Fecal Microbial Transplant for Weight Loss in Adults Living with Obesity and Diabetes

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Fecal Microbiota Transplant for Weight Loss in Adults Living with Obesity and Diabetes

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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>aMD</td>
<td>Adjusted mean difference</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>BP</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>CDI</td>
<td>Clostridioides difficile Infection</td>
</tr>
<tr>
<td>DALY</td>
<td>Disability-adjusted life years</td>
</tr>
<tr>
<td>DEXA</td>
<td>Dualenergy x-ray absorptiometry</td>
</tr>
<tr>
<td>DM</td>
<td>Diabetes Mellitus</td>
</tr>
<tr>
<td>FMT</td>
<td>Fecal Microbial Transplant</td>
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<tr>
<td>GI</td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>HDL</td>
<td>High density lipoprotein</td>
</tr>
<tr>
<td>HEC</td>
<td>Hyperinsulinemic euglycemic clamp</td>
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<tr>
<td>Hgb a1c</td>
<td>Hemoglobin a1c</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>Homeostatic Model Assessment of Insulin Resistance</td>
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<tr>
<td>HOMA-IS</td>
<td>Homeostatic Model Assessment of Insulin Sensitivity</td>
</tr>
<tr>
<td>IBD</td>
<td>Inflammatory Bowel Disease</td>
</tr>
<tr>
<td>IBS</td>
<td>Irritable Bowel Syndrome</td>
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<tr>
<td>LDL</td>
<td>Low density lipoprotein</td>
</tr>
<tr>
<td>LSI</td>
<td>Lifestyle Intervention</td>
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<tr>
<td>NAFLD</td>
<td>Non-alcoholic fatty liver disease</td>
</tr>
<tr>
<td>PPI</td>
<td>Proton pump inhibitors</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomized Clinical Trial</td>
</tr>
<tr>
<td>RMR</td>
<td>Resting Metabolic Rate</td>
</tr>
<tr>
<td>TEE</td>
<td>Total Energy Expenditure</td>
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<tr>
<td>TEF</td>
<td>Thermic Effect of Food</td>
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ABSTRACT

Obesity remains a pressing health concern amongst the US population. Currently, behavior modification is the primary approach to weight management. However, this strategy is not always successful and many are relegated to medications or surgery to achieve weight loss. Research associating the gut microbiome with obesity has fostered interest in microbiome manipulation as a means for weight loss. One method of manipulation, fecal microbial transplant (FMT), has received particular attention. While early inquiry into FMT and weight loss has been unrevealing, heterogeneous methodologies and lack of long term trials limits the ability to draw conclusions. To investigate the potential adjunctive role of FMT in weight loss, we propose a randomized, double-blind clinical trial in which the traditional lifestyle interventions of diet and exercise are supplemented with lean-donor FMT. At 12 months, percent weight change from baseline will be assessed. If successful, FMT may present a novel approach to obesity treatment.
CHAPTER 1: INTRODUCTION

1.1 Background

1.1.1 Current State of Obesity and Diabetes

The socioeconomic and health-related tolls of obesity are well described.\textsuperscript{1-4} With a complex interplay of social, psychological, environmental and genetic factors implicated in its pathogenesis, obesity present an ongoing challenge to public health.\textsuperscript{1,5,6} Despite growing efforts to combat the obesity epidemic at the federal and state level, prevalence rates have yet to show any decline, with the most recent National Health and Nutrition Examination Survey data (2017-2018) citing a prevalence of 40\% or more amongst all adult age groups.\textsuperscript{3} Between 1999 and 2018, the age-adjusted prevalence of obesity rose from 30.5\% to 42.4\% (Figure 1-A).\textsuperscript{3} Future projections are equally alarming, with prevalence expected to reach 48.9\% by 2030.\textsuperscript{7} It is evident that further interventions are needed to stem the ever-growing epidemic.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1a.png}
\caption{Figure 1-A: Trends in U.S. Age-Adjusted Obesity Prevalence} \textsuperscript{3}
\end{figure}
Among the many conditions associated with obesity, type 2 diabetes is one of the most significant.\textsuperscript{1,4,5,8} A 2018 meta-analysis reviewed more than 100 risk factors for type 2 diabetes and found “metabolically unhealthy obesity” was the factor supported by strongest evidence.\textsuperscript{9} Furthermore, a majority of other identified associations were considered “proxies” of obesity or elevated body mass index (BMI).\textsuperscript{9} Indeed, it has been estimated that for every 5 point increase in BMI over 25 kg/m\textsuperscript{2}, diabetes-related mortality increases by 210\%.\textsuperscript{1}

As of 2018, an estimated 27 million US citizens live with a diagnosis of diabetes, approximately 8.2\% of the total population.\textsuperscript{10} The true value is likely greater, with an additional 7.3 million Americans thought undiagnosed.\textsuperscript{10} As the 8\textsuperscript{th} leading cause of death and 4\textsuperscript{th} leading cause of disability-adjusted life years (DALY), diabetes is estimated to cost the public $237 billion dollars in direct medical costs each year, and an additional $90 billion when productivity loss is accounted for.\textsuperscript{11-13} While the age-adjusted incidence of diabetes has declined over recent years, rates are largely unchanged from 20 years ago – 6.2 in 2000 compared to 6.7 in 2018 (Figure 1-B).\textsuperscript{10} However, rates are not forecasted to remain stable, with a 2018 model predicting the number of diagnosed to rise to 39.7 million by 2030 and 60.6 million by 2060.\textsuperscript{14}

![Figure 1-B: Trends in U.S. Incidence of Diagnosed Diabetes, Age-adjusted\textsuperscript{10}](image)
The percentage of foreign-born individuals living in the United States has nearly tripled in the past 50 years, rising from 4.7% to 13.7%. Therefore, it is important to briefly note the global state of these conditions. Diabetes is the leading cause of death and reduced life expectancy worldwide, and of all risk factors, high BMI contributes the most to deaths (30.8%) and DALYs (45.8%) in those with the condition. The global prevalence and incidence of diabetes have risen substantially over the past 30 years, going from 211.2 and 11.3 million in 1990, to 476.0 and 22.9 million in 2017, respectively. Without effective prevention, the Internal Diabetes Federation projects 693 million individuals will be living with diabetes by the year 2045. Hence, a novel approach to obesity management is needed to avert a worsening health crisis, both in the US and abroad.

1.1.2 The Gut Microbiome and Obesity

The gut microbiome has been an area of medical interest for hundreds of years. However, up until the turn of the century, technologically limited analytic techniques left scientific investigation seemingly impossible. With the help of new technology, research into the human microbiome has exploded over the last 20 years, and today, the gut microbiome is appreciated as an immunologic and metabolic organ. Heavily implicated in various human disease, the gut microbiome has now been associated with diseases of the central nervous system, cancer protection, metabolic function, and drug metabolism. Today, obesity is amongst those conditions associated with the microbiome.  

Notable differences have been found amongst the gut flora of lean and obese organisms. First noted in mouse models, these differences have more recently been shown to occur between obese and lean humans as well. A 2019 review compiled a list of 28 studies which had investigated the influence of obesity on the microbiome. In all but five studies, an association was
seen between various bacteria and either: BMI itself; obesity-related conditions; or changes in BMI. The directionality and significance of these relationships has been explored. Starting in the mid-2000s, research by Bäckhed and colleagues revealed a physiologic relationship between circulating gut microbial factors and fat metabolism. Around the same time, a landmark study by Turnbaugh et al. revealed that the microbiome of obese mice was more efficient at energy extraction compared to their lean littermates and, that by colonizing germ free mice with the “obese” microbiome, obesity could be “transmitted”. Groundbreaking at the time, these studies fostered speculation that microbial alterations could in fact lead to weight change.

### 1.1.3 Probiotics and Weight Change

Probiotics, or ingestible sources of live bacteria, were the first microbiome-altering intervention to elicit study. However findings to date have largely been equivocal. A 2019 meta-analysis utilized a random effects model to assess the effect of oral probiotic supplementation on body weight, BMI, and waist circumference. Analyzing 28 randomized and quasi-randomized parallel studies, researchers found that while probiotics were shown to induce a modest reduction in waist circumference, there was no association with decrease in BMI.

A 2015 meta-analysis employed much stricter inclusion criteria, including only randomized controlled trials (RCTs) in which study groups were treated identically aside from the probiotic intervention. Across nine included studies, no relationship between probiotics and BMI or obesity was shown. In contrast, a 2018 meta-analysis of 15 studies found that the administration of probiotics did lead to significantly more weight loss when compared to placebo. However, effects were modest, with a weighted mean difference of only -0.6 kg [-1.19, -0.01] kg.

A separate 2017 meta-analysis took an interesting approach, assessing the effect of probiotics on weight change not only in adults, but pediatrics as well. Across 13 adult, 17
pediatric, and 23 infant RCTs, researchers found that, while lactobacilli-based probiotics led to significantly more weight loss among adults (standardized mean difference of -0.54 [-0.83, -0.25]), lactobacilli probiotics led to minor weight gains in the pediatric and infant populations, with respective standardized mean differences of 0.20 [0.04, 0.36] and 0.30 [-0.01, 0.62].

In perhaps the most inclusive meta-analysis to date, a 2019 study conducted by Koutnikova et al. assessed 105 articles spanning 1990-2018, analyzing the impact of probiotics on 15 variables associated with obesity, diabetes and non-alcoholic fatty liver disease. Koutnikova et al. found probiotics to have a beneficial impact on total body weight, BMI, waist circumference, body fat mass and visceral fat mass – but only in the overweight, non-obese population. Importantly, probiotics did bestow metabolic benefit in particular populations; subjects with type 2 diabetes saw improvements in glycemic control, while those with liver disease showed improvements in certain liver enzyme levels.

Given the large, heterogeneous literary base of probiotic trials and discordant results of meta-analyses, the true effectiveness of probiotics on weight and metabolic outcomes is still unknown.

1.1.4 A New Form of Microbiome Manipulation: Fecal Microbial Transplantation

With the utility of probiotics still uncertain, the use of fecal microbial transplant (FMT) as an alternative method for microbiome manipulation has received increased attention. FMT has been formally described as a “method to directly change the recipient's gut microbiota to normalize the composition and gain a therapeutic benefit”. Arguably, FMT has found greatest utility in treating *Clostridioides difficile* infections (CDI), where it has become the standard of care for refractory cases. Having shown success in CDI, FMT is currently being explored in other microbiome-related conditions, including inflammatory bowel disease (IBD) and obesity.
To date, there is animal research illustrating the potential efficacy of FMT in inducing metabolic changes. In a particularly revealing set of studies, mice receiving fecal transplants from either lean or obese humans went on to mirror the adiposity phenotypes of their respective human donors.\textsuperscript{40,41} FMT has also shown an ability to induce weight loss in animals. In a murine model of Roux-en-Y Gastric Bypass, mice that underwent surgery showed marked alterations in the makeup of their microbiome, and when their stool was introduced into non-operated mice, significant weight loss ensued in the recipients.\textsuperscript{42} In a separate study, intestinal samples were taken from a wild boar – an organism that displays high lean and low fat mass – and infused into mice. Recipient animals showed a marked ability to maintain weight in the setting of a high fat diet and stave off the lipid disorders expectant of a high-fat diet.\textsuperscript{43} Finally, in a study investigating FMT in an adjunctive role, mice subjected to a high fat diet for 12 weeks were placed on either a 25% calorie-restricted diet alone, or in conjunction with an autologous or heterologous FMT.\textsuperscript{44} After 6 weeks, animals receiving adjuvant autologous FMT showed a significantly lower weight than mice receiving diet restriction alone, though both FMT types led to greater results than diet restriction alone. Particularly relevant to our proposal, these study results suggest improved outcomes when using FMT as an adjunct to lifestyle intervention \textit{i.e.} diet.

To date, few studies have been completed in humans, the quality and specifics of which will be subject of our literary review. In summary, early results have largely been unrevealing; however, this area of study is in its infancy and many avenues regarding the use of FMT in weight loss remain unexplored. Additionally, given the remarkable heterogeneity amongst existing studies, it would be irresponsible to draw any conclusions at this time. It is upon these inadequacies our proposal is based.
1.1.5 Gut Microbial Diversity

Central to our study proposal is the idea of “gut microbial diversity”, a concept referring to the number and abundance of different microbial species in the gut, and how evenly the species are distributed.\textsuperscript{45,46} An individual’s bacterial profile is as unique as their fingerprint, and is the result of both innate and environmental factors.\textsuperscript{47} Colonization of the human intestine begins \textit{in utero} and is influenced by the mother’s diet, health and own intestinal flora.\textsuperscript{48-50} At delivery, the infant’s gestational age, mode of delivery, and first form of feeding drastically influence initial colonization.\textsuperscript{48,50} During early childhood, the intestinal microbiome develops quickly and is influenced by diet, medication use and genetics. By year 3-4 of life, the human microbiota is fully mature and will largely remain stable for the rest of life.\textsuperscript{48,50,51} However, numerous factors have still been found to impact the adult microbiome, including geographic location, age, food supply, lifestyle factors (smoking, exercise, diet, household characteristics), stress (physiologic, psychologic) and medication use.\textsuperscript{52-55} Though the impact of these factors is believed small in comparison to those occurring in early life, these variations can upset the microbial balance of the gastrointestinal tract, leading to a state of “dysbiosis”.

Dysbiosis is associated with many different disease states, including irritable bowel syndrome (IBS), IBD, mental health conditions, liver disease, heart disease and obesity, among others. In fact, some conditions have been associated with disruptions occurring within specific microbial species.\textsuperscript{54} A decrease in gut diversity is viewed as an indicator of dysbiosis, and reduced diversity has itself been associated with adverse health conditions. These include IBD, celiac disease, psoriatic arthritis, diabetes type 1 and 2, metabolic syndrome and obesity, though some of these associations are still challenged.\textsuperscript{45,51,56-61} Given its relationship with disease, diversity has been described as a marker of a “healthy gut”.\textsuperscript{45,50,62}
Measuring gut diversity is commonly accomplished through use of diversity indices. In the assessment of gut microbial diversity, one of the most widely used measures is the Shannon Diversity Index (Eqn. 1-A), a specific measurement of $\alpha$-diversity, or diversity within a single community.\cite{46,63-65} Shannon diversity equally accounts for the number of species in a community (richness) and degree to which species are evenly distributed (evenness).\cite{46,64}

\[
H' = -\sum_{i=1}^{s} p_i \ln p_i
\]

**Equation 1-A:** Shannon Diversity Index. *Where $p_i$ is the proportion of organisms found for the $i$th species, and $s$ is the total number of species.*

Despite their widespread use, major limitations to the use of diversity indices exist. Firstly, measurements obtained using different equations are not comparable. Additionally, diversity indices inherently exhibit bias – they are dependent on “how hard one looks, and also how many species there are and in what relative abundances”.\cite{66} In the context of measuring microbial diversity, this statement alludes the importance of the microbial sampling methods chosen; a sample obtained via shallow analytical means will typically reveal fewer bacterial species than one obtained using a more comprehensive approach. For each additional bacterial species found, the relative abundance of each individual species decreases, thereby changing the diversity value. As such, analytical considerations, such as the sequencing depth used during metagenomics sequencing (i.e. how hard one looks), exhibit substantial influence over diversity measurements.\cite{64,67} Oftentimes, specifics used in metagenomic sequencing are not published, making any appraisal of the diversity metrics used in a study difficult. Finally, microbial diversity can be influenced by many of the same factors that influence bacterial colonization – age, geography, disease and exposure to medications.\cite{60,68} This largely bars the comparison of different
populations. In summary, despite routine use in microbial studies, diversity measurements are limited by a lack of generalizability and high reliance on investigator-specific techniques.

1.2 Statement of the Problem

There is burgeoning research to support the use of FMT in non-CDI related conditions. While initial investigation into FMT and weight loss has been unrevealing, a limited number of trials, heterogeneity of methodologies and lack of long-term follow-up leaves much to explore.\(^{69}\) In particular, empiric research suggests the utility of FMT in specific population groups. As research into the respective microbiomes of obese and lean individuals continues, microbial diversity has revealed itself a feature of significance, with lower gut diversity routinely seen in those with obesity.\(^{51,59,70-73}\) While the relevance and relation of gut diversity to obesity has not been fully elucidated, the nature of diversity indices has made it difficult to compare studies and draw conclusions.\(^{56,74}\) Despite this limitation, there remains opportunity for a stratified study approach, wherein the baseline gut diversity of subjects is accounted for. However, this type of analysis has not yet been performed. This offers the potential for an intriguing proposal, as past sub-analyses have shown those with lower baseline gut diversity derive greater metabolic benefit from FMT when compared to their more diverse cosubjects.\(^{75,76}\)

Additionally, it has been long known that diet impacts the composition of the gut microbiota.\(^{56,77-82}\) More recently, evidence has surfaced indicating physical activity can lead to improved gut diversity.\(^{83,84}\) Taken together, it is reasonable to posit that exercise and diet could impact FMT-induced microbial change. In fact, numerous research groups have deemed the combination of FMT and lifestyle change an intervention worthy of investigation.\(^{57,85,86}\) Even so, the use of FMT in conjunction with lifestyle intervention remains an unfrequented area of study.
To date, only 3 studies have investigated FMT alongside a form of lifestyle intervention, all of which were published in the last year. 86-88

1.3 Goals and Objectives

The goal of our study is to examine the effects of FMT on weight loss when used in an adjunctive role to lifestyle intervention; and, specifically whether FMT confers greater benefit to individuals possessing lower baseline gut diversity. Additionally, placebo interventions will be used to help determine the relative benefit those with lower, or higher, baseline diversity could expect from supplemental FMT. The overall objective is to measure, and compare, the average percent weight change experienced by two intervention and two placebo groups to determine if FMT has increased efficacy in those who exhibit more profound dysbiosis at baseline, and if the benefit is substantial when compared to lifestyle intervention alone.

