their Roman number:

- I Faecal microbiota transplantation in patients with *Clostridium difficile* and significant comorbidities as well as in patients with new indications: A case series. Lahtinen P, Mattila E, Anttila VJ, Tillonen J, Teittinen M, Nevalainen P, Salminen S, Satokari R, Arkkila P. *World J Gastroenterol*. 2017 Oct 21;23(39):7174-7184. doi: 10.3748/wjg.v23.i39.7174
- II Randomised clinical trial: faecal microbiota transplantation versus autologous placebo administered *via* colonoscopy in irritable bowel syndrome. Lahtinen P, Jalanka J, Hartikainen A, Mattila E, Hillilä M, Punkkinen J, Koskenpato J, Anttila VJ, Tillonen J, Satokari R, Arkkila P. *Aliment Pharmacol Ther*. 2020 Jun;51(12):1321-1331. doi: 10.1111/apt.15740. Epub 2020 Apr 28
- III Fecal microbiota transplantation for the maintenance of remission in patients with ulcerative colitis: A randomized controlled trial. Lahtinen P, Jalanka J, Mattila E, Tillonen J, Bergman P, Satokari R, Arkkila P. Published online May 7, 2023. doi: 10.3748/wjg.v29.i17.2666
- IV Effectiveness of Fecal Microbiota Transplantation for Weight Loss in Patients With Obesity Undergoing Bariatric Surgery: A Randomized Clinical Trial. Lahtinen P, Juuti A, Luostarinen M, Niskanen L, Liukkonen T, Tillonen J, Kössi J, Ilvesmäki V, Viljakka M, Satokari R, Arkkila P. *JAMA Netw Open*. 2022 Dec 1;5(12):e2247226. doi: 10.1001/jamanetworkopen.2022.47226.

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ABBREVIATIONS

AE:	Adverse event
AGA:	American gastroenterology society
BAI:	Beck Anxiety Inventory
BDI:	Beck Depression Inventory
BIA:	Bioelectrical impedance analysis
BMI:	Body mass index (kg/m ²)
CDI:	<i>Clostridioides difficile</i> infection
DM1:	Type 1 diabetes
%EBMIL:	Percentage of excess BMI loss
ESBL:	Extended-spectrum beta-lactamase
FDA:	Federal Drug Agency (USA)
Fimea:	The national competent authority for regulating pharmaceuticals and blood and tissue products
FMT:	Fecal microbiota transplantation
FODMAP:	Fermentable Oligo-, Di-, Monosaccharides and Polyols
GABA:	Gamma-aminobutyric acid
GLP-1:	Glucagon-like peptide 1
GVHD:	Graft versus host disease
HMP:	Human Microbiome Project
IBD:	Inflammatory bowel disease
IBDQ:	Inflammatory bowel disease quality of life questionnaire, a disease specific assessment.
IBS:	Irritable bowel syndrome
IBS-SSS:	Irritable bowel syndrome symptom severity scale
IBS-QoL:	Disease-specific quality of life questionnaire for irritable bowel syndrome patients
IgA:	Immunoglobulin A
LPS:	Lipopolysaccharides
MAM:	Microbial anti-inflammatory molecule
OMV:	Outer membrane vesicle
PYY:	Peptide YY
rCDI:	Recurrent <i>Clostridioides difficile</i> infection
rRNA:	Ribosomal ribonucleic acid
SCFA:	Short-chain fatty acid
SIBO:	Small intestinal bacterial overgrowth
TMAU:	Trimethylaminuria
%TWL:	Percentage of total weight loss

UC:	Ulcerative colitis
UHGG:	Unified human gastrointestinal genome
UHGP:	Unified human gastrointestinal protein
VLCD:	Very low calorie diet
QoL:	Quality of life
15D:	15-Dimensions general quality of life questionnaire
5-ASA:	5-Aminosalicylic acid

1. INTRODUCTION

In recent decades, we have witnessed a reduction in the prevalence of many infectious diseases and a concurrent rise in noninfectious diseases such as allergies, asthma, type I diabetes, and inflammatory bowel disease (IBD) ¹. Additionally, metabolic syndrome, type II diabetes, and obesity are becoming increasingly prevalent ^{2,3}. The process is in its earlier phase in developing countries compared to urbanized countries with higher income ⁴.

The development of vaccines⁵ as well as the invention of antibiotics during and after the Second World War has enabled the cure of many lethal infections and has increased life expectancy. Simultaneously, changes in living environment and nutrition have decreased exposure to pathogens. According to the biodiversity hypothesis, decreased diversity in the microbial environment surrounding us impairs the development and homeostasis of normal immunity and metabolic functioning ^{6,7}. The increase in noncommunicable inflammatory diseases in urbanized areas with less diversity of nature and a poor microbial environment is in line with the biodiversity hypothesis. Academics in Finland have suggested adopting the relationship between the microbial environment and human health as a central theme of research ⁸.

While providing undeniable health benefits, the use of antibiotic treatments has brought about the problems of antibiotic-resistant bacteria and *Clostridioides difficile*, a pathogen causing antibiotic-related diarrhea and epidemics in health care settings. During the past decade, fecal microbiota transplantation (FMT) has been found to be a viable option to treat *Clostridioides difficile* infection (CDI) ⁹. Although FMT is currently accepted as a routine treatment for recurrent *Clostridioides difficile* infection (rCDI), there is still room for further development to find optimal protocols, patient groups and timing of the treatment. The excellent results in rCDI patients have encouraged investigators to study FMT in many noncommunicable diseases that are associated with altered gut microbiota or dysbiosis ¹⁰. Dysbiosis is a controversial term because a healthy individual may harbor an aberrant gut microbiota and vice versa, but it is used in the lack of a more precise term and incomplete understanding of what constitutes a healthy microbiota ¹¹.

In this dissertation, I present the results of four studies investigating FMT for different conditions. Three of the studies were randomized controlled trials investigating FMT for irritable bowel syndrome (IBS), ulcerative colitis (UC), and obesity. One of the four studies is a retrospective case series presenting patients with rCDI and comorbidities as well as patients with other conditions. FMT as a therapeutic method is reviewed in terms of rCDI, IBS, UC and

1. INTRODUCTION

obesity. Finally, FMT is discussed both as a treatment method by renewing gut microbiota after perturbation and as a method to explore the myriad interactions between gut microbiota and the host.

2. REVIEW OF THE LITERATURE

2.1. OVERVIEW OF GASTROINTESTINAL MICROBIOTA

2.1.1 THE COMPOSITION OF GASTROINTESTINAL MICROBIOTA

Our living environment is covered with microbes, as is the human body. Microbes constitute complex communities of interacting microbes, and these microbiomes are distinctive to the location they grow in¹². The healthy human microbiome consists of more than 30 trillion (30×10^{18}) microorganisms, including bacteria, viruses, yeast, and protozoa. The number of human cells in the body is close to the number of bacteria carried by an individual¹³. However, the gut metagenome, i.e., the genetic code of all the microbes in the human intestine combined vastly outnumbers the genetic code of the host¹⁴. The individual microbiome of each individual originates from the mother at childbirth¹⁵. From then on, the microbiome develops as a result of diet, environmental factors, and genetics simultaneously affecting the development of the individual, especially their immune system^{16,17}. The mature gut microbiota is unique to each individual and has a tendency to remain stable over time¹⁸; however, different microbial exposures impact the gut microbiota throughout life, and the extent to which the gut microbiota may be altered remains to be explored.

The current knowledge on the structure of the gut microbiome is mostly established through the development of sequencing techniques and two large projects mapping the microbiome: the Human Microbiome Project (HMP)¹⁹ and METagenomics of the Human Intestinal Tract (MetaHIT)²⁰, which identified 2172 bacterial species in humans. The species were classified into 12 different phyla. Four main phyla, Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes, comprised 93.5% of the identified species, whereas three of the 12 phyla contained only one species. As an example, *Akkermansia muciniphila* is the only known member of the Verrucomicrobia phyla in humans. Of note, this bacteria draws much attention due to its potential and documented health benefits.²¹ Of the 2172 bacterial species identified from different body sites of humans, 18% are strictly anaerobic²², and given the anaerobic conditions of the colon, the majority of fecal bacteria are anaerobes²³.

The vast majority of human microbes reside in the gastrointestinal tract. The oral cavity, including the teeth, tonsils, and tongue, harbors microbiomes distinctive to each surface. Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Spirochaetes, and Fusobacteria are the six major phyla in the oral cavity, comprising 96% of oral microbes. The predominant genera in saliva are *Gemella*, *Veillonella*, *Neisseria*, *Fusobacterium*, *Streptococcus*, *Prevotella*, *Pseudomonas*, and *Actinomyces*^{24,25}. The oral microbiota may be overlooked; however, oral microbiota assessment may assist in predicting disease in the future. Rheumatoid arthritis patients, even in early onset, have a distinct oral bacteriome abundant with *Prevotella pleuritidis*, *Treponema denticola*, *Porphyromonas endodontalis*, and *Filifactor alocis* species²⁶. Food mixed with saliva travels rather rapidly from the mouth to the stomach; thus, there is a low number of microbes living on the esophageal epithelium. The predominant phylum of the esophageal epithelium is Firmicutes, and the most abundant genus is *Streptococcus*²⁴.

The stomach secretes pH-lowering gastric acid and proteolytic enzymes, providing an environment that inhibits the growth of many microbes. Despite the lower number of microbes, the stomach has a distinct microbiota, and common taxa include *Bacillales incertae sedis*, *Streptococcaceae*, *Enterobacteriaceae*, *Leptotrichiaceae*, *Veillonellaceae*, and *Pseudomonadaceae*. The stomach epithelium is also the site in which *Helicobacter pylori* may be found. Approximately 4.4 billion individuals were estimated to carry *H. pylori* infection worldwide in 2015²⁷. The carriers of *H. pylori* also tend to have a higher abundance of Proteobacteria and overall lower alpha diversity in the stomach compared to noncarriers²⁴.

Human beings can survive without other parts of the gastrointestinal track but not without the small bowel. Due to the villous architecture of the epithelium, the surface area for nutrient absorption and immunological reactions is enormous in the small intestine. The small bowel is composed of the duodenum, jejunum, and ileum. In the duodenum, pancreatic fluids, bile acids, rapid bowel transit, and oxygen impact the microbiota and reduce the bacterial density and diversity. Firmicutes and Actinobacteria are the predominant phyla in the duodenum. The bacterial density and diversity increase along the jejunum, presenting mostly gram-positive aerobes and facultative anaerobes, including *lactobacilli*, *enterococci*, and *streptococci*. In the ileum, from the proximal part to the distal region, the bacterial density rises, the oxygen pressure decreases, and anaerobes and gram-negative organisms typical of the colon begin to predominate^{24,25}.

The large bowel functions as fecal storage and simultaneously serves as the main reservoir of the human microbiota, an assembly of microorganisms unique to each individual and rather stable over time, although it is affected by diet and

distinctive to the living environment ¹⁶. Complex undigested foodstuffs feed the abundant and diverse colonic microbiota. The predominant colonic bacterial phyla are Bacteroidetes, Firmicutes, Verrucomicrobia, Proteobacteria, and Actinobacteria, with a few differences in composition between the ascending and descending colon. Typical families from the colon are *Clostridiaceae*, *Lachnospiraceae*, and *Bacteroidaceae*, which include anaerobic species ²⁴.

The epithelium of the colon is covered with a mucus layer consisting of two parts. The inner mucus layer is dense and practically impermeable to bacteria. The outer mucus layer is loose and colonized by microbes. In addition to the mucus layer, the colonization of the epithelium by microbes is restricted by antimicrobial agents secreted by the epithelial cells. Another innate defense mechanism involves dendritic cells as they take up bacteria from the bowel lumen, migrate to the mesenteric lymph node, and induce B cells to secrete immunoglobulin A (IgA), which is further secreted to the epithelial surface, where it inhibits the association of bacteria with the epithelium ²⁵.

The outer mucus layer is colonized by numerous microbes, such as *Akkermansia*, *Ruminococcus*, and some *Bacteroides* species. Microbes that prioritize dietary starches and nutrients rather than mucins as their nutrient source reside within the colonic lumen. In addition, the oxygen content decreases from the epithelium to the lumen as well as from the cecum to the rectum, which also has an impact on the microbiota composition. Certain bacteria may be found in the large bowel of almost every human being. The core bacterial genera that are shared by all adults are *Bacteroides*, *Eubacterium*, *Faecalibacterium*, *Alistipes*, *Ruminococcus*, *Clostridium*, *Roseburia*, and *Blautia*, and the top three species are *Faecalibacterium prausnitzii*, *Oscillospira guillermontii*, and *Ruminococcus obeum* ^{24,28}.

2.1.2 FUNCTIONS OF THE GASTROINTESTINAL MICROBIOTA

Although the composition of the microbiome greatly varies between individuals, different microbiomes may execute similar functions. Microbes exert their function, e.g., by secreting metabolites, competing with each other and thereby providing colonization resistance, and exerting immunomodulative effects by various mechanisms and molecules, including both structural components of their cells and metabolites.

