The effects of sweeteners on the intestinal microbiota among individuals with eating disorders

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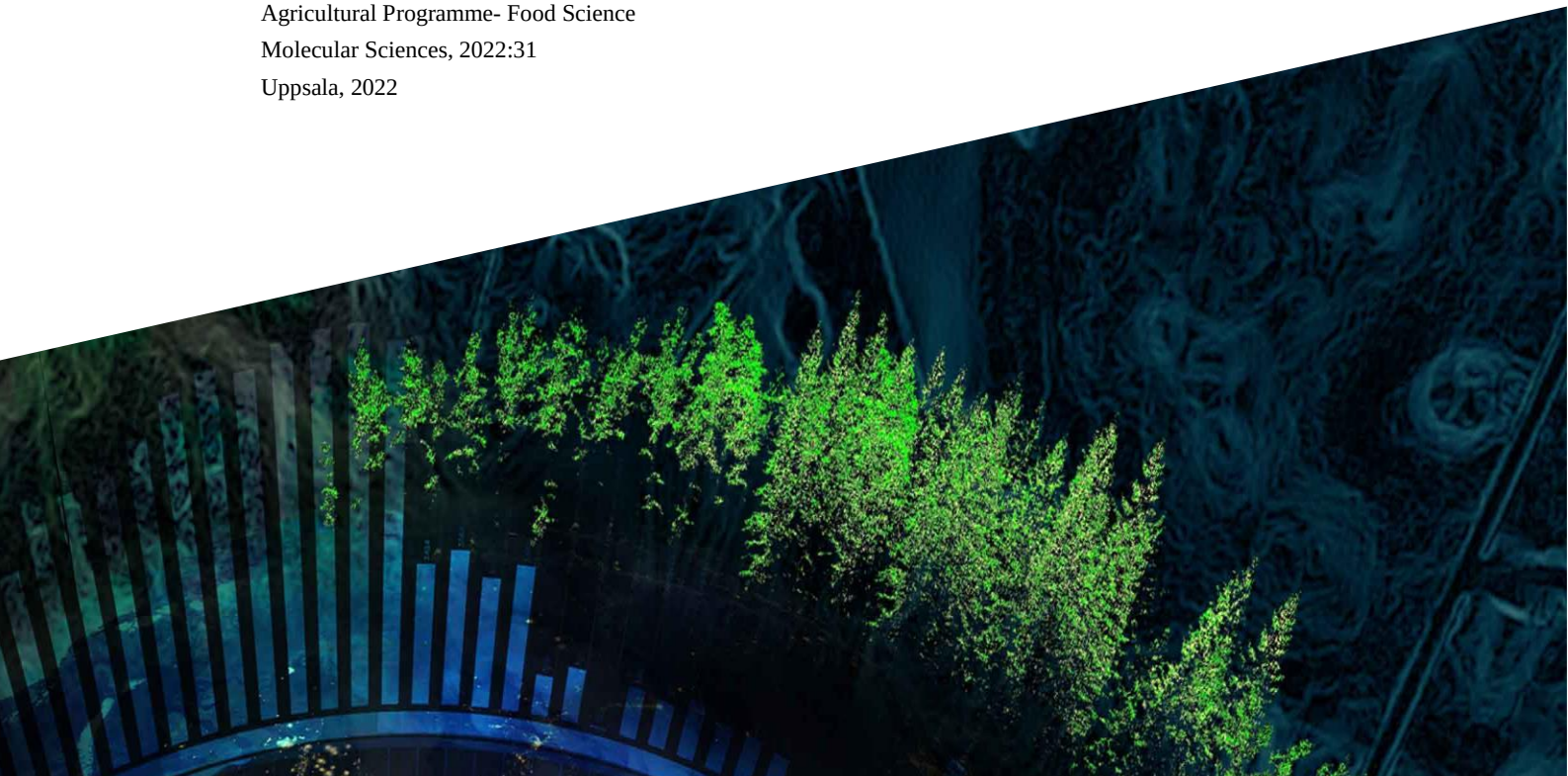

The effects of sweeteners on the intestinal microbiota among individuals with eating disorders

Effekter av sötningsmedel i tarmmikrobiotan hos individer diagnostiserade med olika ätstörningar

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Abstract

In today's society, the number of people who fall ill with various eating disorders is growing at a furious pace, the queues for treatment are getting longer at the same time as younger people are affected. In addition, it is a common phenomenon with ineffective treatments, where setbacks and relapses are common. Sometimes the affected individuals lose many valuable years or even an entire life dedicated to the hard grip of the eating disorder. Concurrently, the options on the food shelves are increasing with low-calorie options in the form of sweeteners. Studies show that individuals diagnosed with an eating disorder tend to choose those options to satisfy the craving for sweets, prevent weight gain, or because of the laxative effect. Due to the extent usage of sweeteners, sweeteners may have a major impact on the microbiota in individuals with eating disorders. It has been partly investigated how sweeteners, sugar, and eating disorders affect the intestinal microbiota separately, but they have not been linked to each other. This literature review aims to answer the following questions: How do sweeteners affect the intestinal microbiota and how do individuals with eating disorders consume sugar and other types of sweeteners? Previous research clearly shows that eating disorders affect the microbiota and bring it into the state of dysbiosis. The most research has been focused on one eating disorder, namely anorexia nervosa. Regarding sweeteners, it has been found that some affect the microbiota and others do not. For instance, aspartame is absorbed quickly, which leads to almost no calories, while the majority of sugar alcohols are slow and fermented further in the colon, which have an effect on the microbiota. Sugar has an inflammatory effect in higher intakes than recommended. The Nordic recommendations advise a diet with less than 10% sugar to avoid an inflammatory effect. The results of the literature study showed clear dysbiosis in both eating disorder studied. In the case of binge-eating disorder, this might be linked to irregular eating habits and episodes of binge eating which can lead to increased intake of sugar and sugar alcohols, resulting in low-grade inflammation. Regarding bulimia nervosa, further research is needed to draw some conclusions. However, there are 12 published studies regarding the microbiota in individuals with anorexia nervosa that indicate dysbiosis, either caused or consequence of malnutrition and starvation. Decreased serotonin levels and depression enchanted the pathways to synthesize serotonin even without the precursor. How sweeteners interact with the microbiota in individuals with eating disorders are also discussed in this thesis. Henceforth more studies are needed about the effect of sugar and sweetener consumption on the intestinal microbiota in individuals with eating disorders.