1.4 Hypothesis

Among adults with obesity and type 2 diabetes undergoing lifestyle modification for 1 year, those supplemented with 50g lean-donor FMT capsules every 3 months will display, on average, a percent weight change significantly different from that of those receiving placebo. This difference will be greatest in the stratum consisting of subjects with lower baseline gut diversity, as measured by Shannon Index, rather than the stratum comprised of more-diverse subjects.
1.5 Definitions

➢ *Adults*: Individuals aged 18-65

➢ *Obesity*: BMI >30 mg/kg²

➢ *Lifestyle Modification*: Consists of 2 components:
  o *Diet*: calorie restricted, with additional guidance on specific eating behaviors
  o *Exercise*: 150–300 minutes of moderate, or 75–150 minutes of vigorous activity a week

➢ *Lean-Donor FMT*: Fecal transplant from an individual with a BMI >18.5 to < 25 mg/kg²

➢ *Gut Diversity*: variability in the number and abundance of different microbial species

➢ *Gut Diversity Stratification*: Upper or lower 50th percentile of baseline Shannon Index values, based on the values amongst study subjects

References

34. Borgeraas H, Johnson LK, Skattebu J, Hertel JK, Hjelmesaeth J. Effects of probiotics on body weight, body mass index, fat mass and fat percentage in subjects with overweight or obesity: a


CHAPTER 2: REVIEW OF THE LITERATURE

2.1 Introduction

To evaluate the practicality and novelty of our initial proposal, a cursory review of the literature was performed in July 2021 using the PubMed and Ovid research databases. A more thorough review was conducted in December 2021, followed by a final review in March 2022 to capture any studies published shortly before final presentation.

In December 2021, a methodical literary review regarding FMT, obesity and metabolic outcomes was performed across several online databases – Ovid-Medline, Ovid-Embase, Scopus and Cochrane Library. Primary searches utilized various combinations of the following key MeSH terms: “obesity” (morbid or unspecified), “microbiota”, “fecal microbial transplantation”, “FMT”, “clinical trial” and “weight loss”. Ovid-Medline search results were manageable, with fewer than 100 articles appearing for each individual query. On Scopus and Medline-Embase, particular searches yielded hundreds of results, and a restricted search using the “article” qualifier returned more refined results. In surveying Cochrane Library, primary search terms “obesity” and “diabetes mellitus” were paired with secondary terms “fecal microbiota transplant” and “fecal microbial transplant”. Searches yielded only trial results; no Cochrane reviews or meta-analyses were identified. The number of trials ranged from one to several dozen. Similarly, a singular search term of “fecal microbial transplant” yielded 188 trials.

Additionally, a search for relevant, ongoing studies was conducted through clinicaltrials.gov. Using the same surveying approach in the Cochrane Library, primary conditions queried were “obesity” and “diabetes mellitus”, and these were then paired with secondary terms of “fecal microbiota transplant” and “fecal microbial transplant”. Over a dozen trials involving FMT, weight loss and metabolic outcomes are listed as ongoing at this time.
Results were filtered by their relevance and restricted to those involving human subjects. The principal studies discussed herein were obtained using the above approach. Supporting articles were obtained either using a more directed search on Ovid and PubMed platforms, or through citations included in principal papers.

2.2 Review of Empirical Studies

A majority of FMT researchers investigate metabolic and weight-based outcomes concurrently, often prioritizing one or the other. Most often, researchers compare the effects of placebo or autologous FMT, with allogenic FMT. In the context of FMT research, allogenic FMT refers to the transfer of another organism's stool or microbial specimens into a subject, while autologous FMT refers to the reintroduction of stool or microbial specimens that were harvested from a subject prior. For the purpose of structuring this review, we will describe studies according to primary outcome of interest.

2.21 Glycemic Control Outcomes

Of those researchers investigating the impact of FMT on metabolic outcomes, the overwhelming majority have chosen to focus their efforts on glycemic control. With a groundwork in animal research laid by Turnbaugh and Bäckhed, FMT researchers turned their attention to human trials in 2012.1-3 In the first trial to investigate the metabolic impact of FMT in humans, Vrieze et al. conducted a randomized, double blind trial in which 18 males with obesity and features of metabolic syndrome received a small intestinal infusion (SI infusion) comprised of microbial samples from either a lean-donor, or the subject themselves.4 At 6 weeks, the allogenic group showed significant improvement in peripheral insulin sensitivity, as measured by the median rate of glucose disappearance using hyperinsulinemic euglycemic clamp (HEC), changing from a
rate of 26.2 \( \mu \text{mol/kg/min} \) at baseline to 45.3 \( \mu \text{mol/kg/min} \) (\( p < 0.05 \)). No significant change in HEC values was observed in the autologous group. At the same time, Vrieze et al. noted study subjects had displayed less microbial diversity at baseline compared to the lean donors, and after allogenic infusion, the microbiomes of these individuals sustained a significant rise in \( \alpha \)-diversity levels as measured by Simpson reciprocal diversity index (178 to 234 species; \( p < 0.05 \)).

In 2017, a similar outcome was recorded by Kootte et al., using the same intervention and primary outcome.\(^5\) Aiming to reproduce the results of Vrieze et al. in a larger cohort, Kootte et al. recruited 38 males with obesity and metabolic syndrome, more than twice the sample of the former study. At 6 weeks, the allogenic group again showed significant improvement in peripheral insulin sensitivity, as measured by HEC (25.8 to 28.8 \( \mu \text{mol/kg/min} \), \( p < 0.05 \)). These improvements in insulin sensitivity were also associated with a modest improvement in hemoglobin \( a_1c \) (39.5 to 38.0 \( \mu \text{mol/mol} \), \( p < 0.01 \)). Notably, Kootte et al. tracked results for a period of 18 weeks compared to the 6 weeks of Vrieze et al, and at the end of 18 weeks the improvements in insulin sensitivity were no longer statistically significant.

While short term improvement in insulin sensitivity was seen in both trials, Kootte et al. did not appreciate the changes in gut diversity that Vrieze et al. did; however, it is important to note Kootte et al. used a different index – Shannon Index – to quantify diversity levels. While bacterial diversity of the allogenic group did not change over the course of their trial, Kootte et al. conducted a sub-analysis that was absent from the study of Vrieze et al. When comparing the microbiomes of those showing >10% improvement in insulin sensitivity with those who did not, Kootte et al. noticed the baseline gut diversity of responders had been significantly lower than that of non-responders (\( p<0.05 \)). In turn, both the results of Kootte et al. and Vrieze et al. suggest a relationship exists between gut diversity and metabolic outcomes. Importantly, diversity was not
the only microbial metric examined by the two studies, and both groups noticed shifts within
certain bacterial species as well. Vrieze et al. saw a relative increase in several butyrate-producing
species, while Kootte et al. saw an increase in acetate-producing species. These compounds have
been associated with glycemic metabolism and insulin sensitivity, respectively.\textsuperscript{6,7} The studies of
Kootte and Vrieze are summarized in table 2-A.

Though both studies demonstrated statistically significant results, both authors later
reported in a review that they had seen a wide range of metabolic responses amongst their cohorts.\textsuperscript{8} This observation was examined further by Li et al. in 2016.\textsuperscript{9} In a study published shortly before
the work of Kootte et al., Li et al. further analyzed 55 stool specimens originating from the subjects
of Vrieze et al. From these specimens, Li et al. discovered that the microbial interactions occurring
between donor and recipient flora had varied across donor-recipient pairs. In some recipients,
donor strains largely replaced the indigenous bacteria, but in other subjects, the donor and recipient
strains co-existed. Moreover, these compositional changes persisted more than 3-months. The
results suggested differing degrees of microbial compatibility and raised the question if individuals
with certain microbial features may be more amenable to FMT than others. If true, this would raise
prospects of developing microbiome-targeted interventions. However, the microbial features
contributing to compatibility differences were unclear.

In 2020, additional research would partially reinforce the findings and conjectures of the
prior studies. Seeking to augment the results of Vrieze and Kootte by employing a multiple FMT
approach, Yu et al. conducted a 12-week double-blind randomized pilot trial in which 24 adults
with obesity and mild-moderate insulin resistance were randomized to receive 6 weeks of either
placebo or lean donor FMT capsules.\textsuperscript{10} Using the same primary outcome as the two
aforementioned studies, Yu et al. also assessed 6-week changes in insulin sensitivity as measured
by HEC. While the FMT group did show improvement in HEC values at 6 weeks, results were non-significant. However, akin to Vrieze and Kootte et al., metabolic responses were again highly variable amongst subjects. No significant changes in gut richness or Shannon index values were recorded in the FMT-treated group.

While the primary endpoints of Yu et al. did not align with those of Vrieze or Kootte et al, several observations made by Yu et al. did support the findings of these prior studies. Of the multiple stool donors used in the study by Yu et al., one in particular displayed markedly higher engraftment rates than the others, with a median autonomic sequence variant (ASV) engraftment rate of 47%. For comparison, the median ASV engraftment rate of other donors was only 9.5%. Reasons behind this finding were unclear, but consistent with an underlying occult relationship between donor and recipient flora. Additionally, though a baseline microbial comparison of the two groups was not published, Yu et al. did conduct a sub-analysis amongst subjects with low baseline gut diversity, defined as those having a baseline Shannon index value below the study median. Here, low-diversity FMT subjects (n=4) showed greater improvement in Hgb A1c and fasting glucose levels than low-diversity placebo subjects (n=11). Though a low subject number largely limited this analysis, the findings suggested that individuals with lower diversity benefited more from FMT. The study by Yu et al. is summarized in table 2-B.

None of the reviewed studies discussed thus far have incorporated any adjunctive form of lifestyle intervention (LSI) into their FMT protocol, and until 2021 no such trial had been published. However, this is not to say the combination of FMT and LSI was a foreign concept. Indeed, for many years different researchers had expressed this was the logical next step for FMT research to take. In one of the first to do so, Mocanu et al. supplemented lean-donor FMT with dietary fiber. Different fiber types promote the growth of various bacterial species and Mocanu
et al. therefore questioned if supplementation could enhance the effects of FMT on glycemic outcomes.\textsuperscript{12,13} In this double-blind, randomized trial, 70 subjects with obesity, impaired glycemic control and features of metabolic syndrome were randomized to receive a daily fiber packet (33g for males, 27g for females) comprised of either high fermentable fiber sources (soluble corn fiber, resistant wheat starch, and acacia gum) or low fermentable fiber sources (cellulose). In addition, subjects were randomized to receive either lean-donor FMT capsules or placebo, leaving four study groups in total. The primary outcome was 6-week change in insulin sensitivity and resistance as measured by homeostatic model assessment (HOMA2-IR/IS).

At the end of 6 weeks, findings of Mocanu et al. were similar to those of prior studies – the low fiber, FMT group (LF-FMT+) showed significant improvement in HOMA2-IR values (3.77 to 3.16; \( p = 0.02 \)), with changes losing significance by week 12. Notably, the diet intervention had ceased at 6 weeks, suggesting the diet intervention had indeed influenced outcomes. In addition to the glycemic changes, microbial findings also aligned with those of prior studies; the gut bacterial \( \alpha \)-diversity of the LF-FMT+ group significantly increased by week 6 and statistical significance was maintained at week 12. Of note, Mocanu et al. used the chao1 richness index to measure \( \alpha \)-diversity, which differs from Shannon index in that it does not account for evenness.\textsuperscript{14} Shannon index values were also calculated, but not significant. In addition to diversity changes, improvements in insulin sensitivity were positively associated with the number of bacterial taxa engrafted from donors. The study by Mocanu et al is summarized in table 2-B.

While the aforementioned studies offer support for the role of lean-donor FMT in treating insulin resistance, as well as for a relationship between glycemic outcomes and microbial features, these findings are not unchallenged in the literature.
In 2020, Craven et al. also aimed to investigate the effects of FMT on insulin sensitivity, albeit in a different population. Craven et al. recruited 21 adults with non-alcoholic fatty liver disease (NAFLD), a condition strongly associated with metabolic syndrome. In a 3:1 ratio, subjects were randomized to either an allogenic, lean-donor FMT, or an autologous FMT, delivered by endoscope to the distal duodenum. As the primary outcome, changes in insulin sensitivity were measured at 6 weeks using the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR). At 6 weeks, neither the allogenic nor the autologous group had significant change in HOMA-IR values. Additionally, no difference in baseline gut diversity was seen between intervention groups. Taking their microbial analysis further, Craven et al. conducted a similar sub-analysis to that of Kootte et al., comparing the microbiomes of those showing >10% improvement in insulin resistance with those who did not. On investigation, baseline diversity values did not differ between responders and non-responders, and the diversity levels of responders did not significantly increase during the course of the trial, though contrastingly the HOMA-IR non-responder group did sustain increased diversity over the course of the trial \( (p = 0.0078) \). The study of Craven et al. is summarized in Table 2-B.

While this concludes the review of studies incorporating glycemic control as a primary outcome, several trials incorporating glycemic control as a secondary endpoint have shown substantial results. In 2021, Koopen et al. enrolled 24 male subjects with obesity and metabolic syndrome and, prior to FMT administration, subjects adhered to a Mediterranean diet for 2 weeks. At the end of these two weeks, subjects saw significant improvement in fasting blood glucose \( (p=.02) \) and borderline significant improvement in insulin resistance as measured by HOMA-IR \( (p=0.056) \). Subjects were then randomized to autologous or lean-donor microbial SI infusion. While the primary aim was to assess if the 2-week dietary intervention could improve
subsequent FMT engraftment, secondary outcomes included changes to glycemic parameters. Six (6) weeks following FMT administration, while the FMT group saw a modest, but significant improvement in Hgb A1c values (p=.01), both hepatic and peripheral insulin sensitivity rates were unchanged. These results stood in stark contrast to the prior findings of Kootte, Vrieze and Mocanu et al. This led Koopen et al. to postulate that the dietary intervention and its resultant metabolic benefits, “rendered hosts-less susceptible to FMT-based intervention”; that is, the benefits of the dietary intervention reduced how much “room” there was for the lean-donor FMT to work. For their microbial analysis, Koopen et al. first measured gut diversity levels at the end of the 2-week diet intervention. This level was then compared to stool samples taken 3, 6, and 12 weeks post-FMT. At end, no significant changes in α-diversity levels were recorded in either group. A summary of the trial by Koopen et al. is available in table 1-A

In a 2020 trial, Allegretti et al. investigated FMT safety as a primary outcome. In this double-blind, placebo-controlled study, 22 subjects with obesity and no prior history of diabetes, metabolic syndrome or nonalcoholic steatohepatitis (NASH) were randomized to intermittently receive either allogenic lean-donor capsules or placebo capsules over a course of 12 weeks (Table 2-D). Given a primary outcome of safety, the effect of FMT on blood insulin and glucose concentrations was not investigated, however Allegretti et al. would later conduct a secondary analysis of their study data, which was published a year later. In this subsequent analysis, the impact of FMT on post-prandial glucose and insulin levels was assessed by area under curve analysis (AUC in mg/dl x 60 min.) following administration of a mixed-meal test.

At 12 weeks, Allegretti et al. discovered that FMT subjects had significantly lower AUC glucose levels than those receiving placebo (579 mg/dl x 60 min vs. 1978 mg/dl x 60 min, p=0.03). Six (6) week values were also lower in the FMT group, but not statistically significant. Similarly,
the FMT group had significantly lower AUC insulin levels at 6 weeks compared to placebo (137 μU/ml x 60 min vs. 2728 μU/ml, p=0.02). Twelve (12) week values were also improved for the FMT group, but not statistically significant. The findings of this secondary analysis by Allegretti et al. are profound. While it revealed glycemic improvements similar to those of the aforementioned studies, these results differed in that a benefit was demonstrated in metabolically healthy subjects, suggesting benefit is not restricted to only those with baseline metabolic dysfunction.