Some bacterial metabolites can act as signaling molecules for interbacterial communication. In a process known as quorum sensing or quorum signaling, bacteria respond to changes in microbiota by secreting molecules that affect the growth or function of certain microbes ²⁹. The microbiome may also act directly by secreting microbial neuromodulators such as gamma-aminobutyric

acid (GABA), which has an influence on the enteric or central nervous system³⁰. Through microbial-derived immunomodulators such as histamine, the microbiome has the ability to interact with intestinal immune cells³¹. Other examples of microbial-derived immunomodulators are serpins, protease inhibitors that suppress inflammatory responses by inhibiting elastase activity, and lactocepins, which are bacterial enzymes with the ability to degrade proinflammatory signals²⁵.

Intestinal microbes breakdown complex carbohydrates, producing short-chain fatty acids (SCFAs) such as acetate, butyrate, and propionate. SCFAs have a large variety of functions; they nourish epithelial cells and affect mucus production but also regulate many local and systemic metabolic pathways. Butyrate, for example, is characterized as an essential energy source for colon epithelial cells and contributes to their proliferation and maintenance of the gut barrier. A more recent discovery is the role of butyrate in contributing to anaerobic conditions near the epithelium by communicating with colonic cells, thus inhibiting the more proinflammatory facultative anaerobes from entering from the colonic lumen in close proximity with the epithelium. SCFAs support the innate immune system by stimulating antimicrobial molecules, and they also contribute to gut motility and transit^{32,33}.

SCFAs impact metabolic health *via* several mechanisms. Propionate and butyrate bind epithelial receptors, inducing the production of gut hormones that affect satiety and glucose homeostasis, i.e., peptide YY (PYY) and glucagon-like peptide 1 (GLP-1). Butyrate activates intestinal gluconeogenesis, and released glucose initiates neural signaling by portal nerves to the brain, generating satiety and increasing insulin sensitivity. Gut-derived SCFAs affect the metabolism of peripheral tissues *via* circulation, and acetate and propionate may contribute to intracellular lipolysis of adipose tissue. SCFAs may inhibit the secretion of proinflammatory cytokines and chemokines and thus reduce macrophage infiltration. SCFAs may increase thermogenesis and browning in adipose tissue. SCFAs enhance lipid oxidation and decrease inflammation in the liver. SCFAs may even improve β -cell function and affect glucose-stimulated insulin secretion³³. Through their effects on epithelial integrity and immunity, SCFAs also play a role in inflammatory bowel disease (IBD), and dysbiosis in IBD is characterized by decreased proportion of butyrate-producing bacteria³⁴. SCFAs and other byproducts of bacterial fermentation may be harvested and used for energy by the host, thus enabling the utilization of otherwise inaccessible nutrients. Increased energy supply *via* this mechanism may contribute to obesity³⁵.

Selected bacteria secrete several vitamins: biotin (B7), cobalamin (B12), folate (B9), niacin (B3), pantothenic acid (B5), pyridoxine (B6), and riboflavin (B2), which may contribute to the micronutrient supply of the host²⁵. An additional

communication route for bacteria is outer membrane vesicles (OMVs), which can carry versatile cargos of effector molecules, penetrate the mucus layer, and interact with the epithelium as well as cells of the innate and adaptive immune systems ^{25,36}.

In addition to bacterial metabolites, bacterial structures may execute functions such as escalating or inhibiting inflammation. Lipopolysaccharides (LPS) are an example of proinflammatory bacterial structures. LPSs are large molecules found on the outer membrane of gram-negative bacteria. A higher abundance of LPS-producing proteobacteria is associated with inflammatory conditions such as IBD ³⁷ and metabolic syndrome ³⁸.

An example of a bacterial structure associated with health benefits is microbial anti-inflammatory molecule (MAM) of *Faecalibacterium prausnitzii* and polysaccharide A of *Bacteroides fragilis*, which both have anti-inflammatory properties and have ameliorated colitis in animal models ³⁹.

A balanced microbial community has the ability to resist pathogen colonization. This phenomenon is known as colonization resistance, and it entails indirect as well as host-mediated inhibitory mechanisms. The host can, for example, facilitate microbial production of antimicrobial sulfides by modulating bile acid secretion ⁴⁰. Colonization resistance was first demonstrated in animal models, in which injuring the gut microbiota with antibiotics was associated with increased susceptibility to Salmonella infection ⁴¹. In humans, a well-defined example of failed colonization resistance is *Clostridioides difficile* infection, which affects antibiotic-treated patients or patients with perturbed microbiota for other reasons ⁴².

The diet is a central modulator of the gut microbiota and its functions. Certain poorly absorbed carbohydrates are prone to induce functional gastrointestinal symptoms, and these short-chain carbohydrates are collectively termed FODMAPs (Fermentable Oligo-, Di-, Monosaccharides And Polyols) ⁴³. Carbohydrate fermentation by intestinal bacteria results in gas production, and in FODMAPs it is associated with gastro-intestinal symptoms in a proportion of people. Thus, a low-FODMAP diet may be used to control symptoms of IBS ⁴⁴. A low-FODMAP diet decreases gut bifidobacterial and overall bacterial count as well as gas production ⁴⁵. However, a regular supply of FODMAPs may enhance the tolerance of the gut microbiota to these compounds. For example, lactose feeding increases the proportion of bacteria capable of hydrolyzing lactose, which may alleviate symptoms caused by excessive lactose consumption ⁴⁶.

2.1.3 THE RESEARCH METHODS OF MICROBIOTA

Before the advent of novel sequencing methods, knowledge of the gut microbiota was based on labor-intensive cultivation methods. A large portion of microbes cannot be cultured, and the availability of fast and low-cost sequencing methods has started to expand the knowledge of the gut microbiota ²².

A common sequencing method to study the gut microbiome has been targeting the bacterial 16S ribosomal ribonucleic acid (rRNA) gene. This gene is found in all bacteria and archaea and contains nine highly variable regions (V1–V9), allowing species to be distinguished. Traditionally, species identification by 16S rRNA gene sequencing relied on sequencing the entire gene from individual species obtained in pure cultures. In microbiota profiling, 16S rRNA sequencing is conducted as massively parallel high-throughput sequencing, where instead of the entire 16S rRNA gene, a shorter subregion of the gene is sequenced in greater depth and from the whole population consisting of different species. The utilization of shorter read lengths usually reduces resolution (i.e., the capacity to distinguish bacteria at the species or even at the genus level) but provides a comprehensive overall picture of the microbiota composition ²². In contrast, shotgun metagenomic sequencing aims to produce genomic information of the whole microbial population and all its genes and can provide more in-depth knowledge of microbiota due to the possibility of studying potential functions of the microbiota by studying its overall genetic capacity ⁴⁷. Thus far, the use of metagenomic sequencing in studying microbiomes has been restricted by its higher price compared to 16S rRNA gene sequencing. As prices have decreased, metagenomic sequencing methods have become more accessible to research groups ²².

Comprehensive reference genomes are essential for functional characterization and taxonomic assignment of the human gut microbiota. A catalog with 9 879 896 human gut microbial genes was published in 2014 ⁴⁸. Recently, 204,938 genomes and 170,602,708 genes were compiled and analyzed from human gut microbiome datasets to generate the Unified Human Gastrointestinal Genome (UHGG) and Protein (UHGP) catalogs ⁴⁹. Additionally, catalogs of the genes of gut viruses are being developed, which will expedite the understanding of the vast gut phageome ⁵⁰.

2.2 FECAL MICROBIOTA TRANSPLANTATION

Fecal microbiota transplantation (FMT) refers to inserting living, feces-derived microbiota into the gastrointestinal track of a recipient to heal or alleviate dysbiosis-associated conditions ⁵¹.

The first appearance of FMT in written history is from China, where Doctor Ge Hong (284-364) presented the oral administration of fecal suspension for food poisoning or severe diarrhea in his emergency therapy handbook “Handy therapy for emergencies”⁵². In the 16th century, Li Shizhen described the use of fermented and fresh fecal solutions as well as dry fecal products and infant feces for severe diarrhea, fever, pain and constipation⁵². The first report of FMT in the context of modern medicine appeared in 1958 as gastrosurgeon Ben Eiseman and his colleagues published a series of four patients with pseudomembranous colitis who were treated successfully with fecal enemas⁵³. Pseudomembranous colitis is a severe form of *Clostridioides difficile* infection.

2.2.1 CURRENT METHODS OF FECAL MICROBIOTA TRANSPLANTATION

FMT may be administered as capsules or *via* colonoscopy, gastroscopy, naso-enteral tube, or rectal enema. Although FMT as orally consumed capsules would be the preferred method of administration for patients, FMT capsules are not yet available in Finland. The results of treating rCDI with FMT capsules have been good, with cure rates of 85% to 93% depending on the duration of the therapy^{54,55}.

In Finland, FMT is mainly administered *via* colonoscopy, and the transplants are produced according to an established protocol⁵⁶. Approximately 30 g of donor feces was mixed with saline to a total volume of 150 mL, and glycerol was added as a cryoprotectant to a total concentration of 10%. The ready transplants were then stored at -80 °C. The donor brings the donation to the laboratory within a maximum of two hours after evacuating. All phases of the process are performed as rapidly as possible to avoid excess oxygen exposure.

2.2.2 FECAL DONORS

Analogous to blood and tissue donation, fecal donation should be voluntary, and the donor may not benefit financially. However, reimbursement to surpass the costs of travel and invested time may be appropriate⁵⁷. The donors are expected to be physically and mentally healthy with normal body weight. Potential donors are interviewed systematically to assess the risk of contagious diseases, and a comprehensive set of laboratory tests is obtained to exclude infectious as well as noninfectious diseases (Table 1). All laboratory examinations that are tested prior to donation are taken again after three months or earlier. At each donation time, the donor is reviewed for any acute gastrointestinal symptoms, signs of infection, medications, and risk behaviors⁵⁷. Precise detectability of each

transplant for the donor and donation time is mandatory to ensure traceability to allow rescreening of the transplant in case of an infectious complication. However, the identity of the fecal bank donor may not be revealed to the recipient. The COVID-19 epidemic has highlighted the constant evolution of infectious diseases, which is essential to acknowledge by fecal banks recruiting donors. The impact of the COVID-19 pandemic on FMT services was recently addressed by an expert panel ⁵⁸.

Finding a donor with an optimal gut microbiota would be desirable; however, we do not yet know how to define an optimal microbiota and which components of stool induce the desired effects on the host. Thus far, we have had to choose donors by external features and lifestyle factors. The diversity of the factors impacting the success of FMT is illustrated in Figure 1.

Table 1. Laboratory tests to screen fecal donors ^{51,58}

	Laboratory test	Method
Stool pathogens	Common fecal pathogens: <i>Salmonella</i> , <i>Shigella</i> , <i>Campylobacter</i> , <i>Yersinia enterocolitica/pseudotuberculosis</i> and diarrhea causing <i>E. coli</i> -strains	PCR
	<i>Clostridioides difficile</i>	PCR
	<i>Helicobacter pylori</i>	EIA
	Norovirus, SARS-CoV-2	PCR
	Parasites: <i>Giardia lamblia</i> , <i>Cryptosporidium spp. (C. parvum or C. hominis)</i> , <i>Entamoeba histolytica</i> , <i>Dientamoeba fragilis</i> .	PCR
	Ova and parasites	Light microscopy
	Multi drug resistant bacteria: VRE, CPE, ESBL, multiresistant <i>Pseudomonas Aeruginosa</i> , multiresistant <i>Acinetobacter</i>	Culture and PCR
Serum pathogens	<i>Treponema pallidum</i>	EIA
	HIV 1 and HIV 2	Anti-HIV antibodies by EIA
	Hepatitis B	HBV surface and core antigens
	Hepatitis C	Anti-HCV antibodies by EIA
	Hepatitis A	Anti-HAV IgM antibodies
	Hepatitis E	Anti-HEV IgM and IgG antibodies
	Cytomegalovirus	PCR
	Epstein-Barr virus	PCR

	Laboratory test	Method
Serum general	Albumin	
	Creatinine	
	Sodium	
	Potassium	
	Glucose	
	Lipids (Cholesterol, LDL, HDL, TG)	
Stool general	Occult blood	
	Calprotectine	
Perineum	MRSA	Culture and PCR

EIA, enzyme immunoassay; PCR, polymerase chain reaction; VRE, vancomycin-resistant *enterococcus*; CPE, carbapenemase-producing enterobacteria; ESBL, extended-spectrum beta-lactamase (producing bacteria); LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglycerides; HIV, human immunodeficiency virus; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; MRSA, meticillin-resistant *Stafylococcus aureus*.

