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INTEGRATED STUDY MASTER'S THESIS

Gut Microbiota in Obese Patients

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List of Abbreviations:

- WHO- World Health Organization
- BMI -Body Mass Index
- HMP- Human Microbiome Project
- 16S rRNA-16S ribosomal RNA
- SCFA- Short chain fatty acid
- F/B- Firmicute/ Bacteroidetes
- HLA- Human Leukocyte Antigen
- MHC- major histocompatibility complex
- TLR- toll-like receptors
- Spp.- species
- PCR- polymerase chain reaction

1. Summary

The composition of hosts gut microbiome has been thought to play a role in obesity and weight management overall. This review aims to identify that the composition of the gut microbiome influences obesity through its role in digestion, metabolism, and immune system regulation. A healthy microbiome must be highly diverse, and most importantly consists of beneficial bacteria that aid in maintaining gut integrity and function. Microbes that make up the majority of the population are facultative anaerobes and aerotolerant anaerobes that aid in digesting of food when consumed by the host. Historically, the Firmicutes/Bacteroidetes ratio was suggested as an indicator of obesity, stating that obese individuals having an altered balance between these two bacterial groups were more likely to be obese or overweight. However, current evidence points to this ratio being an oversimplified biomarker when it comes to the gut microbiota as it lacks consistency amongst different study populations, mainly due to factors such as, diet, genetics, and environment. This review also highlights environmental, genetic, and dietary factors that significantly influenced gut microbiota composition and diversity. Unhealthy dietary patterns, specifically those seen in high-fat, low-fiber Western diets, were associated with reduced microbiome diversity. In contrast, diets rich in plant-based fibers promoted microbial richness and improved metabolic performance. A consistent finding across a variety of populations was reduced gut microbial diversity amongst obese individuals. Reduced microbial diversity was seen to be associated with impaired metabolic function, increased energy extraction from diet, and heightened inflammatory response. Additionally, the promotion of microbial diversity through dietary modifications, lifestyle interventions, and potentially probiotic supplementation was discussed as a promising strategy for managing obesity and improving metabolic health.

2. Introduction

Obesity is a complex and multifactorial metabolic disorder and a major global health crisis. The World Health Organization (WHO) classifies obesity as a chronic disease defined by excessive fat accumulation that can impair ones health (1).The Body Mass Index (BMI) scale is used to define obesity at a BMI of 30 or more, and according to the WHO 1 in 8 people worldwide live with obesity(1). Obesity not only manifests as a change in appearance with excess fat accumulation, but as increased pathologies that can decrease the quality of life and contribute to early mortality. It is important then to investigate the causes of obesity to better treat patients and improve outcomes. Important obesity factors include diet, genetics, behavioral factors, and environmental factors.

The gut microbiota refers to the microorganism population living within the human gastrointestinal tract that have a role in digestion, metabolism, and health. The gut microbiota consists of trillions of microorganisms which generally include bacteria, viruses, archaea, and fungi which vary greatly amongst individuals based on diet, environment, and genetics. A controlled balance within the microbiota plays a vital role in the overall health of human hosts, and imbalances due to poor diet, stress, or comorbidities can cause an overgrowth of these pathogenic organisms. This imbalance in the microbiota is referred to as dysbiosis which can play a role in a multitude of pathologies including, gastrointestinal disorders, inflammatory disorders, central nervous system disorders, metabolic disorders, and cancer.

The role of the gut microbiota, and in turn gut dysbiosis has been theorized to have a causative or associated effect on obesity. This review will investigate the gut microbiota's effect on obesity by examining the composition of the microbiota amongst different populations.

Methodology and Selection Criteria

To identify sources for this narrative review the PubMed database was used. Search terms used included "gut microbiota," "obesity," "dysbiosis," "short-chain fatty acids," "dysbiosis," "Body Mass Index," "Firmicutes," "Bacteroidetes," and "Probiotics." These terms were searched individually and in various combinations to have a comprehensive and relevant collection of the studies.

Inclusion criteria included the study design of clinical trials, randomized controlled trials, journal articles, and comparative studies. The participant types in the relevant studies had to be adult, obese subjects or lean subjects in comparative studies that were otherwise healthy. All studies were published within 15 years of the research, no later than 2010. Only studies published in English were considered.

Exclusion criteria included reviews unless they were meta- analysis or systemic reviews. Animal populations were excluded in data selection as were participants with significant comorbidities. Articles not published in English and before the year 2010 were excluded. The process of study selection is outlined in a PRISMA flowchart, which is available in Annex 1.

3. The Healthy Gut Microbiota

To identify a dysbiotic or altered microbiome, a healthy gut microbiome must first be identified. The term healthy microbiome must be defined, as it can vary amongst different populations, and it can be challenging to set a single benchmark for what is healthy. Individual microbiomes can vary significantly due to an array of factors such as a host's genetic composition, different environmental

stressors, diet, and age. To help with this identification, the Human Microbiome Project (HMP) studies the human microbiota as whole, not only that of the gut, but also nasal, urogenital, and integumentary microbiotas. The HMP has used 16S ribosomal RNA (16S rRNA) gene sequencing to investigate the human microbiota from over 31,596 different samples in an effort to identify a healthy human microbiome(2).

Defining a healthy microbiota can be challenging, as it suggests a specific microbial composition, and as stated before there are a vast array of factors that can determine a healthy microbiota composition. Therefore, it is important to define healthy microbiota in the context of this research. The term healthy microbiota will be used when referring to functions of the microbiome that promote health such as short chain fatty acid producers (SCFA), while the term normal microbiota will refer to the microbial composition of the general population. When speaking about the microbial diversity of obese patients, that will be referred to as normal microbiota of obese populations.

The healthy human gut microbiota is predominantly composed of the following four bacterial phyla Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria.(3)

Recent taxonomic changes by the National Center for Biotechnology Information as of 2021 have reclassified these bacterial phylum(4):

- Firmicutes to Bacillota
- Bacteroidetes to Bacteroidota
- Actinobacteria to Actinomycetota
- Proteobacteria to Pseudomonadota

When discussed in this paper the terms will be used interchangeably based on the publication, date of research, and whether proper nomenclature was used. These bacteria are the basis for the composition and function of a healthy microbiome and will be discussed in further detail in later sections. The Firmicute spp. include *Lactobacillus* and *Clostridium*, which function in SCFA production and energy metabolism(3). This occurs though the fermentation of dietary fiber leading to production of SCFA which provide energy to colonocytes and aid in metabolism(5). The Actinobacteria spp. include the genus *Bifidobacterium*, though they constitute a small minority of the gut microbiota, has a crucial role in gut health through fermentation of dietary fiber and synthesis of B vitamins(6). The Proteobacteria spp. function in Nitrogen and Sulfur metabolism which participate in influencing nutrient cycling within the gut, in addition they regulate gut oxygen levels(7). A diverse microbiome of these four phyla of bacteria play an important role in the healthy microbiota, in later sections dysbiosis of these organisms and their effects will be discussed.

3.1 The Healthy Gut Microbiota: Firmicute/ Bacteroidetes Ratio

The Firmicutes spp. and Bacteroidetes spp. are two of the dominant bacterial phyla making up about 90% of the microbiota population. In literature and in research the Firmicute/ Bacteroidetes (F/B) ratio has been a commonly used metric to describe a balanced and healthy microbiota. The F/B ratio has major implications in obesity research with a disbalance of the F/B ratio perhaps aiding in obesity which will be discussed in later sections. In the healthy microbiome Firmicutes including *Lactobacillus* and *Clostridium* aid in fiber fermentation, SCFA production, and gut barrier maintenance. Bacteroidetes including *Prevotella* species breakdown complex carbohydrates and aid in bile acid metabolism. A balance in the F/B ratio may be necessary for metabolic homeostasis and immune function(8).

3.2 The Healthy Gut Microbiota: Digestion

The functions of a healthy gut microbiota include playing a major role in digestive and metabolic processes. The human gastrointestinal tract lacks the enzymes needed to metabolize dietary fiber, in turn gut bacteria are essential in fermentation of dietary fiber within the human gut. Fiber fermentation occurs mainly in the colon where gut bacteria, mainly Bacteroidetes and *Bifidobacterium* break down non- digestible polysaccharides and oligosaccharides. These bacteria utilize glycoside hydrolase, glycosyltransferases, carbohydrate esterase and polysaccharide lyases to break these carbohydrates into simple sugars(9). Due to the variety of carbohydrate binding molecules and enzymes within gut bacteria, hydrolysis of a wide variety of fibers is possible and beneficial. Fibers including cellulose, hemicellulose, pectin, gums, and fructans that contain various monosaccharide units are preferable to be consumed in the diet and provide for a far more diverse microbiota(9).Once these simple sugars are created via carbohydrate lyases they are then aerobically fermented by bacteria resulting in the production of SCFA, most importantly acetate, propionate and butyrate. Acetate the primary SCFA within microbial circulation is used as an energy source by peripheral tissue and is involved in lipid and cholesterol metabolism(10). Propionates metabolic functions may include the reduction of lipogenesis, lowered serum cholesterol levels, and decreased carcinogenesis in other tissues(11) and may even help regulate hepatic gluconeogenesis(12). Butyrate's function is to be the primary energy source for colonic enterocytes ,and in addition provide to the integrity of the gut barrier and aid in mucous production(13).