Further support for the impact of FMT on glycemic parameters came in a 2020, New Zealand trial. As the only pediatric FMT trial to date, Leong et al. elected to investigate the impact of FMT on BMI in an adolescent population. In this study, dubbed “The Gut Bugs Trial”, 87 children (age 14-18) with obesity were randomized to receive either lean-donor FMT capsules, or saline placebo. While no changes in insulin sensitivity were seen on primary analysis, a post-hoc analyses produced substantial results. This analysis involved subjects in the FMT group who had been found to have underlying, undiagnosed metabolic syndrome (n = 18). These were children who had no known history of metabolic syndrome prior to enrollment, and were identified only as a result of initial study measurements. At 6 weeks, these children had sustained a 34% improvement in HOMA-IR values (adjusted mean difference (aMD) 0.66; p = 0.018), a 29% decrease in fasting insulin levels (aMD 0.71; p = .042), and a 7% decrease in fasting glucose levels (aMD −0.38; p = 0.033). Though these improvements were no longer statistically significant by week 12, they were still substantial enough that by week 26, 14 of the 18 subjects no longer met criteria for metabolic syndrome. Leong et al. proceeded to compare the microbiomes of those with resolved and non-resolved metabolic syndrome, but no significant differences in composition were seen. A summary of the study by Leong et al. is provided in Table 2-C.
Finally, in a study focusing on the effectiveness of FMT in preventing weight regain following rapid loss, Rinott et al. first randomized subjects to 6 months of exercise and dietary intervention.21 Following weight loss, subjects were randomized to receive autologous FMT capsules, or placebo capsules, every 3-weeks for 6 months while also continuing previously assigned lifestyle interventions. Changes in fasting insulin levels were measured as a secondary outcome. Within one of the diet intervention groups, subjects receiving autologous FMT capsules sustained a significant change in fasting insulin levels between months 6 and 14 compared to those from the same group receiving placebo (FMT −1.46 μIU/mL vs placebo +1.64 μIU/mL, p =0.04).
<table>
<thead>
<tr>
<th>Author</th>
<th>Population</th>
<th>Intervention</th>
<th>Relevant Outcomes</th>
<th>Relevant Results &amp; p-values</th>
<th>Notable Findings</th>
</tr>
</thead>
</table>
- Adult males, age 40-55  
- BMI <30 kg/m² OR  
- Waist circumference < 102 cm  
- Fasting plasma glucose level > 5.6 mmol/L  
  n = 18 | SI microbial infusion:  
- autologous infusion  
OR  
- allogeneic infusion, lean-donors | 1. ▲ in insulin sensitivity at 6 weeks post FMT  
2. ▲ in particular small/large intestinal microbial species | 1^a: ▲ peripheral insulin sensitivity in autologous group at 6 weeks  
  p < 0.02  
2^a: 10 bacterial groups ▲ gut diversity  
  ▲ in allogeneic group  
  p < 0.02  
- No ▲ in total fecal bacteria | - Obese microbiome characterized by ▲ microbial diversity compared to lean donor  
- Gut diversity significantly ▲ after allogeneic microbe transfer, but not autologous transfer |
- Adult, age 21-69  
- BMI ≥30 kg/m² AND  
  met criteria for metabolic syndrome  
  n = 38 | SI microbial infusion:  
- autologous infusion  
OR  
- allogeneic infusion, lean-donors (1 or 2) | 1. ▲ in insulin sensitivity at 6 and 18 weeks post FMT  
2. ▲ in microbial composition at 6 post FMT | 1^b: ▲ peripheral insulin sensitivity in autologous group at 6 weeks  
  (p < 0.05); no significant change in either group at 18 weeks  
2^b: No ▲ in microbial composition of either group:  
- Autologous p = 0.29  
- Allogeneic FMT p = 0.49 | - Changes to insulin sensitivity lost significance by 18 weeks  
- Sub-analysis: those who responded most to intervention had significantly ▲ gut diversity at baseline |
- Adult, age 21-69  
- Omnicore  
- BMI ≥30 kg/m² AND  
  met criteria for metabolic syndrome  
  n = 20 | SI microbial infusion:  
- autologous infusion  
OR  
- allogeneic infusion, vegan, lean-donors | 1. ▲ in postprandial TMAO production from di-functional and di-carnitine  
- ▲ in gut microbial composition at 2 weeks  
2. ▲ in vessel wall inflammation at 2 weeks | 1^c: No ▲ in postprandial TMAO production:  
- Autologous p = 0.57  
- Vegan FMT p = 0.51  
- No ▲ in gut diversity.  
- Autologous p = 0.72  
- Vegan FMT p = 0.26  
2^c: No ▲ in vessel wall inflammation.  
- Autologous p = 0.28  
- Vegan FMT p = 0.90 | - In allogeneic group, the microbe of only certain recipients shifted towards vegan profile, others in the group did not show this change |
- Adult, age 21-65  
- BMI <30 kg/m² AND  
  met criteria for metabolic syndrome  
  n = 24 | 2 week run-in period of Mediterranean diet  
- SI microbial infusion:  
- autologous infusion  
OR  
- allogeneic infusion, lean-donors | 1. ▲ in gut microbial composition after diet intervention and at 3, 5, 12 weeks post FMT  
2. ▲ in insulin sensitivity (hepatic, peripheral) | 1^d: No ▲ in Shannon Index at 3, 6 or 12 weeks. Respective p-values:  
- Autologous: 0.61; 0.59; 0.59  
- Allogeneic: 0.88; 0.66; 0.31  
2^d: No ▲ in insulin sensitivity.  
  p-values: hepatic, peripheral  
- Autologous: 0.88; 0.35  
- Allogeneic: 0.48; 0.33 | - Microbial changes resulting from dietary intervention may coincide with those of lean-donor FMT; there may be a reduction in the relative benefit of lean-donor FMT if paired with diet |

Table 2-A: FMT and Metabolic Primary Outcomes (1 of 2)
<table>
<thead>
<tr>
<th>Author</th>
<th>Population</th>
<th>Intervention</th>
<th>Relevant Outcomes</th>
<th>Results</th>
<th>Notable Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yu, E. et al. (2020)</td>
<td>- US adults, age 25-60&lt;br&gt;- BMI ≥30 kg/m²&lt;br&gt;- Mild-moderate insulin resistance (HOMA-IR 2.0-8.0)&lt;br&gt;<strong>n = 24</strong></td>
<td>Weekly capsules, x6 weeks either:&lt;br&gt;- Healthy, lean donor FMT capsules&lt;br&gt;- Placebo capsules</td>
<td>1°: Percent Δ in insulin sensitivity at 6 weeks using HEC&lt;br&gt;2°: Δ in weight, other metabolic parameters at 6 and 12 weeks</td>
<td>1°: Non-significant improvement in HEC values in FMT group as compared to placebo group:&lt;br&gt;- FMT: +5% ± 12%&lt;br&gt;- Placebo: −3% ± 32%&lt;br&gt;p = 0.16</td>
<td>- FMT engraftment rates of one donor far exceeded rates of other donors&lt;br&gt;- Sub-analysis: subjects with lower gut diversity benefited more from FMT than placebo; results not significant (low subject #)</td>
</tr>
<tr>
<td>Craven, L. et al. (2020)</td>
<td>- Canadian adults, age &gt;18&lt;br&gt;- Diagnosis of non-alcoholic fatty liver disease (criteria American Association for the Study of Liver Diseases)&lt;br&gt;<strong>n = 21</strong></td>
<td>Randomized in 3:1 ratio to SI microbial infusion:&lt;br&gt;- Allogenic infusion, lean-donors&lt;br&gt;- Autologous infusion (control)</td>
<td>1°: Δ in insulin sensitivity at 6 weeks using HOMA-IR&lt;br&gt;2°: Δ in intestinal permeability at 6 weeks using lactulose:mannitol urine test&lt;br&gt;- Δ in hepatic fat fraction at 6 months</td>
<td>1°: Non-significant improvement in HOMA-IR for FMT group:&lt;br&gt;- FMT: 3.88 → 3.45 (p = 0.22)&lt;br&gt;- Control: 4.88 → 5.09 (p = 0.69)</td>
<td>- Previous findings regarding effects of FMT on insulin sensitivity not corroborated&lt;br&gt;- Previous findings regarding association between gut diversity and outcome responses not corroborated</td>
</tr>
<tr>
<td>Noccmu, V. et al. (2021)</td>
<td>- Canadian adults, age 18-65&lt;br&gt;- BMI ≥30 kg/m²&lt;br&gt;- Hgb alc ≥ 5.5%, OR fasting plasma glucose &gt;5.6 μmol/l&lt;br&gt;- OR; diabetic medication use&lt;br&gt;- plus 1 additional criterion of metabolic syndrome&lt;br&gt;<strong>n = 70</strong></td>
<td>1x oral lean-donor FMT + high or low fermentable fiber diet&lt;br&gt;- 4 groups:&lt;br&gt;- Oral FMT + High Fiber Oral FMT + Low Fiber Sham FMT + High Fiber Sham FMT + Low fiber</td>
<td>1°: Δ in insulin sensitivity and resistance at 6 weeks using HOMA2-IR/IS&lt;br&gt;2°: Δ in weight, other metabolic parameters at 12 weeks</td>
<td>1°: Only FMT + low fiber group had significant improvement in HOMA2-IR at 6 weeks (p = 0.02)&lt;br&gt;2°: Significant weight ↓ in sham + low fiber group at week 12 (p=0.03)&lt;br&gt;- Significant ↓ BP in all groups (except Sham FMT + High fiber)&lt;br&gt;- no Δ in other metabolic parameters</td>
<td>- Only those in FMT + low fiber group had significant improvement in insulin resistance and gut bacterial richness, effects lasted 6-12 weeks</td>
</tr>
</tbody>
</table>

**Table 2-B:** FMT and Metabolic Primary Outcomes (2 of 2)
2.22 Cardiovascular Outcomes

Dyslipidemia is a common characteristic amongst FMT cohorts. Most commonly, this is a result of studies prioritizing recruitment of individuals with metabolic syndrome or its features, of which dyslipidemia is a criterion. Cardiovascular parameters are typically analyzed as a secondary outcome and, in contrast to glycemic outcomes, the evidence regarding FMT’s ability to influence cardiovascular outcomes is much weaker.

To date, only one study has primarily focused on the ability of FMT to induce change in cardiovascular outcomes. In this 2018 trial, Smits et al. conducted a study in which 20 males with metabolic syndrome were supplemented with either autologous or allogenic SI infusion, with allogenic infusions arising from lean, vegan donors. The goal of Smits et al. was to investigate if the diet of the donor had the potential to impact cardiovascular outcomes. Specifically, researchers assessed if vegan FMT could influence the ability of the gut microbiota to create trimethylamine-N-oxide (TMAO) – a compound implicated in atherosclerosis – following ingestion of a meal. Additionally, researchers evaluated the impact vegan FMT had on vessel wall inflammation, as measured by uptake of F-fluorodeoxyglucose (FDG) on positron emission tomography - computed tomography (PET/CT). At the end of this 2-week pilot study, changes in both postprandial TMAO and FDG-uptake were non-significant.

Importantly, the researchers involved in this trial by Smits et al. arose from the same research group as Vrieze et al., Li et al. and Kootte et al. As a result, Smits et al. was similarly invested in determining how baseline gut diversity predicts outcomes, and to this end questioned if baseline microbial diversity had influenced their results. However, in contrast to the prior studies which showed lower microbial diversity in subjects with obesity, the analysis by Smits et al. revealed baseline levels of gut diversity in lean-donors and recipients with obesity to be quite
similar, displaying near identical Shannon index values (5.9 vs 6.0, p=.08). Potentially, lack of
difference in baseline diversity contributed to the nonsignificant difference in outcomes.

Though no further studies have prioritized cardiovascular outcomes, several researchers
have measured lipid changes as a secondary endpoint, the results of which are mixed.

Perhaps the most favorable results regarding FMT and lipid outcomes were seen in a 2021
trial conducted by Ng et al. In this 24-week double-blind, placebo-controlled trial, Ng et al.
randomized 61 adults with obesity and diabetes to one of three interventions: lean-donor microbial
SI infusion + lifestyle intervention (FMT+LSI); lean-donor microbial infusion without LSI (FMT
only); or sham infusion + LSI (LSI only). LSI was defined as physical activity (amount/type not
specified) and a calorie restricted diet, details of which were individualized by a dietitian. FMTs
were delivered on a recurring basis (Table 2-D). At 24 weeks, those in the FMT+LSI group had
sustained a reduction in both their total cholesterol and LDL (p = 0.046, 0.011, respectively),
compared to FMT alone (p=0.837, 0.955) and LSI only (p= 0.333, 0.275). The fact this
improvement only occurred in the FMT + LSI group, but not the FMT or LSI only groups was a
remarkable finding. Alone, neither FMT nor LSI had effected lipid levels, and only when the two
intervention were combined did a health benefit manifest.

However, the findings of other FMT trials have not been as robust. In the previously
described trial by Yu et al. – in which subjects received either 6 weeks of either placebo or lean
donor FMT capsules – the primary glycemic outcomes had been nonsignificant. However, as
discussed, a sub-analysis showed those with lower baseline gut diversity had improvement in
several metabolic parameters, including Hgb A1c and fasting glucose. In addition to these
glycemic parameters, FMT subjects in the sub-analysis also had improved levels of total
cholesterol compared to the low diversity subjects receiving placebo. Much like the glycemic
improvements, results were non-significant, though the small sample size largely limits the ability to draw conclusions. In contrast, no improvements in LDL, triglycerides (TG), high density lipoprotein (HDL) were shown in either the primary analysis or the sub analysis.

While cardiovascular outcomes (largely lipid parameters) have been assessed in several other of the aforementioned studies, none have shown significant results.

2.23 Weight-based outcomes

Prior to 2020, no FMT trial prioritizing a weight outcome had been conducted. The aforementioned “Gut Bugs Trial”, conducted by Leong et al. was the first to do so.20 Children with obesity are 5 times as likely to be obese in adulthood compared to normal weight children.26 Given the importance of early-life intervention, Leong et al. elected to investigate if FMT use in children could promote weight loss. As previously described, 87 children (age 14-18) with obesity were randomized to receive either lean-donor FMT capsules, or saline placebo.20 Subjects were followed for 26 weeks and asked not to change their diet or physical activity. Researchers elected to use 6-week BMI standard deviation score, or z-score, as their primary outcome. Notable secondary outcomes included: BMI z-score at 12 and 26 weeks; body fat percentage and android:gynoid fat ratio (A/G ratio), measured by dual-energy X-ray absorptiometry (DEXA). At 6 weeks, there was no change in BMI z-score in the FMT group, with a reported adjusted mean difference (aMD) of $-0.026$ ($p = 0.291$). Similarly, no change was recorded at 12 or 26 weeks either. However, the FMT group showed significant improvement in A/G fat ratio at 6, 12 and 26 weeks as visualized on DEXA [respective p-values = 0.042, 0.028, and 0.0069]. These changes were associated with microbial changes, specifically a decrease in the relative frequency of \textit{E coli}, and increased frequency of \textit{F prausnitzii} and \textit{Alistipes spp}, all of which have been associated with BMI in past research.27,28 Microbial diversity, as measured by Shannon index, was similar between
donors and subjects at baseline, and no significant rise in diversity was appreciated in any group by the end of the trial. Although, in the FMT group, improvements in A/G ratio were weekly correlated with higher diversity values. The trial by Leong et al. is summarized in Table 2-C.

A second trial prioritizing weight outcomes was conducted a year later. In this aforementioned study by Rinott et al., a slightly different approach was taken. Instead of focusing on FMT as a primary means of weight loss, Rinott et al. rather chose to study the effectiveness of FMT in preventing weight regain following rapid loss. Two hundred ninety four (294) adults with either abdominal obesity or dyslipidemia were first randomized to 6 months of exercise (45-60 min. aerobic and resistance training 3-4 times/wk) and 1 of 3 dietary interventions. These included “healthy diet guidelines”, which was described as non-specific nutritional counseling; a calorie-restricted Mediterranean diet, which was supplemented with 28g of walnuts; or a “Green-Mediterranean diet”, which provided the same amount of calories as the Mediterranean diet, but differed in that it provided less red, processed meat and included more polyphenol diet sources (green tea and Wolffia globosa shake). After 6 months, those sustaining weight loss ≥3.5% could advance to phase-2, a level chosen to maximize statistical power. Stool specimens were collected from the remaining subjects (n=90). Participants were then randomized to receive either their own stool specimens in capsulated form, or placebo capsules, every 3-weeks during months 8-14 (Table 2-D) while simultaneously continuing their assigned LSI. This study design is illustrated in Figure 2-A. Primary outcome was percent weight regain, or how much of the weight lost in the first 6 months was regained during month 6-14. Relevant secondary outcomes included changes in waist circumference between months 6-14. The trial by Rinott et al. is summarized in Table 2-C.
At the end of 14 months, no difference in weight regain (as a percent) was seen across diets (Healthy guidelines +39.1%; Mediterranean +36.1%; Green-Mediterranean +33.6%, P =0.92). Similarly, no difference in weight regain was seen between the two capsule interventions (Autologous FMT +30.4% [2.2 kg] vs. Placebo +40.6% [2.6 kg], p = 0.28). However, within the Green-Mediterranean group, those receiving autologous FMT regained less weight than those receiving placebo (FMT +17.1% [1.6 kg] vs placebo +50% [3.6 kg]; P = 0.02). Similarly, FMT subjects in this diet group had a significantly attenuated regain in waist-circumference (FMT +1.89 cm vs. placebo +5.05 cm, p=0.01).