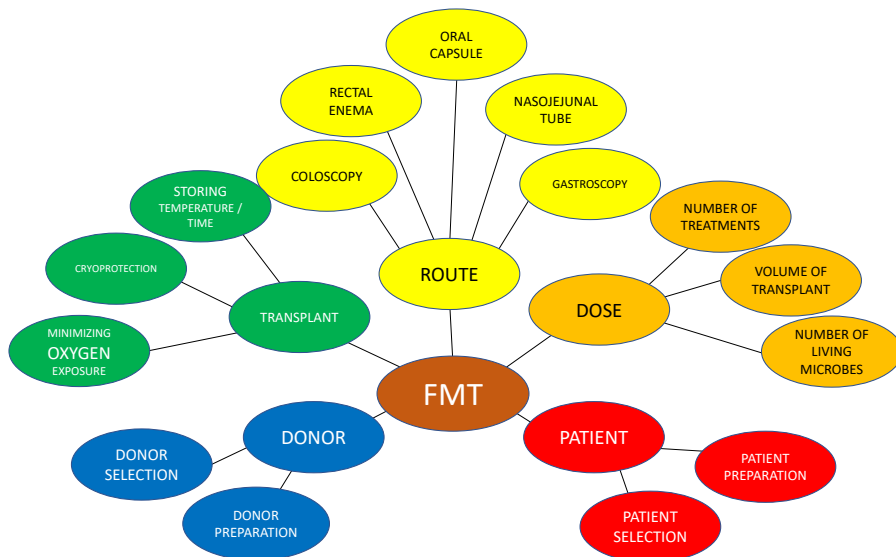


Figure 1. Factors affecting the success of fecal microbiota transplantation (FMT)

2.2.3 REGULATION OF FECAL MICROBIOTA TRANSPLANTATION

Despite arguments by FMT-providing experts, FMT is considered a drug and biologic agent by the Federal Drug Agency (FDA) in the USA, although investigational new drug requirements are not necessary when FMT is given for *Clostridium difficile* infection that is unresponsive to standard treatments^{59,60}. Europe-wide regulation for FMT is lacking; however, experts in the field have joined forces to formulate European best practice guidance in tune with the European Union Tissue and Cells Directive⁶¹⁻⁶³. Although FMT regulation between European countries varies, the centers providing FMT in Europe operate with high standards; however, the FMT availability for rCDI patients remains inadequate and varies across regions⁶⁴. In Finland, Fimea, the national competent authority for regulating pharmaceuticals and blood and tissue products, has stated that stool is not a drug, and FMT does not fall under their regulation. Therefore, FMT does not have specific regulations in Finland thus far and is therefore governed by the existing rules and regulations affecting health care.

2.3 CLOSTRIDIODES DIFFICILE INFECTION

Clostridioides difficile is a gram-positive, anaerobic bacillus. Approximately 5% of the adult population is colonized by *C. difficile*, and the bacillus is abundant in the environment, especially in health care settings. The ability to form resilient spores makes it more difficult to eradicate these bacteria from the human intestine as well as from hospital surfaces. The main risk factors for *C. difficile* infection are antibiotic exposure, older age, and hospitalization. Antibiotics, especially those with a wide spectrum, devastate the microbiota, leading to weakened colonization resistance against pathogens. Additionally, old age is associated with reduced diversity and weaker colonization resistance capacity of microbiota. Other defined risk factors include inflammatory bowel disease, gastrointestinal surgery, immunodeficiency caused by malignancies, organ transplants, chronic kidney disease, or immunosuppressive medication⁶⁵. The spores formed by *Clostridioides difficile* are resistant to heat, acid, and antibiotics, and they are transmitted by the fecal-oral route. *C. difficile* is not an invasive pathogen and seldom leads to extraintestinal manifestations. The virulence of *C. difficile* is caused by enzymes and toxins (A and B) that damage the epithelial cell cytoskeleton, disrupt tight junctions, and cause inflammation. The symptoms of CDI vary from mild diarrhea to severe pseudomembranous colitis and death^{66,67}.

2.3.1 TREATMENT OF *CLOSTRIDIODES DIFFICILE* INFECTION

Milder forms of CDI resolve a few days after withdrawing the predisposing antibiotic. Initial options for the treatment of CDI are antibiotics: metronidazole, vancomycin or fidaxomicin, which in high-risk patients may be combined with bezlotoxumab, a monoclonal antibody against toxin B secreted by *C. difficile*^{68,69}. A proportion of patients remain resistant to medical treatments or relapse after every medical intervention. The most effective treatment after failure of medical treatments is FMT⁷⁰.

During the past decade, FMT has become an accepted and recommended therapy for rCDI. In 2012, a Finnish observational cohort study demonstrated over 90% efficacy of FMT for rCDI⁷¹. Since 2012, the superiority of FMT over antibiotics has been demonstrated in controlled trials^{55,72,73}. In 2017, a meta-analysis was published that included 37 studies of which 7 RCTs had a mean pooled overall response for FMT in rCDI of 92%. The studies included 1973 patients, with 428 patients enrolled in RCTs⁹. FMT is approved as a viable therapy for rCDI by expert statements and guidelines in Europe and America^{51,61,74-77}. Currently, FMT is a recommended treatment option for rCDI after two relapses⁷⁰.

FMT was recently investigated after the first or the second CDI episode in a controlled trial by a Danish group⁷⁸. This trial was halted prematurely after an interim analysis due to significantly lower rates of symptom resolution in the placebo group (33% vs. 90% in the FMT group) at two months after the intervention. All 42 patients received a course of vancomycin preceding FMT or placebo.

Approximately 10% of patients treated with FMT for rCDI relapse within three months⁷⁹. Antibiotic usage, IBD, and poor bowel lavage are predictors of FMT failure. Patients with rCDI who relapse after FMT may be managed with antibiotics and/or repeat FMT⁷⁹.

FMT has been successfully used for rCDI patients with comorbidities, such as IBD⁸⁰, liver cirrhosis⁸¹ and immunocompromised patients⁸²⁻⁸⁴. In severe CDI and when refractory to antibiotics, FMT in combination with *C. difficile* targeted antibiotics has shown good efficacy, which further increases with repeated FMT treatments⁸⁵. FMT restores a balanced gut microbiome and its functionality. The action modalities of FMT in treating CDI include direct competition of *C. difficile* with delivered commensals but also recovery of secondary bile acid metabolism and restoration of the intestinal barrier by influencing the mucosal immune system⁸⁶.

2.4 IRRITABLE BOWEL SYNDROME

Irritable bowel syndrome (IBS) affects approximately 10% of the world's population, although estimations of the prevalence of IBS vary depending on the population studied and the applied diagnostic criteria^{87,88}. The diagnostic criteria according to the Rome foundation (Rome III–IV) divides the IBS phenotypes according to the dominant symptom: diarrhea predominant, constipation predominant, mixed and unsubtyped IBS⁸⁹.

The etiology of IBS is unknown. Many possible etiological factors have been associated with IBS: altered gut motility, epithelial hyperpermeability, low-grade inflammation, visceral hypersensitivity, genetics, disturbances of gut-brain interaction, psychological stressors, and altered gut microbiota⁸⁷. IBS patients have an increased risk of suffering from depression and anxiety compared to the general population⁹⁰. Psychological factors may contribute to the IBS symptoms for a proportion of IBS patients⁹¹.

The gut microbiota may be involved in the pathogenesis of IBS in multiple ways⁹². Many pieces of evidence suggest that the gut microbiota is an important etiological factor in IBS. The risk of IBS increases after infectious gastroenteritis^{93,94}. There are differences between the gut microbiota composition of IBS patients and healthy controls^{92,95,96}. Furthermore, some antibiotics⁹⁷ as well as some probiotics⁹⁸ may alleviate IBS symptoms. Additionally, rCDI patients treated with FMT are less likely to experience severe gastrointestinal symptoms over the long term than rCDI patients who are only treated with antibiotics⁹⁹.

2.4.1 FECAL MICROBIOTA TRANSPLANTATION IN IRRITABLE BOWEL SYNDROME

The placebo-controlled trials investigating FMT in IBS published to date (Table 2.) have yielded conflicting results¹⁰⁰⁻¹⁰⁶. In two of these trials, a single FMT was administered *via* colonoscopy^{100,102}, three of the studies applied fecal capsules for 3 to 12 days^{101,103,106} and in one trial FMT was administered *via* gastroscopy¹⁰⁵, while in another trial FMT was given *via* nasojejunal tube¹⁰⁴. In summary, FMT *via* colonoscopy is associated with a modest, but transient, decrease in IBS symptoms^{100,102}. The FMT capsules did not exert symptom relief^{101,103,106}, whereas FMT *via* nasojejunal tube decreased IBS symptoms¹⁰⁴, and as an outstanding result, FMT *via* gastroscopy achieved a clear benefit with up to 89.1% response rate¹⁰⁵. Although all of these administration routes altered the microbiota of IBS patients toward that of the donor¹⁰¹, a concurrent decrease in the symptom severity was associated only with FMT administered *via* colonoscopy, enteral tube, or gastroscopy^{102,104,105}. The attained clinical effects in our experience seem mild and transient. However, the above reviewed

best results by El-Salhy and colleagues¹⁰⁵ were reported to be maintained for at least three years¹⁰⁷. These results need to be confirmed by other groups.

FMT results in a long-lasting change in the gut microbiota of IBS patients, and in preliminary investigations, the clinical efficacy is associated with the extent of the microbial shift toward that of the donor after having a higher relative abundance of Firmicutes and a lower relative abundance of Bacteroidetes prior to FMT¹⁰⁸. FMT has also increased the fecal SCFA levels in IBS patients, and the increase in the butyric acid level has been inversely correlated with IBS symptoms¹⁰⁹.

TABLE 2. Controlled trials investigating FMT for IBS

First author, publication year	Follow-up time	Sample size, allocation	FMT Route	Dosage of FMT Group	Control Group	Primary measure	Response at 12 weeks
Aroniadis 2019 ¹⁰³	3 months	<i>n</i> = 48, 1:1	Oral capsules for 3 days	25 FMT capsules, 10 g/day, 3 days	Placebo capsules	IBS-SSS	No difference between the groups
El-salhy 2020 ¹⁰⁵	3 months	<i>n</i> = 165, 1:1:1	Gastroscopy	30 g FMT or 60 g FMT	Autologous feces	IBS-SSS	FMT 30 g 77%, FMT 60 g 89%, placebo 24%
Halkjær 2018 ¹⁰¹	6 months	<i>n</i> = 52, 1:1	Oral capsule for 12 days	25 FMT capsules, 50 g/day, 12 days	Placebo capsules	IBS-SSS	FMT 36%, placebo 79%
Holster 2019 ¹⁰²	6 months	<i>n</i> = 17, 1:1	Colonoscopy	30 g FMT	Autologous FMT	GSRS-IBS	No difference between the groups
Holvoet 2020 ¹⁰⁴	3 months	<i>n</i> = 62, 2:1	Naso-jejunal tube	Not known	Autologous FMT	Unvalidated symptom questionnaire	FMT 56%, placebo 26%
Johnsen 2018 ¹⁰⁰	12 months	<i>n</i> = 90, 1:1:1	Colonoscopy	Frozen or fresh 50–80 g FMT	Autologous FMT	IBS-SSS	FMT 65%, placebo 43%
Singh 2022 ¹⁰⁶	10 weeks	<i>n</i> = 44, 1:1:1, FMT, CM-FMT, R-FMT, Placebo	Oral capsules, single dose	19 FMT capsules (14 g)	Placebo capsules	IBS-SSS	No difference between the groups

FMT, fecal microbiota transplantation; IBS, irritable bowel syndrome; IBS-SSS, IBS symptom severity score; GSRS-IBS, gastrointestinal symptom rating scale -IBS; CM-FMT, FMT with ciprofloxacin and metronidazole pretreatment; R-FMT, FMT with rifaximine pretreatment.

2.5 ULCERATIVE COLITIS

Ulcerative colitis (UC) is a chronic inflammatory bowel disease of unknown etiology. The pathophysiology of UC is suspected to involve an exaggerated inflammatory response to gastrointestinal bacteria in genetically predisposed individuals ¹¹⁰. The prevalence of UC is highest in North America and Northern Europe, and in Finland, the yearly incidence is over 25/100 000 ¹¹¹. The typical symptoms of active UC include bloody diarrhea and abdominal cramps. Patients with UC have a lower quality of life than the general population, even if the disease is quiescent ¹¹². The risk of colon cancer is higher than that of the general population, and the risk of colectomy is elevated despite the new immune response targeting medications ¹¹³. Patients with UC have decreased gut microbial diversity and stability, and the function of the gut microbiota is altered compared to that of healthy individuals. The two major phyla in healthy individuals, Bacteroidetes and Firmicutes, appear to be decreased in IBD patients, and the phylum Proteobacteria, including many gram-negative bacteria with inflammatory properties, tends to be increased compared to healthy individuals ¹¹⁴. Functional changes may be more important than taxonomic changes in IBD. For example, the reduction in *Fecalibacterium prausnitzii* and other SCFA producers in IBD patients suggests a central role of SCFAs in IBD. SCFAs possess many immunoregulatory functions; they improve epithelial barrier integrity and facilitate the differentiation of T-regulatory cells and T-effector cells. The absence of this regulatory function leads to an increase in the activity of proinflammatory cytokines ¹¹⁵. The UC-associated dysbiosis, together with good results from FMT in rCDI, has encouraged researchers to investigate FMT in UC.