Keywords: Microbiota, sweeteners, eating disorders, bulimia nervosa, binge-eating disorder, anorexia nervosa, depression, psychopathology, bacteria, microbes, archaeon

Sammanfattning

I dagens samhälle växer antalet som insjuknar i olika ätstörningsdiagnoser i rasande takt, köerna till behandling blir allt längre samtidigt som allt yngre drabbas. Dessutom är det ett vanligt fenomen med ineffektiva behandlingar, där bakslag och återfall är vanligt. Ibland mister den drabbade många värdefulla år eller till och med ett helt liv tillägnad ätstörningens hårda grepp. Samtidigt, ökar alternativen i livsmedelshyllorna med låg kalori alternativ i form av sötningsmedel. Studier visar att individer diagnostiserade med en ätstörning tenderar att välja dessa alternativen för att tillfredsställa sötsuget, motverka att gå upp i vikt eller på grund av den laxerande effekten. Det är delvis undersökt hur sötningsmedel, socker och ätstörningar påverkar mikrobiotan var för sig, men de har inte satts i samband med varandra förut. Denna litteraturstudie avser att besvara följande frågor: Hur påverkar sötningsmedel mikrobiotan samt hur konsumerar individer med ätstörningar socker och andra sorters sötningsmedel? Tidigare forskning visar tydligt att ätstörningar påverkar mikrobiotan och för den in i tillståndet dysbios. Mest forskning har fokuserat på endast en ätstörning, närmare bestämt anorexia nervosa. Angående sötningsmedel har man funnit att vissa påverkar mikrobiotan och andra inte. Aspartam absorberas upp fort, vilket leder till få kalorier, medan majoriteten sockeralkoholer är långsamma och fermenteras vidare i tjocktarmen varav de flesta påverkar mikrobiotan. Nordiska rekommendationerna råder en kost innehållande mindre än 10% socker för att undvika en inflammatorisk effekt. Resultatet av litteraturstudien visade på en tydlig dysbios hos de indvidier med anorexia nervosa och hetsätningsstörning. Vid hetsätningsstörning är detta troligen kopplat till oregelbundna ätvanor och episoder av hetsätning. Detta ledde till ökat sockerintag och intag av sockeralkoholer, vilket resulterade i låg-gradig inflammation. Angående bulimi nervosa krävs ytterligare forskning för att dra några slutsatser. Däremot finns det 12 publicerade studier angående mikrobiotan hos individer med anorexia nervosa som tyder på dysbios antingen orsakad eller en konsekvens av undernäring och svält. Utökade vägar att syntetisera serotonin på grund av minskade serotonin-nivåer och depression var också tydligt. Hur sötningsmedel interagerar med mikrobiotan i individer med ätstörning är också diskuterat i denna uppsatsen. Framöver behövs fler studier om effekten av socker- och sötningsmedelskonsumtion på tarmmikrobiotan hos individer med ätstörningar.

Nyckelord: *Mikrobiota, sötningsmedel, ätstörningar, bulimia nervosa, hetsätningsstörning, anorexia nervosa, depression, psykopatologi, bakterier, mikrober, arkeon*

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Abbreviations

AN	Anorexia nervosa
AN- BP	Anorexia nervosa subtype binge/purging
AN- R	Anorexia nervosa subtype restrictive
BED	Binge-eating disorder
BN	Bulimia nervosa
BMI	Body mass index
CNS	Central nervous system
FMT	Fecal microbiota transplantation
GABA	Gamma-aminobutyric acid
SCFAs	Short-chain fatty acids
SLU	Swedish university of agricultural sciences
OCD	Obsessive-compulsive disorder

1. Introduction

1.1 Research issue

Microbes are of great importance. For several decades, microbes have been feared, hated, and ignored by human beings as the culprit behind spoilage and diseases. Finally, the time has come to appreciate microbes in order not to deplete ourselves. If we could trust their beneficiaries, and understand and learn how to modify our microbiotas, new possibilities could emerge. There is a limited number of studies on eating disorders. The microbiota of anorexia nervosa is mainly characterized, although, the other kinds of eating disorders are often forgotten in this context. Additionally, sweeteners' impact on the microbiota has been investigated in part, but not specifically in eating disorders. Since there are strong indications that individuals with eating disorders consume sweeteners to a greater extent, particularly low-calorie sweeteners, this thesis is desired (Klein *et al*., 2006).

1.2 Aim of the thesis

This study aims to perform a literature review, focusing on the effects of sweeteners on the microbiota among individuals with eating disorders. This literature review intends to provide answers to the following questions: How do sweeteners affect the microbiota and how do individuals with eating disorders consume sugar and other kinds of sweeteners?

1.3 Method and material

The method was primarily based on a literature review. Scientific articles were retrieved from specific databases such as Google Scholar, Scopus, Science Direct, Web of Science, and PubMed via the search platform for the university- Primo. The following search terms were used to locate relevant literature: “eating disorders”, “anorexia nervosa”, “bulimia nervosa”, “binge-eating disorder”, “sweeteners, “polyols”, “artificial sweeteners”, “microbiota”, “probiotics”, bacteria”. Other physical literature was used, for example, American Psychiatric Assoc. Diagnostic and statistical manual of mental disorders (5th ed.). Arlington, VA: American Psychiatric Publishing; 2013 was used to characterize the most common eating

disorders. Meanwhile, Bergey's manual (Whitman *et al.*, 2009) was used for the characterization of bacteria.

1.4 Demarcations

Research on the role of microbiota in eating disorders is relatively new and requires further studies. There are multiple articles about microbiota in anorexia nervosa while other eating disorders are lacking. Binge-eating disorder (BED) had only one article published at the time of writing this thesis, meanwhile, there are no published articles about bulimia nervosa (BN). Although there were around 100 participants in the BED study, the results were uncertain. Another demarcation is drawing the conclusion that all individuals with BED are overweight, which is not the case. Obesity is a common consequence of being ill long-term, as those diagnosed with BED are 3-6 times more likely to be obese. However, this does not occur in all diagnosed individuals (McCuen-Wurst *et al*., 2018).

In addition, the terminology within the field of microbiota has its limitations because it excludes genomes, which the microbiome does include, those are used interchangeably. This thesis focuses on the gut microbiota, not the microbial populations in or on different sites of our body. Sequential questions can be asked. Does it matter that we know which microorganisms live in the intestines of specific people? The sole information tells us what and where, but not what might be more important such as why or how the specific microbe ends up there. The time limit and outline of this thesis did not allow for further discussions.

The concept of BMI has its limitations as well as different eating disorder diagnosis, which exclude some individuals which do not fulfill all criteria for the diagnosis nor the concept of BMI. Its demarcations have been shown in consideration during this work. Neither diagnosis nor body weight is the answer to how the life quality of an individual is affected by the eating disorder. There was little research on how individuals with eating disorders consume specific sweeteners and sugars. Additionally, problems deciding and controlling an individual's whole diet and energy intake were an obvious issue during the studies.

1.5 Background

1.5.1 Binge-eating disorder

According to the Diagnostic and Statistical Manual of Mental Disorders' criteria (DSM-5), the main characteristics of binge-eating disorder (BED) is repetitive periods of binge-eating (eating major quantities for two hours, under the same consideration and time). Additionally, a feeling of loss of control is felt. Those periods should be connected to at least three of the following characteristics. Subsequently, feeling shame, guilt, or even depression after binge-eating. Individuals prefer eating separately due to embarrassment over the amount of food

consumed and even if hunger is absent, a large amount of food is consumed at a faster rate than usual until the individual is painfully full. The main key symptoms are anxiety around binge-eating, which appears to occur once a week for a period of three months. The binge eating itself should not be connected to compensatory action and should not be during the period of the diagnosis of anorexia nervosa or bulimia nervosa. The severity level is determined by the frequency of self-abusive compensatory behaviors. Mildly (1-3 times), moderate (4-7 times), severe (8-13 times), extreme (<14) per week (American Psychiatric Association & American Psychiatric Association, 2013).