Several explanations were proposed regarding the weight-based changes seen in the Green Mediterranean group. Firstly, of those within the Green Mediterranean diet group, subjects receiving autologous FMT displayed significant changes in the relative abundance of 6 specific bacterial species during months 6-14, including *A. putredinis*, *B. vulgatus*, and *Bacteroides uniformis*, all of which have been associated with leanness in past research. Additionally, as part of the green-Mediterranean diet, this group consumed a higher amount of polyphenols. As a
probiotic, polyphenols may have promoted the growth of the aforementioned lean-associated bacteria. However, those in the Green-Mediterranean group receiving placebo had actually regained the most weight of any placebo group. While the degree of regain was not significantly different when compared to other placebo groups, the fact those consuming this diet gained the most weight argues against the significance of the polyphenols. In contrast, this observation suggests inherent importance to the supplemental FMT, as only those receiving both the dietary intervention and FMT had protection against weight regain. In many ways, these findings mirror those of the study by Ng et al. – in which those receiving FMT alongside LSI had improvement in LDL, but those receiving either of these interventions alone did not.25

Similar to Rinott et al., the final two studies which showed significant changes in weight based outcomes also incorporated a lifestyle intervention alongside FMT. One of these was the aforementioned 2021 trial conducted by Mocanu et al. – in which subjects were provided one of two fiber types (high fermentable vs low fermentable) alongside FMT.11 In this study, BMI and hip/waist circumference were tracked as secondary outcomes. At the end of 12 weeks, those receiving a low-fermentable fiber supplement alongside FMT lost a significant amount of weight from baseline (p=.03). This was the only group in the study to do so.

The last FMT trial to show significant impact on weight outcomes was that of Ng et al., a study which is both most recent, and most relevant to our proposal.25 As detailed earlier when discussing the cardiovascular outcomes of Ng et al., 61 adults with obesity and diabetes were randomized to lean-donor SI infusion + lifestyle intervention; lean-donor infusion without LSI; or sham infusion + LSI. Importantly, FMTs were delivered intermittently over the course of this 24 week trial (Table 2-D). The primary focus of this trial was to investigate how repeated FMTs and
LSI influenced donor engraftment rates over time. Accordingly, the primary endpoint was the proportion of subjects who acquired ≥20% of the lean-donor microbiome at 24 weeks.

At week 24, 100% of subjects in the FMT + LSI group had retained ≥20% of the transplanted donor microbiota. Comparatively, only 88.2% of subjects in the FMT-only group, and 22.2% of subjects in the LSI-only group had obtained ≥20% of the FMT donor microbiota. This difference in engraftment rates was profoundly significant (p < 0.0001). Similarly, the percentage of lean-associated bacteria (bacteria from the donor) was highest in the FMT + LSI group, followed by the FMT-only and LSI-only groups (29.7%, 29.0% and 13.6%). Finally, the donor engraftment rates of those in the FMT+ LSI were significantly higher at week 16 and 24, when compared to week 4 (p < 0.01, < 0.05, respectively), suggesting additional FMTs had an cumulative effect. On microbial analysis, there were no significant differences in gut diversity across groups, as measured by Shannon index, though there was an increase in richness in the FMT +LSI group at week 4 and 16 (p< 0.01, < 0.05, respectively). Similar to the Vrieze et al., the FMT recipients had significantly higher relative abundances of butyrate-producing bacteria.

As a secondary outcome, Ng et al. monitored the proportion of subjects achieving ≥10% weight loss. At 24 weeks, 2 subjects in the FMT group lost ≥10% of weight, compared to zero in the placebo group, a non-significant difference. However, in an intriguing sub-analysis, Ng et al. looked specifically at those in the FMT+LSI group who had been ≥50% compliant with LSI measures. These individuals sustained significantly more weight loss than those <50% compliant with LSI (p <.05). A microbial analysis was conducted on this subgroup, but no difference in the percentage of lean-microbiota acquired was found between groups.

The results of Ng et al. are compelling. As previously noted, the outcomes regarding LDL and total cholesterol suggest that life style intervention maximizes the effects of FMT, as
neither LSI or FMT alone produced significant change, rather only the combination did. The results of the compliance-based sub-analysis support this conclusion, as those who had been the most compliant with LSI had reaped the greatest improvement in weight. In addition to these findings, Ng et al. demonstrated that recurrent FMT leads to increasing donor engraftment. If indeed the degree of metabolic benefit received from FMT is correlated with engraftment rate, recurrent FMT administration would theoretically lead to superior outcomes.
<table>
<thead>
<tr>
<th>Author</th>
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<th>Results</th>
<th>Notable Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leong K. S. 2020</td>
<td>New Zealand adolescents, age 14-18 - BMI ≥30 kg/m² - No pre-diagnosed chronic diseases n = 87</td>
<td>lX administration of: - Healthy, lean-donor FMT capsules OR - Saline capsules</td>
<td>1°: Δ BMI z-score at 6 weeks 2°: Δ BMI z-score at 12 &amp; 26 weeks - Δ gut diversity - Δ % body fat - Δ android gynoid fat ratio - Δ insulin sensitivity</td>
<td>1°: No Δ in BMI z-score at 6 weeks; adjusted mean difference (aMD): aMD = -0.026; p = 0.291 2°: Significant Δ A/G ratio FMT group: 6 week aMD: -0.021 p = 0.042 12 week aMD: -0.023 p = 0.028 26 week aMD: -0.029 p = 0.0069 - No Δ in any other 2° outcomes</td>
<td>- Post-Hoc analysis: Children with undiagnosed metabolic syndrome in FMT group had significant improvements in glycemic control at 6 weeks</td>
</tr>
<tr>
<td>Allegretti, J. R. (2020)</td>
<td>U.S. adults, age 30-60 - BMI ≥35 kg/m² - Metabolically healthy (no diabetes, NASH, metabolic syndrome) n = 22</td>
<td>Intermittent provision of: - Healthy, lean-donor FMT capsules OR - Placebo capsules</td>
<td>1°: Safety, adverse effects 2°: 12 week Δ in: - GLP-1 levels - Gut diversity - Bile acid profiles</td>
<td>1°: No difference between adverse effect rates of FMT, placebo at 26 weeks 2°: FMT group had significant Δ stool taurocholic acid level (p &lt; 0.05) - No significant Δ in GLP-1, diversity</td>
<td>- Side effects of FMT capsules were non-different from those of placebo  - Significant ↑ β-diversity in FMT subjects, suggesting engraftment of donor flora</td>
</tr>
<tr>
<td>Rimmert, E. (2021)</td>
<td>Israeli adults, age ≥30 - Having abdominal obesity - Waist circumference &gt;102 cm (♂) / &gt;88 cm (♀) OR dyslipidemia - TG: &gt;150 mg/dL - HDL: ≤40 mg/dL (♂) ≤50 mg/dL (♀) n = 90</td>
<td>Weight loss phase 6 months physical activity + 1 of following diets: - Healthy diet guidelines - Mediterranean - Green-Mediterranean FMT phase 6 months of autologous FMT vs placebo capsules</td>
<td>1°: Regain of lost weight over months 8-14 (FMT phase) 2°: Δ in: - waist circumference - metabolic parameters - gut microbiome (Between months 8-14)</td>
<td>1°: No difference in weight regain across diets (p=0.92) or FMT types (p=0.28) In green Mediterranean group, FMT attenuated weight regain (p=0.02) 2°: In green Mediterranean group, FMT attenuated Δ in waist (p=0.02) and improved fasting insulin (p=0.04) - No significant Δ in gut composition</td>
<td>- In a particular diet group, FMT subjects had ↓ fasting insulin and lesser rise in waist circumference, while those receiving placebo regained more weight than any other placebo group</td>
</tr>
<tr>
<td>Ng, S. C. (2021)</td>
<td>Chinese adults, age 18-70 - BMI ≥24 and &lt; 45 kg/m² - Diagnosed DM2 for ≥3 months n = 61</td>
<td>Lifestyle intervention (LSI) = intermittent SI infusion over 24 weeks: 3 groups: FMT alone Sham donor FMT + LSI Lean donor FMT + LSI</td>
<td>1°: Proportion acquiring ≥20% microbiota from lean donors at week 24 2°: Proportion attaining ≥10% weight loss at 24 weeks - Δ lipid, glycemic parameters at 24 weeks - Adverse outcomes</td>
<td>1°: Proportions acquiring ≥20% lean-associated microbiota at week 24: • 100% FMT + LSI • 88.2% FMT alone • 23% sham FMT+ LSI P &lt; 0.0001 2°: No significant weight loss - FMT+LSI group had improved LDL (p = 0.03) and Total Cho. (p=0.04)</td>
<td>- LSI + FMT led to greater improvement in bacterial richness and lipid levels compared to either alone - Sub-analysis: those ≥50% compliant with LSI lost significantly more weight</td>
</tr>
</tbody>
</table>

Table 2-C: FMT and Other Primary Outcomes
2.3 Review of Potential Confounding Variables in Current Literature

Due to the relatively few number of studies regarding FMT and metabolic outcome, we will widen our discussion to FMT use in other conditions.

2.31 Diet and Activity level

The impact of diet and exercise on weight and metabolic parameters in those with obesity is well described.\textsuperscript{31-34} Often, FMT studies ask subjects to maintain baseline diet and activity levels to avoid any confounding effects; that is, unless lifestyle change is incorporated as an independent variable.\textsuperscript{10,18,20}

2.32 Donor characteristics

The notion that superior stool-donor characteristics exist is regarded in FMT literature as the “super-donor phenomenon”, and the existence of such a donor remains a source of ongoing debate and study.\textsuperscript{8,35-37} This topic is particularly prevalent in research involving FMT and IBD. In one profound example, 7 of 9 subjects achieving IBD remission after FMT had all received the same donor’s stool.\textsuperscript{38} In a large meta-analysis assessing FMT use in IBD, the evidence supporting a correlation between donor relationship (related vs nonrelated) and remission was described as “moderate”, which authors defined as 25-75\% of studies supporting a relationship.\textsuperscript{39} While investigation into the super-donor phenomenon is limited, gut diversity seems to be a “key theme”.\textsuperscript{37} It is possible a similar interaction occurs between FMT and metabolic outcomes. Importantly, several of our aforementioned trials saw variability in outcomes depending on the stool donor, though this relationship was not consistent across our studies.\textsuperscript{9-11} Notably, super-donor evidence is not as robust in other disease states. The donor-outcome relationship has been explored in CDI, but is largely unsupported by meta-analysis.\textsuperscript{40,41}
2.33 Baseline Microbial Composition & Diversity

A review of the relationship between obesity and the microbiome is discussed in our introductory chapter. Briefly, a majority of studies support the association of lower baseline gut-diversity in obesity.42,43 However, as described, there is contention that this difference contributes to metabolic outcomes.5,10,15 Notably, there is CDI research supporting a relationship between pre-FMT microbial composition and outcomes. In a recent trial involving fulminant CDI, those responding to FMT had a significantly different pre-FMT microbial composition compared to non-responders (p <.001).44 Outside of FMT, baseline microbial features have even predicted the weight outcomes and glycemic responses of dietary interventions.45-47 Whether baseline microbial composition affects the metabolic outcomes of FMT remains unclear and will be a focus of this study proposal.

2.4 Review of Relevant FMT methodology

Presently, no set standards exist regarding the optimal dosage, preparation, administration, and frequency of FMT in the treatment of obesity, and this methodology is variable across published studies.36,48-50 Given the lack of a standardized protocol for FMT and metabolic research, FMT researchers often incorporate common FMT practices used in the treatment of CDI. However, even in context of CDI treatment, heterogeneity exists amongst FMT protocols.51,52 FMT methodologies implemented by trial groups included in our review are listed in Table 2-D.
<table>
<thead>
<tr>
<th>Study author (year)</th>
<th>Total stool (g) provided [study duration]</th>
<th>Vehicle for transplant</th>
<th>Administration protocol</th>
<th>Donor #</th>
<th>Targeted Area of GI tract</th>
<th>Required Bowel Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ng, S. C. et al (2021)</td>
<td>200g [24 weeks]</td>
<td>Direct Infusion</td>
<td>100-200 ml infusion (30g stool) over 2-3 min Provided at baseline, every 4 wks thereafter</td>
<td>6</td>
<td>Duodenum</td>
<td>Nose</td>
</tr>
<tr>
<td>Mocenni, V. et al (2021)</td>
<td>50g [6 weeks]</td>
<td>Oral Capsule</td>
<td>1x administration of 20 capsules</td>
<td>4</td>
<td>N/A</td>
<td>24-hr clear liquid fast + 2 doses bowel prep</td>
</tr>
<tr>
<td>Rinott, E. et al (2021)</td>
<td>100g [6 months]</td>
<td>Oral Capsule</td>
<td>100 capsules (1g ea) 10 capsules at start, 10 capsules at 3 wks there after</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Allegretti, J. R. et al (2020)</td>
<td>40.5g [12 weeks]</td>
<td>Oral Capsule</td>
<td>54 capsules (.75g ea) 30 capsules at start, 12 more at wks 4 and 8</td>
<td>1</td>
<td>Colon</td>
<td>No bowel prep; ppi taken for 72 hrs before each dose</td>
</tr>
<tr>
<td>Leong, K. S. et al (2020)</td>
<td>22g [26 weeks]</td>
<td>Oral Capsule</td>
<td>28 capsules Administered over 2 consecutive mornings (1st day, 12 2nd day)</td>
<td>8</td>
<td>Colon</td>
<td>Overnight fast + bowel cleanse (70g lavage)</td>
</tr>
<tr>
<td>Yu, F. W. et al (2020)</td>
<td>168g [12 weeks]</td>
<td>Oral Capsule</td>
<td>105 capsules* 15 capsules on two consecutive days at start, 15 capsules once weekly x5 wks thereafter</td>
<td>4</td>
<td>N/A</td>
<td>4 hr fast before, +1 hr after each administration</td>
</tr>
</tbody>
</table>

* Study utilized autologous stool transplant as opposed to allogenic
\* Full study was 14 months. First 6 months was weight loss phase, followed by 2 months for stool collection. FMT capsules began at month 8
\# 20mg osmgolide
\† The same research group was responsible for this trio of studies. Near identical FMT methodology was utilized for each
\§ An exception to this is Kootte et al, who delivered a second FMT to a subset of the allogenic FMT group
\γ Total stool amount provided was not published. Studies involved both allogenic and autologous stool
\* Reported there was 24g stool provided with every 15 capsules

**Table 2-D:** Comparison of FMT methodology
2.41 FMT Capsules

Numerous FMT administration methods exist, including infusion via nasogastric tube or colonoscopy, oral capsules and retention enema. FMT capsules have been shown to be non-inferior to colonoscopy-delivered FMT in the treatment of CDI.\textsuperscript{53} Indeed, a large recently published meta-analysis of 26 studies utilizing FMT to treat CDI found the cure rate of capsule-delivered FMT (92.1%) to be quite comparable to that of colonoscopy-delivered FMT (94.8%), with both methods being superior to enema (87.2%) and nasogastric/nasoduodenal delivered FMT (78.1%).\textsuperscript{54}

2.42 FMT Donors and Screening Protocols

FMT donors need are vigorously vetted to ensure no pathogens are transferred to FMT recipients. Additionally, if the same FMT donors are to be used for an extended period, routine screening is required. Recommendations regarding interval length between screenings vary from as little as 4-8 weeks, to as long as 3-6 months\textsuperscript{55-57}

In regards to the specific safety parameters evaluated, no standardized list has been created. Several donor-screening protocols have been developed for use in CDI treatment, and currently those investigating FMT and metabolic outcomes seem to either incorporate these CDI protocols or use own, internally developed protocols.\textsuperscript{4,10,11,22,58} Donor screening information is not routinely published, and details regarding donor criteria and selection protocols are absent from many existing studies. Additionally, no consensus regarding the optimal age and BMI of donors has been established, and optimal donor characteristics remains an area of ongoing area research.\textsuperscript{8,52,59}

Given the lack of published FMT donor protocols outside of CDI, a group of Canadian researchers came together to establish a FMT screening protocol specifically for use in treating metabolic syndrome-associated diseases.\textsuperscript{57} The group included several prolific publishers in the field of FMT, of which had backgrounds in infectious disease, microbiology, and immunology.
2.43 FMT Capsule Preparation

Historically, both fresh and frozen stool specimens have both been utilized in capsule preparation and there is considerable evidence supporting noninferiority.\textsuperscript{53,54,56,60} The adverse effects on anaerobic species with aerobic preparation has not been shown to be clinically significant.\textsuperscript{56,61}

2.44 FMT Capsule Formulation

Modern FMT capsules utilize colonic-targeted, delayed release formulations, and these have shown increased efficacy in treating CDI when compared to gastric-released capsules.\textsuperscript{62} New film coating technology allows for a combination of enzymatic and pH-triggered capsule release. Capsules utilize a high amylose starch film that resists degradation by pancreatic amylase, but remain susceptible to the variety of amylases produced by colonic microbiota. This mechanism has led to reliable colonic capsule release.\textsuperscript{63}

2.45 FMT Capsule Dosing

The optimal amount of stool for FMT is unknown.\textsuperscript{62} As described previously, in studies investigating impact of FMT on weight-based and metabolic outcomes, both total and average weekly stool volumes provided (in studies involving multiple administrations) is wide-ranging (Table 2-D). In the context of CDI treatment, specifics regarding FMT dosage is not always published. However, based on available information, stool amounts vary greatly for this indication as well, influenced by delivery method, practice group and number of FMT administrations in the regimen.\textsuperscript{51,52,54,63} Fueling further uncertainty, specific stool dosing may not even be of particular importance, as a 2018 study found no difference in CDI cure rates between low (7.5g) and high dose therapy (22.5g) when utilizing colonic-released oral capsules.\textsuperscript{62} Nevertheless, various
professional groups have published guidelines for FMT dosing. In a 2017 statement put forth by the European Consensus Conference on faecal microbiota transplantation, a single induction dose of 30-50g was recommended in the treatment of CDI. However, the following year, a meta-analysis challenged the generalizability of this guideline. This 15-study analysis, which involved some of the same authors, found fecal dosages ≤50g to be less efficacious when used in the treatment of recurrent CDI. Similarly, that same year a 50g minimum was advised by the British Society of Gastroenterology and Healthcare Infection Society. This recommendation was based on studies showing increased CDI recurrence when treating recurrent CDI with lower amounts, and as such may not be applicable when considering FMT for weight loss.