2.5.1 FECAL MICROBIOTA TRANSPLANTATION IN ULCERATIVE COLITIS

The results of controlled trials on FMT for UC (Table 3.) have been promising, but the FMT protocols and study designs have been diverse ¹¹⁶⁻¹²⁴. A unifying feature of trials investigating FMT for UC is that the treatment has been given in multiple sessions. In Australian trials, FMTs have been prepared from the feces of multiple donors ^{118,119}. In one of the trials, FMTs were prepared in anaerobic conditions ¹¹⁹, and in another, the FMT enema was given in up to 40 sessions ¹¹⁸.

Two controlled trials have combined FMT with a dietary intervention ^{125,126}. In a study from Israel, dietary intervention was more effective than FMT in inducing remission of mild to moderate UC ¹²⁵. In an Indian trial, repeated FMT together with a dietary intervention was more effective than optimized standard

medical therapy alone in inducing remission ¹²⁶. With continued nutritional intervention, the treatment group was more likely to maintain remission for one year.

One of the trials investigated pediatric patients ¹²⁰. Two small trials exclusively applied FMT capsules with promising results ^{121,122}. One trial had mesalamine enemas as the control treatment for FMT enemas; in this trial, repeated FMT enemas were not inferior to mesalamine enemas in the induction of remission in patients with active left side colitis ¹²³.

In the trial by Paramsothy and colleagues, UC patients who responded to FMT had increased post-FMT levels of *Eubacterium hallii* and *Roseburia inulivorans* compared with patients who did not achieve remission. SCFA biosynthesis and secondary bile acids also increased among the responders. Patients who did not reach remission after FMT had enrichment of *Fusobacterium gonidiaformans*, *Sutterella wadsworthensis*, and *Escherichia* species together with increased levels of heme and lipopolysaccharide biosynthesis ¹²⁷.

Before our study (III), only one randomized placebo-controlled trial investigated the efficacy of FMT in the maintenance of remission of UC ¹²⁴. The study population included selected patients who had reached UC remission with FMT treatments. While in remission, FMT was given to the patients *via* colonoscopy six times in one year. The patients receiving repetitive FMTs were more likely to maintain remission than the placebo group.

2. REVIEW OF THE LITERATURE

TABLE 3. Controlled trials investigating FMT for active UC

Study	N, allocation	Intervention	Control	Endpoint	Main outcome
Moayed 2015 ¹¹⁷	n=75, 1:1	FMT enema once per week for 6 weeks	Water enema	Endoscopic remission at week 7	FMT 24% vs. placebo 5% (p = 0.03)
Rossen 2015 ¹¹⁶	n=48, 1:1	FMT <i>via</i> nasogastric tube twice in three weeks	Autologous FMT	Combined endoscopic and clinical remission at week 12	FMT 30% vs. placebo 20% (p = 0.51)
Paramsothy 2017 ¹¹⁸	n=85, 1:1	FMT once <i>via</i> colonoscopy + FMT enema 5 times per week for eight weeks	Saline based enema	Clinical remission and endoscopic response at 8 weeks	FMT 27% vs. placebo 8% (p = 0.02)
Costello 2019 ¹¹⁹	n=73, 1:1	FMT once <i>via</i> colonoscopy + two enemas within a week	Autologous FMT	Clinical remission and endoscopic response at 8 weeks	FMT 32% vs. placebo 9% (p = 0.02)
Brezina 2021 ¹²³	n=45, 1:1	FMT enemas 5 times in the first week + FMT enemas weekly for 5 weeks	5-ASA enemas	Clinical remission at 12 weeks	FMT 52% and 36% 5-ASA (p = N/A) FMT was noninferior
Pai 2021 ¹²⁰	n=25, 1:1	FMT enema twice per week for 6 weeks	Saline enema	Clinical improvement at 6 weeks	FMT 92% vs. placebo 50% (p = N/A) (RR, 1.8; 95% CI, 1.1-3.7)
Crothers 2022 ¹²²	n=12, 1:1	FMT once <i>via</i> colonoscopy +12 weeks daily FMT capsules	Placebo capsules	Clinical remission at 12 weeks	FMT 33% vs. placebo 0% (p = 0.45)
Haifer 2022 ¹²¹	n=35, 1:1	FMT capsules for 8 weeks + antibiotic pretreatment	Placebo capsules	Clinical remission, endoscopic response at week 8	FMT 53% vs. placebo 15% (p = 0.03)
Kedia 2022 ¹²⁶	n=66, 1:1	FMT <i>via</i> colonoscopy 7 times weekly + AID	Standard medical therapy	Clinical remission at 8 weeks	FMT-AID 60% vs. control 32.3% (p = 0.02)

Study	N, allocation	Intervention	Control	Endpoint	Main outcome
Sarbagili-Shabat 2022 ¹²⁵	n=62, 1:1:1	FMT <i>via</i> colonoscopy + enemas on Days 2 and 14; Group 2: FMT + UCED	UCED alone	Clinical remission at 8 weeks	FMT 12%, FMT + UCED 21%, UCED 40% (nonsignificant)

The UC severity of the included studies was mild to moderate. Abbreviations: FMT, fecal microbiota transplantation; UC, ulcerative colitis; 5-ASA, 5-aminosalicylic acid; UCED, ulcerative colitis exclusion diet; AID, anti-inflammatory diet; N/A, not applicable; RR, risk ratio; CI, confidence interval

2.6 OBESITY

Obesity is a large and constantly growing global health problem ^{2,3}. In particular, the proportion of individuals with severe obesity is rapidly increasing ². Morbid obesity is associated with several comorbidities, decreased quality of life (QoL), and increased mortality ¹²⁸. Behavioral and, in particular, medical therapies have progressed in recent years; however, surgery remains the most effective method to reduce severe obesity ¹²⁸. Bariatric surgery reduces mortality^{129,130} and increases QoL ¹³¹. Nevertheless, some patients achieve inadequate weight reduction after bariatric surgery or regain weight after an initially adequate reduction in body weight ^{132,133}.

Due to accumulating knowledge of the gut microbiota and its interaction with the host, the intestinal microbiota has been regarded as a potential target for the treatment of obesity ¹³⁴.

2.7 GUT MICROBIOTA IN OBESITY

The intestinal microbiota of obese individuals is altered compared to that of lean individuals ¹³⁵. In particular, patients with severe obesity harbor an intestinal microbiota with decreased bacterial diversity and microbial gene richness compared to healthy controls ^{135,136}, but bariatric surgery may improve gut microbial gene richness ¹³⁶. The causal relation between the intestinal microbiota and body weight has been established in animal models ^{137,138}. Additionally, in humans, changes in the gut microbiota and its function may contribute to body weight through several mechanisms. Increased levels of *Desulfovibrio* and a reduction in Clostridia may result in increased lipid absorption ¹³⁹. Although SCFAs harbor many beneficial properties, an overly high SCFA content is associated with gut dysbiosis, gut permeability, excess

adiposity, and cardiometabolic risk factors¹⁴⁰. Microbiota-derived SCFAs may be an additional source of energy not only for colon epithelial cells but also for the host and contribute to obesity.

2.8 FECAL MICROBIOTA TRANSPLANTATION IN OBESITY

Preliminary controlled trials have investigated FMT for obesity or metabolic syndrome¹⁴¹⁻¹⁵¹ (Table 4). In some trials, FMT resulted in slight improvements in insulin sensitivity^{141,143}. In one trial investigating adolescent patients with metabolic syndrome, a reduction in abdominal adiposity after FMT was observed¹⁴², and in another trial, FMT was associated with positive changes in lipid metabolism¹⁴⁴. However, in controlled human studies to date, a reduction in body weight has not been associated with FMT^{142,145,147}. In an Israeli study, autologous FMT, collected during the diet-induced weight-loss phase and administered afterward in the weight regain phase, preserved weight loss and retained weight loss-induced metabolic benefits^{152,153}.

In the study by Leong and colleagues, the diversity of the gut microbiota of obese adolescents increased six weeks post FMT, but interestingly, this was only detected among female participants. Positive metabolic outcomes were associated with a reduction in the relative abundance of *E. coli* and an increase in *Faecalibacterium prausnitzii*, *Bacteroides ovatus*, *Bacteroidales bacterium ph8*, *Alistipes onderdonkii*, *Alistipes finegoldii*, and *Alistipes shahii*¹⁴².

TABLE 4. Controlled trials investigating FMT for metabolic diseases

Study	N	Participants, randomization ratio	Intervention, Control, Randomization ratio	Main endpoint	Follow-up	Main outcomes
Vrieze 2012 ¹⁴¹	n=18	Adult males with metabolic syndrome	FMT once <i>via</i> nasoduodenal tube, Allogeneous FMT, 1:1	Insulin sensitivity	6 weeks	No difference between the groups. Improvement in insulin sensitivity vs. baseline in FMT group
Kootte 2017 ¹⁴⁶	n=38	Adult males with metabolic syndrome, 2:1	FMT once <i>via</i> nasoduodenal tube, Allogeneous FMT, 2:1	Insulin sensitivity	18 weeks	No difference between the groups. Improvement in insulin sensitivity vs. baseline in FMT group at 6 wk, but not 18 wk

Study	N	Participants, randomization ratio	Intervention, Control, Randomization ratio	Main endpoint	Follow-up	Main outcomes
Smits 2018 ¹⁴⁸	n=20	Adult males with metabolic syndrome	FMT once <i>via</i> nasoduodenal tube, Allogeneous FMT, 1:1	TMAO production	2 weeks	Vegan FMT did not change TMAO production
Allegretti 2020 ¹⁴⁵	n=22	Obese adults , without metabolic syndrome	Monthly FMT capsules trice, Placebo capsules, 1:1	Safety	26 weeks	No difference between the groups in adverse events or BMI at week 12
Craven 2020 ¹⁴⁹	n=21	Adult NAFLD patients	Single FMT <i>via</i> gastroscopy, Autologous placebo, 3:1	Insulin sensitivity, gut permeability	26 weeks	No difference in insuline resistance; FMT showed potential to reduce small intestinal permeability
de Groot 2020 ¹⁵⁰	n=22	Adult males with metabolic syndrome	Gastric bypass donor FMT, metabolic syndrome donor FMT: 1:1	Insulin sensitivity	8 weeks	FMT using donor with metabolic syndrome decreased insulin sensitivity
Leong 2020 ¹⁴²	n=87	Adolescents 14-18 years, BMI>30	Single course FMT capsules, Placebo capsules, 1:1	BMI at 6 weeks	26 weeks	No difference between the groups in BMI. Abdominal adiposity reduced in FMT group
Witjes 2020 ¹⁵⁰	n=21	Adults with hepatic steatosis	FMT trice <i>via</i> nasoduodenal tube in 8 week intervals, Allogeneous FMT, 1:1	Liver histology at 24 weeks	24 weeks	No statistically significant differences in histology scores, a tendency toward improvement in necro-inflammation score.

Study	N	Participants, randomization ratio	Intervention, Control, Randomization ratio	Main endpoint	Follow-up	Main outcomes
Yu 2020 ¹⁴⁷	n=24	Obese adults with insulin resistance (HOMA-IR 2.0 and 8.0)	FMT capsules weekly for 6 weeks, Placebo capsules, 1:1	Insulin sensitivity at 6 weeks	12 weeks	No difference between the groups in HOMA-IR or weight
Mocanu 2021 ¹⁴³	n=70	Severely obese adults with metabolic syndrome	FMT (single dose capsules 50 g) + LF or FMT + HF, LF and HF, 1:1:1	Insulin sensitivity at 6 weeks	6 weeks	FMT-LF improved insulin sensitivity compared to baseline. No change in other groups.
Rhinott 2021 ¹⁵²	n=90	Obese or dyslipidemic adults	Diet 6 months + autologous FMT capsules months 8-14, Diet + placebo capsules, 1:1	Weight regain	FMT 26 weeks	Autologous FMT collected during Green Meditarrean Diet and transferred after the diet, preserved weight loss

TMAO, trimethylamine N-oxide; DM, diabetes mellitus; NAFLD, nonalcoholic fatty liver disease; BMI, body mass index; HOMA-IR, homeostatic model assessment for insulin resistance; LF, low-fermentable fiber supplements; HF, high-fermentable fiber supplements

2.9 FECAL MICROBIOTA TRANSPLANTATION IN OTHER CONDITIONS

FMT has been investigated in case series and small controlled trials for many other indications in addition to those mentioned above. The rationale for these studies is provided by underlying investigations indicating that gut dysbiosis is associated with the conditions and could thus be an etiological factor ¹⁵⁴.

FMT may be an option in some liver diseases. A single FMT enema and antibiotic pretreatment reduced episodes of hepatic encephalopathy for five months in a trial with 20 cirrhosis patients who were on lactulose and rifaximin therapy for recurrent hepatic encephalopathy episodes. Half of the patients in the control group (5/10) experienced a hepatic encephalopathy episode during the follow-up versus none of the treatment group (0/10) ¹⁵⁵. In a small

preliminary RCT, FMT was safe and reduced alcohol consumption for 15 days in cirrhosis patients with an alcohol use disorder ¹⁵⁶.