BED affects both normal weight and obese individuals (50/50). Although, help is most often sought after by obese individuals. Great amounts of food lead to disabilities, increased distress, and a poorer standard of living. This mostly occurs in people who want to lose weight through dieting (Females 1.6%, males 0.8% (American Psychiatric Association & American Psychiatric Association, 2013).

1.5.2 Bulimia nervosa

The main characteristics of bulimia nervosa (BN) include similar criteria as for BED, repetitive periods of binge-eating (eating major quantities for two hours faster than other individuals, under the same consideration and time; DSM-5). The food is seen as an overwhelming amount, rather than cravings for nutrients. Individuals are most likely to choose the food they would normally avoid. Additionally, a feeling of loss of control is experienced, which results in the usage of compensatory appearance to avoid gaining weight. This can be in the form of self-inflicted vomiting, the usage of diuretics/ laxatives/ fasting/ medication/ an overwhelming amount of exercise. The mentioned behaviors existed at any rate once a week for three months. Weight and body size have an overwhelming influence on self-evaluation. The disruption however does not occur during anorexia nervosa. The severity level is determined by the frequency of self-abusive compensatory behaviors. Mildly (1-3 times), moderate (4-7 times), severe (8-13 times), extreme (<14) per week (American Psychiatric Association & American Psychiatric Association, 2013).

This results in consequences such as negative self-evaluation, body dysphoria, anxiety, aggregation, and depression. BN affects mainly young adults, 1-1.5% of young females with an approximate duration of a year. 10:1 female: male ratio. BN mostly starts after losing weight (dysfunctional dieting). Mortality risk gradually increases during the diagnosis (2% per decade). Purging causes electrolyte imbalances and loss of fluid and gastric acid. The enamel of the teeth can also be broken down (American Psychiatric Association & American Psychiatric Association, 2013).

1.5.3 Anorexia nervosa

The main characteristics of anorexia nervosa (AN) include major energy restriction and starvation resulting in extremely low weight and BMI (under 18.5; DSM-5).

The disorder can be life-threatening. Another criterion is the noticeable fear of gaining weight and becoming fat, despite very low body weight at the point. The weight and shape affect the self-evaluation. There are two different subtypes: 1. restrictive, AN-R (fasting, dieting, or extreme exercise) or 2. binge/purging, AN-BP (overuse of diuretics, enemas, laxatives, or self-inflicted vomiting). These occur for at least three months. In contrast to the BED- diagnosis, AN severity is evaluated based on the BMI as follows: Mild ($\geq$ 17 kg/m^2), moderate (16-16.99 kg/ m^2), severe (15-15.99 kg/ m^2), and extreme (<15 kg/ m^2). Depression, insomnia, and obsessive- compulsive disorder (OCD) as well as other functional abnormalities are other key symptoms. The prevalence of AN is most common in young females (0.4%). Here as well, females are more affected than males. 10:1 female: male ratio. The illness is commonly caused by a tough and stressful life situation. The older you are at the first onset; the longer period of illness is likely. The mortality rate for AN is 5% during ten years, which is connected to medical issues. In total 12 people of 100 000 die from the illness annually (American Psychiatric Association & American Psychiatric Association, 2013).

1.5.4 Gut microbiota

The terminology microbiota refers to a broad and constantly evolving community of microbes, living under specific conditions. In a standard weight man, there are alike many bacteria as cells in the body; 3.9 x 10^{13} (Rastelli *et al*., 2019). Every surface of the body is colonized by microbes, including the intestinal tracts. The majority of the microorganisms in the intestine are bacteria. In healthy individuals, the two most frequent phyla of bacteria are *Firmicutes* and *Bacteriodetes* (Satokari, 2020). However, there are not only bacteria living in the intestine. Viruses (phages), fungi, and archaea enrich the environment further (Rastelli et al. 2019). "All disease begins in the gut." (Hippocrates ho Kőos: c. 460–c. 370 BCE). All diseases, according to Hippocrates, begin in the gut. Imbalances and dysbiosis as a result of the chosen diet are the foundation of diseases. The composition of the microbiota influences neurotransmitters, endocrine functioning, metabolic regulation (energy and glucose), immunity, appetite, and wellbeing via the microbiota-gut-brain axis (Wu *et al*., 2020).

Microbiota-gut-brain axis works through different pathways, for example through neurotransmitters. Neurotransmitters are signaling molecules that enable crosstalk between the microbiota and the brain. One example is the important neurotransmitter serotonin, but there are more neurotransmitters, such as dopamine and gamma-aminobutyric acid (GABA). Serotonin activates neurons and determines mood, depression, and anxiety. Approximately 95% of the serotonin is synthesized in the intestinal tract, reaching the lumen and later the blood. Several subtypes of receptors specified for serotonin are identified in the wall of the gut (mucosa) where the local level of serotonin is high (Gershon & Tack, 2007). If the serotonin levels are low, for example during eating disorders, impaired function will arise. Therefore, the gut microbiota has a huge impact on every single individual daily and is on the cusp of future diseases' respective well-being (Rastelli *et al.*, 2019).

Unabsorbed dietary components such as sugar alcohols, complex sugars, fibers, and carbohydrates are fermented by intestinal bacteria. These create gas, metabolites, and a variety of bioactive substances being either beneficial or damaging to one's health. Short-chain fatty acids (SCFAs), for example, are seen as the energy source to intestinal cells in the form of acetate, butyrate, and propionate. The metabolic influence of SCFAs on host health is directly dependent on the microbiota as they can affect the energy metabolism and hunger (Rastelli *et al.*, 2019).

1.5.4.1 Methodology for studying microbiota/ microbiome

The methodologies used to sequence gut microbes are through PCR amplification of either one specific gene (16S rRNA gene sequencing) or the whole metagenome (shotgun metagenomics sequencing). The two methodologies have both pros and cons. 16S rRNA sequencing is the most common methodology for both the identification and classification of bacteria in studies and is used in majority of the studies included in this thesis. As it only uses one gene, based on the used primer, it only identifies a limited portion of the gut bacteria. Another problem is distinguishing between different bacteria within the same species, because of either high or low abundant taxa. The choice often goes to 16S rRNA because it is a cheap methodology, with a low risk for false-positive results, and applicable for all kinds of microbiomes (Durazzi *et al.*, 2021).

Metagenomics is another methodology based on the whole genome, which allows further analyses as well as a whole picture of the interested genome. It is better at identifying taxa and functional genes. However, it is less common, more expensive, and tends to have a higher risk for false-positives (Durazzi *et al.*, 2021).