Regarding frequency of dosing, the majority of FMT studies in CDI utilize a colonoscopy-delivered approach and deliver 1-2 infusions, with additional infusions administered in the case of relapsing disease. While the optimal frequency of FMT dosing in CDI is unknown, multiple FMT infusions have been shown to increase efficacy and remission rates.

When used to induce metabolic change, FMT regimens have comprised anywhere from 1 to 10 administrations. This number includes all forms of administration, including capsules (Table 2-D). While the evidence for multi-dose FMT is not as strong in studies outside CDI treatment, a recent study did show a significant increase in both the level and duration of lean-microbiome engraftment when multiple FMT administrations were utilized. In trials involving multiple FMTs, the largest duration between individual administrations has been 4 weeks. Two studies have incorporated this interval length, however neither provided any rationale for doing so.

It is largely unknown how long the effects of a single FMT lasts, and individual responses seemingly vary. As alluded to in our literary review, this may be a consequence of variances in donor-recipient microbiome compatibility, a relationship that is described as “idiosyncratic” and
likely to be reliant on a multitude of unknown factors. Nevertheless, there is evidence suggesting FMT-induced changes are present for much longer than 4 weeks, as many as 3-6 months and perhaps longer. Indeed, in one study, researchers assessing FMT in recurrent CDI found that, at 1 year post-FMT, recipient microbiome profiles still showed more similarity to that of the donor, rather than the subject’s own pre-FMT profile.

2.46 Pre-FMT Bowel Preparation

There is no consensus regarding the need for bowel preparation prior to FMT administration. While a variety of bowel preparatory methods have been utilized in the existing literature, the use of bowel preparation is not ubiquitous (Table 2-D). In 2017, the European Consensus Conference on faecal microbiota transplantation commented on the use of prokinetics and proton pump inhibitors (PPIs) in upper-GI FMT administration, noting a benefit of these agents has not yet been established. Furthermore, cautionary use was recommended given the “microbiome modifying effects” associated with the use of prokinetics and PPIs. Despite this, in their 2018 guidelines, the joint British Society of Gastroenterology (BSG) and Healthcare Infection Society (HIS) recommended a low dose of these agents be considered, though the strength of this recommendation was graded as “weak:”.

In CDI treatment, antibiotics are commonly administered a couple days prior to FMT to reduce the abundance of C. difficile. Similarly, in IBD treatment, pre-FMT antibiotics have been reported to improve FMT engraftment and increase remission rates. However, antibiotics modify microbial composition. Moreover, these modifications do not present uniformly across individuals, and responses in gut microbial communities can vary. Given their known impact, FMT trials exploring metabolic outcomes almost ubiquitously incorporate recent antibiotic use into the exclusion criteria for subjects and stool-donors, alike.
In regards to pre-FMT bowel cleansing, a Canadian research group has found it obsolete, citing advances in capsule-release technology (Section 2.34).\textsuperscript{62,63} Similarly, in the treatment of CDI, FMT has not always been accompanied by bowel preparation and this practice does not appear to lead to a reduction in efficacy.\textsuperscript{61}

No evidence could be found regarding the need for fasting prior to FMT administration. The fasting protocols of studies included in this review varied (Table 2-D). Of capsule studies, fasting protocols were: 24 hour clear liquid fast; overnight fast; 4 hour pre/1 hour post-FMT fast.

\textbf{2.5 Conclusion}

In our review of the literature, results are mixed, and the heterogeneous nature of FMT protocols makes comparisons difficult. However, several different researchers, using different protocols and arising from different countries, have noted improvements in glycemic parameters with FMT administration, even if benefit was transient.\textsuperscript{4,11,19,20,22} Given recurrent support, this is certainly a topic deserving of further attention, specifically in the subject group who would derive most benefit – those living with diabetes.

The current findings supporting a use for FMT in weight-based outcomes are less robust. However, it is imperative to note only two existing studies have utilized weight as a primary outcome.\textsuperscript{20,21} As such, a majority of studies lack the power needed to detect weight-based change. Additionally, nearly all trials have looked at FMT in isolation, despite numerous researchers voicing that combining FMT with life-style intervention is an attention-worthy pursuit.\textsuperscript{8,50} Indeed, 2 of 3 studies employing a combined approach resulted in findings suggesting improved outcomes by pairing FMT with lifestyle intervention.\textsuperscript{11,25}

Finally, the significance of baseline microbial composition is still unclear, with studies both in support of a relationship and others refuting it.\textsuperscript{5,10,15} Differing results may be in part to the
complex nature of metagenomics sequencing methods and the variations of approaches.\textsuperscript{69,70}

Similarly, the use of differing diversity indices further impairs the ability to compare different study populations.\textsuperscript{69-71} A trial specifically investigating outcomes in participants with reduced microbial diversity would aid in further elucidating the relationship between baseline microbial characteristics and the impact of lean donor FMT on weight-based and metabolic outcomes.

**References**


CHAPTER 3: STUDY METHODS

3.1 Study Design and Key Variables

To specifically investigate the impact of reduced microbial diversity on FMT and weight loss outcomes, we propose a year-long multisite trial, of both double-blind and placebo-controlled design. After enrollment, subjects will be stratified into parallel arms based upon their respective microbial diversity levels. To this end, we will use initial microbial sampling to assess each subject’s baseline Shannon Diversity level, with the upper and lower 50% being grouped together. Within these two strata, subjects will be randomized to receive an intervention, either FMT capsules or placebo. During the 12-month intervention phase, participants will attempt weight loss through diet and exercise, with the intervention (FMT vs placebo capsules) being supplemented every 3 months. Immediately prior and during the intervention phase, subjects will meet with a dietitian and health fitness specialist to review diet and exercise goals, as well as troubleshoot any obstacles that arise. Laboratory tests, vital signs and anthropometric measures will be measured every 90 days. Table 3-A provides a summary of the key variables while Figure 3-A provides an overview of the study design, as well as an estimated timeline.

<table>
<thead>
<tr>
<th>Independent Variable:</th>
<th>FMT vs Sham capsules (placebo-control variable)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dependent Variable:</td>
<td>Mean, percent weight change from baseline</td>
</tr>
<tr>
<td>Confounding Variable:</td>
<td>Baseline gut diversity</td>
</tr>
</tbody>
</table>

Table 3-A: Key Study Variables
Figure 3-A: Study Overview and Timeline

- All enrolled subjects $n = 253$
- Baseline microbial samples collected
- Enrollment 4 months
- Assessment of baseline gut diversity 2 months
- Stratification
- Randomization
- Concealed Allocation
- High Diversity Strata $n = 126$
  - Random # gen.
  - 50th Percentile Shannon Values
- Low Diversity Strata $n = 126$
  - Random # gen.
- FMT capsules (HD-f+) $n = 63$
  - FMT capsules (LD-f-) $n = 63$
  - Initial data collection + subject orientation 2 months
  - Intervention phase 12 months
  - Final data collection + result publishing 3 months
- Sham capsules (HD-f-) $n = 63$
  - Lower 50% Shannon Diversity
  - Initial labs drawn, anthropometrics measured + Lifestyle education
  - Upper 50% Shannon Diversity
  - Lifestyle intervention + FMT vs placebo
  - Final labs drawn, anthropometrics measured and microbial samples collected
3.2 Study population and recruitment

The study population will consist of weight loss seeking adults, age 18-65, who are living with a diagnosis of diabetes and possess a BMI of 30-40 kg/m². Subjects will be recruited through outpatient weight loss clinics that reside within Connecticut. Eligible facilities include those that are associated with a tertiary medical center and specialize in helping patients pursue weight loss through multiple modalities, including pharmacotherapy and lifestyle intervention. Weight loss does not need to be the sole purpose of the center, but it must be its primary focus with >50% of the center’s patient population being seen for this reason. Commercial weight loss clinics, *i.e.* Jenny Craig and Weight Watchers, are not eligible. Subjects will be recruited from eligible sites through convenience sampling. All new referrals presenting to the center over a course of 4 months will be screened according to eligibility criteria (see next section). The research team will approach and discuss potential enrollment with those eligible.

Stool donors will be recruited through local advertising, *i.e.* newspaper, fliers. In order to attract attention, a financial incentive will be listed. Applicants will undergo a stringent screening protocol to determine eligibility (see section 3.8, FMT Donors).

3.3 Inclusion/Exclusion Criteria

Eligibility criteria were developed with similar study protocols in mind. Specifically, we focused on studies involving FMT in conjunction with lifestyle measures. Both past and ongoing studies were reviewed and the most common criteria were incorporated into our protocol.

When considering eligibility, we will use the BMI taken at the subject’s initial clinic visit. For initial screening purposes, researchers will review candidate charts for a diagnosis of diabetes. However, if the subject is interested in enrollment, a Hgb A1c result no more than 3 months old
will need to be presented to confirm an active diagnosis. Our Hgb A1c range was subjectively chosen as a proxy for risk of noncompliance.\textsuperscript{1} Finally, to ensure the safety of participants, all subjects will need to receive medical clearance from their primary care physician, stating they are in suitable health for weekly intensive exercise of a year’s duration.

Regarding exclusion criteria, efforts were made to eliminate potential confounding as a result of medications or subject comorbidities, as well as reduce possible selection bias due to a subject’s inability to maintain regular contact. Females will be asked to complete a urine pregnancy test prior to enrollment and at 6 months. Specific endocrine disorders of concern include those that are known to impact weight, such as hyper/hypothyroidism, Cushing’s syndrome, and Polycystic Ovary Syndrome.\textsuperscript{2,3} Additionally, to ensure subjects start on a level field, we will exclude those who are already actively pursuing weight loss, as well as those who had recently attempted to lose weight, as they may still be displaying physical or metabolic effects related to the attempt.

**Figure 3-B: Inclusion and Exclusion Criteria**

### Inclusion Criteria
- Age 18-65
- BMI $\geq$ 30 kg/m$^2$ and $<40$ kg/m$^2$
- Hemoglobin A1c 6.5 – 10%
- Diagnosis of diabetes, made no earlier than 6 months prior to enrollment
- Willingness to adhere to both components of lifestyle intervention (diet and exercise)
- Ambulatory
- Able to safely partake in moderate/vigorous physical activity
- Capacity to provide informed consent

### Exclusion Criteria
- Active or recent weight loss attempt (past yr)
- Currently pregnant, or seeking pregnancy
- History of GI disease (IBD, Celiac, etc.)
- History of bariatric or GI altering surgery
- Uncontrolled endocrine disorders that may influence weight loss attempts
- Ongoing use of any medications known to impact weight (gain or loss)
- Lack of reliable transportation, telephone access
- Use of antibiotics/probiotics in last 3 months
- Inability or unwillingness to maintain diet and exercise diaries
- Immunosuppression
3.4 Sample Size

Due to lack of data regarding the expected effect of FMT in weight loss, sample size calculations utilized the results from Ng et al., the only study to date utilizing a combination of FMT and lifestyle measures and demonstrating improved weight loss with supplemental FMT.\(^4\) As noted in our literary review, Ng et al. conducted a subgroup analysis of their 24-week study, focusing solely on subjects who were ≥50% compliant with lifestyle intervention. Because weight loss was not a primary outcome of interest, explicit outcome values for the sub-analysis were not published. However, they can be reasonably estimated using the box plots provided (Figure 3-C). At 24 weeks, the FMT group showed an approximate median loss of 3.3% bodyweight compared to 2.1% in the placebo group.\(^4\) While the standard deviations of these results were not published, they can estimated using interquartile range.\(^5\) This effect size of 1.2% at 24 weeks was extrapolated to our control group data, which arises from the recent Semaglutide phase 3 clinical trial conducted by Davies et al.\(^6\) Data from the Semaglutide trial was chosen due to its recency, large sample size, and nearly identical subject population and lifestyle interventions. At 52 weeks, the Semaglutide control group, exposed to exercise and diet only, lost 3.4% bodyweight on average. Using this information, we estimated a 5.3% effect of FMT at 52 weeks.
Finally, we accounted for dropout rate. While Ng et al. accounted for 30% drop out, their FMT intervention was much more invasive, requiring conscious sedation for direct duodenal infusion. Notwithstanding, they incurred an attrition rate of only 15%. Alternately, Mocanu et al., who utilized capsules for the FMT intervention, had a dropout rate of only 13% after accounting for 10% during sample size calculations. Hence, we will account for 15% dropout. Based on these calculations, a sample size of 253 subjects is required to achieve 80% power with a 5% level of significance (Appendix B).

### 3.5 Baseline Analysis

Immediately following enrollment, baseline demographic, biochemical and anthropometric measurements will be collected. Demographic information of interest includes age, sex, and ethnicity. The physical measurements to be taken include BMI, weight, and hip and waist circumference, from which a hip-waist ratio will be calculated. Blood pressure will also be
documented. Initial laboratory studies will include a lipid panel (total cholesterol, TG, LDL, HDL) and diabetic measures (fasting glucose, hemoglobin A1c). Finally, we will assess how subjects are currently managing their diabetes: lifestyle only, medications (no insulin) or a combination of medications and insulin. Table 3-B lists how variables will be operationalized and the statistical test that will be used to compare results amongst groups.