Manipulation of gut microbiota may have an effect on the course of type I diabetes (DM1). In a controlled trial with 20 new-onset DM1 patients, autologous FMT *via* nasoenteral tube halted the decline in endogenous insulin production. The stimulated C peptide levels were significantly better preserved in the autologous FMT group than in the healthy donor FMT group at 12 months.¹⁵⁷

Even in cancer patients, FMT has yielded preliminary positive results. FMT has been associated with increased tolerance to cancer medication. In a placebo-controlled trial of 20 metastatic renal cell carcinoma patients, FMT reduced diarrhea induced by tyrosine-kinase inhibitors in eight weeks of follow-up ¹⁵⁸. Even increased efficacy of cancer medication has been associated with FMT. FMT promoted response in checkpoint inhibitor (anti-PD-1) immunotherapy-refractory melanoma patients, as in a case series of 10 patients FMT, the immunotherapy response improved in three patients ¹⁵⁹.

Graft-versus-host-disease (GVHD) is a condition in which an immune system transplanted in stem cell transplantation recognizes its own tissues as nonself, causing inflammation that often affects the gastrointestinal track. Case series investigating FMT for acute steroid refractory or steroid-dependent GVHD have yielded positive results ¹⁶⁰⁻¹⁶². Controlled trials investigating FMT for GVHD have not yet been published.

Additionally, several neurologic and psychiatric conditions are associated with gut dysbiosis¹⁶³ and promising preliminary case series investigating FMT in diseases such as Parkinson's disease¹⁶⁴ and autism¹⁶⁵. The results from controlled trials are forthcoming.

Negative or null results in FMT trials build up scientific knowledge, as do positive results. Although certain probiotics have been effective for chronic pouchitis ¹⁶⁶, a single FMT was ineffective for alleviating chronic pouchitis compared to autologous placebo ¹⁶⁷. Additionally, significantly negative results in controlled FMT trials may guide science on tracks of etiological factors of diseases. For example, FMT administered once *via* duodenal tube was associated with an increased number of treatment failures in a 26-week follow-up in a placebo-controlled trial with 30 psoriatic arthritis patients ¹⁶⁸. Although negative, the result suggests an association between the gut microbiota and psoriatic arthritis.

Large controlled FMT trials are preferred over case series to establish causal relations between diseases and microbiota; however, some diseases or conditions are so sporadic that a controlled trial would be practically impossible. This dissertation comprises placebo-controlled FMT trials, case series, and singular case reports.

3 AIMS OF THE STUDY

The aim of this dissertation was to investigate the safety and efficacy of fecal microbiota transplantation in different dysbiosis-associated conditions. The specific aims of the substudies were as follows:

- I The aim of study (I) was to investigate the efficacy and safety of FMT for new indications and to study FMT for rCDI in immunocompromised patients.
- II The aim of study (II) was to investigate the potential of FMT to reduce symptoms of irritable bowel syndrome and improve quality of life as well as to assess the potential of FMT to correct gut microbial imbalances.
- III The aim of study (III) was to investigate FMT for the maintenance of UC remission and the effect of FMT on QoL in UC patients.
- IV The aim of study (IV) was to investigate FMT for morbid obesity and to analyze the effects of FMT on the results of obesity surgery. Additional aims were to assess the effect of FMT on QoL, body consistency, and blood chemistry among obese patients.

4 MATERIALS AND METHODS

4.1 STUDY SUBJECTS

The subjects of the four studies were adult patients who received FMT or autologous placebo with different indications.

The subjects of the first study were 21 FMT-treated patients. Thirteen of the patients had rCDI and major immunocompromising comorbidities: human immunodeficiency virus (two patients), hemodialysis (six patients), kidney transplant (two patients), liver transplant (two patients), and chronic lymphatic leukemia (one patient). They were 19 to 80 years old; eight were female, and five were male. The case series also included eight patients with new experimental FMT indications: *Salmonella* carriage (two patients), trimethylaminuria (two patients), small intestinal bacterial overgrowth, lymphocytic colitis, ESBL-producing *Escherichia coli* carriage, and a common variable immunodeficiency patient with chronic norovirus infection. They were 17 to 66 years old, five female and three male. All patients were treated at Helsinki University Hospital.

The subjects of the second trial were 49 adult IBS patients, of whom 51% had diarrhea-dominated IBS, 14.3% had mixed IBS, 28.6% had unsubtyped IBS, and 6.1% had their IBS in remission at the study baseline and were thus not in any subtype category. All patients were treated at Helsinki University Hospital.

The subjects of the third trial were 48 adult patients with ulcerative colitis in remission. At the beginning of the trial, the inclusion criteria accepted only patients with a UC duration of a maximum of six months. Because of slow recruitment, the inclusion criteria were broadened, and an amendment for the ethical board was made. Thereafter, UC patients in remission with any disease duration were accepted for the study. The patients were treated in Helsinki University Hospitals and Päijät-Häme Central Hospital, Lahti, Finland.

The subjects of the fourth trial were 41 adult obese patients who were proceeding to obesity surgery six months after FMT. The patients were treated at Helsinki University Hospital and Päijät-Häme Central Hospital.

Approval by the Helsinki University ethics board was obtained for each of the four included trials (I–V). Every patient in the included placebo-controlled trials (II–IV) provided their informed written consent.

4.2 METHODS

Case series. In trial I, we retrospectively reviewed FMT-treated patients from up to 2016 at Helsinki University Hospital. We included patients with immunosuppressive comorbidities (n=13) as well as patients treated for indications other than *Clostridioides difficile* (n=8). Most of the FMTs were administered *via* colonoscopy and in some instances *via* gastroscopy. Some of the patients, the ones treated earlier in time, were treated with fresh FMT from a donor familiar to the patient. The rest of the patients, those treated later, were given frozen and thawed FMT from a general donor.

Randomized controlled trials. Trials II, III, and IV were randomized placebo-controlled trials investigating a single FMT vs. autologous placebo, i.e., FMT was prepared similarly but from patients' own fresh stool for patients with IBS (II), UC (III), or obesity (IV).

In trial II, 49 adult IBS patients received either autologous or allogenic FMT *via* colonoscopy (Figure 2). The primary endpoint was a sustained reduction in IBS symptoms defined as a 50-point reduction in the IBS Symptom Severity Score (IBS-SSS)¹⁶⁹. The IBS subtype was determined by applying the Rome-III questionnaire¹⁷⁰. The secondary outcomes were mental health, quality of life, fecal water content, and gut microbiota. Mental health was assessed with two questionnaires: the Beck Anxiety Inventory (BAI) and the Beck Depression Inventory (BDI)¹⁷¹. Quality of life was assessed with two questionnaires as well: the disease-specific IBS Quality of Life (IBSQoL)¹⁷² questionnaire and the general QoL questionnaire, 15 Dimensions (15D)¹¹². The fecal water content and stool consistency were assessed with stool dry weight analysis, which is an indicator of stool consistency and correlates with the bowel transit time¹⁷³.

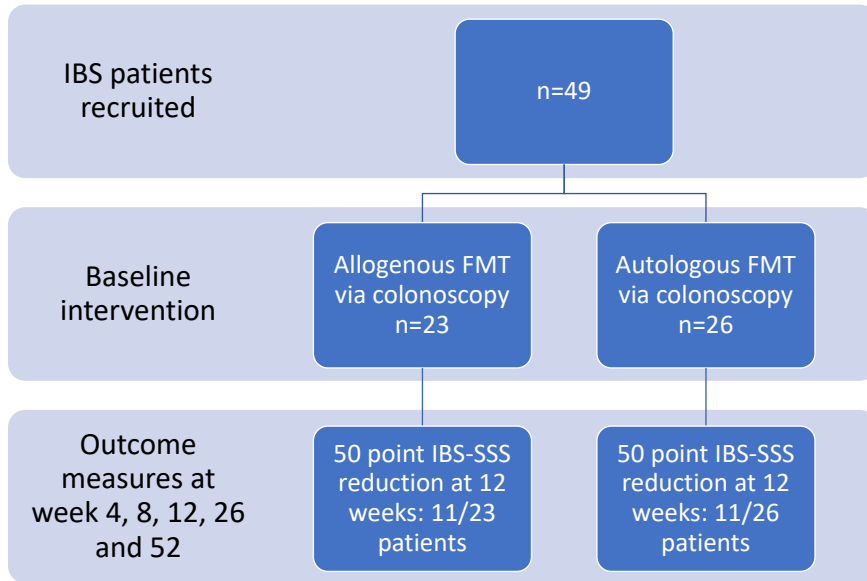


FIGURE 2. The trial design and flow of FMT for the IBS trial.

Abbreviations: FMT, fecal microbiota transplantation; IBS, irritable bowel syndrome; IBS-SSS, IBS-syndrome severity scale.

Microbiota analysis (II). After mechanical lysis¹⁷⁴, high-throughput DNA extraction using KingFisher Flex 96 (Thermo Fisher Scientific) was performed. The analysis of microbial composition based on sequencing the V3–V4 region of the 16S ribosomal gene using MiSeq sequencing. The preprocessing of the reads was performed using the R-package mare pipeline¹⁷⁵. The reads, on average 47029 per sample, were annotated to taxonomic assignments using USEARCH (version 8.1.1756) and SILVA 16S rRNA reference database version 115¹⁷⁶.

In trial III, 48 adult patients with UC in remission were recruited to the study (Figure 3). Eligibility criteria included fecal calprotectin below 100 µg/g and a clinical Mayo score < 3 at the time of screening. However, after the screening visit and before the baseline intervention, some patients experienced minor activation of the disease; thus, we also analyzed the patients in subgroups: participants with fecal calprotectin ≥ 200 µg/g or a clinical Mayo score ≥ 3 were analyzed separately as “subgroup B” (n=15), and the participants without signs of disease activity at baseline were included in “subgroup A” (n=33). The patients were allocated 1:1 to receive FMT or autologous placebo once in colonoscopy. The study follow-up lasted for one year, and the primary endpoint was maintenance of remission. A relapse was defined as a clinical Mayo score

≥ 3 or fecal calprotectin level $\geq 200 \mu\text{g/g}$ or if a participant experienced overt activation of colitis.

The initial inclusion criteria regarded patients with new-onset disease (< 6 months) as eligible. However, due to slow recruitment, the inclusion criteria were changed to include patients with any duration of the disease. The ethical board accepted the amendment to the study protocol.

The secondary endpoints were QoL, fecal calprotectin, and blood chemistry, and an endoscopic assessment was carried out at 12 months. General QoL was assessed with the 15D questionnaire, and disease-specific QoL was assessed with the Inflammatory Bowel Disease Quality of Life Questionnaire (IBDQ) (McMaster University, Hamilton, Canada, license No. IBDQ22-o81)¹¹².

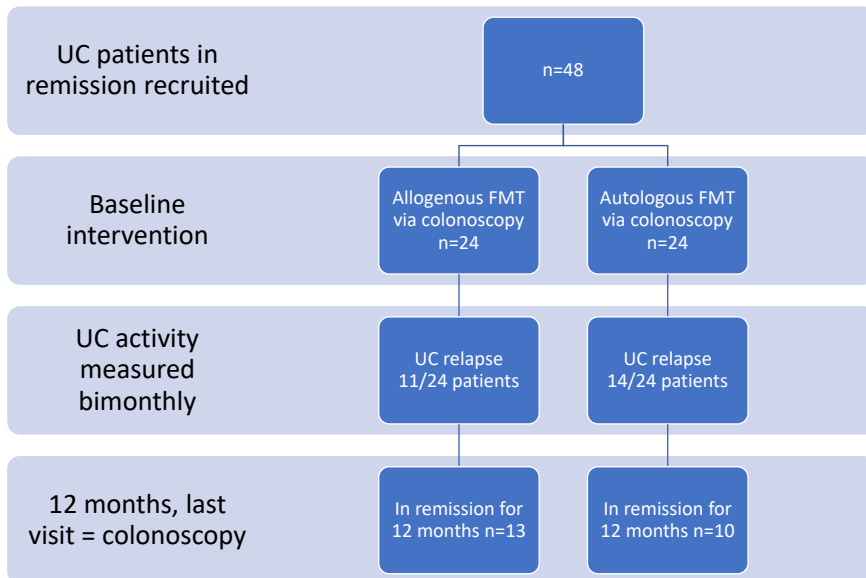


FIGURE 3. The study design and flow of FMT for maintenance of remission in UC patients trial (III)

Abbreviations: FMT, fecal microbiota transplantation; UC, ulcerative colitis.

In trial IV, 41 obese patients who were enrolled in obesity surgery were recruited to the trial (Figure 4). The inclusion criteria were the same as the criteria for bariatric surgery: a BMI $> 40 \text{ kg/m}^2$ or $> 35 \text{ kg/m}^2$ and comorbidities related to obesity such as type 2 diabetes, hypertension, sleep apnea, nonalcoholic fatty liver disease, osteoarthritis, or hyperlipidemia¹²⁸. The participants were allocated 1:1 to receive a lean donor FMT or autologous placebo once *via* gastroscopy half a year prior to obesity surgery. All participants

were appointed to obesity surgery, and the follow-up lasted for one year after the surgery.

The primary outcome of the study was the reduction in body weight: the change in body mass index (Δ BMI) compared to the baseline, the percentage of total weight loss (%TWL), and the percentage of excess BMI loss (%EBMIL) ¹⁷⁷.