1.5.5 Microbiota among individuals with different eating disorders

Twelve studies did a characterization of the microbiota of anorexia nervosa (AN). All articles indicated a clear dysbiosis in people with AN. At the phylum level, nearly half of the studies found increased levels of *Bacteroidota,* although there were different kinds of genera. Furthermore, nearly half of them found increased levels of the class *Clostridia,* again of different kinds of genera. Mörkl *et al.* (2017) and Kleiman *et al.* (2015) found increased levels of the family *Coriobacteriaceae* (Kleiman *et al*., 2015; Mörkl *et al*., 2017). Both of these studies had study populations of less than 20 participants diagnosed with AN. Within the order *Enterobacteriales,* bacteria were of higher occurrence. An increase in the genera *Enterococcus, Escherichia,* and *Shigella* was seen in individuals with AN. Decreased levels at the genus-level of *Clostridia* were found in more than half of the studies. Especially lower levels of *Roseburia* (Mack *et al*., 2016; Borgo *et al.*, 2017; Hanachi *et al.,* 2019; Lambertova *et al.*, 2019). Generally, different kinds of genera belonging to the order *Clostridia* increased (*Ruminococcus*) respectively decreased (*Roseburia, Faecalibacterium, Ruminococcus*) in AN. Although *Ruminococcus* was found to both increase and decrease in different studies, which indicates further research is needed or individual inequality.

All studies that tested for the presence of archaea found a significant increase in the specie *Methanobrevibacter smithii* in AN compared to the healthy controls (Armougom *et al.*, 2009; Million *et al*., 2013; Mack *et al*., 2016; Borgo *et al*., 2017). One restriction was that all references did not include tests for the archaeon, only four of them did. However, shotgun metagenomics should have found *M. smithii.*

A connection between AN and lower levels at genus-level of *Lactobacillus* was seen, especially in the two species *L. reuteri* and *L. plantarum* (Armougom *et al*., 2009; Million *et al*., 2013; Morita *et al*., 2015).

Eleven novel species from only one individual diagnosed with AN. The conclusion was drawn that there are more novel bacteria in AN compared to the healthy controls, as a result of the loss of key microbiota (Pfleiderer *et al*., 2013). In several studies, they found less microbial diversity (alpha diversity) in AN. Monteleone *et al*. (2021) and Prochazkova *et al.* (2021) discovered minor changes in microbiota even after treatment and recovery. Even months later the microbiota still looked more like ill individuals than the healthy controls (Monteleone *et al*., 2021; Prochazkova *et al*., 2021) Hanachi *et al.* (2019) found FID (functional intestinal disorder) predominant in malnourished individuals with AN. Altered host microbes' symbiosis between bacteria with pro-inflammatory and protective assay within the genera *Roseburia*, was seen as a consequence (Hanachi *et al.*, 2019).

Characterization of the microbiota in individuals with BED is published in only one article from Belgium (Leyrolle *et al*., 2021). The study had a relatively large study group (n = 42 cases with BED), compared to other studies of microbiota in eating disorders. The study analyzed fecal samples with 16S rRNA, HPLC, MS/MS, and ESI. The study determined that BED is characterized by changes in microbiota, biological markers and metabolites. The observations showed significantly increased numbers at the genus-level of *Bifidobacteria, Anaerostipes* and *Roseburia.* But also, decreased numbers of the genera *Akkermansia, Sutterella, Desulfovibrio,* and *Intestinimonas.* Other results include lower levels of Bisphenol A bis, Isovalerylcarnitine, and abnormal balance in the form of self-regulation (Leyrolle *et al.*, 2021). See *Table 1* for more information about the relevant microbes, occurring in individuals with eating disorders in more than one study, and in which article/ eating disorder they are highlighted.

Table 1. Information about enriched members of the gut microbiota

Bacteria	Phylum	Article highlighted	Eating disorder found
Akkermansia	Verrucomicrobia	Leyrolle *et al*., Everard *et al.* & Mack *et al*.	Decreased in BED and obesity
Anaerostipes	Bacillota	Leyrolle *et al.*, Heil *et al*., Kleiman *et al.*, Lambertova *et al* & Hanachi *et al*.	Produces butyric acid. Lower values in AN

M.smithii	Euryarchaeota	Armougom *et al*., Borgo *et al.*, Mack *et al.* & Million *et al.*	Increasing in AN
Lactobacillus	Firmicutes	Morita *et al.*, Armougom *et al.*, & Million *et al.*	Increase in obese individuals. AN had lower amounts of *L.reuteri* and *L.plantarum*
Ruminococcus	Firmicutes	Prochazkova *et al*., Kleiman *et a.*, Borgo *et al.*, Lambertova *et al.*, Hanachi *et al.* & Pfleiderer *et al.*	Lower values in AN
Roseburia	Firmicutes	Heil *et al.*, Borgo *et al.*, Lambertova *et al.*, Mack *et al.* & Hanachi *et al.*	Lower values in AN

1.5.6 Relevant microbes

Akkermansia muciniphila is an anaerobic but nano oxygen tolerant bacterium that degrades mucin in the mucus layer of the intestinal tract in mammals. The availability of mucin as the only carbon source provides a probiotic assay, the optimal habitat is in a mucin enriched environment. *Akkermansia* is a producer of short-chain fatty acids, SCFAs, through fermentation of mucin. For example, propionate, sulfate, and acetate are produced. Those are responsible for the regulation of inflammations and the production of gut peptides. Therefore, *Akkermansia* strengthens and protects the gut function. The bacteria reduce the risk of low-grade inflammation and improve the sensitivity to insulin which reduces obesity. The bacteria colonize in an early stage in the human microbiota as it is found in breast milk, with 8 species identified. Unbalanced, high energy, and high-fat diets decrease the levels of *Akkermansia*, on the other hand, polyphenols, cinnamon, and specified unsaturated fatty acids increase the levels (Zhai *et al.*, 2019).

Anaerostipes (a stick not living in the air) consists of only one bacterial species, *Anaerostipes caccae.* Out of traditional tests, with bacterial culture, the bacteria are acid-producing if D-glucose, maltose, sucrose, and sorbitol are available. On the other hand, it cannot create acid from lactose and other sugars. In an AP150 test, sugar alcohols also produced acetic, butyric acid respectively lactic acid out of glucose (Whitman *et al.*, 2009).

In the human microbiota, *Methanobrevibacter smithii* (*M. smithii)* is the dominant archaeon. In healthy individuals, *M. smithii* can contribute up to at least 10% of the total content of anaerobes in the colon as a hydrogen-consuming methanogenic and heterotrophic archaeon. The archaeon can digest complex sugars, polysaccharides and consume hydrogen. Elimination of hydrogen results in a more oxidized final product which allows higher energy extraction of nutrients and

optimal exchange of energy in foods. In this way, *M. smithii* can regulate the harvest of calories, influencing host metabolism. Studies indicate greater gene expression, increased polysaccharide digestion, and higher levels of the archaea in lean individuals (Buck *et al.,* 2007).