Determining baseline microbial diversity is pivotal to our hypothesis. To ensure optimal and standardized collection methods, stool samples will not be subject-provided, rather 200-300 mg of stool will be obtained by a trained provider at the outpatient clinic. All samples will be flash frozen and delivered to the Clinical Microbiology Laboratory at the Yale School of Medicine for shotgun metagenomic sequencing. DNA will be extracted from the samples by way of mechanical lysis (bead beating). Both extraction and library preparation will be completed using proprietary kits (pending cost analysis). Stool samples should provide enough microbial DNA material for amplification-free library preparation and sequencing. Sequencing will utilize the Illumina platform with lengths of 2x150 or 2x300 bp and a targeted sequencing depth of approximately 5-10 Gb per sample. Following sequencing, metagenomic assembly will be carried out with the use of proprietary software. Once the taxonomic data is identified and sorted, a post-processing analysis will generate the Shannon Index of each sample.
Table 3-B: Baseline Data and Analysis

<table>
<thead>
<tr>
<th>Baseline Subject Characteristics</th>
<th>Variable Of Interest</th>
<th>Operationalization</th>
<th>Statistical Method of Comparison*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>Continuous (mean, standard deviation)</td>
<td>Within strata: Student t-test  All groups: ANOVA</td>
<td></td>
</tr>
<tr>
<td><strong>Sex:</strong></td>
<td></td>
<td></td>
<td>Within strata: $\chi^2$  All groups: $\chi^2$</td>
</tr>
<tr>
<td>– Male</td>
<td>Dichotomous (proportion)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>– Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
<td>Within strata: $\chi^2$  All groups: $\chi^2$</td>
</tr>
<tr>
<td>– Caucasian</td>
<td>Nominal (proportion)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>– African American</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>– Hispanic</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>– Asian American</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>– Other</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Body Measurements</strong></td>
<td>Continuous (median, IQR)</td>
<td>Within strata: Mann Whitney-U  All groups: Kruskal-Wallis</td>
<td></td>
</tr>
<tr>
<td>– Weight (kg)</td>
<td>Continuous (mean, standard deviation)</td>
<td>Within strata: Student t-test  All groups: ANOVA</td>
<td></td>
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<tr>
<td>– BMI (kg/m²)</td>
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<tr>
<td>– Waist Circumference (cm)</td>
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<td>– Hip Circumference (cm)</td>
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<tr>
<td>– Hip-Waist ratio</td>
<td></td>
<td></td>
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<tr>
<td><strong>Vitals:</strong></td>
<td>Continuous (mean, standard deviation)</td>
<td>Within strata: Student t-test  All 4 groups: ANOVA</td>
<td></td>
</tr>
<tr>
<td>– Systolic blood pressure (mmHg)</td>
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<tr>
<td>– Diastolic blood pressure (mmHg)</td>
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<tr>
<td><strong>Laboratory Measurements</strong></td>
<td>Continuous (mean, standard deviation)</td>
<td>Within strata: Student t-test  All groups: ANOVA</td>
<td></td>
</tr>
<tr>
<td>– Fasting plasma glucose (mmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>– Hgb A1c (%)</td>
<td></td>
<td></td>
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<tr>
<td>– Fasting plasma Cholesterol:</td>
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<td></td>
<td></td>
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<tr>
<td>– Total Cholesterol (mmol/l)</td>
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<tr>
<td>– TG (mmol/l)</td>
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<td>– LDL (mmol/l)</td>
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<tr>
<td>– HDL (mmol/l)</td>
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<td></td>
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<tr>
<td><strong>Current form of diabetes control:</strong></td>
<td>Nominal (proportion)</td>
<td>Within strata: $\chi^2$  All groups: $\chi^2$</td>
<td></td>
</tr>
<tr>
<td>– Lifestyle only</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>– Medication (no insulin)</td>
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<td></td>
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<tr>
<td>– Medication + Insulin</td>
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</table>

*All comparisons for similarity will be made both within strata (HD vs LD) as well as between all 4 groups.

3.6 Stratification, Randomization and Concealed Allocation

Subjects will be stratified into two groups based on their relative Shannon Index values, “High diversity” (HD) and “Low Diversity” (LD). The HD group will comprise of the subjects displaying the uppermost 50% Shannon Index values, while those with the lower 50% of values will fall into the LD group. No baseline variables other than diversity will dictate stratification.
Individuals in each strata will then be randomly allocated to an intervention (FMT capsules or placebo capsules) by way of random number generator. After subjects are assigned a number, the numbers will be randomly assigned to one of the interventions. Concealed allocation to intervention groups will be 1:1 as subject number allows. Subjects will receive cards with their respective number, which they will present each time the intervention is dispensed. A master list of subject numbers will be kept, but only utilized in the event a subject card is lost. The master list will be kept in a safe in the research office, to which select few have access.

3.7 Behavioral Modification

To assess the added benefit of FMT when used as an adjunct to lifestyle intervention, all study subjects will pursue weight loss through diet and exercise while concomitantly receiving the intervention – FMT or placebo capsules. Education regarding behavioral modification will be delivered in a group setting, at time of initial data collection. Education will consist of two successive 1-hour sessions, one dedicated to diet and the other to exercise. Diet and exercise education will be delivered by registered dietitians and health fitness specialists, respectively. During these sessions, a comprehensive overview of the lifestyle interventions will be provided. Verbal education will be accompanied by written materials. During the intervention phase, subjects will be asked to complete and submit an exercise and diet log for ≥10 days each month, both to ensure compliance with the intervention and aid specialists in personalizing education sessions (Appendix E). Logs can be completed on any 10 days during the month and do not need to be performed sequentially.

During the intervention phase, follow-up education and monitoring will occur at 45 day intervals, either taking place in clinic or by telephone. During these 20 minute sessions, nutrition and exercise experts will review each individual’s progress towards their diet and exercise goals,
reeducate as needed and troubleshoot any barriers to meeting the guidelines. Additionally, it is
during these sessions that adverse outcomes and protocol adherence will be assessed (see section
3.11 and 3.12). Specifics regarding each form of lifestyle modification are detailed below.

3.71 Calorie Restricted Diet

Dietary intervention will focus on calorie reduction, while ensuring the Daily
Recommended Intake values of each nutrient are met. Specifically, each subject’s total energy
requirements (TEE) will be estimated. Several factors comprise an individual’s TEE: resting
metabolic rate (RMR), or energy used at rest; thermal effect of food (TEF), or energy needed to
digest food; and physical activity (PA), or the energy needed to perform our daily routine activities.
Below, the TEE equation and respective weight of each component is shown.9

\[
TEE = \left[ RMR \left( \sim 60-75\% \right) + TEF \left( \sim 5-10\% \right) \right] \times [PA \left( 15-30\% \right)]
\]

Physical Activity Coefficients:
- Sedentary: \( \geq 1 \) to \(< 1.4 \)
- Low active: \( \geq 1.4 \) to \(< 1.6 \)
- Active: \( \geq 1.6 \) to \(< 1.9 \)
- Very Active: \( \geq 1.9 \) to \(< 2.5 \)

\textbf{Equation 3-A: Total Energy Expenditure Equation}

Men: \( RMR = \left[ 10 \times \text{weight (kg)} \right] + \left[ 6.25 \times \text{height (cm)} \right] - \left[ 5 \times \text{age (y)} \right] + 5 \)
Women: \( RMR = \left[ 10 \times \text{weight (kg)} \right] + \left[ 6.25 \times \text{height (cm)} \right] - \left[ 5 \times \text{age (y)} \right] - 161 \)

\textbf{Equation 3-B: Mifflin-St. Jeor Equation}

RMR will be calculated using the Mifflin-St. Jeor equation, a formula that has been
validated in our population.10-12 The thermic effect of food will be estimated at 10%, and to account
for subject activity level, a factor of 1.8 will be utilized.

Subjects will be instructed to consume 500-750 calories less than estimated by their EER,
with a weight loss goal of 1-2 lbs per week.13 Personal calorie goals will be disclosed to subjects
at initial education session. If necessary, adjustments to calorie goals may be made at the dietitian’s discretion, after accounting for an individual’s weight trajectory or inability to maintain a level of calorie restriction. The initial education session will focus on reviewing the key macro and micronutrients associated with each food group, as well as simple, diet behaviors to support weight loss (Figure 3-D). Additionally, subjects will receive instruction on completing dietary logs. To ensure subjects do not incur a nutritional deficiency secondary to diet restriction, we will use proprietary software to assess diet quality during the intervention phase.

**Dietary Intervention Overview**

- Caloric intake goal = EER – (500-750 kcals)
- Education overview
  - “MyPlate” eating method & portion control
  - Avoidance of fast food & sugar-sweetened beverages
  - Importance of adequate fluid intake

Figure 3-D: Dietary Intervention Overview

3.72 Physical Activity

Exercise goals will be consistent with the 2018 recommendations set forth by the U.S. Department of Health Human Services (Figure 3-E). Subject will receive educational handouts derived from these guidelines (Appendix F). Verbal education sessions will primarily serve to review the importance of physical activity on human health, as well as explore the different exercises and activities that can be used to meet the activity requirement. Education regarding injury-prevention will also be provided. Additionally, subjects will receive instruction on completing activity logs.
3.8 Intervention: Overview

The intervention will be conducted over a span of 12 months (Figure 3-F), with the intervention (FMT capsules vs placebo) administered every 3 months. Education on lifestyle intervention will be provided at baseline, and then routinely reinforced and monitored throughout the intervention. Specifics regarding the FMT intervention are sequentially described.

**Physical Activity Guidelines**

150 – 300 minutes of moderate-intensity exercise a week

**OR**

75 – 150 minutes of vigorous-intensity aerobic physical activity a week

**OR**

An equivalent combination of moderate and vigorous aerobic activity

Figure 3-E: Exercise Intervention Overview

Figure 3-F: Intervention Overview
3.81 FMT Capsules:

Given the previously described inconsistencies in FMT methodology and practice, our protocol also considers feasibility, tolerability and the protocols of past, similar studies.

**FMT Capsule Dosing:**

Given the lack of consensus regarding stool dosing, we chose to utilize conservative dosing. For each FMT, subjects will be provided 50g of stool in the form of 50 capsules (1g stool each) and delivered over a two day period (25 capsules on each day).

As described, the optimal frequency of FMT dosing is unknown. However there is evidence suggesting FMT-induced changes are present for 3-6 months or longer.16-19 Both in light of this research and to increase the feasibility and tolerance of our protocol, we will pursue a multi-dose FMT protocol, with a 3 month interval between administrations.

**FMT Bowel Preparation:**

Our protocol will forego the use of any pre-transplant pharmacotherapy. This includes antibiotics, proton pump inhibitors and prokinetics. In regards to bowel cleansing, with four FMT administrations occurring across the 12-month intervention, the use of laxative or cleansing agents will likely precipitate subject drop out. Our protocol will forego the use of these agents.

Our subject population must be considered when determining a need for pre-FMT fasting. Among those using insulin and hypoglycemic medications, prolonged fasting could invite unnecessary health risk. However, given a theoretical risk that capsules could become entrapped within gastrointestinal chyme, an overnight fast (~6-8 hour) will be implemented. Similarly, subjects will be asked to wait 1 hour before eating post-FMT. This conservative degree of fasting
should offer some element of protection against the aforementioned scenario, but not be drastic enough to hinder adherence or increase risk of hypoglycemia.

**FMT Capsule Administration**

On the day of intervention administration, following an overnight fast, subjects will present to the clinic to receive their assigned capsules. To streamline the process, appointments will be scheduled prior to start of normal clinic function, approximately 6-8 am. Following routine data collection, subjects will present the clinic nurse their subject number. The nurse will then retrieve the assigned 2-day course of capsules. At this time, the subject will take the first capsule dose (25 capsules). Water may be used to help consumption. To prevent abdominal discomfort, a light snack will be provided (crackers with jelly). Afterwards, subjects will be advised to wait 1 hour before eating. Prior to departing, the nurse will convey the same instructions for the second set of capsules, which will be taken at the subject’s home.

**FMT capsule Formulation**

Following procurement, donor stool specimens will be flash-frozen upon collection and delivered to the Clinical Microbiology Laboratory at the Yale School of Medicine. Capsules will be prepared by members of the research team. For simplicity, capsules will be prepared under aerobic conditions. Capsules will utilize colonic-targeted, delayed release formulations.

**FMT Donors**

Given the aforementioned lack of FMT donor protocols, we will utilize the previously described criteria developed by a Canadian research group.\(^{20}\) Presently, this is the only FMT donor protocol specifically developed for use in metabolic-syndrome associated diseases, and given the FMT-research background of its authors, this criteria is most appropriate for our protocol.
We will seek to enroll two donors, with the second to be used only in the event the primary donor is not available. For convenience purposes, we have elected to rescreen stool donors at 6 months. As such, donor screening will only need to be completed twice during the study, once shortly before the intervention phase, and the second shortly before the 6 month mark of the intervention phase.

3.82 Placebo capsules

To prevent placebo-related glycemic changes, placebo capsules will be filled with saline as opposed to starch, flour or other carbohydrate-based substance. Placebo capsules will be identical in weight and color to FMT capsules. Internal sampling will be completed by the research team to ensure smell and aftertaste are non-distinguishable from that of FMT capsules.

3.9 Blinding of intervention

Numerous precautions will be taken to maintain the integrity of our blinding. Subjects will be asked to keep their assigned numbers confidential. Additionally, as noted above, the subject numbers will be randomly sorted to an intervention. Hence in the event that a subject’s number is known, there will be no way of associating a subject to an intervention type.

FMT and placebo capsules will be of identical weight, color and size. Both sets of capsules will be prepared 1-2 days in advance by a designated member of the research team. The capsules will then be delivered to the weight loss clinics, only being labeled as ‘Batch A’ or ‘Batch B’. The individual involved in creating the capsules will leave the facility immediately after delivery. As subjects arrive to collect their capsules, the clinic nurse will ask for the subject’s numbered card. The nurse will refer to a list to determine the batch the subject is to receive and then provide the subject with their assigned 50 capsule course. The nurse will witness the subject ingest 25 of these
capsules, with the rest leaving with the subject for ingestion the subsequent day. These same proceedings will occur each time the intervention administered. At the end of the trial, the researcher who prepared the interventions will disclose the identity of all batches. The master list of subject numbers will then be used to assess what group the participants had been assigned to.

3.10 Study outcomes and Data collection

Our primary outcome of interest is percent weight change at 12 months. Secondary outcomes will include change in metabolic measures, anthropometrics and gut diversity. Anthropometric measures will include waist circumference and hip-to-waist ratio. Metabolic parameters will include Hgb A1c, fasting plasma glucose, total cholesterol, TG, LDL and HDL. Additionally, we will compare adverse effects and protocol adherence amongst the four groups. For more detail regarding primary and secondary outcomes, please refer to Table 3-C.

Anthropometric and laboratory data will be obtained during all clinic visits. At 2 visits, a stool specimen will be obtained. As previously described, in-person visits will occur on intervention days 0, 90, 180, 270 and 360. For each site visit, participants will all be asked to fast 12 hours prior to arrival, and also void/defecate immediately before being officially weighed. To reduce measurement bias, only one individual at each clinic will be responsible for assessing the weights of study subjects. The same brand scale will be used for all study subjects, and only one specific scale will be utilized at each individual clinic.
### Study Outcomes

<table>
<thead>
<tr>
<th>Primary Outcome</th>
<th>Assessment time frame</th>
<th>Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent weight change from baseline</td>
<td>6, 12 months</td>
<td>Student t-test</td>
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</table>

<table>
<thead>
<tr>
<th>Secondary Outcomes</th>
<th>Assessment time frame</th>
<th>Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycemic Control</td>
<td></td>
<td></td>
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<tr>
<td>Absolute change in Hgb A1c from baseline</td>
<td>3, 6 months</td>
<td>Student t-test</td>
</tr>
<tr>
<td>Absolute change in fasting plasma glucose</td>
<td>3, 6 months</td>
<td>Student t-test</td>
</tr>
<tr>
<td>Average Hgb A1c</td>
<td>12 months</td>
<td>Student t-test</td>
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</table>

<table>
<thead>
<tr>
<th>Anthropometric measures</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Absolute change in waist circumference (cm)</td>
<td>6, 12 months</td>
<td>Student t-test</td>
</tr>
<tr>
<td>Absolute change in waist-to-hip ratio</td>
<td>6 months</td>
<td>Student t-test</td>
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<thead>
<tr>
<th>Metabolic Parameters</th>
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<tbody>
<tr>
<td>Absolute change in lipid levels from baseline:</td>
<td></td>
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<tr>
<td>- Total Cholesterol</td>
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<tr>
<td>- Triglycerides</td>
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<td>- LDL</td>
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<tr>
<td>- HDL</td>
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<td></td>
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<tr>
<td>6, 12 months</td>
<td>Student t-test</td>
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<table>
<thead>
<tr>
<th>Gut Diversity</th>
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<tbody>
<tr>
<td>Shannon Index values</td>
<td>6, 12 months</td>
<td>Student t-test</td>
</tr>
<tr>
<td>Absolute change in Shannon Index (within individual)</td>
<td>6, 12 months</td>
<td>Paired t-test</td>
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<thead>
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<th>Adverse Effects</th>
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<td>Reported rates of the following:</td>
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<td></td>
</tr>
<tr>
<td>- Upset stomach, nausea and/or vomiting</td>
<td>3, 6, 9, 12 months</td>
<td>X²</td>
</tr>
<tr>
<td>- Diarrhea</td>
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<tr>
<td>- Constipation</td>
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<tr>
<td>- Belching or Flatulence, to degree of impacting daily life or behaviour</td>
<td></td>
<td></td>
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<tr>
<td>- Other</td>
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<td></td>
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<tr>
<td>- No adverse effects reported</td>
<td></td>
<td>X²</td>
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<tr>
<th>Protocol Monitoring</th>
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<tbody>
<tr>
<td>Adherence with diet and exercise logs</td>
<td>12 months</td>
<td>X²</td>
</tr>
<tr>
<td>Adherence to assigned intervention (# missed doses)</td>
<td>12 months</td>
<td>X²</td>
</tr>
</tbody>
</table>

*Outcomes will be assessed both by diversity level (HD vs LD) and intervention type (FMT capsule vs placebo).