Secondary outcomes included changes in body composition measured with a bioelectrical impedance analysis (BIA, Tanita MC-980, Tanita Corporation, Tokyo, Japan), blood chemistry, and QoL. Disease-specific QoL was measured with the Moorehead-Ardelt QoL questionnaire ¹⁷⁸, and health-related QoL was assessed with the 15D questionnaire ¹⁷⁹.

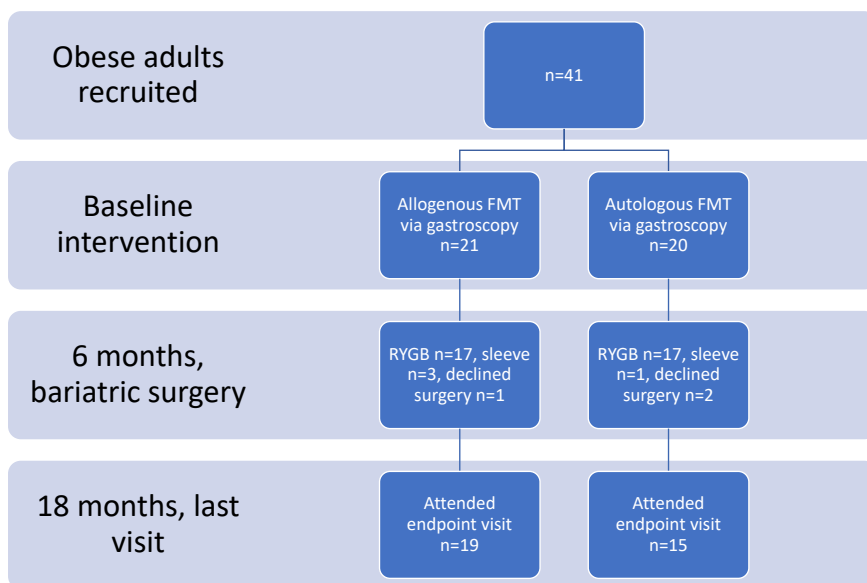


FIGURE 4. The study design and flow of the FMT for obesity trial

Abbreviations: FMT, fecal microbiota transplantation; RYGB, Roux-en-Y gastric bypass surgery; sleeve, sleeve gastrectomy

4.3 STATISTICAL METHODS

The results are presented as the means and standard deviations (SD) or 95% confidence intervals (CI) for continuous variables and as numbers and percentages for dichotomous variables.

The applied statistical tests were two-sided t tests for continuous data and the χ^2 test for nominal data. ANOVA, estimated marginal means, and repeated

measures analysis of variance were applied for multiple testing, or the Wilcoxon signed rank test or Kruskal–Wallis test was used for nonparametric data. Levene’s F test was used to analyze the variance of weight loss (IV).

Microbiota correlation with symptoms and the correlation between donor and recipient microbiota were assessed with Spearman correlation and visualized with principal coordinate analysis (PCoA) (II).

The Kaplan–Meier method was applied to assess the maintenance of remission (III, Figure 6). The associations of baseline characteristics with the maintenance of remission were analyzed with Cox regression (III). P values below 0.05 were considered statistically significant for all analyses.

The statistical analyses were performed using the SPSS software program (IBM Statistics versions 26 and 27) (II–IV) and R software program (R core team, version 3.5.2.)(II–III).

In Study II, the sample size was calculated assuming that 40% of the placebo group and 80% of the FMT group would respond to the treatment. The calculated sample size was 52 participants (CI 95%, $\alpha = 0.05$ and $\beta = 0.1$).

In Study III, the relapse rates during the one-year follow-up period were estimated to be 50% in the placebo group and 15% in the FMT group. The calculated sample size was 66 (CI 95%, $\alpha=0.05$ and $\beta=0.1$), and the original aim was to recruit 80 participants.

In Study IV, the sample size was calculated assuming that 40% of the FMT group and 10% of the placebo group would reach a weight reduction of 10% by week 24 after FMT. The calculated sample size was 40 patients, with 20 patients in both groups (CI 95%; $\alpha =.05$ and $\beta = 0.1$).

5 RESULTS

5.1 FECAL MICROBIOTA TRANSPLANTATION FOR RECURRENT *CLOSTRIDIoidES DIFFICILE* INFECTION IN PATIENTS WITH IMMUNOCOMPROMISING COMORBIDITIES (I)

Among the patients with different immunocompromising conditions who were treated with FMT for rCDI, eleven out of thirteen patients were successfully cured, and they did not have further recurrences of CDI. Six of these patients experienced major adverse events, and two patients died within 5 months after FMT. Four patients experienced adverse events within one month after FMT (Table). The observed adverse events were considered not directly attributable to FMT.

Table 5. Immunocompromised rCDI patients treated with FMT (Study I)

Immunocompromised rCDI patients treated with FMT	Adverse events in 1 month
28-year-old male with HIV, antiviral medication and viruses undetectable, earlier suspicion of ulcerative colitis.	No
59-year-old female with HIV, depression, continuous heavy smoking and consumption of alcohol.	No
60-year-old female, rheumatoid arthritis and in hemodialysis due to amyloidosis.	No
19-year-old female, hemodialysis due to Goodpasture syndrome complicated with pulmonary hemorrhage. Immunosuppressive therapy.	No
77-year-old male, hemodialysis after renal carcinoma operation.	1 week after hospitalized due to edema and cystitis
80-year-old male, hemodialysis because of chronic glomerulonephritis.	No
66-year-old male, hemodialysis due to microscopic polyangiitis.	No
79-year-old female, hemodialysis due to episode of rhabdomyolysis.	2 weeks after <i>C. difficile</i> reinfection
78-year-old female, kidney transplant due to polycystic renal disease.	Gastroenteritis 3 days after FMT Hospitalized 12 days after FMT
61-year-old female, kidney transplant due to polycystic renal disease.	No
56-year-old female, liver transplant due to mushroom intoxication.	No
69-year-old male, liver transplantation due to alcohol cirrhosis after which renal insufficiency and hemodialysis.	No
65-year-old female, 2003 autologous stem cell transplantation for CLL, 2009–2011 cytostatic interventions, 2011 chronic norovirus infection and rCDI.	CDI and norovirus related diarrhea continued.

Abbreviations: CDI, *Clostridioides difficile* infection; FMT, fecal microbiota transplantation; HIV, human immunodeficiency virus; CLL, chronic lymphocytic leukemia; rCDI, recurrent CDI.

5.2 FECAL MICROBIOTA TRANSPLANTATION FOR IRRITABLE BOWEL SYNDROME (STUDY II)

The primary endpoint was a sustained reduction in IBS symptoms, defined as a 50-point reduction in IBS-SSS at every measurement point. This was not achieved in either group. In the FMT group, there was a clinically significant, over 50-point reduction in the mean IBS-SSS at 12 weeks after FMT compared

to baseline ($P = 0.01$). However, there were no statistically significant differences between the groups (Figure 5, Table 6).

After allogeneic FMT, the stool microbial composition changed to resemble the donor's stool microbiota, but not after autologous FMT. Additionally, the microbial richness increased in the FMT group, but there was no change in diversity (Figure 6). The stool water content decreased compared to baseline, and the difference was only statistically significant for the FMT group.

There was no difference in depression or anxiety scores between the groups. However, a subgroup analysis was made of the responders, i.e., the patients whose IBS symptoms decreased after the intervention. Only in the responders to FMT did the depression score on the BDI decrease and the quality of life score, 15D, significantly increase. The corresponding changes were insignificant among the responders to placebo.

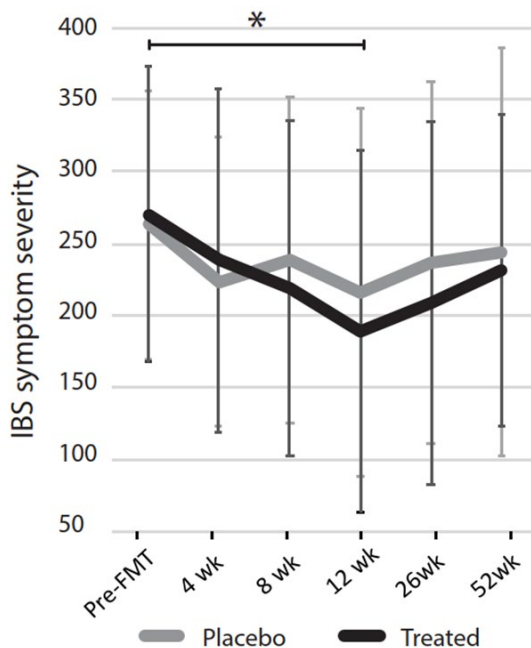


FIGURE 5. IBS symptoms between the groups at different timepoints (Study II)

The IBS-SSS mean total score (vertical axis) decreased significantly* in the FMT group from baseline to the 12-week time point. Abbreviations: IBS, irritable bowel syndrome; IBS-SSS, IBS symptom severity score; FMT, fecal microbiota transplantation.

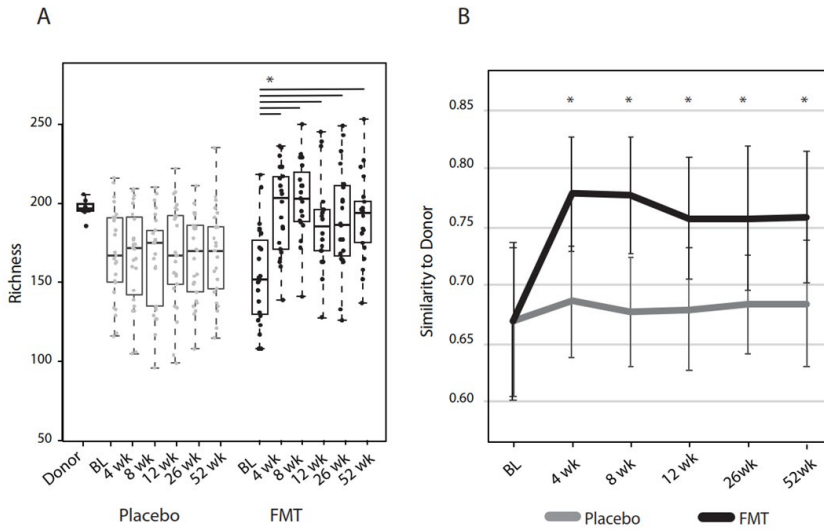


Figure 6. Microbiota changes in IBS patients after FMT or placebo (Study II)

A) The species richness of the gut microbiota increased in the FMT group from baseline to subsequent time points. No change was observed in the placebo group. B) The gut microbiota composition changed toward that of the donor after FMT. The changes persisted through the follow-up of one year. Abbreviations: IBS, irritable bowel syndrome; FMT, fecal microbiota transplantation.

5.3 FECAL MICROBIOTA TRANSPLANTATION FOR MAINTENANCE OF REMISSION OF ULCERATIVE COLITIS (STUDY III)

The main endpoint of the study was sustained remission, including *f*-calprotectin < 200 µg/g and a clinical Mayo score < 3. The primary endpoint was achieved by 13/24 (54%) patients in the FMT group and by 10/24 (41%) patients in the placebo group ($P = 0.660$). QoL decreased in the FMT group at four months after FMT compared to the placebo group ($P = 0.017$). Simultaneously, the disease-specific QoL was higher in the placebo group than in the FMT group ($P = 0.003$). There were no differences between the groups in blood chemistry, fecal calprotectin, or endoscopic findings at 12 months. The adverse events were similar in both groups, and they were mild and infrequent.

At baseline, some of the patients had a clinical Mayo score of three or more or fecal calprotectin above 200 µg/g; thus, two subgroups were formed, including only those with mild baseline activity (subgroup B) and those with

no signs of activity (subgroup A). There were no differences between the FMT and placebo groups within the subgroups (Figure 7).

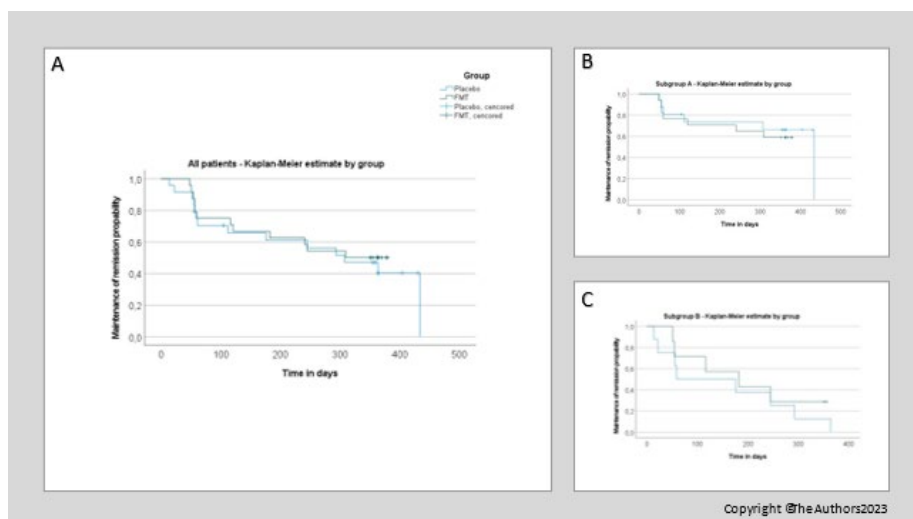


FIGURE 7. Maintenance of remission in UC patients (Study III)

A) Maintenance of remission included all participants. B) Maintenance of remission in Subgroup A, the patients in remission at baseline. C) Maintenance of remission in Subgroup B, the patients with mild colitis activity at baseline.