3.3 The Healthy Gut Microbiota: Immune System Regulation

The gut microbiota has a crucial role in the regulation of the immune system by maintaining its activation through communication with immune cells. The Gut- Associated Lymphoid Tissue is the principal component of the mucosal immune system and plays a crucial role in epithelial gut barrier integrity and immunity. The intestinal epithelium consists of enterocytes, goblet cells, Paneth cells, and enteroendocrine cells whose function is to absorb nutrients, secrete mucous, and regulate hormones. Within the lamina propria of the intestines are the immune cells like Peyer's patches and macrophages which work by presenting microbial antigens to T cells thus forming the immune response which has shown to be aided by a highly developed microbiota(14).

3.4 The Healthy Gut Microbiota: Genetic Factors

The hosts genetics can influence the makeup of the microbiota by affecting host immune system genes such as Human Leukocyte Antigen (HLA) genes, which are genes on major histocompatibility complexes (MHC) that help differentiate between self and non-self-components aiding in pathogen recognition (15). These variations in HLA alleles may influence susceptibility to certain bacteria thus shaping the microbiota composition (16). Additionally toll- like receptors (TLR) serve to detect microbial associated molecular patterns of bacteria within the epithelium of the gut, for example detection by TLR-4 recognizes lipopolysaccharides of Gram- negative bacteria. This recognition of microbe associated molecular patterns activates intracellular signaling pathways and regulates the maintenance of gut homeostasis contributing to a healthy microbiota. (17) Studies on over 416 twin pairs carried out by Goodrich et al. 2014 found the abundance of specific bacteria within the feces of the test subjects had an effect on subjects BMI. Notably, the family Christensenellaceae was found to be abundant in individuals with low BMI(18).

3.5 The Healthy Gut Microbiota: Environmental Factors

Environmental factors play a crucial role in the composition and maintenance of the healthy human gut microbiota. Diet and medications have stood out as the primary environmental factors influencing microbiota richness(19). High fiber, plant-based diets as seen commonly with the Mediterranean diet have been investigated, and seem to promote the growth of commensal bacteria(20). Alternatively Western diets or energy dense diets with few nutrients have been shown to contribute to microbial resistance and in turn inflammation(20). The beneficial diet has microbiota accessible carbohydrates allowing for the production of SCFA's which promote balanced microbiota development. Dietary protein is cleaved into peptides and free amino acids to be metabolized

elsewhere. Medications, specifically a wide range of antibiotics, proton pump inhibitors, and diabetes medication such as metformin have been found to reduce microbial diversity(21,22). The maintenance of a healthy gut microbiome requires an adequate diet and can be negatively affected by excess and prolonged medication use. It is important then to note, that the modern western diet, which has evolved from advancement in farming, provides excess calories in the form of sugars and fats while lacking healthy carbohydrates and protein, can play a pivotal role in gut dysbiosis and obesity. Excess medication use, especially polypharmacy, seen in older and obese populations may have detrimental effects on the healthy microbiota(23).

4. The Firmicutes to Bacteroidetes Ratio in Obesity

The Firmicutes and Bacteroidetes constitute the major bacterial phyla found within the human gut microbiome, by accounting for more than 90% of the bacteria population within the gut(24). Early studies done on microbial populations of gut microbiota reported that obese individuals tended to have an increased F/B ratio compared to those with lower BMI's. A higher or increased F/B ratio describes an elevation in the number of Firmicutes and/ or the decrease in the number of Bacteroidetes within the gut. This was first seen in studies with animal populations mainly in mice, but in recent years human gut biome studies have shown similar results. In a study done measuring F/B levels using 16s RNA sequencing, it was shown that obese subjects had a lower proportion of Bacteroidetes than their lean counterparts. Furthermore the Bacteroidetes level increased while Firmicutes level decreased, which in turn increased the F/B ratio when obese subjects lost weight due to maintaining a low calorie diet(25). The hypothesis that the F/B ratio might be, what came to be known as the biomarker of obesity, was further tested amongst non-obese subjects. Subjects underwent weight loss via calorie restriction and in addition to weight loss in these subjects, the F/B ratio became normalized(8). Though showing promising results the F/B ratio can vary widely amongst both healthy and obese subjects. Many factors can influence the F/B ratio even amongst obese subjects with similar BMI's, these factors include age, gender, and dietary habits which can drastically change the ratio. It is important then to note that the F/B ratio unfortunately cannot be used as a direct biomarker of obesity or even accurately gauge gut microbiota health(26). To be an accurate measurement of microbiota diversity all exogenous factors must be considered and calculated to even be considered clinically useful.

In addition to positive evidence in favor of the F/B ratio difference amongst obese subjects, there is an abundance of evidence that there is no significant difference, and, in some cases, an inverse relationship has been seen. One large analysis of a combined 1,600 subjects found that the F/B ratio

was lower in obese patients than the non-obese control group(27). A study performed with 151 overweight but not all obese subjects, did not show any association between F/B ratio and obesity though it did show a predictive value for excess body weight gain based on the F/B ratio(28). In a meta- analysis performed in 2016 from 10 independent studies it was concluded that an F/B ratio did not show a consistent pattern in the pattern of obesity, though gut microbial diversity as a whole was shown to be significant(29). Obese and lean subjects can have similar F/B ratios and the F/B ratio cannot be extrapolated into measuring gut microbiota diversity.

5. Link Between F/B ratio and Obesity

The gut microbiome plays a crucial role in regulating the hosts metabolism, as discussed, alterations in the F/B ratio may be implicated in obesity through multiple mechanisms. One hypothesis suggests that a higher F/B ratio enhances energy harvest, due to the Firmicutes species production of enzymes that break down complex carbohydrates into fermentable sugars and SCFA, thus increasing caloric absorption. Studies in both mice and humans have shown that an obese microbiota, characterized by a higher proportion of Firmicutes, extracts more energy from food(30,31). SCFA are then critical in the discussion of dysbiosis, most importantly butyrate, acetate, and propionate.

5.1 Short Chain Fatty Acids- Butyrate

Butyrate is the primary energy source for colonocytes and works to maintain the intestinal barrier by enhancing tight junction proteins and aiding in mucin production. Tight junctions work by facilitating the movement of proteins and lipids between apical and basolateral cell membranes(32). Butyrate exerts anti-inflammatory effects by suppressing the activity of nuclear factor kappa B which works as a transcription factor in the inflammation response. Inhibition of nuclear factor kappa B reduces expression of cytokines, mainly tumor necrosis factor- alpha and interferon gamma. Nuclear factor kappa B is directly inhibited by butyrate, due to its function as a histone deacetylase inhibitor which leads to hyperacetylation of histones(33). Butyrate activates G protein coupled receptors specifically GPR43, GPR41, and GPR 109A which all have proven effects of changing cytokine levels and promoting anti- inflammatory responses(13). Through GPR 109A butyrate promotes differentiation of regulatory T cells which suppress excessive inflammation responses and maintains immune tolerance(13). Butyrate also increases insulin sensitivity through activation of AMP activated protein kinase, by promoting glucose uptake and fatty acid oxidation while inhibiting lipid synthesis(13). Butyrate may even increase levels of phosphorylated AMPK on GLUT 4 transporters

in adipose tissue as shown in a study when given exogenously to mice(34). These discoveries of butyrate's effect on increased insulin sensitivity cannot be understated, especially for the obese patient. Improved insulin sensitivity reduces the risk for type 2 diabetes as exogenous insulin becomes more effective. With improved insulin sensitivity excess glucose isn't stored as fat which can aid in weight management, and in addition to reduction of high blood pressure, lowers the risk of metabolic syndrome(35).

Alongside butyrate's protective role when it comes to gut microbiota it's potential obesogenic effects should also be mentioned. Butyrate is a high energy molecule and its production within the gut can depend on the microbiota composition including the F/B ratio. The Firmicute spp. have been associated with increased energy or caloric intake from food, leading to weight gain. Butyrate-producing species like *Roseburia spp.* and *Faecalibacterium prausnitzii* break down carbohydrates that otherwise would be indigestible by human enzymes, thus providing more substrate for weight gain(36). This highly complex relationship of the SCFA butyrate with the gut microbiota cannot be understated, as mentioned before the positive and protective effects of butyrate are beneficial and even necessary for proper gut function. It's important to emphasize that dysbiosis and an overabundance of butyrate may be obesogenic and can depend on the metabolic state of the host and their diet(36).

5.2 Short Chain Fatty Acids- Acetate

Acetate the most abundant SCFA in the body's circulation acts as a binding molecule for lipogenesis and cholesterol synthesis in the liver. Acetate appears to exhibit both a protective and obesogenic role based on the hosts metabolism, hosts diet, and microbiome composition much like butyrate. Acetate is synthesized like all SCFAs through microbial fermentation by *Bacteroides spp., Bifidobacterium, Clostridium,* and *Lactobacillus.* In addition high levels of acetate contribute to cross feeding interactions that increase butyrate production(37). This occurs via primary fermenters such as *Bacteroides spp.* and *Bifidobacterium spp.* which produce lactate and acetate which are crucial in this interaction. Secondary fermenters or butyrate producing bacteria then convert the lactate and acetate to butyrate via the butyryl- CoA pathway(38). This pathway is important due to the production of butyrate, acetate is the most abundant SCFA within the microbiome and during dysbiosis levels of butyrate may be low and lead to metabolic dysregulation. Excess acetate may contribute to the cross- feeding interaction and even with an altered microbiota composition this production of butyrate can help support biome health and maintain gut integrity(39).