**Table 3-C: Study Outcomes**

#### 3.11 Monitoring Adverse Events

Vital measurements and laboratory work will be obtained at each clinic visit. In addition to providing outcome data, these tests will be used to monitor for metabolic derangement, which may result from the drastic lifestyle changes. Laboratory safety parameters will be developed in conjunction with weight loss clinic physicians, and are likely to include electrolytes, renal function, liver chemistries and CBC. Additionally, in the event of acute safety concerns, subjects...
will be provided a phone number with which they can reach a research team member at any time to seek guidance.

To assess for intervention-related side effects, subjects will be asked a series of standardized questions approximately 45 days after each intervention course (Appendix D). These questionnaires will be administered by the dietitians and health fitness specialists at the time of the subject’s lifestyle intervention (LSI) check-in. Subjects will be asked to use their discretion and separate intervention-related adverse effects from those of any acute illness. Adverse effects of interest include nausea, vomiting, constipation, diarrhea and flatulence.

3.12 Monitoring Adherence

Adherence to lifestyle interventions will be assessed by the nutrition and exercise specialists. As previously described, subjects will be asked to record diet and exercise ≥10 days each month and submit this information at their next quarterly clinic visit. Once submitted, the diet and exercise logs will be reviewed by the dietitian and health fitness specialist, respectively. Software will be used to assess each subject’s diet for protocol adherence (correct number of calories). In addition, during each LSI check-in, the nutrition and fitness specialists will be asked to document any instances of noncompliance they uncover during the course of their meetings.

Intervention adherence will be assessed through two methods. Subjects who fail to pick up their capsules on disbursement date will be documented by a researcher on each site. Additionally, as described, participants will be asked standardized questions regarding intervention administration (Appendix- D). Participants will be asked to describe how they took their prescribed capsules, if any doses were missed and any side effects they experienced. Instances of missed doses or incorrect administration will be documented.
3.13 Final Statistical Analysis

All continuous, normally distributed baselines variables, will be reported as mean ± standard deviation. Groups in each strata will be compared using a student’s t-test. Additionally, ANOVA will be utilized to compare all continuous, normally distributed variables across the four groups. Our sole continuous, non-parametric variable (weight) will be reported as median with interquartile range, and will be compared between the two groups in each strata using a Mann-Whitney U test. Weight will also be compared across the four groups using a Kruskall-Wallis test. Qualitative baseline variables (dichotomous, nominal) will be reported as proportions and compared using chi-square analysis. This information is available in Table 3-B.

Primary and secondary outcomes will be compared across diversity level and intervention type (Figure 3-G). Average percent weight change from baseline will be reported as mean ± standard deviation and compared using a student’s t-test. Significance of our two-tailed hypothesis will be defined as p <0.05. All secondary anthropometric, glycemic and metabolic outcomes will be reported and compared in a similar fashion.

![Figure 3-G: Target Comparisons of Primary and Secondary Outcomes](image)

Gut diversity will be quantified using Shannon index and overall values will be compared between groups using a student’s t-test. Furthermore, the change in each individual’s own gut
diversity from baseline will be assessed using a paired t-test. Depending on the distribution, these values will be reported as a mean ± standard deviation or as a median with interquartile range.

Adherence to protocol will be assessed on a group level and will be based on the number of missed capsule administrations and diet/exercise logs in each group. Adherence to each parameter will be reported as a proportion \( i.e. \frac{\text{[all capsules} – \text{missed capsules]}}{\text{all capsules}} \) and compared between groups with a chi-square test. Finally, adverse effects will also be reported as frequencies and compared using chi-square. All above information is available in Table 3-C.

3.14 Subject Protection and Confidentiality

Institutional review board (IRB) approval will be sought through the Yale Human Research Protection Program. As a multi-site study, we will abide by the human research policies of all cooperating sites and financial funders, and submit additional IRB documentation as requested.

All research team members and assisting clinic staff will undergo Health Insurance Portability and Accountability Act (HIPPA) training prior to subject enrollment. Subject records will be stored on encrypted, study-dedicated laptops and desktops. The use of personal devices for study purposes will not be tolerated. Similarly, any printed documents will first be de-identified. Following publication of finalized data, all subject information will be destroyed.

Study participants will be subject to routine safety monitoring. As described, subjects will have unfettered access to a research team member in the event of safety concerns. At clinic visits, subject vitals and bloodwork will be monitored by participating clinic physicians.

Informed consent is required of all seeking to participate. A research team member will meet with all interested individuals to review the Authorization and Consent form (Appendix A), in which all the above is further detailed.
References


CHAPTER 4: CONCLUSION

4.1 Study Strengths and Limitations

Our proposal is supported by a number of unique strengths. To date, our study would incorporate the largest sample of any FMT trial investigating metabolic outcomes. Additionally, few studies have tracked outcomes beyond a few months, while our design allows for prolonged monitoring and follow-up. Thirdly, we utilize a combined approach of repeat-FMT and lifestyle intervention, a methodology that has garnered interest among FMT researchers, but has not yet been widely studied.1-6 Similarly, few studies have utilized weight-based primary outcomes, and those incorporating them as secondary outcomes lack the statistical power needed to adequately assess change. Therefore, by prioritizing weight loss, we would contribute valuable data to this field. Finally, the ability of an individual’s microbial composition to influence outcomes is an exciting prospect. Indeed, findings of select metabolic and CDI-related FMT trials have supported a relationship.7-10 Deserving of further attention, our study is designed to examine this potential relationship and is the first to focus on a low diversity population.

Our proposal is not without limitations. As stated, due to a lack of past research to draw on, our sample size is derived from the results of a sub-analysis.1 In order to convert the results of this analysis into the values needed for our calculation, we assumed the outcomes of Ng et al. were normally distributed. However, the FMT and placebo results for Ng et al. exhibited respective leftward and rightward shift (Figure 3-C). Therefore, our sample size calculation likely overestimates true effect size. Although, by focusing on those with low microbial diversity at baseline, we anticipate a stronger effect size than Ng et al., one which offsets the estimates made in calculating sample size. Finally, our protocol is inherently subject to selection bias, as we utilize...
participant-reported exercise and diet diaries. Due to a large sample size, prolonged duration, and inclusion of multiple sites, this approach was the only feasible.

4.2 Study Significance

Our findings would be relevant not only to healthcare providers, but healthcare practice in general. With obesity only increasing in prevalence, primary care providers will increasingly find themselves tasked with caring for patients with an elevated BMI. With the deleterious effects of obesity well established, it is paramount that health providers address weight loss with these patients. While bariatric surgery and newer pharmacotherapies have shown efficacy, these options may not align with a patient’s personal goals. Indeed, a large U.S. survey demonstrated the public perception surrounding weight loss surgery is largely negative, with responses revealing continued stigmatization. Similarly, newer pharmacotherapies are not without their own unique barriers. As one example, the recently approved weight loss medication Semaglutide is currently only available as a weekly injectable. Certain to impact acceptance, needle-fear is a known obstacle to treatment initiation and has been cited as a top barrier to starting insulin therapy. Additionally, as with most pharmacotherapies, rare but serious side effects are possible with Semaglutide, including pancreatitis and thyroid cancer. In contrast, existing FMT literature suggests the safety and adverse effect profile of FMT is comparable to that of placebo.

If FMT is shown efficacious in treating specific microbial phenotypes, practitioners would have yet another potential intervention to offer their patients with obesity, one that may give hope to those who have failed lifestyle intervention, or better appeal to those hesitant to pursue surgery or injectable medications. As a whole, FMT may offer a novel tool for health providers in combating an ever-growing obesity epidemic.
References


Appendix A: Study Consent

COMPOUND AUTHORIZATION AND CONSENT FOR PARTICIPATION IN A RESEARCH STUDY

YALE UNIVERSITY SCHOOL OF MEDICINE
YALE-NEW HAVEN HOSPITAL

Study Title: Fecal Microbial Transplant for Weight Loss in Adults living with Obesity and Diabetes
Principal Investigator: Rosemarie Fisher, MD
Co-Investigator: Nathan Franks RD, PA-S II
24-Hour Phone Number: [In event of safety concerns, contact number for subjects will be listed here]

Research Study Summary:
- We are asking you to join a research study.
- The purpose of this research study is to investigate if the use of fecal microbiota transplant, in along with diet and exercise, leads to greater weight loss over a year’s time than diet and exercise alone.
- Study procedures conducted as part of the study will include:
  - Initial wellness examination
  - Stool collection
  - Routine collection of bloodwork and vitals
  - Routine measurement of weight and hip/waist circumference
  - Regular engagement with dietitian and exercise specialist
  - 50 oral capsule intervention
- 6 in-person (includes 1 initial onboarding session) and 4 tele-visits are required.
- These visits will take approximately 12 hours total.
- There are some risks from participating in this study:
  - Gastrointestinal upset or infection (related to consumption of donor fecal specimens)
  - Pain, bruising, or less likely infection (related to blood draws)
  - Hypoglycemia (related to fasting periods)
  - Electrolyte abnormalities, dehydration (related to diet/exercise changes)
- The study may or may not benefit you. It is possible the intervention will improve the results of your diet and exercise regimen, or it may have no effect. Regardless, this study will aid in the development of new, alternative treatments to help those suffering from obesity.
- Taking part in this study is your choice. If you do not wish to enroll, you can still proceed with diet and exercise interventions alone. If you do enroll, you can withdraw at any time. Whatever choice you make, you will not lose access to your medical care or give up any legal rights or benefits.
- If you are interested in learning more about the study, please continue reading, or have someone read the rest of this document to you. Take as much time as you need before you make your decision. Ask the study staff questions about anything you do not understand. Once you understand the study, we will ask you if you wish to participate; if so, you will have to sign this form.
Why is this study being offered to me?
We are asking you to take part in a research study because you are currently living with a diagnosis of obesity and diabetes, and to improve your health, you have been newly referred to this specialty weight loss clinic. Additionally, you have not actively attempted to lose weight in the last year. We are looking to enroll approximately 253 individuals across several specialty weight loss clinics inside Connecticut.

Who is paying for the study?
We are still seeking a source of funding

Who is providing other support for the study?
- Yale New Haven Hospital
- Participating specialist weight loss clinics located in Connecticut

What is the study about?
Obesity has been associated with changes in an individual’s gut bacteria. The purpose of this study is to determine if transplanting gut bacteria from a lean, healthy individual into an individual with obesity, who is attempting diet/exercise, can lead to better weight loss than diet/exercise alone.

What are you asking me to do and how long will it take?
If you agree to take part in this study, this is what will happen:

1: Study Onboarding:
- Certification of eligibility: To confirm you meet the characteristics of the population we wish to enroll, we will request a hemoglobin a1c lab result from the last 3 months. Additionally, we will ask females to provide a negative urine pregnancy test.
- Initial wellness examination: We will ask you undergo a physical examination by your primary care provider, with an understanding you will be partaking in a year long, intensive exercise regimen. We ask they consent that your participation would not pose a health risk.

2. Subject Assignment:
- Stool sample: We believe those with certain gut bacterial features may benefit more from a fecal microbiota transplant. To study this, we will divide subjects into two groups based on these features. The easiest way to detect these features is by analyzing the bacteria types in one’s stool.
- Randomization: After stool samples are analyzed, you will be assigned a random number using a computer program. This number will determine which of the two capsule types you receive during the study. You will be asked to keep this number confidential.

3. Intervention preparation (One 3 hour visit)
- Baseline measurements: To establish your baseline, we will obtain bloodwork and measure your blood pressure, weight and hip/waist circumference. Additionally, you will be asked questions regarding your age, ethnicity, and how you currently manage your type 2 diabetes.
- Diet and Exercise Education: As part of the study, all subjects will be attempting to lose weight using the same calories restricted diet and exercise methods. To explain these methods, you will need to attend 2, 1-hour sessions to receive instruction. At this time, you will be told what your daily calorie goal is.
4. Intervention Phase (1 year)

- **Capsule intervention**: You will be given 4 administrations of 50 capsules over the course of the study, with 90 days in between each administration. You will be asked to consume the capsules over 2 consecutive mornings (half in clinic, half at home). You will be asked not to eat or drink 6-8 hours before each administration, and 1 hour after. However, you will be provided water and snack with capsules. Capsules will either be a placebo – a “fake” intervention – or contain donor stool specimens. Researchers and subjects will not know which intervention a subject receives.

- **Exercise and Diet meetings (20 min each)**: During the trial, you will actively attempt to lose weight with the diet and exercise methods you were taught. To help you through this time, we ask you meet a dietitian and exercise specialist every 45 days, either in person or by telephone. These experts will track your progress and provide recommendations to help you meet your goals. At these meetings, you will also be asked about any side-effects you experienced after taking the capsules. Additionally, they will ask questions about the steps you took when consuming the capsules at home.

- **Clinic Visits (60-90 min)**: After the trial begins, you will be asked to come to the clinic every 90 days for measurements. We will measure weight and hip/waist circumference, and draw blood. At two of these visits, you will be asked to provide an additional stool sample for bacterial analysis. Also, you will meet with a diet and exercise expert and be given your capsules at these visits.

**What are the risks and discomforts of participating?**

Possible risks associated with participation are detailed below.

- **Capsule-related**
  - Gastrointestinal upset, including: nausea, vomiting, bloating, abdominal pain, diarrhea or constipation.
  - Low blood sugar: related to periods of fasting before and after capsule consumption

- **Blood draw-related**
  - Potential pain, bleeding or bruising at site of blood draw. Less likely, infection is possible.

- **Exercise or diet-related**
  - Low blood sugar, electrolyte changes or dehydration, which can result from radical changes in diet and activity level

**How will the potential risks be managed?**

- **Capsule-related**
  - Subjects will be provided snacks and water to take with capsules. If needed, subjects experiencing significant upset can immediately reach the research team for guidance.
  - For fasting periods, clinic physicians will make changes to your insulin and oral medications to minimize the risk of low blood sugar

- **Blood draw-related**
  - In an effort to minimize discomfort, trained blood-draw technicians will draw samples. Additionally, any immediate complications will be managed by clinic staff and site physician.

- **Exercise or diet-related**
  - In addition to providing study data, the laboratory work drawn at clinic visits will also assess safety parameters, including electrolyte levels and kidney function. These results will be reviewed by clinic physicians. Additionally, subjects will be counseled on the signs of these complications.
**How will I know about new risks or important information about the study?**
We will tell you if we learn any new information that could change your mind about taking part in this study.

**How can the study possibly benefit me?**
We theorize individuals with certain bacterial features may benefit more from fecal microbiota transplant. If you are randomly assigned to this intervention, it is possible you will receive additive benefit from planned diet and exercise, including additional improvements in blood pressure, fat or sugar levels, and weight control. However, it is also possible you will receive no benefit.

**How can the study possibly benefit other people?**
The benefits to science and other people may include a better understanding of how changes in the gut bacteria impact body weight. This information will aid in the development of new, alternative treatments to help those suffering from obesity.

**Are there any costs to participation?**
You will not have to pay for taking part in this study. The only costs include transportation and your time coming to the study visits.

**Will I be paid for participation?**
You will not be paid for taking part in this study. However, all reasonable attempts will be made to account for any extra costs you incur, including parking and food during longer clinic visits.

**What are my choices if I decide not to take part in this study?**
Instead of participating in this study, you have some other choices.
- Continue your planned diet and exercise regimen without being in the study. In this case, you would be followed exclusively by clinic staff. Your care would have no impact on the study and vice versa.
- Elect to pursue a separate weight loss intervention entirely (other than diet and exercise)

**How will you keep my data safe and private?**
We will keep the information we collect about you confidential. We will share it with others if you agree to it or when we have to do it because U.S. or State law requires it. For example, we will tell somebody if you learn that you are hurting a child or an older person.

To best keep your health information safe, all research team and participating clinic staff members will undergo confidentiality training. Additionally, all study records will ONLY be stored on encrypted computers. Any printed study documents will not have any information that could identify you if it were lost. Following publication of our study data, all subject information and lab specimens will be destroyed.

When we publish the results of the research or talk about it in conferences, we will not use your name. If we want to use your name, we would ask you for your permission.