5.4 FECAL MICROBIOTA TRANSPLANTATION FOR OBESITY (STUDY IV)

All measurements, body weight, body consistency, blood chemistry, and QoL, were measured at seven timepoints during the 18-month follow-up period. Single gastroscopy-administered FMT did not reduce weight compared to autologous FMT.

The primary outcome was weight reduction. There were no statistically significant differences in body weight between the FMT and placebo groups at any time point. At 6 months, immediately after a period of VLCD and before the surgery, the mean body weight was reduced by 4.8% ($P < 0.001$) in the FMT group and 4.6% ($P = 0.006$) in the placebo group. At 18 months from the baseline intervention, 12 months after bariatric surgery, the mean weight was reduced by 25.3% ($P < 0.001$) in the FMT group and by 25.2% ($P < 0.001$) (Figure 8, Table 6). There were no differences between the groups in QoL, body composition, or blood chemistry (Figure 9).

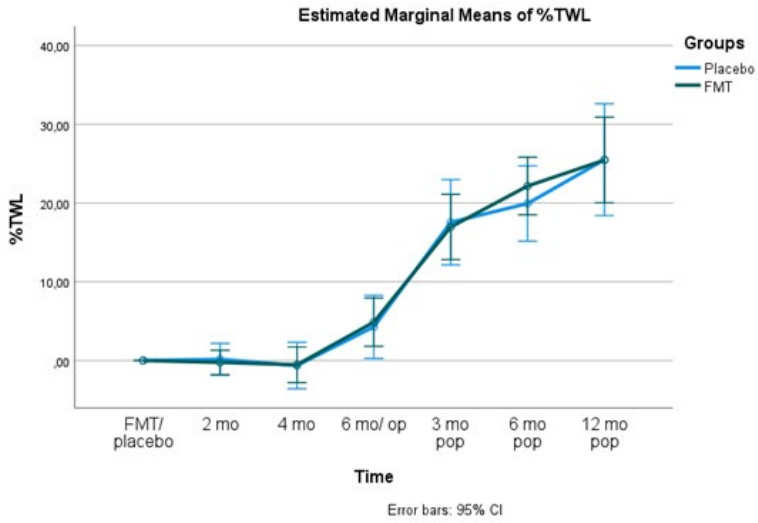
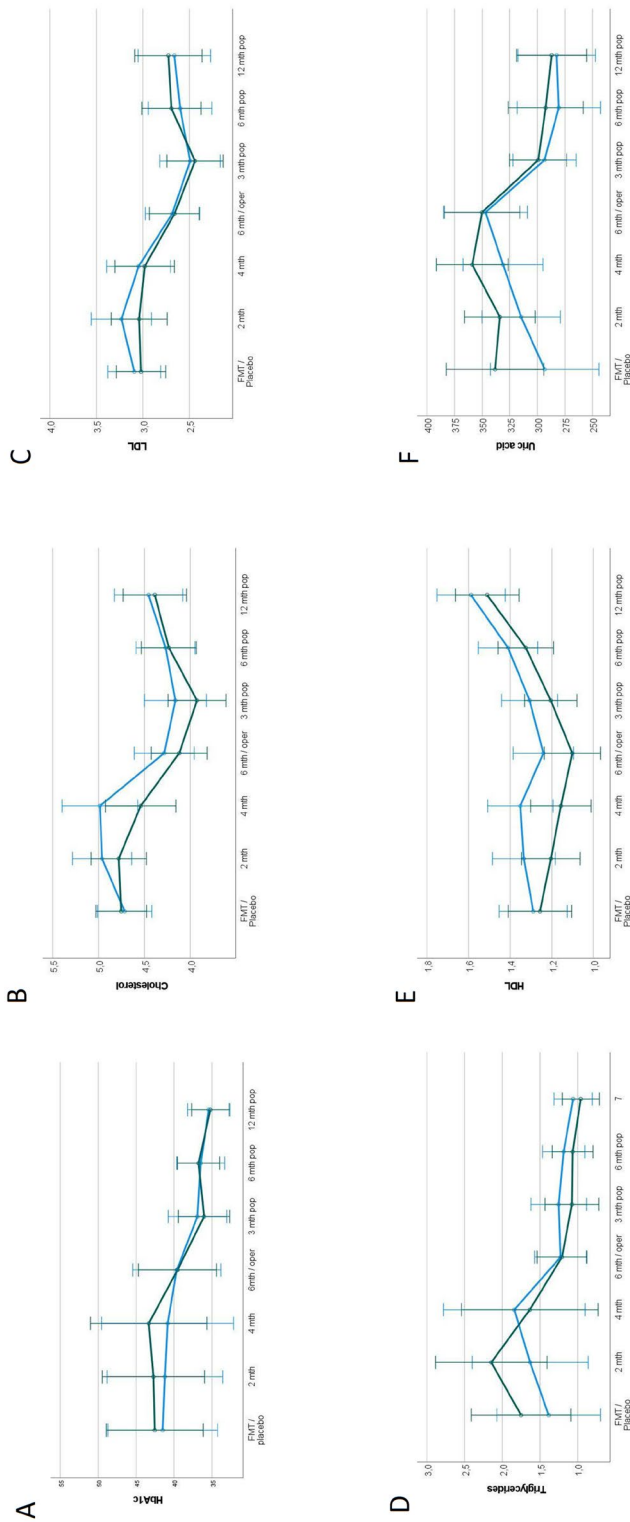


FIGURE 8. The estimated marginal means of %TWL between the groups (Study IV)

Abbreviations: TWL%, percentage total weight loss; FMT, fecal microbiota transplantation; mo, months; op, operation; pop, postoperation; CI, confidence interval.

Estimated Marginal Means

Groups
 — Placabo
 — FMT



Error bars: 95% CI

FIGURE 9. Estimated marginal means of blood metabolic parameters (Study IV)

A) HbA1c. B) Cholesterol. C) LDL, low-density lipoprotein. D) Triglycerides. E) HDL, high-density lipoprotein. F) Uric acid. Abbreviations: FMT, fecal microbiota transplantation; mo, months; op, operation; pop, postoperation; CI, confidence interval.

TABLE 5. Randomized controlled trials presented in this dissertation

Trial	Indication	N	Intervention	Control	Follow-up	Outcome measures	Results
Study II	IBS	n=49, 1:1	Single frozen 30 g FMT <i>via</i> colonoscopy	Single fresh 30 g autologous FMT <i>via</i> colonoscopy	1 year	IBS symptoms (IBS-SSS), QoL, microbiota composition	No difference in IBS symptoms between the groups. In FMT group significant reduction of symptoms at 3 months compared to baseline. The microbiota of the patients changed after FMT to resemble that of the donor.
Study III	UC	n=48, 1:1	Single frozen 30 g FMT <i>via</i> colonoscopy	Single fresh 30 g autologous FMT <i>via</i> colonoscopy	1 year	Clinical symptoms (Mayo score), f-calprotectin	No difference between the groups in maintenance of remission of UC or QoL
Study IV	Obesity	n=41, 1:1	Single frozen 30 g FMT <i>via</i> gastroscopy; bariatric surgery at 6 months	Single fresh 30 g autologous FMT <i>via</i> gastroscopy; bariatric surgery at 6 months	1,5 year	Body weight, QoL	No difference between the groups in body weight or QoL, both groups reduced weight equally well after bariatric surgery.

Abbreviations: IBS, irritable bowel syndrome; FMT, fecal microbiota transplantation; IBS-SSS, IBS symptom severity score; QoL, quality of life; UC, ulcerative colitis.

5.5 FECAL MICROBIOTA TRANSPLANTATION IN INFREQUENT DYSBIOSIS-ASSOCIATED CONDITIONS (I)

We reviewed the treatment results of eight patients with conditions other than rCDI, to whom FMT was given as an experimental treatment (Table 6). The conditions were *Salmonella* carriage (two patients), trimethylaminuria (TMAU, two patients), small intestinal bacterial overgrowth (SIBO, one patient), lymphocytic colitis (one patient), common variable immunodeficiency patient with chronic norovirus infection (one patient) and ESBL-producing *Escherichia coli* (*E. coli*) carriage (one patient). The two *Salmonella* infections and ESBL-producing *E. coli* were eradicated. For the other five patients, there was no benefit of FMT, or the benefit was indefinite.

TABLE 6. Patients with infrequent special conditions treated with FMT (Study I)

	Patient and diagnosis	Age at 1st FMT and gender	Route of administration	Outcome	FMT related complications
1	A carrier of <i>Salmonella</i> #1	17-year-old male	Colonoscopy	Successful eradication of <i>Salmonella</i>	No
2	A carrier of <i>Salmonella</i> #2	52-year-old female	Colonoscopy	Successful eradication of <i>Salmonella</i>	No
3	A patient with TMAU #1	24-year-old male	Gastroscopy	Mild transient self reported relieve of symptoms	No
4	A patient with TMAU #2	49-year-old female	Gastroscopy	No change in self-reported symptom severity	No
5	A patient with SIBO	66-year-old male	Gastroscopy (FMTx3)	Self-reported decrease in symptom severity	No
6	A patient with lymphocytic colitis	21-year-old female	Colonoscopy	Mild transient self reported relieve of symptoms	No
7	A carrier of norovirus	32-year-old female	Colonoscopy	No change in self-reported symptom severity, no success in virus eradication	No
8	A carrier of ESBL-producing	31-year-old female	Colonoscopy	Successful eradication of ESBL-producing <i>E. Coli</i>	No

Abbreviations: FMT, fecal microbiota transplantation; TMAU, Trimethylaminuria; SIBO, small intestinal bacterial overgrowth; ESBL, extended-spectrum beta-lactamase.

6 DISCUSSION

6.1 SHORT REVIEW OF RESEARCH FINDINGS AND THEIR MEANING

The main finding of this dissertation is that FMT showed no clear benefit over autologous placebo in a controlled trial with IBS, UC, and obesity patients (II–IV). However, microbiota analysis showed some degree of colonization of donor-like bacteria lasting for one year in IBS patients. Other positive findings were the treatment success in rCDI among immune compromised patients as well as success in eradicating ESBL *E. Coli* and *Salmonella* carriage (I).

Our positive findings with immunocompromised rCDI patients are in line with previous data, as other groups have published similar results as they have treated rCDI with FMT among immune deficient patients⁸². However, there are many different types and severities of immune deficiencies, and more knowledge of the theme is needed.

The results of controlled FMT trials for IBS have mixed results ranging from statistically significant results favoring placebo to a positive result favoring FMT (Table 2). Our trial yielded average results: no difference between the groups but a statistically significant transient improvement in IBS symptoms compared to baseline. Not only the donors but also many other aspects of the FMT protocol vary among these trials, such as route of administration, number of treatments, and patient selection. Thus, comparing the trials is not straightforward.

Maintenance of remission of UC was previously explored in one trial with positive results¹²⁴. In this trial, FMT was performed colonoscopically every two months, and the patients of this trial had previously reached remission with FMT treatments. Thus, our initial attempt to include recently diagnosed UC patients may have been a good idea (Study III). Unfortunately, our trial suffered from slow recruitment, and we had to expand the inclusion criteria and recruit patients with a longer duration of the disease.

Our obesity study was the first controlled trial to investigate FMT in bariatric surgery patients (IV). The controlled FMT trials for metabolic syndrome and obesity have applied the upper route of administration (Table 4). However, there is much variation between the trials in many aspects, such as patient selection, outcome measures and follow-up time. A sufficiently long follow-up time is necessary when investigating interventions for obesity. The follow-up

time in our trial was 18 months (IV), longer than the controlled FMT trials for metabolic syndrome and obesity (Table 4).

The negative results of our randomized trials may be caused by several factors. For one, it may be that microbiota does not have a remarkable role in the etiology of the diseases studied; however, some trials have reached clear differences between the treatment groups, indicating a causality between the gut microbiota, IBS, and UC ^{105,121}. Limited sample size, low volume or number of FMT treatments, donor selection, aerobic handling of the transplant, or the lack of pre-FMT antibiotic treatment are some of the possible factors that might have affected the lack of efficacy of FMT in our controlled trials.

6.2 STRENGTHS OF THE CLINICAL TRIALS

Our trials had clear advantages and novelty for the research field as well as for the clinic. The development of the FMT protocol has had a remarkable impact on FMT practices nationally and internationally ⁵⁶. The successful introduction of FMT to the clinic in several hospitals paved the way for further FMT research in Finland ⁷¹. The trials presented in this dissertation builds on these foundational studies. Treating rCDI patients with severe comorbidities benefitted the treated patients and provided grounds to consider FMT treatments among immunocompromised patients when drug approaches fail. Treating patients with special indications was innovative as well, and experiences in eradicating resistant pathogens were especially intriguing (I). The controlled clinical trials were well designed with proper blinding, and the follow-up times from one to one and a half years (II–IV) were longer compared to many other FMT studies ^{101,117,142}. The greatest advantage of our trials is the multidisciplinary approach involving clinicians of different specialties as well as microbiologists and bioinformatics. This liaison enables clinically relevant sample sets to be analyzed with the highest quality methods, which brings us closer to personalized medicine solutions.