Acetate's protective affects have a major role in microbiome health. Acetate contributes to energy homeostasis by serving as an energy source for peripheral tissue mainly the liver and skeletal muscle, unlike butyrate's effect on primarily colonocytes. Acetate's protective role includes stimulation of glucagon-like peptide- 1 and peptide YY release(40). Done through free fatty acid receptors like GPR43 and GPR41. Glucagon-like peptide-1 increases insulin secretion promoting satiety through delayed gastric emptying which in turn reduces food intake by the host, while peptide YY slows gastric motility(41). Although acetate generally exhibits anti-inflammatory effects, certain conditions including obesity and metabolic disease can induce a pro-inflammatory response(42).

Acetate's role in fat storage and energy balance has been studied, it serves as a substrate in de novo lipogenesis which may potentially increase fat synthesis. Once absorbed into circulation, acetate is converted into acetyl-CoA by acetyl-CoA synthetase within the hepatocytes, forming the foundation for fatty acid synthesis. Acetyl-CoA then enters the lipogenic pathway, leading to the production of malonyl-CoA via acetyl-CoA carboxylase, which is further elongated by fatty acid synthase into long-chain fatty acids and ultimately stored as triglycerides in adipose tissue(43). In one study acetate may have been linked to ghrelin stimulation, ghrelin is an orexigenic hormone that stimulates appetite and promotes the storage of energy. According to this theory increased plasma acetate would promote excess ghrelin secretion thus stimulating appetite(44). Conclusions made showed that endogenously given acetate had a higher turnover rate amongst lean individuals vs the obese, however this turnover rate of acetate did not have a significant effect on ghrelin secretion in neither group(44). Though inconclusive this study shows that further research is needed into acetate and its effects of ghrelin and their effects on obesity. The overall effect of acetate is highly dependent on gut microbiota and though requiring more research, it's affects can be obesogenic in a dysbiotic gut.

5.3 Short Chain Fatty Acids- Propionate

Propionate's effect according to most research and data is considered to have mainly anti-obesity effects unlike acetate or butyrate. Though excess of propionate and its bacterial precursors can potentially be dysbiotic, propionate helps with appetite regulation, fat metabolism, and improvement of insulin sensitivity. Enhanced fat oxidation occurs through de novo lipogenesis due to propionate, and helps with limiting fat storage by inhibiting key enzymatic activity of acetyl CoA carboxylase within the fatty acid synthesis cycle (45).

6. F/B Ratio Use

While a high F/B ratio has been associated with many of these pro-obesity mechanisms, it is a simplified marker of a far more complex microbiome-host relationship. Not all Firmicutes promote obesity, and not all Bacteroidetes are protective, as discussed, no specific bacterial species and functional pathways can determine metabolic outcomes as seen with the end production of SCFA. Therefore, research should move beyond the F/B ratio as a singular biomarker, instead focusing on microbial diversity, SCFA profiles, and inflammatory pathways to better understand how gut dysbiosis contributes to obesity. The F/B ratio may still have a use in future research especially since so much research has already been done, future research can build upon what has been learned and established without over relying on it.

7. Microbial Diversity

Reduced microbial diversity is consistently seen in obese individuals, reduced diversity simply means fewer species of bacteria living within the microbiome(46). This decline in microbial diversity has been linked to higher energy extraction from food, chronic low inflammation, and metabolic dysregulation(47). A diverse gut microbiome plays a crucial role in the maintenance of overall host health by improving metabolic efficiency and immune function(47). Higher microbial diversity has been shown to increase gut stability, making it more resistant to antibiotic use or dietary changes(48). Additionally, a wide variety of microbial species contribute to efficient nutrient metabolism, which includes the break down of dietary fiber into SCFA's, and the synthesis of essential vitamins like vitamin B and K. When diversity is reduced, metabolic consequences arise, such as impaired insulin sensitivity, increased fat storage, and chronic inflammation(49). This means that microbial diversity is essential for maintaining metabolic health and the reduction in the risk of chronic conditions such as obesity. It is important then to discuss some microbes within the gut that aid in a healthy microbiome and how the gut is affected by their absence.

7.1 *Akkermansia muciniphila* and its role in Obesity

Akkermansia muciniphila is a bacterium that resides within the human gut, its responsibility lies in the maintenance of the gut barrier via mucous production and improvement of metabolic health markers such as reduced adiposity and improved insulin control. It is a Gram-negative, anaerobic bacterium, of the gut epithelium. Research shows that higher levels of *A. muciniphila* are often associated with leanness and improved gut barrier function in regard to tight junction proteins. On the other hand lower levels have been observed in individuals with obesity and metabolic disorders

suggesting an association with decreased *A. muciniphila* levels and dysfunction(50). Supplementation with some strains of *A. muciniphila* has demonstrated a potential benefit in the improvement of metabolic health, this includes improving insulin sensitivity and reducing inflammatory markers such as interleukins. In an unbalanced microbiota, low levels of *A. muciniphila* may lead to increased gut permeability, this phenomenon known as leaky gut, allows the harmful gastrointestinal bacterial components into the bloodstream and turn leads to inflammation. The mechanism of this leaky gut phenomenon begins with degradation of the mucosal layer of intestinal epithelium then followed by the degradation of tight junctions, which then leads to a condition known as metabolic endotoxemia(51).

Metabolic endotoxemia is a chronic, low-grade inflammatory state caused by an increase in circulating endotoxins, mainly lipopolysaccharides from any Gram- negative bacteria(51). These endotoxin producing, opportunistic Gram- negative bacteria include *Escherichia coli*, *Pseudomonas spp*., and *Desulfovibrio spp*., which is a sulfate-reducing genus that produces lipopolysaccharides and hydrogen sulfide(52). The mechanism of metabolic endotoxemia occurs once lipopolysaccharides come into circulation, they bind to toll-like receptor 4 on immune cells, activate pro-inflammatory signaling pathways like NF-κB and MAPK, which then leads to the release of specific cytokines TNF-α, IL-6, IL-1β(53). These lipopolysaccharides may then reach the liver via the portal vein and portal system, when in the liver they promote hepatic inflammation and in time the development of non-alcoholic fatty liver disease(54). In conclusion a lack of *A. muciniphila* due to gut dysbiosis can be detrimental to the overall healthy microbiome and highlights the necessity of microbial diversity.

7.2 *Faecalibacterium prausnitzii* and its role in Obesity

Faecalibacterium prausnitzii is a butyrate-producing bacterium that has a substantial population within the gut, which belongs to the Firmicutes phylum. Its positive role in gut health is due to its anti-inflammatory effects, production of SCFA's notably butyrate, and its role in the stability of gut epithelium(55). Butyrate plays a key role in regulating energy metabolism, glucose balance, and fat storage. Dysbiosis and lowered levels of *F. prausnitzii* and thus reduced butyrate production have been associated with increased visceral fat, higher insulin resistance, and increased incidence of metabolic syndrome(56). *F. prausnitzii* increases the production of anti-inflammatory cytokines such as interleukin 10 and reduces pro-inflammatory markers like interleukin- 6 and tumor necrosis factor alpha, with these effects *F. prausnitzii* has been shown to help against chronic low-grade inflammation commonly seen in obesity and metabolic disorders(57). *F. prausnitzii* levels in obese

individuals tend to be lower than leaner individuals as shown in several studies(58). This was shown in a study comparing the gut microbiota of obese and lean Japanese participants by using 16S rRNA gene sequencing(58). The research involved 10 obese participants and 10 lean participants and key findings importantly showed a significantly high Shannon diversity index amongst the lean subjects compared with the obese subjects. The Shannon diversity index is a statistical calculation that summarizes and quantifies the population of a group, in this case the lean individuals had a high diversity, while the obese group had low diversity(59). Notably in this study, bacteria with anti-inflammatory properties such as *F. prausnitzii* were increased in lean subjects and decreased in obese subjects(58).

7.3 *Lactobacillus spp.* and its Role in obesity

Unlike *Akkermansia muciniphila* and *Faecalibacterium prausnitzii*, which are generally beneficial for metabolic health, *Lactobacillus spp*. have strain-dependent effects on obesity. Some strains have been linked to weight gain and obesity, while others have a proposed link to weight loss and leanness(60,61).