Lactobacillus (milk rodlet) is fermentative bacteria with specific nutritional needs. Fifty percent of the end product is lactate, but the bacteria can also produce acetate, formate as well as CO_2. Various parts of the diet such as sourdough, water, sauerkraut, pickled vegetables, wine, beer, meat, fish as well as dairy products contain these bacteria, as these bacteria often live in low pH. There are many different species in the genus, some of them are gas producing and can either be homofermentative or heterofermentative, creating different end products (Whitman *et al.,* 2009).

Ruminococcus (coccus of the rumen/large bowel/caecum in mammals) is strictly anaerobic bacteria. To survive, it needs carbohydrates to ferment and synthesize acetate, ethanol, formate, lactate, and succinate (Whitman *et al.,* 2009).

Roseburia is named by the American microbiologist Theodor Rosebury who found the bacteria naturally occurring in the intestinal tract of mammals. The bacteria belong to the cluster XIVa of the subphylum *Clostridium* whereas two species are identified (*Roseburia intestinalis* and *Roseburia cecicola)*. Strictly anaerobic bacteria are very sensitive to oxygen and die after only two minutes exposed to air. The bacteria are of interest because it ferments and hydrolyses starch (*D-glucose*, *D-maltose*) and out of this, it does produce CO_2, H_2, and huge amounts of butyrate. *Roseburia* can also produce formate, lactate, and sometimes ethanol (Whitman *et al.,* 2009).

1.6 Dietary patterns- sweeteners

1.6.1 How individuals with eating disorders consume sugar and other sweeteners

In addition to eating disorders, microbiota as well as diet and energy intake, have a big influence. As there are significant signs that people with eating disorders consume more sweeteners, mainly low-calorie sweeteners like aspartame and sugar alcohols, it would be interesting to investigate if there are any links between the consumption of sweeteners and the intestinal microbiota. A study using self-report questionnaires found a difference in the use of sweeteners between healthy individuals and those with eating disorders - including individuals with both subtypes of anorexia nervosa (both restrictive and binge/purging subtype) and one group diagnosed with bulimia nervosa (Klein *et al*., 2006).

The findings suggested that those diagnosed with bulimia nervosa and anorexia nervosa subtype binge/purging consume more artificial sweeteners in the form of

low-calorie beverages, diet sodas, light yogurt, chewing gum, and other foods in comparison to health individuals. In general, those with eating disorders had a higher tendency to use those products compared to the controls, to maintain weight or more easily lose weight. Additionally, there was a connection between BMI and the use of each product. There were no variations in the intake of diet drinks between the groups, although those with purging subtypes used diet beverages mostly. The conclusion was drawn that females with eating disorders more frequently used artificial sweeteners to stimulate their sweet tooth (Klein *et al*., 2006).

Primarily, sorbitol, in sugar-free gum, was used by 86% of the participants. Out of 21 participants, 18 of them used quantities of sorbitol each day, whereas some of them used huge quantities. On the other hand, aspartame was commonly used among individuals with BN. In the study, they demonstrated that carbohydrates together with aspartame increased the amino acid levels in the central nervous system (CNS) (Klein *et al*., 2006).

If this is a result of those diagnosed with BN and AN-BP being better in reporting usage of chewing gums and sweetener packets, or if they do use more sweeteners than AN-R is still a question. Gum and diet soda use was lower in the restrictive subtype of anorexia compared to the ones binging and purging. Instead, the ones with AN-R used artificial sweeteners in other forms, for example, sweetener packets, and reported flatulence (Klein *et al*., 2006). Other sweeteners are used for laxative purposes, primarily by BN and the AN-BP subtype. Sweeteners with laxative effects are those consisting of at least 10% sugar alcohols, including sorbitol, polyglycerol syrup, maltitol, erythritol, lactitol, isomalt, xylitol, and mannitol (Livsmedelsverket, 2022).

There is a lack of information about the intake of ordinary table sugar (saccharose) in eating disorders. However, hypotheses can be drawn. During AN, the total carbohydrate intake was lower in one study (Borgo *et al.*, 2017). A general decrease in 29 g carbohydrates/ diet corresponds to nearly 15% of the daily carbohydrate intake, which may result in lower values of glucose as the carbohydrates are broken down to glucose (Borgo *et al*., 2017). On the contrary, during recovery nutrient drinks are added to individuals with low BMI to gain weight. Nutrient drinks contain pure saccharose (*Fresubin ® Energy/ Energy Fibre DRINK*, 2019).

One conclusion can be drawn, that sugar is the first product to be absorbed rapidly by the intestinal tract (Stümpel *et al.*, 2001). Individuals with BN and BED normally avoid sugar products and tend to binge eat and crave those products. Therefore, larger amounts of saccharose are consumed during binge eating (American Psychiatric Association & American Psychiatric Association, 2013). For those with BN, even if they purge or vomit, the sugar has already been taken up by the blood vessels in the intestine (Stümpel *et al.*, 2001).

1.6.2 How sweeteners affect the microbiota

Sweeteners can be degraded and used by certain bacteria, yeasts, and other microorganisms in the microbiota. It might be for the better or the worse, depending on which microbes and sweeteners are used and how they interact. During the period of eating disorders, individuals tend to consume excessive levels of sugar alcohols and artificial sweeteners, especially aspartame. The dipeptide **aspartame** is 200-fold sweeter than sugar. Although it does not affect the insulin concentrations, the glucose concentration increased during consumption of aspartame compared to the controls without aspartame. A decrease in sugar intake was observed associated with aspartame intake. Aspartame is quickly absorbed in the small intestine, and broken down into two peptides, aspartic acid, and phenylalanine. These are connected to the production of SCFAs, mainly propionate. As it absorbs quickly, it does not have time to go out into the blood and large intestine. In a study performed in a rat model of obesity, obese rats were divided into four different treatments for a period of eight weeks. One group with a standard pellet diet, another one with supplemented water or a low dose of aspartame added. The last group was a high-fat diet. The study resulted in 10% higher values of *Clostridium leptum* and *Enterobacteriaceae* being seen as a consequence of aspartame intake, otherwise, no bigger changes in the microbiota were observed. Similar results were obtained in another study performed in humans (Palmnäs *et al.*, 2014). Aspartame resulted in the products aspartic acid, phenylalanine, and methanol (Grembecka, 2015).

On the other hand, the effect of sugar alcohols depends greatly on how they are metabolized and which effect they have on the individual's glucose level. Generally, they are low and slowly metabolized carbohydrates, resulting in fewer calories**.** For example, all parts of the polyol are not absorbed through passive diffusion in the small intestine. SCFAs are produced by bacteria in the colon of the not absorbed residues. The nutritive polyol with 5-carbons, **xylitol** (E967), is absorbed to 50% in the small intestine and fermented further in the large bowel, respectively liver metabolized directly (Grembecka, 2015). Uebanso *et al.* (2017) investigated the effects of xylitol on the intestinal microbiota in mice. Out of a medium-dose and high-dose xylitol added to the diet, the study found decreased values of *C. difficile* and *Bacteriodetes*, increased *Firmicutes* as well as higher numbers of *Bifidobacteria* because of a medium-dose xylitol together with a diet high in fat, which promotes A*naerostipes spp.* to grow (Uebanso *et al.*, 2017).