**What Information Will You Collect About Me in this Study?**
The information we are asking to use and share is called “Protected Health Information.” It is protected by a federal law called the Privacy Rule of the Health Insurance Portability and Accountability Act (HIPAA). In general, we cannot use or share your health information for research without your permission. If you want, we can give you more information about the Privacy Rule. Also, if you have any questions about the Privacy Rule and your rights, you can speak to Yale Privacy Officer at 203-432-5919.
The specific information about you and your health that we will collect, use, and share includes:

- Yale New Haven Hospital electronic health records (used to establish study eligibility)
- Research study records
- Medical and laboratory records of only those services provided in connection with this study
- Records about phone calls made as part of this research
- Records about your study visits
- Information obtained during this research regarding:
  - Your sex, date of birth, ethnicity, past medical history
  - Your current medical conditions and how you manage them i.e. medications
  - Weight and body measures
  - Information regarding the bacteria in your gut
  - Laboratory, x-ray, and other test results
  - Diaries and questionnaires

How will you use and share my information?
We will use your information to conduct the study described in this consent form.
We may share your information with:

- The U.S. Department of Health and Human Services (DHHS) agencies
- Representatives from Yale University, the Yale Human Research Protection Program and the Institutional Review Board (the committee that reviews, approves, and monitors research on human participants), who are responsible for ensuring research compliance. These individuals are required to keep all information confidential.
- Health care providers who provide services to you in connection with this study.
- Principal Investigator, Co-Investigators and other investigators of the study
- Study Coordinator and Members of the Research Team
- Data and Safety Monitoring Boards and others authorized to monitor the conduct of the Study

We will do our best to make sure your information stays private. But, if we share information with people who do not have to follow the Privacy Rule, your information will no longer be protected by the Privacy Rule. Let us know if you have questions about this. To better protect your health information, agreements are in place with these individuals and/or companies that require they keep your information confidential.

Why must I sign this document?
By signing this form, you allow researchers to use and disclose your information (as detailed above) for study purposes. This is to ensure that the information related to this research is available to all parties involved in the study. You always have the right to review and copy your health information in your medical record. However, this is a single/double blinded treatment study and if you sign this permission form you will not be allowed to look at or copy your study related information until after the research is completed.

What if I change my mind?
The authorization to use and disclose your health information collected during your participation in this study will never expire. However, you may withdraw or take away your permission at any time. You can easily withdraw your permission by talking with study staff.

If you withdraw your permission, you will not be able to stay in this study, but the care you get from your doctor outside this study will not change. No new health information identifying you will be gathered after the date you withdraw. Information that has already been collected may still be used and given to others until the end of the research study to insure the integrity of the study and/or study oversight.
Who will pay for treatment if I am injured or become ill due to participation in the study?
Yale New Haven Health does not normally provide any form of compensation for injury or lost income. Commonly, if a for-profit organization funds a study, it is expected they will cover the cost of any adverse effects you sustain. However, if a non-profit organization funds the study, you or your insurance may be billed for costs of adverse events. We will communicate this information to you once funding is secured.

What if I want to refuse or end participation before the study is over?
Taking part in this study is your choice. You can choose to take part, or you can choose not to take part in this study. You also can change your mind at any time. Whatever choice you make, you will not lose access to your medical care or give up any legal rights or benefits.

We would still treat you with standard therapy or, at your request, refer you to a clinic or doctor who can offer this treatment. Not participating or withdrawing later will not harm your relationship with your own doctors or this institution.

To withdraw from the study, you can call a member of the research team at any time and tell them that you no longer want to take part.

The researchers may withdraw you from participating in the research if necessary. Certain circumstances under which this may occur include:

- You have a severe side effect to the intervention and it is dangerous for you to continue
- If you are female and become pregnant
- If you continually miss study visits, phone calls or have great difficulty following the study protocol, you may be asked to withdraw so that your results do not impact the rest of the study

What will happen with my data if I stop participating?
If you decide to withdraw from the study, measurements already collected may be included in the final analysis. All electronic data will be destroyed at the end of trial along with that of other subjects.

Who should I contact if I have questions?
Please feel free to ask about anything you don't understand. If you have questions later or if you have a research-related problem, you can call the Principal Investigator at [PI phone number will be listed here]

If you have questions about your rights as a research participant, or you have complaints about this research, you call the Yale Institutional Review Boards at (203) 785-4688 or email hrpp@yale.edu.

Authorization and Permission
Your signature below indicates that you have read this consent document and that you agree to be in this study.

We will give you a copy of this form.

Participant Printed Name _______________________________ Participant Signature _______________________________ Date _______________________________

Person Obtaining Consent Printed Name _______________________________ Person Obtaining Consent Signature _______________________________ Date _______________________________
Appendix B: Sample Size calculation using Power and Precision Software
## Appendix C: Donor Selection Criteria

### Exclusion Criteria

**General**
- Age: <18 or >65 years
- BMI: <18 kg/m² or ≥25 kg/m²

**Medical:**
- Any history of:
  - Type 2 diabetes or Insulin sensitivity
  - Autoimmune disease
  - Any kind of gastrointestinal disorder or liver disease
  - Hypertension, hyperlipidemia or atherosclerosis
  - Psychiatric conditions
  - Infection with HIV, syphilis, hepatitis B or C
  - Exposure to HIV or viral hepatitis in last 12 months
  - Malignancy
  - Major gastrointestinal surgery
  - Family history of early onset coronary disease, gastrointestinal disease or liver disease
  - Family history of colorectal carcinoma
- Taking chronic medications
- Use of antibiotics, antifungals or antivirals in past 3 months
- Use of probiotics in last 3 months
- Hospitalization in last 3 months

**Social History:**
- Travel to an area high risk for infectious diarrhea in last 3 months
- Tattoo or body piercing in last 6 months
- High risk sexual behavior
- Illicit drug use
- Alcohol intake >10g/day (women) or >20g/day (men)

### Clinical Evaluation

#### Stool Testing
- Ova, cysts and parasites
- Microscopy and culture
- Norovirus, Rotavirus, Adenovirus
- Clostridium Difficile Toxin
- Vancomycin-resistant *Enterococcus* screen
- Carbapenem-resistant *Enterobacteriaceae*
- Extended spectrum β-lactamase-producing *Enterobacteriaceae*
- Fecal antigen: *Giardia* and *Cryptosporidium*
- Microsporidia

#### Blood Testing
- Complete blood count and liver function tests
- Electrolytes, urea and creatinine
- Fasting lipids and blood glucose
- HIV 1 and 2
- Human T-cell lymphotropic virus 1 and 2
- *H. pylori*
- *Listeria*
- *Treponema pallidum* screening cascade
- Serologies:
  - Anti-TTG antibody
  - Hepatitis C antibody
  - Hepatitis A virus IGM
  - Hepatitis B: virus surface antigen, virus core antibody (IgM and IgG), virus surface antibody
  - Cytomegalovirus IGM, Epstein Barr virus IGM
  - *Strongyloides stercoralis, Entamoeba histolytica, Helicobacter pylori*

#### Other:
- Nasal swab: *Methicillin-resistant Staphylococcus aureus*
- Urine Testing: Gonorrhea and chlamydia

### FMT Donor Screening
**Appendix D:** Standardized Questionnaires Regarding Adherence, Adverse Effects,

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**Post-Intervention Standardized Questionnaire**
*(To be delivered verbally to subjects by research staff)*

The day you were seen in clinic, you were provided your first set of capsules and were instructed to take the second set the following day. Did you:

<table>
<thead>
<tr>
<th>Question</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Did you fast from midnight up to the time you ingested your capsules?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Did you take your capsules between 6-8 am the following morning? If not, when did you ingest them?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Did you consume all 25, 1g capsules? If no, why?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Did you take your capsules with a water and light snack?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Following consumption, did you wait 1 hour prior to eating anything else?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
45-Day, Post-FMT Assessment of Side effects, Adverse Events  
*(To be delivered verbally to subjects by research staff)*

In the 45 days following intervention ingestion, have you experienced any of the following side effects, or adverse events?

<table>
<thead>
<tr>
<th></th>
<th>Question</th>
<th>Yes</th>
<th>No</th>
<th># of days?</th>
<th>If yes, how would you rate symptom severity?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bloating, sensation of fullness?</td>
<td></td>
<td></td>
<td></td>
<td>1  2  3  4  5  6  7  8  9  10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Excessive flatulence or eructation?</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Nausea or Vomiting?</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Diarrhea or loose stool?</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Constipation or inability to pass stool?</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Abdominal cramping, aches or pain?</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Headache?</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>8</td>
<td>Fever?</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>No</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>9</td>
<td>Fatigue, malaise or change in appetite?</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Other – Please Describe:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix E: Diet and Exercise Diaries (samples)

Sample Sources:
- CDC free online Exercise Diary: https://www.cdc.gov/healthyweight/pdf/physical_activity_diary_cdc.pdf
- Vertex free online Food Diary Template: https://www.vertex42.com/ExcelTemplates/food-diary-template.html
Appendix F: Exercise Education Handouts (3 pages)

2008 Physical Activity Guidelines for Americans
Fact Sheet for Health Professionals on Physical Activity Guidelines for Adults

How much physical activity do adults need for health benefits?
Adults who are active are healthier, are less likely to develop many chronic diseases, and have better aerobic fitness than adults who are inactive. Adults need to do two types of physical activity each week to improve health – aerobic and muscle-strengthening activities.

Aerobic Activities
For substantial health benefits, adults need to do at least:
• 2 hours and 30 minutes (150 minutes) each week of moderate-intensity* aerobic activity.
  OR
• 1 hour and 15 minutes (75 minutes) each week of vigorous-intensity* aerobic activity.
  OR
• An equivalent mix of moderate- and vigorous-intensity aerobic activity.

Aerobic activity should be performed for at least 10 minutes at a time, preferably spread throughout the week.

*Intensity is the level of effort required to do an activity.
A person doing moderate-intensity aerobic activity can talk, but not sing, during the activity.
A person doing vigorous-intensity activity cannot say more than a few words without pausing for a breath.

Muscle Strengthening Activities
Muscle strengthening should be done 2 or more days a week.
• All major muscle groups should be worked. These are the legs, hips, back, abdomen, chest, shoulders, and arms.
• Exercises for each muscle group should be repeated 8 to 12 times per set. As exercises become easier, increase the weight or do another set.
How can adults get additional health benefits?

**Aerobic Activities**

For greater health benefits, adults should do:

- 5 hours (300 minutes) each week of moderate-intensity aerobic activity, OR
- 2 hours and 30 minutes (150 minutes) a week of vigorous-intensity aerobic activity, OR
- An equivalent mix of moderate- and vigorous-intensity aerobic activity.

**Health Benefits from Regular Physical Activity**

Participating in regular physical activity provides many health benefits, as summarized below. Reducing risk of some of these conditions may require years of participation in regular physical activity. Other benefits, such as increased heart and lung—or cardiorespiratory—fitness, may require only a few weeks or months of participation.

**Strong Evidence for Health Benefits**

- Lower risk of:
  - Early death
  - Coronary heart disease
  - Stroke
  - High blood pressure
  - High cholesterol or triglycerides
  - Type 2 diabetes
  - Metabolic syndrome
  - Colon cancer
  - Breast cancer

- Prevention of weight gain
- Weight loss, particularly when combined with reduced calorie intake
- Improved cardiorespiratory (aerobic) fitness and muscular strength
- Prevention of falls
- Reduced depression

**Aerobic Activities by Level of Intensity**

There are different ways to classify intensity of exercise. Absolute intensity is the amount of energy expended per minute of activity. Moderate-intensity activities expend 3.0 to 5.9 times the amount of energy expended at rest. The energy expended in vigorous-intensity activities is 6.0 or more times the energy expended at rest.

Relative intensity is the effort required for an individual to do an activity. Relative intensity of aerobic activity is related to cardiorespiratory fitness. Less fit people generally require a higher level of effort than fitter people to do the same activity. Relative intensity can be estimated using a scale of 0 to 10, where sitting is 0 and the highest level of effort possible is 10. A moderate-intensity activity is a 5 or 6. A vigorous-intensity activity is a 7 or 8.
For most people, light daily activities such as shopping, cooking, or doing the laundry do not count toward the guidelines. Here are some examples of aerobic activities that require moderate-intensity and vigorous-intensity effort:

### Level of Intensity: Moderate-Intensity
- A person doing moderate-intensity aerobic activity can talk, but not sing, during the activity.
- Brisk walking (3 miles per hour or faster, but not race walking)
- Water aerobics
- Bicycle riding slower than 10 miles per hour
- Tennis (doubles)
- Ballroom dancing
- General gardening

### Level of Intensity: Vigorous-Intensity
- A person doing vigorous-intensity activity cannot say more than a few words without pausing for a breath.
- Race walking, jogging, or running
- Swimming laps
- Tennis (singles)
- Aerobic dancing
- Bicycling 10 miles per hour or faster
- Jumping rope
- Heavy gardening (continuous digging or hoeing with heart rate increases)
- Hiking uphill or with a heavy backpack

## Muscle-Strengthening Activities

Adults also need to do muscle-strengthening activities **at least 2 days a week**, at a moderate to high level of intensity. These activities should **work all the major muscle groups**: the legs, hips, back, chest, abdomen, shoulders, and arms.

No specific amount of time is recommended for muscle strengthening, but exercises should be performed to the point at which it would be difficult to do another repetition. A repetition is one complete movement of an activity, like lifting a weight or doing a sit-up. Adults can do activities that strengthen muscles on the same or different days that they do aerobic activity, whichever works best. Muscle-strengthening activities do not count toward the aerobic activity total.

Below are some examples of muscle-strengthening physical activities for adults:

### Types of Muscle-Strengthening Activity
- Lifting weights
- Working with resistance bands
- Doing exercises that use body weight for resistance (push-ups, sit-ups)

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**Sample Sources:**
Appendix G: Diet Educational Handout (samples)

Sample A

Eat Healthy, Lose Weight, Exercise, Change Your Lifestyle...

...and Have FUN While Doing It!

1. Explore and get to know the MyPlate website.
   - Choose meals to match the MyPlate image.
   - Make sure to include all food groups on your plate, plenty of fruits and vegetables!
   - It is all about balance!

2. Involve your family.
   - Go grocery shopping together, make it a fun outing.
   - Read food labels
   - Plan and cook fresh meals together and find recipes.
   - Limit food eaten from restaurants, fast-food, etc.
   - Eat regular meals and make sure to NOT to skip breakfast!
   - Prepare your own lunches and snacks.
   - Reduce portion sizes, reduce salt and sugar, increase your daily water intake.
   - If your school offers healthy food, especially, funded breakfast and lunch, participate.

   - Make time to get some sort of daily exercise in (taking the stairs, sports, swimming, yoga, etc.)
   - Take the stairs instead of the elevator and get involved in structured sports activities.
   - Involve your family or friends in your activities, such as walking. Accountability and company is very encouraging!
   - Change your lifestyle! Instead of watching TV, take a walk or a bike ride. Join a workout class.
   - Limit the time you watch TV and have screen time (i.e. computers, social media, video games, etc.) ESPECIALLY while you are eating.

4. Get support!
   - You are not alone. Don't feel overwhelmed. There are many resources that can help you on this road.
   - If your school offers a nutrition class, sign up!
   - Join support groups, as a way to get encouraged, be held accountable for, and know that you are not alone. Lots of support groups allow you to stay in contact via text, email, or phone.

Additional Resources

- http://www.choosemyplate.gov

Sample Source
Abuzeid, Yasmine, "Eat Healthy, Lose Weight, Exercise, Change Your Lifestyle… And Have FUN While Doing It!" (2018). All Student-Created Educational Resources. 103.
https://dune.une.edu/an_studedres/103
Sample B

1. Eat foods from all food groups. Use MyPlate to help you eat a variety of foods from all food groups. Include low-fat dairy, whole grains, and colorful fruits and vegetables daily.

2. Cut back on the sweet stuff. You’re already sweet enough! Try replacing soda with low-fat milk or water. Replace cookies, candies, and cakes with fresh fruits.

3. Eat the rainbow. Eating a variety of colorful fruits and vegetables helps your body to get the nutrients it needs to stay healthy and strong.

4. Keep moving. Do at least 1 hour of physical activity per day. This includes activities such as riding your bike, walking briskly, swimming and rollerblading. Don’t worry, you don’t have to do it all at once! Try being active for 10 minutes, 6 times per day. That’s nothing!

5. Our bodies need fat, but not too much. Try eating foods that are baked instead of fried. Replace whole-fat dairy with low-fat. Try eating leaner meats more often, such as chicken breast or lean pork instead of steak.

6. Stick to “Handy” portion sizes. Not sure how much to eat? Use your hand as a guide when deciding how much of a certain food to eat at one meal.


Additional Resources:
www.choosemyplate.gov
WHO Activity Guidelines:
www.who.int/dietphysicalactivity/factsheet_young_people/en/
USDA Teen Nutrition Programs
https://www.nal.usda.gov/fnic/teen-nutrition

Sample Source
Bibliography


Fadda HM. The Route to Palatable Fecal Microbiota Transplantation. AAPS PharmSciTech. 2020;21(3):114.


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Meldrum DR, Morris MA, Gambone JC. Obesity pandemic: causes, consequences, and solutions—but do we have the will? Fertility and Sterility. 2017;107(4):833-839.