Certain *Lactobacillus spp.* have demonstrated anti-obesogenic effects, by contributing to improved microbial balance and reduced inflammation. Among these, *Lactobacillus gasseri* is one of the most extensively studied strains for its potential anti-obesogenic effects. Clinical trials have demonstrated that supplementation with *L. gasseri* lead to significant reductions in participants BMI, visceral fat, body weight, and waist circumference(62). During a 12-week randomized, controlled trial, participants who consumed *L. gasseri* fermented milk experienced a 4.6% reduction in abdominal fat compared to those of the control group(63). Similarly, *Lactobacillus rhamnosus* has been linked to weight regulation and appetite control, which suggests its potential role in obesity management. The beneficial effects of *L. rhamnosus* may be due to several mechanisms which include reduction of inflammation and an increase of leptin sensitivity. Studies have shown that *L. rhamnosus* supplementation may counteract leptin resistance, which is helpful in those with high fat diets as leptin resistance is common(64). This effect on leptin enhances the gut's ability to regulate appetite and reduce food intake thanks to leptins innate hormonal ability in satiety. Clinical evidence of *L. rhamnosus* effect on weight regulation was seen in a randomized, double-blind, placebo-controlled trial involving 125 obese men and women. Participants followed a 12-week calorie-restricted diet, supplemented with either *L. rhamnosus* supplementation or a placebo(65). Specifically, women in the *L. rhamnosus* supplemented group experienced a significantly greater weight loss compared to the placebo group. In addition, during a 12-week weight maintenance phase,

women in the *L. rhamnosus* supplemented group continued to lose weight, while the placebo group showed subsequent weight gain. Notably the male group showed no changes and no significant effects during this study(65).

In contrast other *Lactobacillus spp.* have demonstrated pro-obesogenic effects, these strains have been linked to weight gain and the increase of subcutaneous fat storage. This research suggests that the mechanism by which this species contributes to higher fat mass accumulation is by increasing energy extraction from food, thus being able to extract more calories. One example includes *Lactobacillus reuteri* which has been associated with increased adiposity in both animal and human studies. According to the study this could potentially be due to *L. reuteri* effect on metabolism and the hosts energy balance between calories consumed and expended. In this study done with school aged children living in Mexico City, a higher abundance of *L. reuteri* was found to be directly associated with greater adiposity, which was measured by BMI and waist circumference of subjects. Notably this association persisted amongst subjects even after adjustment for dietary fructose intake, indicating that the presence of the organism *L. reuteri* itself may be the link to increased adiposity within this population(66). Effects of *Lactobacillus fermentum* on body weight and obesity are strain-specific and have not been accurately discovered. Some strains have been associated with weight gain in animal studies, while others have demonstrated protective effects against obesity or having no significant impact on weight. Research in mice with *L. fermentum* LM1016 showed a reduction in the risk of diet-induced obesity and the subsequent fat accumulation in the liver caused by increased weight(67). In another study investigating the effects of *L. fermentum* ZJUIDS06 on hypercholesterolemic hamsters, while the strain improved lipid profiles and increased short-chain fatty acid levels, it did not prevent body weight gain in the animals(68). These inconclusive findings highlight the importance of considering specific bacterial strains, host species, and experimental conditions when evaluating the role of all strains and species of bacteria. To fully understand the potential benefits and risks of *L. fermentum* well-designed, safe human clinical trials must be made.

The role of *Lactobacillus spp.* in obesity is highly varied and dependent, with certain species exhibiting anti-obesogenic effects while others are associated with weight gain and increased adiposity. Strains like *L. gasseri* and *L. rhamnosus* have demonstrated potential benefits in weight regulation through mechanisms such as improved gut balance, reduced inflammation, and enhanced leptin sensitivity. On the other hand, *L. reuteri* has been linked to greater fat accumulation in both animal and human studies, suggesting its role as an obesogenic organism. The effects of *L. fermentum* remain inconclusive, with some strains contributing to weight gain while others have shown protective metabolic benefits.

7.4 *Bacteroides* and *Prevotella* and their Role in Obesity

The two dominant genera that reflect different nutritional habits and diet of the host are *Bacteroides spp.* and *Prevotella spp. Bacteroides* is more prevalent in populations consuming a Western diet, which is high in fat and protein but low in fiber, whereas *Prevotella* is commonly found in individuals following plant-based, fiber-rich diets. A comparative analysis of African children and European children's gut microbiota diversity showed that diet had a strong influence on the composition(69). The gut microbiota of children from Africa specifically rural Burkina Faso, who consumed a high-fiber, plant-based diet, was dominated by *Prevotella* and lacked *Bacteroides spp.* comparatively. In contrast European, specifically Italian children, who consumed a Western diet rich in animal proteins, fats, and sugars, had *Bacteroides*-dominant microbiotas with little to no *Prevotella spp.*(69). In addition the gut microbiota of the Burkina Faso children exhibited greater bacterial diversity, which is generally considered beneficial for gut health and resilience against disease(69). This study emphasized that diet is a major factor shaping the gut microbiota specifically *Bacteroidetes spp*. and *Prevotella* and suggested that dietary changes could alter microbial composition in ways that impact long-term metabolic health. In addition, this study showed the importance of a fiber-rich diet in maintaining microbial diversity and gut health.

The role of these bacteria in obesity is complex and to better understand these bacteria the *Prevotella/ Bacteroides* ratio has been introduced(70). *Bacteroides* species specialize in breaking down animal proteins and fats, leading to higher energy absorption and fat deposition, which may contribute to weight gain. In contrast, *Prevotella* ferments dietary fiber to produce SCFAs, which can help regulate metabolism, reduce inflammation, and support gut health. A post-hoc analysis or a statistical analysis specifically done after a study has been concluded, investigated how the *Prevotella*-to-*Bacteroides* ratio influences weight loss outcomes on diets varying in macronutrient composition and dietary fiber over 24 weeks. The study involved 80 overweight participants who were randomized to follow a 500 kcal/day energy-deficit diet, either high or low in dairy products, with macronutrient compositions of 30% fat, 52% carbohydrate, and 18% protein. Participants were categorized into high and low *Prevotella*-to-*Bacteroides* ratio groups based on fecal sample analyses(70). Findings concluded that individuals with an increased *Prevotella*-to-*Bacteroides* ratio lost an average of 3.8 kg more in both body weight and body fat compared to those with decreased *Prevotella*-to-*Bacteroides* ratio, regardless of the specific diet followed. In addition, those participants following a high fiber diet lost significantly more weight when looking at the participants with a high *Prevotella*-to-*Bacteroides* ratio. This research and its results show that the *Prevotella*-to-*Bacteroides* ratio may be used as a predictive biomarker , but just like the F/B ratio depends on many

factors and must be used carefully(70). Given the findings from recent data, research suggests that modulating the balance between *Bacteroides* and *Prevotella* through dietary interventions could be a potential strategy for personalized nutrition and obesity management.

7.5 *Roseburia, Adlercreutzia,* and *Blautia* and their Role in Obesity

Roseburia of the Firmicute phyla is a key butyrate-producing bacterium in the gut, and its abundance in the gut microbiota is associated with better insulin sensitivity, reduced systemic inflammation, and overall metabolic stability. Production of butyrate strengthens the intestinal barrier, while decreased *Roseburia* and thus decreased butyrate leads to leaky gut and chronic inflammation. In a Hong Kong study the stool samples of 100 subjects (43 lean and 57 obese subjects) were collected and sequenced(71). The result of the study made the conclusion that *R. hominis* was depleted in subjects with obesity based on the stool sample analysis. Additionally, the abundance of *R. hominis* showed a negative correlation with BMI and serum triglyceride levels, meaning that higher BMI and triglyceride levels were associated with reduced *R. hominis* presence. Based on this new research it is theorized that this bacterium may play a protective role in obesity and other metabolic disorders. It is important to note that this cohort study looked at a human population, but also analyzed an animal population, specifically mice. Thus, further research is needed to establish a direct causal relationship in humans(71).

In an Australian study by Dekker Nitert et al. 2020 the relationship between gut microbiota composition and back pain amongst overweight and obese individuals was explored(72). The findings revealed that participants experiencing back pain had a higher abundance of certain bacterial genera, including *Adlercreutzia, Roseburia*, and unclassified *Christensenellaceae*, compared to those without back pain. The term unclassified used for the *Christensenellaceae* family refers to the fact that the bacterial species or genus within the family could not be precisely identified or assigned to a known, well-defined taxonomic group. Additionally, *Adlercreutzia* levels were positively correlated with BMI, serum adipsin, and serum leptin levels, suggesting a potential link between gut microbiota alterations, obesity-related inflammation, and musculoskeletal pain(72). While the study does not establish causality, it suggests that alterations in gut microbiota, may be linked to inflammation and pain in the context of obesity. This information could provide a broader perspective on the implications of *Roseburia hominis* both in metabolic health and its potential connections to musculoskeletal conditions like back pain. Thus, this information considers not only the increased morbidity associated with obesity but also the reduced quality of life that accompanies it (72).

Research suggests that *Adlercreutzia* is more abundant in obese individuals compared to those who are lean and even those who are overweight, indicating a possible link between its presence and increased adiposity(73). In a study by Yun et al. 2017 the increase of *Adlercreutzia* was significant amongst the obese group compared with those in the overweight group, linking its effect on host lipid metabolism(73). Additionally, higher *Adlercreutzia* levels have been correlated with inflammatory markers such as leptin and adipsin, which are often elevated in obesity, suggesting a potential role in systemic inflammation and metabolic dysfunction(72).

Blautia much like *Roseburia* is part of the Firmicute phyla, unlike *Roseburia*, which is often reduced in obesity, *Blautia* had been found in higher abundance in obese individuals(74,75). Some research suggests that *Blautia* may contribute to energy extraction from food, potentially promoting fat storage and weight gain. However in recent years substantial research has come out linking the relationship between *Blautia* and obesity as complex and neither beneficial or harmful(76). The 2024 review by Chanda et al. concluded that current evidence does not definitively identify *Blautia* as a pathogenic microbe when it comes to the development or progression of obesity.