Isomalt (E953), consisting of glucose-sorbitol and glucose-mannitol has the lowest absorption of them all (less than 10%), resulting in high amounts of fermentation by bacteria. On the contrary, the majority of yeasts and microbes cannot ferment isomalt. The final products of fermentations are SCFAs (butyrate), H_2, CO_2, and CH_4. Those products promote the growth of *Bifidobacteria*, as seen in vitro studies in 19 humans (Grembecka, 2015). In healthy individuals, the effects of isomalt intake on intestinal and metabolic markers were investigated. During four weeks, 19 study participants ate a base diet enhanced with 30 g isomalt or 30 g sucrose. It was discovered that isomalt slowly ferments metabolized residues in the colon, which is beneficial in mucus and gives higher values of *Bifidobacteria* (Beards *et al.*, 2010). *Bifidobacteria* can ferment isomalt, producing butyrate in high amounts

and ending up in a healthy gut. This is because butyrate can undergo metabolization by tissues and epithelium. Additionally, 50% of the energy requirements of the mucosa are generated by butyrate (Beards *et al.,* 2010). An example of low absorbed sugar alcohol is the disaccharide **maltitol** (E965) made of glucose and sorbitol. It needs to be hydrolyzed before absorption and the range of absorption can be between 5% and 80%. During two weeks 40, individuals consumed chocolate sweetened with maltitol, or maltitol supplemented with polydextrose or maltitol supplemented with resistant starch. Significant changes in the microbiota were obtained. The ones consumed with only maltitol had increased levels of *Bifidobacteria* and *Lactobacilli* as well as higher levels of SCFAs (Beards *et al.,* 2010).

Lactitol (E966) is another sweetener affecting the microbiota. Lactitol is a disaccharide of sorbitol and galactose. Lactitol acts as a dietary fiber and can have a laxative effect, having a positive effect on and acting as an energy source for *Lactobacillus spp.* and *Bifidobacteria*, which decreases the pH (Grembecka, 2015). The microbiota, pH, and SCFA levels were investigated during a controlled research study of 75 healthy individuals both before and during low consumption of lactitol. The study participants were given tablets with 10 g of sweeteners in the form of chocolate. *Bifidobacteria* had seen considerable alterations. While there was a pH drop as a result of a large increase in butyric and propionic acid. The conclusion was drawn that low consumption of lactitol can be categorized as prebiotics, according to the findings (Finney *et al.*, 2007).

Three sugar alcohols where no effect has been demonstrated on the composition of the microbiota are mannitol, sorbitol, and erythritol. **Erythritol** (1,2,3,4-Butanetetrol, E968) is naturally occurring in vegetables, fruits, and fermented products. The sugar alcohol is non-fermentable and does not affect the glucose level (Grembecka, 2015). The 6 carbon **sorbitol** (D-glucitol, E420) can be synthesized out of either sucrose or glucose (Grembecka, 2015). Sorbitol increases the water concentration in the intestine and has an osmotic laxative effect, resulting in diarrhea and other gastrointestinal abnormalities (Ruiz-Ojeda *et al.*, 2019). The 6-carbon sugar alcohol **mannitol** (E421) is an isomer to sorbitol and those isomers go through either partly absorption or passive absorption, going further to the colon where the residues are metabolized to increase blood sugar (Grembecka, 2015). Interestingly *Lactobacillus reuteri* produces mannitol when it cultivates on saccharose or pure fructose (Carvalheiro *et* al., 2011). These sugar alcohols are novel and continue to develop further. Because of this, all sugar alcohols' effect on the microbiota as well as the metabolism requires further studies (Grembecka, 2015).

Of the daily energy intake, added sugars should not transcend 10%, according to the Nordic nutrition recommendations (Björck & Tetens, 2012). If the daily intake transcend this intake, the consequence is a changed composition of the microbiota, quite similar to dysbiosis (Björck & Tetens, 2012). Ultra-processed food and high intake of fat and sugars have a clear connection to diseases, and obesity through different concentrations of bile acid, destroying the gut barrier. Lower diversity of *Bacteriodetes* and higher levels of *Proteobacteria* originate from non-absorbed

monosaccharides, feeding and making *Proteobacteria* grow rapidly. The growth of *Proteobacteria* disrupts the balance of the microbiota and results in abnormal immunological homeostasis (Satokari, 2020).

2. Discussion

This discussion will further investigate the microbiota in the main eating disorders and its connection to sweeteners. At the time of writing this thesis, there was just one published study about the microbiota in individuals with binge-eating disorder, BED (Leyrolle *et al.*, 2021) This needs to be kept in mind, as it may be a statistical issue. As this study is from Belgium, there might be differences between countries as well. If individuals diagnosed with BED did the same study in for example USA, other results could have been obtained. Further studies are required to conclude if these are general characteristics in individuals diagnosed with BED, and its connection to psychopathology such as loss of control, ashamed guilt, depression and anxiety (American Psychiatric Association & American Psychiatric Association, 2013).

Changes in the microbiota of individuals with BED are shown in *Figure 1.* The microbiota is characterized by a clear dysbiosis, which might have associations with irregular eating habits, binge eating and episodes of high sugar, fat, and processed food intake. This kind of diet leads to increased low-grade inflammation, which was seen to have a connection to the decrease of the bacteria *Akkermansia* (seen in individuals with BED). *Akkermansia* was previously shown to protect the gut through synthesizing SCFAs as a product of the fermentation of mucin (Zhai *et al.*, 2019). Additionally, findings indicated that *Akkermansia* is associated with the eating behaviors, and psychopathology in individuals diagnosed with BED. Furthermore, increased values of *Bifidobacteria* and *Clostridium* (especially *Anaerostipes* and *Roseburia*) were observed, whereas there were decreased values of *Akkermansia, Sutterella, Desulfovibrio,* and *Intestinimonas.* The rise in *Anaerostipes* is opposite to a decrease in this bacteria observed in AN (Leyrolle *et al*., 2021).