6.3 LIMITATIONS OF THE CLINICAL TRIALS

The disadvantages and difficulties of the studies were mainly related to the lack of prior FMT trials when planning these studies. We applied a single administered FMT in each of these trials, although data are compiling that some nonrCDI indications may need repeated FMT sessions to achieve adequate outcomes ¹¹⁸. Additionally, it may be that increasing the dose of the transplant is beneficial in FMT for noninfectious diseases ¹⁰⁵. A clear disadvantage is the

level of dietary surveillance among the donor and the study participants, which will be addressed more comprehensively in our future studies. When planning studies, one must struggle between defining strict inclusion criteria to make the study population homogenous and to assure that the inclusion criteria are open enough to allow prompt recruitment. Our trials had a limited size (II–IV); however, the number of participants is comparable to FMT trials performed by other groups (Tables 2–4). Our study with colitis patients (III) suffered from slow recruitment, which further led to alterations in the inclusion criteria. Despite all efforts, the targeted sample size was not reached.

6.4 POSSIBLE MECHANISMS OF ACTION FOR FECAL MICROBIOTA TRANSPLANTATION

One of the main reasons why it is so difficult to find an optimal FMT protocol is the fact that we do not know what in FMT is the vehicle of the treatment effects, and the mode of action is likely to be different in various indications. Eradicating antibiotic-resistant pathogens such as *Clostridioides difficile* from the gut microbiota is an act of balancing the dysbiotic gut microbiota by applying colonization resistance (I). However, UC, IBS, and obesity may involve systemic, immunological, or metabolic responses to gut microbiota, and thus, the possible mechanisms are indirect (II–IV). The transplanted microbiome includes the bacteriome, virome, and mycobiome and, along travels, various metabolites¹⁸⁰. A group from northern Germany reported a successful treatment of five rCDI patients with an FMT of which bacteria had been filtrated, thus indicating that other components of stool, parts of bacteria, bacteriophages, or metabolites, may trigger the necessary change in the host microbiota and its function¹⁸¹. In animal models, “fecal virome transplantation”, a transplant from which other stool components except viruses have been processed apart as well as possible, has induced changes similar to FMT. In a Danish study, diet-induced weight gain and insulin resistance in mice were reversed with fecal virome transplantation from a lean donor¹³⁸. In another trial, transplantation of bacteriophages derived from UC patients worsened colitis in a mouse model¹⁸². In addition to bacteria and viruses, fecal metabolites may induce changes in the host microbiota and its function. The microbes of the gut signal to each other and the host by secreting different substances¹⁸³. Therefore, transplantation of only the metabolites, without the microbes, could induce meaningful changes in the gut microbiota, metabolism, or immunity of the recipient.

In our IBS trial, we detected a sustained shift of gut microbiota after FMT toward that of the donor; however, the resulting clinical effect was modest and transient (II). A possible explanation for this discrepancy is that certain

metabolites may mediate the clinical effect. Another possibility would be that the transplanted bacteria alter their actions after being transferred into a new atmosphere.

6.5 ENGRAFTMENT OF TRANSPLANTED MICROBIOTA

FMT for rCDI alters the microbiota of the recipient in the long term^{184,185}. As we showed in our trial with IBS patients, engraftment seems possible when FMT is performed for non-CDI conditions (II). This, similar to many other previous studies, applied 16S rRNA gene analysis, which lacks taxonomic resolution; therefore, differentiating recipient and donor-derived microbiota members is not possible. FMT studies applying fecal metagenome analysis provide more reliable data on engraftment, and a recent analysis combining data from 14 FMT trials showed that donor strain engraftment is strongly positively correlated with recipient microbiota dysbiosis and that engraftment could be enhanced by antibiotic pretreatment and bowel lavage¹⁸⁶. Our trials did not apply antibiotic pretreatment, but bowel lavage was performed prior to colonoscopy (I–III). Engraftment may be predicted from the abundance and phylogeny of bacteria in the donor and the recipient. Strains within a species seem to engraft in an all-or-nothing manner, and previously undetected strains may colonize the FMT recipient¹⁸⁷. The donor-recipient compatibility varies¹⁸⁸, and “a super microbiota” that would suit all patients is unlikely to exist. Thus, we need new strategies to predict engraftment. Autologous FMT introduces a microbiota, likely with a low degree of resistance by the host. Even though applied in many studies as a placebo, when administered *via* the upper route, the patient’s own fecal microbiota may alter the small bowel microbial environment. In one recent controlled trial, autologous FMT *via* the upper route even slowed the progression of type 1 diabetes¹⁵⁷. Additionally, in our trial with bariatric surgery patients (IV), we applied FMT *via* gastroscopy and autologous FMT as placebo without antibiotic pretreatment or bowel lavage. It will be interesting to analyze the microbiota samples; however, we do not have control samples from the small bowel after FMT or autologous placebo, and thus, we will be assessing the changes in the fecal microbiota of the patients.

6.6 IMPROVING THE EFFICACY OF FECAL MICROBIOTA TRANSPLANTATION

The clinical outcome of FMT is associated with engraftment in some studies¹⁸⁹, whereas in others, it is not¹⁹⁰; however, the final goal of FMT treatment is to induce significant clinical effects. Treating rCDI patients with FMT induces long-lasting clinical benefit for most patients, whereas in other diseases, the achieved effects have thus far been modest and transient^{146,191} despite successful microbial engraftment¹⁴⁷. The duration of the FMT achieved treatment effect may be prolonged with continued FMT treatments¹²². Data on long-term effects, especially in nonrCDI indications, are very limited. In certain settings, the clinical outcomes of FMT may be enhanced and prolonged with dietary intervention¹²⁶. Additionally, the effect of dietary intervention may be prolonged with FMT, as in one study, autologous FMT collected during diet-induced weight reduction and administered after dietary intervention in the weight regain phase could preserve weight loss¹⁵². Yet, this was true for only one diet containing a *Wolffia globosa* “duck weed” product, green tea, and a high amount of polyphenols. For future research, it appears worthwhile to combine FMT or other microbiota manipulations with well-planned and documented dietary interventions.

It may be possible to improve the efficacy of FMT therapy by applying multidonor transplants, that is, to prepare fecal transplants of feces from more than one donor. In Australian controlled FMT trials, this method was successfully used for active UC^{118,119}. The mixing of stool from many donors hinders the traceability of the fecal transplant to the individual donor and increases the risk of pathogen transmission. Therefore, the European panel of FMT experts does not recommend the multidonor approach⁶¹.

In our trial with IBS patients, a single donor was applied (II). However, there may be some reason for applying more than one donor in a study and comparing the outcomes they induce. In a controlled Canadian FMT study with UC patients, a clear difference in the efficacy between donors was found, as transplants from one donor were more effective than transplants from the other five donors¹¹⁷. Identifying the so-called super donors is one step forward in identifying the stool components that induce beneficial outcomes. In our studies with colitis patients and obesity patients, two donors were used (III–IV). The FMT studies to date have not been powered to find differences between the donors; however, this matter will be addressed as some of the ongoing trials are completed, including a Norwegian study investigating FMT for IBS among 450 patients allocated into four groups: three donors each forming one group and autologous transplant as a control group. (ClinicalTrials. Gov; identifier: NCT04691544).

Targeting patient recruitment with knowledge from previous FMT trials may further improve the outcomes. IBS patients enrolled in an FMT study should perhaps be more symptomatic at the study baseline than patients in our trial (I). Additionally, more targeted donor recruitment may be possible in the future. Analysis from the study by Paramsothy and colleagues revealed that *Bacteroides* in donor stool forecasted a positive outcome and that *Streptococcus* species in donor stool predicted a negative outcome in UC patients^{118,127}. Haifer and colleagues identified a clear difference in clinical efficacy for UC between two fecal donors and reported that microbiota stability and species evenness were markers of donor efficacy¹⁹². In a trial by Sood and colleagues, repeated FMTs were more likely to induce remission if the patient was younger, had left-sided colitis, and only moderate disease activity¹⁹³. The same group also found that the shorter the time from stool donation to transplantation, the better the outcome¹⁹⁴. Of note, fresh stool was used in this study instead of frozen stool.

6.7 DEVELOPING MULTIOMICS OF CLINICAL SAMPLES MAY REVEAL MECHANISMS OF ACTION

Controlled FMT studies should aim to serve basic science by providing sufficiently high-quality samples. When controlled trials are performed and when clinical effects are demonstrated, it is necessary to carry out a comprehensive analysis of fecal components and their function before and after treatment. As 16S rRNA gene sequencing has been applied in most recent FMT studies, future studies should aim to perform more precise whole microbiome sequencing. In addition to analyzing the genetic material of stool, it is of great importance to gather information on its action *via* proteomics and metabolomics from both fecal and blood samples. Not only collecting informative samples and applying the best omics possible but also finding meaningful associations between the omics data and clinical data demands the assistance of computerized analysis and artificial intelligence. Machine learning may be applied to build metabolic network models and detect microbiota-host interactions¹⁹⁵.

In our trial with IBS patients (II), we performed microbiota analysis with 16S rRNA gene sequencing, and further analysis applying metagenomic sequencing is ongoing. We are also performing microbiota analysis throughout our trials with colitis patients (III) and bariatric surgery patients (IV). These results will become available later due to the laboratory delays caused by the COVID-19 pandemic.

6.8 SAFETY OF FECAL MICROBIOTA TRANSPLANTATION

In our controlled trials, we did not detect differences in adverse events between the donor FMT and autologous FMT groups (II–IV). FMT also appeared safe for rCDI in immunocompromised patients in our case series (I). Most FMT-related adverse events (AEs) are mild and self-limiting, and FMT appears to be highly safe; however, the methodology should be further improved to reduce both delivery-related AEs and microbiota-related AEs ¹⁹⁶. FMT is not risk free, and there are documented FMT-related deaths, of which one is associated with aspiration due to sedation for colonoscopy ⁸². Sedation is a known risk factor for endoscopy-related adverse events; thus, in Finland, if sedation is used, it is applied in low doses, and the patient stays conscious. Unconscious sedation or general anesthesia is seldomly used.

In North America, one FMT-related death was caused by ESBL *E. coli* sepsis, which was transmitted to an immunocompromised patient from a fecal donor who was not tested for ESBL bacteria ¹⁹⁷. This underlines the importance of adhering to established recommendations in which comprehensive screening of MDR bacteria is included ^{51,61,198}. The epidemiological environment is constantly changing, demanding close monitoring and guidance by the scientific community, and thus, SARS-CoV-2 ⁵⁸ and monkeypox virus ¹⁹⁹ have recently been addressed by an international expert panel.

Expert consensus recommends FMT registries to gain further information on the long-term efficacy and safety of FMT ⁵⁷. One of the first FMT registry trials is ongoing in Finland (ClinicalTrials.Org, identifier NCT03732131). FMT-providing centers collect data from consenting FMT patients; however, the results of this trial have not yet been published. Perhaps the largest FMT registry is in North America, established by the American Gastroenterology Association (AGA). The first results were previously published, indicating a good real-life efficacy and safety profile of FMT for rCDI ²⁰⁰.

6.9 CONCLUSIONS AND STEPS FORWARD

FMT is an established method to cure rCDI ^{55,71}, and its accessibility in different geographical areas should be further improved ⁶⁴. Although the clinical results of our controlled trials were negative, FMT has shown potential in many non-CDI conditions ^{100,155}; however, further proof and standardization are needed. Transferring a whole microbial ecosystem to a new host opens a window to view microbiome-host interactions. To utilize the full potential of FMT studies, we should aim to further standardize the protocols by which FMT is exerted to enable comparison across studies. In addition, utilization of next-generation

sequencing methods, unifying methodology, and establishing data libraries would further precipitate the establishment of associations between the host and the microbiome. A self-explanatory step forward in FMT research is to enlarge the sample sizes when investigating the diseases and methods that have shown promise in earlier stage trials. Well-documented case reports with comprehensive mechanistic examinations will also have their place in the exploration of new innovations in the future.

This dissertation has presented early-stage controlled FMT trials in different conditions (II–IV). While the established benefit of FMT in rCDI was further confirmed and cases of successful eradication of other antibiotic-resistant bacteria were presented (I), the results of our three controlled trials with IBS, UC, and obesity patients, in light of the main endpoints, were negative (II–IV). However, establishing how the host-microbiota interaction functions is as crucial as showing how it does not. In terms of positive findings, we were not left completely empty handed. In the IBS trial, important new data on microbiota engraftment were reported as well as the effect of FMT on stool consistency (II). Due to resources and the COVID-19 pandemic, all planned microbiota analyses from all three controlled trials were not yet exerted; thus, new microbiological data will be reported after completing the analysis. The results presented in this thesis and the upcoming microbiota analysis to be published will inform future FMT trials to further develop the protocols for treatment and to bring FMT research to the next level.

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