In conclusion, the roles of *Roseburia, Adlercreutzia,* and *Blautia* in obesity are complex and multifaceted, with varying effects on metabolic health. *Roseburia*, as a butyrate-producing bacteria, that appears to play a protective role in metabolic stability, with reduced levels associated with obesity and systemic inflammation(71). *Adlercreutzia* has been linked to increased adiposity and inflammatory markers, suggesting a potential role in obesity-related inflammation and metabolic dysfunction(73). Meanwhile, *Blautia* presents an ambiguous relationship with obesity, with some studies associating it with increased fat storage and others indicating a more neutral or even beneficial metabolic role(76). These findings show the intricate dynamic between gut microbiota and obesity, emphasizing the need for further research to determine the causal mechanisms of these bacteria in metabolic health.

8. Relevant Studies Highlighted

The relationship between gut microbiota and obesity has been extensively studied, with numerous cohorts investigating the role microbial composition has on metabolic health and obesity. This section will highlight specific studies, their methodology's, and conclusions made that are relevant to this research topic. Before discussion of these relevant studies, 16S rRNA gene sequencing, which is used in the majority of microbiota research, and its limitations must be discussed. One major limitation of 16S rRNA sequencing is its inability to distinguish between closely related bacterial species or strains. This is due to the 16S rRNA gene being similar across

many bacteria, and when sequencing only a small portion of the gene, not enough differences may be captured to accurately classify bacteria, especially at the species level(77). This can often lead to misclassification or even make it impossible to differentiate between certain bacterial taxa. Additionally, 16S rRNA sequencing can be affected by PCR amplification biases, where some bacterial DNA is amplified more than others, leading to skewed results in the direction of these amplified microbes. Additionally, since this method only looks at a specific gene, it doesn't provide much information about the functions of the microbes based on their strain or their roles in the gut ecosystem, this limits the understanding of how they contribute to health or disease(78). This should be mentioned as some studies fail to understand this limitation and make contradicting statements, or nonfactual conclusions.

8.1 Yun et al. 2017

The study by Yun et al. 2017 investigated the relationship between gut microbiota composition and BMI among 1,463 Korean adults(73). 16S rRNA genes were extracted and amplified from fecal specimens using the MO-BIO PowerSoil DNA Isolation Kit and then analyzed. Findings did not support the F/B ratio hypothesis as a factor in obesity but confirmed the trend of lower microbial diversity in obese individuals. Clustering analysis revealed that the gut microbiota composition of the obese group differed significantly from normal and overweight groups. This study supported the theory of increased energy harvesting in an obesogenic microbiome, since obese subjects had higher SCFA production, which as discussed may contribute to lipogenesis and metabolic changes. Specifically, propionate producing bacteria were enriched in obese versus overweight individuals. These bacteria included *Bacteroidetes spp*. in addition to *Acidaminococcus*, *Megasphaera*, and *Mitsuokella* which utilize lactate in the production of propionate. Lower levels of *Akkermansia* in obese individuals was seen, which is linked to gut barrier dysfunction and systemic inflammation(73). This finding was accompanied by a study that showed successful weight reduction in obese human individuals was accompanied by increased *Akkermansia* numbers in feces(79). This association was linked to increased thermogenesis and energy expenditure amongst those subjects with weight loss(79). Finally, *Actinobacteria*, *Eggerthella* and *Adlercreutzia* which have been linked to lipid metabolism and pro-obesogenic effects were noted. *Eggerthella* showed a negative correlation with both obese and overweight groups, while *Adlercreutzia* was positively correlated with obesity, independent of dietary intake(73).

This study published in 2017 is a noteworthy example of microbiome research evolving over the years, this study looks at a significantly large population which allows for better statistical

significance. In addition to a varied sample size including both overweight, obese, and lean subjects, the study looked at the effect of weight loss on the microbiome of all three of the study groups. In the context of past gut microbiome research this study wasn't able to find relevant data for the F/B ratio, but did find evidence for the importance of SCFA producing bacteria within the microbiome(73).

8.2 Kasai et al. 2015

The study by Kasai et al. 2015 examined the human gut microbiota composition in a Japanese population of 23 non-obese subjects and 33 obese subjects. It should be noted in this study, that non-obese subjects are those with a BMI of <20 kg/m² in contrast to WHO standards of normal weight being 18.5-25 kg/m², the study does not provide a specific rationale for selecting a BMI of 20 kg/m² as the cutoff for the non-obese group. Additionally this study unlike others had participants who actively smoked and/or drank, this should be noted when comparing this study to others like it as other studies of this type and topic tend to exclude smoking and drinking subjects(80).

Initial analysis using terminal restriction fragment length polymorphism revealed that obese subjects had significantly reduced numbers of *Bacteroidetes* and a higher F/B ratio compared to non-obese subjects(80). Terminal restriction fragment length polymorphism is a quicker cost-effective alternative to 16S rRNA sequencing, the former analyzes enzyme digested DNA fragments rather than whole rRNA sequence as done in 16S rRNA(81). Disadvantages of terminal restriction fragment length polymorphism is the inability to identify specific species of bacteria, thus the need for next generation sequencing(82). Furthermore, bacterial diversity was significantly greater in obese subjects. Next-generation sequencing revealed differing microbial profiles between the two groups, obese individuals showed higher abundances of Firmicute spp. such as *Blautia hydrogenotorophica, Coprococcus catus, Eubacterium ventriosum, Ruminococcus bromii,* and *Ruminococcus obeum*, all of which are associated with increased energy harvesting. In contrast, non-obese individuals had higher levels of species like *Bacteroides faecichinchillae, Bacteroides thetaiotaomicron, Blautia wexlerae, Clostridium bolteae*, and *Flavonifractor plautii(80)*.

Findings suggested by this study links gut microbiota composition to obesity, with obese participants microbiota perhaps enhancing energy extraction from food, while non-obese participants microbiota may limit energy absorption. However, the study also noted that microbial diversity was higher in obese subjects, contrary to previous research, highlighting the complexity of the relationship between gut microbiota and obesity. Future research done should use a larger population size which may benefit from revised inclusion and exclusion criteria.

8.3 Andoh et al. 2016

The study by Andoh et al. 2016 compared the gut microbiota of obese and lean individuals in a Japanese population using 16S rRNA sequencing. The study included 10 obese and 10 lean individuals. Exclusion criteria included no medications or supplements that could affect gut microbiota composition. DNA was then extracted from fecal samples and analyzed via 16S rRNA sequencing(58).

The results revealed that lean individuals had a significantly higher Shannon diversity index, indicating greater microbial diversity compared to obese individuals. At the phylum level, Firmicutes spp. and *Fusobacteria* were significantly more abundant in obese individuals, while no significant differences were found in Bacteroidetes spp. abundance or the F/B ratio between the two groups(58).

This study found that the gut microbiota of obese individuals was characterized by reduced diversity and increased Firmicutes and *Fusobacteria*(58). The increased presence of Fusobacteria in obese individuals is a novel finding, and is notable for this research because this phylum is typically associated with inflammation and increased colorectal cancer risk(83). Lean individuals, had higher levels of anti-inflammatory bacteria like *Faecalibacterium prausnitzii*, which may protect against obesity-related inflammation(84).

This study concluded that gut microbiota composition of obese Japanese individuals differs from that observed in Western populations. Unlike in Western studies, Japanese obese individuals did not exhibit a decrease in Bacteroidetes spp. or a lower F/B ratio(25,85,86). Instead, the study identified an increase in *Fusobacteria* amongst obese individuals, a novel finding. Further studies specifically on Japanese populations must be done with larger sample sizes to make foundational conclusions about both Japanese obese populations, and Japanese obese populations compared with Western ones.

8.4 Patil et al. 2012

The study by Patil et al. 2012 investigated the composition of gut microbiota across different BMI categories in an Indian population(87). The study included 20 participants, consisting of 12 males and 8 females, divided into four groups: lean, normal weight, obese, and surgically treated obese. This study employed a different method of categorizing BMI into four groups compared to the WHO classification, so the differences should be acknowledged. The lean group was classified as BMI <19 kg/m², the normal group 18-24 kg/m², the obese group 25-53 kg/m², and the treated obese group 25-36 kg/m². In addition, to ensure accuracy the lean subjects were analyzed with a handheld OMRON HBF-306C body fat analyzer to ensure true leanness. Using this classification:

for men a lean body fat percentage would typically be 8–17%, while for women a lean body fat percentage would typically be 15–24%. The surgically treated groups included individuals who underwent sleeve gastrectomy and adjustable gastric banding surgeries(87).