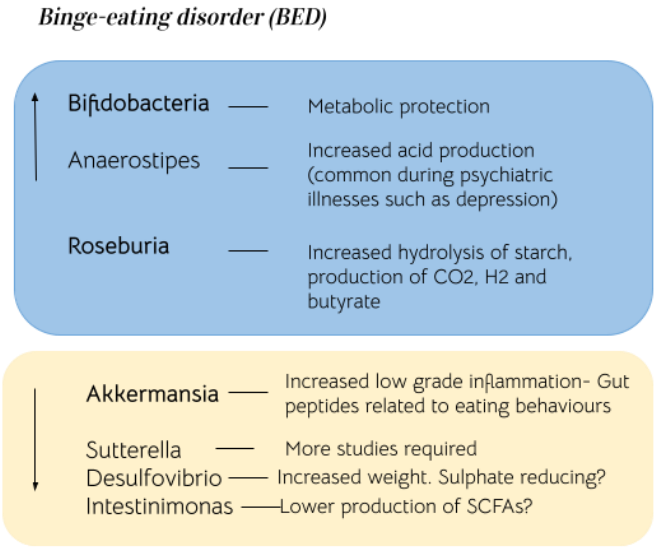

Figure 1. Changes in the relative abundance of bacteria in BED, and its potential effect on the individual. Reference: *(Leyrolle* et al., *2021).* Copyright: Nellie Nyd

There are several studies characterizing the microbiota in obese individuals. It would be worthwhile to compare those studies with studies in BED in terms of the association between microbiota and BMI. However, obese individuals without BED do not necessarily have the same psychopathology as BED. In comparison to obese individuals, similarities are found. They both had increased amounts of *Anaerostipes* (Hiel *et al., 2020;* Leyrolle *et al., 2021).* Also, lower amounts of *Akkermansia* were found (Everard *et al.,* 2013; Hiel *et al*., 2020; Leyrolle *et al*., 2021). Also, increased low-grade inflammation seems to be similar to obese individuals. Additionally, lower levels of metabolites connected to appetite and BMI seem to be a common factor between the two of them (Everard *et al.,* 2013; Hiel *et al*., 2020; Leyrolle *et al*., 2021).

On the other hand, differences between BED and obesity were observed. In BED there were increased levels of *Bifidobacteria* and *Roseburia,* whereas in obese individuals, studies indicated decreased levels of *Roseburia* and more of *Lactobacillus* (Hiel *et al.,* 2020; Leyrolle *et al*., 2021). The increase in *Bifidobacteria* seems to have an association with cardio metabolic protection in individuals with BED, as energy is not added frequently, something that not might be needed in obese individuals as they might have more regular eating habits and a constant supply of energy, without binge-eating (Everard *et al.,* 2013).

The high sugar (saccharose) and sugar alcohol intake during episodes, was observed to lead to bacterial fermentation of complex sugars, and sugar alcohols (for example isomalt) leading to increase flatulence and gastrointestinal abnormalities. However,

BED had increased the growth of *Anaerostipes.* This may be because of the intake of the sugar alcohol xylitol, which promotes *Anaerostipes* to growth (Ruiz-Ojeda *et al.*, 2019). This bacteria, on the other hand, enhances the acid production when additional maltose, sorbitol, or sucrose is available, and more acids are produced (Whitman *et al.*, 2009) More acid production leads to a deeper state of depression and could be connected to other psychiatric illnesses (Leyrolle *et al.*, 2021).

Individuals with anorexia nervosa (AN) were also found to have significant dysbiosis in the microbiota, according to all twelve studies. The dysbiosis might be connected, either caused or a consequence of malnutrition, starvation, and the exclusion of some food groups. This could also be linked to less diversity of species and the respective decreased total amount of bacteria (especially obligate anaerobes, found Morita *et al.*, 2015) in the microbiota, which the majority of studies found (Morita *et al*., 2015; Mörkl *et al.*, 2017; Hanachi *et al.*, 2019; Lambertova *et al.*, 2019; Roubalová *et al.*, 2020; Monteleone *et al*., 2021; Prochazkova *et al*., 2021). Mack *et al.* (2016) referred it to as a little core size in AN (Mack *et al*., 2016).

The bacteria are deprived of nutrients during food restriction periods. Therefore, one possibility is that the ones with the ability to extract energy out of low-calorie food survive this environment. An example is the archaeon *M. smithii*, which seems to be the key microbe in AN. This Archaeon can digest complex sugars, polysaccharides and recycle hydrogen through the elimination of hydrogen. Elimination of hydrogen results in a more oxidized final product which allows higher energy extraction of nutrients and optimal exchange of energy in foods. In this way, *M. smithii* can regulate the harvest of calories (Buck *et al.*, 2007). This may be the reason why all the studies testing for *M. smithii*, found increased levels of the archaeon (Armougom *et al*., 2009; Million *et al*., 2013; Mack *et al*., 2016; Borgo *et al*., 2017). It would have been fascinating to see if the experiment could be repeated and the results were the same.

As seen in *Figure 2, the relative abundance of* several bacteria was altered in individuals with AN. An increase in potentially pathogenic bacteria was seen in individuals with AN, *Enterococcus, Shigella*, etc. Additionally, *Alistipes, Coriobacteriaceae,* and *Firmicutes* were increased. Decreased levels of *Clostridia* were found in more than half of the studies. Especially *Roseburia* (Mack *et al*., 2016; Borgo *et al*., 2017; Hanachi *et al.*, 2019; Lambertova *et al*., 2019). Generally, different kinds of genera of *Clostridia were* increased (*Ruminococcus*) or decreased (*Roseburia, Faecalibacterium*) in individuals with AN. Moreover, lower levels of *Anaerostipes, Ruminococcus, Parabacteriodes,* and *Faecalibacterium* were seen

(Kleiman *et al.*, 2015; Hanachi *et al.*, 2019; Lambertova *et al.*, 2019).

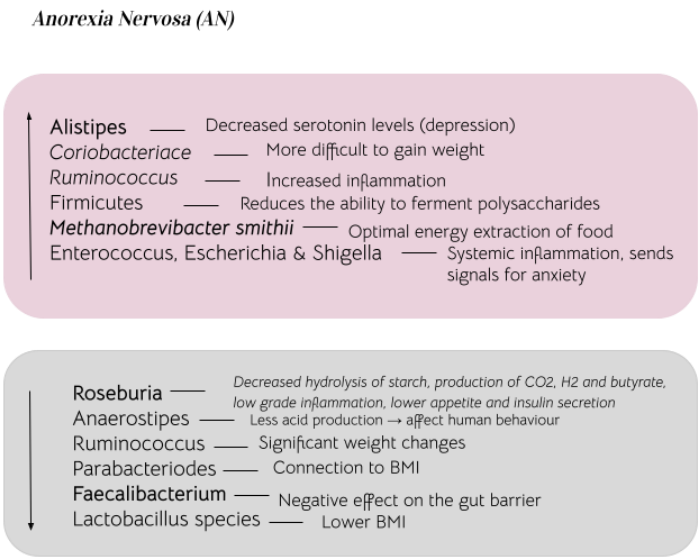

Figure 2. Microbes changed in AN, and its potential effect on the individual. References: *(Armougom* et al., *2009; Million* et al., *2013; Pfleiderer* et al., *2013; Kleiman* et al., *2015; Morita* et al., *2015; Mack* et al., *2016; Borgo* et al., *2017; Mörkl* et al., *2017; Lambertova* et al., *2019; Roubalová* et al., *2020; Monteleone* et al., *2021; Prochazkova* et al., *2021)* Copyright: Nellie Nyd

Lactobacillus is another bacterial specie that decreased in AN. Especially *L. reuteri* and *L. plantarum* (Armougom *et al.*, 2009; Million *et al.*, 2013; Morita *et al.*, 2015) Million *et al.* (2013) suggested that *L. reuteri* is associated with BMI (Million *et al.*, 2013). Kleiman *et al.* (2015) suggests the increase of *Coriobacteriaceae* as a response to stress, caused by eating disorders (Kleiman *et al.* 2015). Pfleiderer *et al.* (2013) found 11 novel species from one individual and mentioned that there are more novel bacteria in AN. Monteleone *et al.* (2021) and Prochazkova *et al.* (2021) discovered that even after weight gain treatment, the microbiota still looked more like an ill individual than the healthy controls, indicating the loss of core microbiota (Monteleone *et al.*, 2021; Prochazkova *et al.*, 2021). This can have associated with the fact that individuals with AN need more energy to maintain the same weight than others, even when they are restored. The loss of key microbiota and decrease of *Roseburia*, who is the bacteria who stands for the important hydrolysis of starch, might cause a chronic disorder in individuals with AN (Hanachi *et al.*, 2019). In the same study, they found FID (functional intestinal disorder) predominant in AN together with altered host microbes' symbiosis (Hanachi *et al.*, 2019).