This study did not observe a consistent trend in the distribution of Bacteroidetes and Firmicutes neither amongst obese nor normal weight subjects. However, Bacteroides spp. were significantly more abundant in obese individuals compared to the other groups, a finding then confirmed by quantitative PCR analysis. Obese individuals also exhibited a notably higher density of archaea and increased fecal SCFA's. In contrast, surgically treated obese individuals showed reduced counts of *Bacteroides* and archaea, along with lower fecal SCFA levels, suggesting that bariatric surgery alters the gut microbial composition in ways that could contribute to metabolic change and improvement(87). This study provides insight into the gut microbiota composition in obese Indian individuals, highlighting differences in microbial diversity, particularly the increased abundance of *Bacteroides* and archaea in obese individuals and their reduction following bariatric surgery. However, the small sample size of only 20 participants in this study limits the generalizability of the results, and further studies with larger cohorts are necessary to confirm these associations between gut microbiota and obesity in varied populations(87).

8.5 Politi et al. 2023

The study by Politi et al. 2023 investigated the association between gut microbiota composition and overweight/obesity in a cohort of 163 adults from southern Italy(88). Participants were categorized based on their BMI into normal weight, overweight, and obese groups. In addition to normal exclusion criteria such as pregnancy, chronic disease, and inflammatory bowel disease, participants were asked to explicitly categorize their diet into groups such as vegetarian or vegan(88). The information gathered by the researchers about participants diets is highly significant, because of the extensive research done on the Mediterranean diet and its impact on weight and gut microbiota. Knowing specific diets followed by the participants of this study allows a better understanding of not only the genetic and environmental effects on microbiota, but the effects diet has amongst these participants. The results revealed significant differences between the groups overweight/obese individuals exhibited higher relative abundances of Bacteroidetes and *Proteobacteria*, and lower levels of Firmicutes and *Verrucomicrobia* compared to their normal-weight counterparts. In addition, the F/B ratio was inversely associated with BMI, suggesting that a lower F/B ratio may be linked to increased susceptibility to obesity(88).

While this study supports the hypothesis of the gut microbiota effecting obesity, once again the F/B ratio was proven to be insignificant in this population. It was noted by the researchers that this insignificance may stem from population heterogeneity and dietary differences. The conclusions made by this study confirmed a relationship between obesity and a dysbiotic gut. It is noted by the researchers that larger population studies are necessary to find the association between gut microbiota dysbiosis and obesity. Additionally, future studies on obese individuals should be conducted to confirm whether the F/B ratio can serve as a reliable biomarker for obesity(88).

8.6 Koliada et al. 2017

The study by Koliada et al. 2017 analyzed the microbiome composition of 61 Ukrainian individuals. The participants were split amongst four BMI categories: <18.5 kg/m², (underweight), 18.5-24.9 kg/m², (normal weight), 25.0-29.9 kg/m², (overweight), and >30.0 kg/m², (obese). Exclusion criteria included history of oncologic disease, anorexia, psychiatric disorders, but notably unlike many other studies of this type did not exclude smoking or alcohol use. Though not excluding smokers in this study, the researchers made a clear distinction between non-smoking participants, smokers, and heavy smokers amongst all weight groups(89). This may be beneficial as it can show how a modifiable and common variable such as smoking may affect microbiota composition amongst various weight groups.

Results showed that as BMI increased, the relative abundance of Firmicutes increased while Bacteroidetes decreased, leading to a higher F/B ratio in overweight and obese individuals. The relative abundance of *Actinobacteria* remained relatively stable across all BMI categories. A logistic regression analysis confirmed a significant positive association between the F/B ratio and BMI, even after adjusting for age, sex, smoking, and physical activity. These findings obtained indicate that obese and overweight persons in this Ukrainian adult population have a significantly higher level of Firmicutes and lower level of Bacteroidetes compared to normal-weight and lean adults(89).

8.7 Ahmad et al. 2019

In the study by Ahmad et al. 2019 the microbiome of 74 Emirati individuals was analyzed using 16S rRNA sequencing. Participants were 18-60 years old otherwise healthy individuals that were either obese or lean(90). It is important to note that obese participants in this study were those with a BMI of ≥35 kg/m²,, according to the World Health Organization and the USA Center for Disease Control a BMI of >30 kg/m², is considered obese, thus the participants in this study would be considered in the category of class 2 obesity or higher(91,92). Obese participants included within the

study had all expressed interest in both weight loss and the idea of future bariatric surgery. Inclusion criteria of lean participants included a healthy participant with a BMI of 18.5–24.9 kg/m^2, that hadn't experienced any weight changes, >5% of body weight or more in the past three months.

Obese participants exhibited lower alpha diversity and the beta diversity profiles were different from those of lean controls(90). Alpha diversity refers to the diversity of microbial species within a single sample and provides a measure of how rich and even the microbiota is in a given environment(93). Beta diversity measures the differences in microbial composition between multiple samples or individuals(93). In the context of this study, lower alpha diversity in obese individuals suggests reduced microbial richness and stability, while the different beta diversity profiles between obese and lean subjects indicate that obesity is associated with significant alterations in the gut microbiota(90).

At the phylum level, both groups were dominated by Firmicutes and Bacteroidetes. Firmicutes spp. accounted for 50% of lean participants gut composition and 47% of obese participants, while Bacteroidetes spp. accounted for 44% of lean participants and 49% of obese participants. The study showed no significant differences in the F/B ratio. In addition, obese individuals had higher levels of *Verrucomicrobia* and *Saccharibacteria* while showing an increase in *Lentisphaerae*. Obese participants had a higher relative abundance (0.5%) compared to lean participants (0.3%) of *Verrucomicrobia*, with a q-value of 0.04, indicating statistical significance. Obese participants had a higher relative abundance (0.003%) compared to lean participants (0.0009%) of *Saccharibacteria*, with a q-value of 0.0002, indicating statistical significance. Obese participants had a lower relative abundance (0.03%) compared to lean participants (0.08%) of *Lentisphaerae,* with a q-value of 0.004, also indicating statistical significance(90).

At the genus level, *Acidaminococcus* and *Lachnospira* were significantly increased with q values of 0.01 and 0.04 respectively in the obese group and positively correlated with adiposity markers(90). Adiposity markers are measurable indicators used to assess body fat accumulation and distribution such as BMI, waist circumference, waist-to-hip Ratio, waist-to-height Ratio, and body fat percentage(94). In this study *Acidaminococcus* and *Lachnospira* were found to be positively correlated with BMI and waist circumference(90).

This study found evidence of differences in microbiota composition between obese and lean subjects at phylum and genus levels. Though it showed no significant differences in Firmicutes or Bacteroidetes, providing more evidence that F/B ratio cannot be used as a universal predictor biomarker for obesity. Limitations of the study include its small sample size, but its significance lies

in examining the gut microbiota of Emirati Arabs, a population that is underrepresented in microbiome research(90).

8.8 Plummer et al. 2020

The study by Plummer et al. 2020 analyzed the gut microbiota composition among 25 Arab Kuwaitis using 16S rRNA gene sequencing of the V3–V4 regions(95). In this study 63% of the study participants were classified as overweight or obese, this finding being significant in this research as this reflects the typical weight distribution within the Kuwaiti population. The results showed the gut microbiome was predominantly composed of Firmicutes (48%) and Bacteroidetes (46%), which together accounted for most sequencing reads. At the genus level, *Bacteroides* was the most prevalent, being the dominant genus in 22 out of 25 participants. Additional phylogenetic analysis further revealed that the *B. dorei* and *B. vulgatus* group was the most abundant phylogenetic cluster and was detected in all 25 individuals in the study. According to the researchers larger studies examining the impact of diet and geography on the microbiome in the Arabian Peninsula are needed, as is a larger population size(95).

8.9 Jinatham et al. 2018

The study by Jinatham et al. 2018 analyzed the composition of gut microbiota amongst 42 Thai participants that were categorized into lean, overweight, and obese groups based on BMI. Fecal samples were then collected, and quantitative polymerase chain reactions were used to measure the relative abundance of bacteria. Additionally metabolic parameters, such as BMI, cholesterol, and LDL levels, were analyzed to find associations between gut microbiota and these obesity related factors. The composition of the 42 person population included 14 males and 28 females aged 20 to 49 years old, with 21 lean, 10 overweight, and 10 obese participants(96).

Lean individuals exhibited an increase of Bacteroidetes (Median = 9.42) compared to obese individuals (Median = 8.18) with a p value of 0.016 indicating significant difference of Bacteroidetes spp. with increasing BMI. In contrast Firmicute spp. though increased in lean participants showed no statistical significance with a p value of 0.127. At the genus level, *Staphylococcus spp*. were significantly higher in lean participants (Median = 2.73) compared to obese individuals (Median = 1.44) with a statistical significant p value of 0.034, which suggests a potential role in the maintenance of a lean phenotype. Additionally, *Akkermansia muciniphila*, a bacterium linked to gut barrier integrity and metabolic health, was more abundant in lean individuals (Median = 5.6) compared to obese individuals (Median = 4.19) with a statistical significant p value of 0.007(96).

This studies key findings indicated that lean participants exhibited higher levels of Bacteroidetes spp., *Akkermansia muciniphila*, and *Staphylococcus spp.*, compared to their obese counterparts, with significant negative correlations between the listed bacterial groups and BMI. This studies small sample size is a limitation and future cohorts may be needed to confirm associations made in this study(96).

9. Analysis of Gut Microbiota Studies on Obesity

This section will discuss the nine relevant studies that were found and analyzed in the previous section. Key findings will be summarized, highlighting comparisons and contrasts across studies while identifying limitations seen in some of the case reports. The case studies are listed as followed with authors name, date of publication, and population studied.

1. Yun et al. 2017 Korea (73)
2. Kasai et al. 2015 Japan (80)
3. Andoh et al. 2016 Japan (58)
4. Patil et al. 2012- India (87)
5. Politi et al. 2023- Italy (88)
6. Koliada et al. 2017- Ukraine (89)
7. Ahmad et al. 2019- Emirates (90)
8. Plummer et al. 2020- Kuwait (95)
9. Jinatham et al. 2018- Thailand (96)

9.1 Sample Size and Reliability

The nine studies and generally studies in this field vary widely in sample size, which affects the strength of conclusions made. Small cohorts for example (~20 subjects)(58)(87)(95) are common and can lead to findings that may not be generalizable due to significant individual differences. For example, the Japanese study by Andoh et al. 2016 studied only 10 obese and 10 lean adults reported an increase of the phylum Fusobacteria in obese participants that hadn't been seen in past studies. This small sample size limits the ability to draw definitive conclusions due to low statistical power and increased potential for bias(58).

In contrast, much larger studies provide more robust statistical power but are more difficult to organize, expensive, and drawn out. A Korean cohort by Yun et al. 2107 investigated 1,463

individuals and in turn was able to detect subtle but significant trends, such as reduced bacterial diversity with higher BMI, that smaller studies may have missed(73).

Medium-sized studies, in this case those with 40–160 participants, find a middle ground between realistic sample sizes and real-world limitations such as budget and time. Important to note these studies are still at risk for skewed results. For instance, the Italian study of 163 participants found a relatively clear association between microbiota and overweight status(88), whereas the Thai study with similar demographics and 40 subjects did not find relevant data or statistical significance, which may be partly attributed to smaller sample size(96).

Overall, studies of all sizes are valuable when looking at research into the gut microbiota. Smaller studies are often exploratory, that provide initial findings that may then lead to the design of larger cohorts in the future. On the other hand, larger studies offer robust statistical power and greater confidence in their results, while unfortunately requiring significant resources and funding. Therefore, a balance of small, medium, and large studies are important to the field of obesity research.

9.2 Population Demographics and Inclusion Criteria

Differences in inclusion criteria and population demographics also appears to have shaped the results as seen in the nine studies. All nine studies in some way focused the study scope on specific ethnic or regional groups. Examples include, all subjects from southern Italy(88), a single Japanese population from a specific province(80), or specifically native Thai volunteers in a single province(96). The lack of population variance in these studies can be both a limitation and an advantage, depending on the context of how the research is analyzed.

Limitation occur when these studies focus on specific ethnic or regional groups when often the findings are often not universally applicable. For example when viewing a single Japanese population as in Andoh et al. 2016, the results may not reflect the diversity of microbiota variations within different parts of Japan let alone different geographical locations, cultures, and genetic backgrounds(58). Extrapolating the results from this study and others like it could lead to overgeneralization and the potential for misleading conclusions if the findings are applied to a wider, more diverse group. The external validity, which is the extent to which a study can be generalized to represent other populations(97), is quite low in these hyper-specific case studies.

Advantages of these types of cohorts includes the ability to know specific data for these populations which can be incredibly valuable for targeted health intervention. For example, a population in New York state that's predisposed to Fusobacterium spp. habitation within its

microbiome is more likely to suffer from colorectal cancer as seen in Rubenstein et al. 2019(83). These cohorts may aid populations by allowing health care professionals and epidemiologists to develop tailored and more effective treatment for the specific population. Additionally, focusing on specific populations allows for an understanding of how and why variations might be more prevalent or impactful within certain groups.

Some studies included participants with varied habits, like smokers and alcohol drinkers, without strict exclusion. In one Japanese cohort(80), around 21% of both obese and non-obese were smokers(80), and within the Italian study 16% of all participants smoked(88). Within these two studies, neither found large microbiota differences attributable to smoking. In contrast, the Ukrainian study explicitly measured and adjusted for smoking status, physical activity, age, and sex in their analysis. By accounting for smoking as a confounder in their analysis, the study was able to clarify that any observed microbiota differences between obese and non-obese participants was not attributable to smoking(89).

Across these studies, several common exclusion factors were applied to ensure that gut microbiota differences were attributed primarily to obesity rather than other health conditions or external influences. Antibiotic use was excluded across almost all studies. Even studies that didn't explicitly state antibiotic use was excluded may have just not explicitly stated so within their exclusion criteria, nonetheless these studies should be noted. Studies that clearly excluded participants included five studies which explicitly excluded antibiotic use anywhere from 4-8 weeks before stool sample collection(88)(80)(58)(87)(73). The remaining studies either did not explicitly mention antibiotic exclusion or included participants regardless of antibiotic use(95)(90)(89)(96).

Some studies excluded participants with chronic metabolic diseases such as type 2 diabetes, cardiovascular disease, and liver or kidney disease, while others included them but accounted for their impact during statistical analysis. Five studies explicitly excluded participants with type 2 diabetes and other major chronic diseases to isolate microbiota differences related specifically to obesity(88)(90)(95)(87)(89). In contrast, the Korean study(73) included individuals with type 2 diabetes and justified this by performing statistical adjustments to determine whether diabetes itself significantly influenced gut microbiota composition. This study found that excluding diabetic participants did not significantly change the overall microbiome within obese subjects, suggesting that obesity driven microbiota changes were detectable regardless of diabetes status. By retaining diabetic participants, this study better reflected real life populations, where obesity and diabetes are often comorbidities, thereby improving the generalizability of its findings(73).

Overall, these nine cohorts differed on whether confounders like smoking, alcohol, diet, and medication were included. Nonetheless all nine studies controlled for these factors, either by design or during analysis, which leads to increased confidence that the observed microbiome differences were actually linked to obesity.

9.3 F/B Ratio Trends

F/B ratio, though commonplace as a legitimate biomarker in the early days of microbiome research, has become outdated in most research cohorts. The hypothesis of F/B ratio was built upon the fact that Firmicutes promote obesity, and Bacteroidetes are protective against obesity. Multitude of research has shown that this isn't true and has not been substantiated by statistical analysis(89)(28). Nonetheless, cohorts when analyzing fecal microbiota samples will still calculate F/B ratio with varying results, and some recent studies have still specifically researched F/B ratio in population groups(28).

In two of the nine studies observed, obese individuals indeed exhibited a higher F/B ratio than lean individuals(80)(89). In the study by Kasai et al. 2015 obese subjects had a high F/B ratio driven by a significantly reduced Bacteroidetes, with a ratio of 2.09(80). In the study by Koliada et al. 2017 obese subjects had a higher F/B ratio of ~1.6 which is significant(89). These findings were supportive of an increased F/B ratio seen in this population of obese patients(89).

In seven of the nine studies observed, obese individuals either showed no difference from lean individuals in F/B ratio, or showed an inverse relation to the F/B ratio meaning that higher BMI was linked to decreased F/B ratio. The Japanese study by Andoh et al. 2016 showed no significant difference in F/B amongst lean and obese subjects , and actually similar proportions of Bacteroidetes and Firmicutes were seen in all subjects(58). Five other studies showed no significant F/B ratio differences and concluded that F/B ratio alone was not a reliable obesity marker for their populations(73)(95)(87)(90)(96). The Italian study by Politi et al. 2023 found an inverse association between F/B, meaning higher BMI was linked to lower F/B(88).

Overall, the F/B ratio was not a consistent biomarker of obesity across studies. Some obese populations exhibited a higher ratio(80)(89), others a lower ratio(95)(68)(80)(83)(89), and many show overlapping ranges. In summary, researchers argue that focusing solely on the F/B ratio is an overly simplistic approach, and its value as a reliable obesity indicator appears limited.

9.4 Obesity Specific Bacterial Species

Across the multiple studies, certain bacterial species and their lack off were consistently linked to obesity. Beneficial butyrate-producing and mucin-degrading bacteria were often reduced in obese individuals.

Akkermansia muciniphila was found at lower levels in overweight/obese hosts in several studies. Often cited as a health-associated bacterium, the effect of decreased levels of *Akkermansia* may lead to a thin mucous layer that allows for increased translocation of bacterial toxins thus leading to inflammation(50,98). Three studies found significant decreases of *Akkermansia* within the obese groups compared to the lean groups(73,88,96). Interestingly in the study by Ahmad et al. 2023 *Akkermansia* was reported to be more prevalent in the obese group but was negatively associated with waist-to-height ratio. A possible explanation for this association with the waist-to-height ratio, is a potential protective role of *Akkermansia(90).* This is because a higher waist-to-height ratio is associated with central obesity and metabolic risk(99), thus a negative correlation may suggest that *Akkermansia* may help regulate fat distribution, particularly visceral fat. Additionally it was noted by the researchers of this case that dietary or genetic factors may have played a role in these results(90). Though findings are not entirely consistent amongst all populations, there seems to be an association with leanness and anti-obesogenic effects seen with higher levels of *Akkermansia muciniphila.*

Faecalibacterium prausnitzii is a major butyrate producer with anti-inflammatory properties seen in lean participants. Two studies showed significantly decreased *F. prausnitzii* levels in obese subjects(58,87), while one study also showed decrease in *F. prausnitzii* in all participants from lean to obese but was not significant(96).

These nine studies though all performed with similar methodologies and populations, have shown to have a variety of results. Sample size was shown to be a critical factor, larger studies tended to agree that obesity correlates with lower overall species diversity, while smaller studies sometimes reported conflicting phylum level changes that cannot be consistently reproducible and may only reflect the specific population studied. The specific population differences such as diet, genetics, and lifestyle played a major role in shaping the microbiota. This was seen as similar BMI participants whether they be obese or lean, seemed to share microbiome makeup with participants from their study cohort, while differing drastically from other populations. The F/B ratio showed no universal trend with obesity across these studies, reinforcing that it is not a reliable standalone indicator of an obese microbiome(53,68,74,81,83). More substantial results were seen with specific bacteria species and their effects on host health, as decrease of beneficial species like *A.muciniphila* and *F. prausnitzii*

was seen in obese participants(58,87). Each of these studies contributes valuable insight into gut microbiota research, and they have laid the groundwork for future studies with standardized methods, larger cohorts, and longitudinal design.

10. Obesity Treatment via use of Probiotics

Probiotics, as defined by the World Health Organization are live microorganisms which, when administered in adequate amounts, confer a health benefit on the host(100). Certain strains of probiotics, such as *Lactobacillus gasseri SBT2055*, *Bifidobacterium breve B-3*, and *Akkermansia muciniphila*, have demonstrated effects in reduction of visceral fat, appetite-regulation, and enhancement of glucose metabolism(101)(102)(103). The hypothesized mechanisms by which probiotics enhance weight loss is via increase of energy metabolism through SCFA production(104), which may regulate the hosts appetite via satiety hormones like ghrelin(105), and additionally the reduction of fat stores via modulation of gene expression and reduction of inflammation(106).

In a review done by Oudat and Okour 2025 clinical research was found for the effects of probiotics for weight loss in obese patients, prevention of weight gain, and visceral fat reduction(107). The effect of the probiotic *Lactobacillus gasseri BNR17* on weight was tested in several studies and seems to have promising results. *L. gasseri BNR17* was discovered in the 1990's within the microbiome of Japanese adults and was found to have bile acid tolerance, and when studied on rats seemed to have anti-obesogenic effects(108).

In one study the effects of *L. gasseri BNR17* supplementation on body fat in obese adults was tested through a randomized, double-blind, placebo-controlled trial. The study included 90 participants aged 20 to 75 years with a BMI between 25-35 kg/m². The participants were assigned to a placebo group, a low dose BNR17 group, or a high-dose BNR17 group for 12 weeks. During the trial, all participants were instructed to reduce their daily caloric intake by 200 kcal and increase their physical activity expenditure by 100 kcal per day. Exclusion criteria included the current use of dietary supplements or medications affecting body weight, recent probiotic use, history of gastric surgery, hypertension, and endocrine disorders such as diabetes or Cushing's(63).

Results after 12 weeks showed that visceral adipose tissue significantly decreased in the high dose BNR17 group (98.9 cm²) compared to that of the placebo group (120.5 cm²). Despite the findings, the study found no significant differences in overall body weight, BMI, or biochemical parameters, such as blood lipid profiles or glucose levels amongst participants. One hypothesis states that these findings may suggest that *L. gasseri BNR17* supplementation may contribute to visceral fat reduction and waist circumference management in obese individuals. However, since the overall

body weight and metabolic biomarkers were not significantly affected, further research is needed to explore the long term metabolic effects and mechanisms of these changes(63).

Another study on *Lactobacillus gasseri* was done on the *SBT2055 (LG2055)* strain to examine its anti-obesogenic effects. The study was a double-blind, randomized, placebo-controlled trial that included 87 Japanese adults (59 males, 28 females) with a BMI between 24.2–30.7 kg/m² and visceral fat area ranging from 81.2–178.5 cm². Exclusion criteria were any chronic diseases, hypertension, diabetes, and dairy allergies. Researchers assessed abdominal fat using CT scans, along with body weight, BMI, waist and hip circumference, body fat percentage, and serum adiponectin levels before, during, and after the 12-week period. Participants were divided into two groups, the active group consumed 200g/day of fermented milk containing *L. gasseri SBT2055* for 12 weeks, while the control group consumed identical fermented milk without *L. gasseri SBT2055* for 12 weeks(101).

The results demonstrated a significant reduction in abdominal fat amongst the active group. Visceral fat area decreased by 4.6%, and subcutaneous fat area decreased by 3.3%, while no significant changes were observed in the control group. In addition to fat loss, the active group experienced a 1.4% decrease in body weight and a 1.5% reduction in BMI (-0.4 kg/m², $p < 0.001$). Waist circumference decreased by 1.8% and hip circumference decreased by 1.5%, with no reduction in the control group. The researchers summarized that *L. gasseri SBT2055* showed anti-obesogenic effects in the form of decreased visceral fat, subcutaneous fat, weight, and BMI(101).

In the study to test the efficacy of *Bifidobacterium breve* B-3 and its obesogenic effects 100 Korean adults with a BMI between 25–30 kg/m² were divided into two groups. The experimental group received 5 billion colony forming units/day of BB-3 while the control group received a placebo pill once a day for 12 weeks. Changes were assessed before, during, and after using dual-energy X-ray absorptiometry, body weight, BMI, waist and hip circumference, visceral and subcutaneous fat, and blood lipid markers(109).

Results showed that body fat mass significantly decreased in the BB-3 group, with no significant reduction in the placebo group. Abdominal and trunk fat were particularly affected, suggesting BB-3's role in visceral fat metabolism. Body weight and BMI also significantly declined in the BB-3 group, while waist and hip circumference were lower compared to the placebo group tough not significant. Although reductions in total cholesterol, LDL, triglycerides, and leptin were observed, they were not statistically significant, and adiponectin levels remained unchanged(109).

The findings of this study confirmed that *Bifidobacterium breve B-3* effectively reduces body fat, body weight, and waist circumference, highlighting its potential as a safe and effective probiotic

supplement for weight management. Researchers in this study recommended future research combining BB-3 supplementation with exercise interventions to potentially optimize fat loss(109).

The use of probiotics as a potential strategy for obesity treatment has shown promising yet varied results. Specific strains such as *Lactobacillus gasseri BNR17, L. gasseri SBT2055*, and *Bifidobacterium breve B-3* have demonstrated significant effects in reducing visceral and subcutaneous fat, waist circumference, and BMI in clinical trials(101)(109). However, while some reductions in fat mass were observed, some studies reported no significant changes in overall body weight or metabolic biomarkers(63). Despite these findings, the mechanisms underlying probiotic-mediated weight regulation remain not fully understood. The influence of SCFA production, appetite regulation via ghrelin, and inflammation modulation must be further researched. As mentioned in several studies, while probiotics hold potential in weight loss intervention and the gut microbiota, they should not replace established weight management and diet.

11. Conclusion

This review looked at the gut microbiota and its role in host metabolism, immune function, and overall health. This complex relationship between gut microbiota and obesity, was highlighted by findings from multiple case studies and research that analyzed microbial diversity, the F/B ratio, SCFAs, and the impact of probiotics on weight and weight management.

Early research suggested that an increased F/B ratio could be the definitive biomarker of obesity, but findings across a variety of studies have shown this hypothesis to be inconsistent. While some studies found higher F/B ratios in obese individuals, others showed no significant differences or even an inverse of this relationship. This lack of reproducibility reinforces that the F/B ratio alone cannot be a reliable biomarker for obesity. Instead, markers like microbial diversity, which have consistently shown to be lower in obese individuals and higher amongst healthy and lean individuals may be used as a more accurate benchmark of gut health. SCFAs, particularly butyrate, acetate, and propionate, are essential metabolic products of the microbiota that have an influence on the metabolism. Butyrate has been shown to support the integrity of the gut barrier and can aid in the reduction inflammation and inflammatory markers. Additionally, butyrate may also contribute to increased energy extraction of food. Acetate works as a substrate for lipogenesis and cholesterol synthesis in the liver, and depending on the hosts metabolism, it may either promote satiety or contribute to fat storage. Propionate appears to have the most consistent anti-obesogenic effects where it works by improving insulin sensitivity and limiting fat accumulation. These SCFA's that are synthesized more so by specific bacteria emphasize the importance of gut diversity when looking at obesity and weight gain.

Finally, the therapeutic potential of probiotic supplantation for the treatment of obesity was discussed. Specific strains had promising results in the reduction of visceral fat, waist circumference, and BMI, in some studies. Other studies, despite showing reductions in fat mass were unable to observe any significant changes in overall body weight. Mixed results from these studies on probiotics indicate that once again probiotics alone may not be sufficient for meaningful weight loss and may only have a small role in the broad scope of obesity management.

Overall, while the gut microbiota and dysbiosis has been shown to contribute to obesity, their relationship is highly complex and influenced by multiple factors, including genetics, diet, lifestyle, and environmental conditions. Rather than relying on single microbial markers like the F/B ratio, future research should focus on comprehensive microbiome profiling of patients and personalized interventions should be made based on host specific factors. Further clinical trials and research must be done with adequate population sizes and demographics to better understand the mechanism of the gut microbiome in obesity.

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13. Annex 1

PRISMA Flowchart for Study Selection