Interestingly, individuals with AN might develop altered serotonin pathways through the gut-brain axis. Out of SCFAs, individuals with AN can synthesize serotonin and dopamine even without tryptophan, the precursor to serotonin. *Alistipes* hydrolyzes tryptophan to indole, which lowers serotonin levels and is linked to depression. One question is if those new serotonin pathways are connected

to the eating disorder. Do individuals feel relieved when they follow the eating disorder or follow obsessive-compulsive thoughts (OCD)? This means that serotonin might be produced individuals with AN by skipping a meal (Prochazkova *et al*., 2021). Therefore, decreasing the amounts of *Alistipes*, that hydrolyze serotonin, or increasing the amount of tryptophan in the diet to enable more serotonin production could be further investigated as novel therapeutic approaches.

Xylitol can create flatulence in BED. In the same way, different sweeteners have their impact on the microbiota, see *Figure 3*.

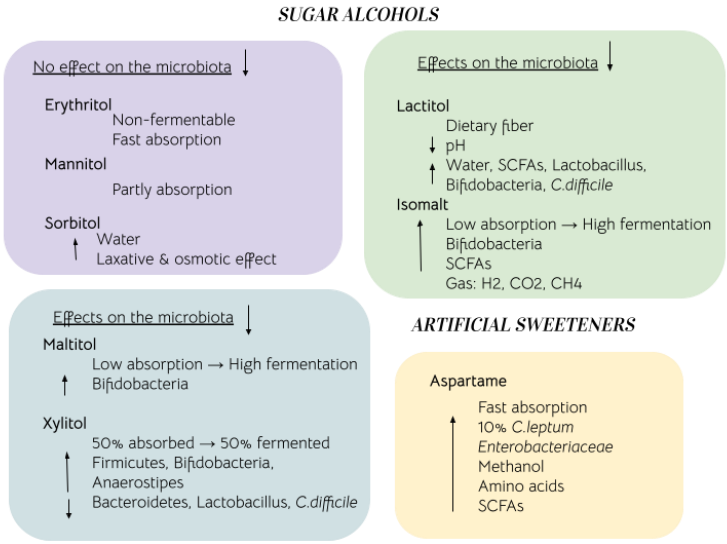

*Figure 3.*Different sweeteners effect or non-effect on the microbiota. Copyright: Nellie Nyd

In the coming part of the discussion, the possible link between sweeteners and eating disorders will be discussed, illustrated in *Table 2*.

Table 2. Link between sweeteners and eating disorders

Bacteria	Kind of sweetener	Changed in which eating disorder	Reference
Enterobacteriaceae	Aspartame	Anorexia nervosa	Palmnäs *et al.*, Borgo *et al.*
Anaerostipes	Xylitol	Binge-eating disorder	Ruiz-Ojeda *et al.*, Leyrolle *et al.* & Heil *et al.*
Bifidobacteria	Maltitol, xylitol, lactitol and isomalt	Binge-eating disorder	Leyrolle *et al*, Uebanso *et al.*, Grembecka *et al.* & Finney *et al.*

Anorexia nervosa is characterized by a lower sugar intake than the other eating disorders (Borgo *et al*., 2017). The patients compensate this low sugar intake with a higher artificial sweetener intake, particularly aspartame. The AN-BP subtype was shown to use significantly increased values of sugar alcohols as well. A lower

sugar intake leads to a more anti-inflammatory diet. As they use artificial sweeteners in higher amount, these are proven to affect the microbiota. Aspartame is rapidly absorbed in the small intestine, production of propionate. An increase in *Enterobacteriaceae* was observed (Grembecka, 2015), at the same time as the study with the artificial sweetener aspartame was seen to increase the numbers of *Enterobacteriaceae* (Palmnäs *et al.,* 2014). Further studies would have been interesting to detect if there is any connection between the high intake of aspartame and an increase in *Enterobacteriaceae.*

There are no specific studies for bulimia nervosa (BN) yet. The coming study BEGIN, from Karolinska Institute, will be the first published study about the microbiota in individuals with BN. The study will additionally add information about the microbiota in binge-eating disorder and its connection to food intake, of different kinds of products. There might be some similarities between BN and BED, as they both perform repetitive binge-eating. Although at the same time there can be similarities between AN and BED, as malnutrition can be a consequence of self-induced vomiting, mainly there will be similarities between AN-BP and BN.

Binge-eating disorder (BED) have been characterized and connections can be drawn. In BED increased levels of *Anaerostipes* where founded, on the other hand does xylitol promote growth of *Anaerostipes,* which results in more of the specific bacteria. This would have been an interesting connection as those who binge-eat consume sugar alcohols to a greater extent. Also a higher level of *Bifidobacteria* where observed, which also was seen during high intake of other sugar alcohol's as for example maltitol, xylitol, isomalt and lactitol.

As dysbiosis has been documented in patients with eating disorders, targeting the intestinal microbiota could provide new and more effective treatment options for these disorders. In this way, the microbiota can be normalized, and the chronicity, relapses, and setbacks can be avoided. There are several ways to target the microbiota. Diet and intake of nutrients is one way; this can be in the form of either prebiotics or probiotics. Bioactive compounds, SCFAs, and diverse nutrients are other ways. Otherwise, it can be through fecal microbiota transplantation. Fecal microbiota transplantation (FMT) is a medicinal method that involves transferring feces from one person to another. One patient with anorexia nervosa (AN) was treated with FMT, which resulted in an improved intestinal barrier, greater species richness, and higher overall SCFA levels, as well as less fungal (Lambertova *et al.*, 2019). Probiotics are live microorganisms (often bacteria) that are supplied in sufficient amounts to provide therapeutic benefits to the host in providing assay or during treatment in the form of either a long time of use or new ways of next-generation probiotics, for example using *Akkermansia*. To sum up, in fact using probiotics with *Bifidobacteria* and *Lactobacillus* boosts *Roseburia* and the synthesis of butyrate, therefore pre- as well as probiotics, could have a therapeutic advantage for individuals undergoing recovery, enhancing the rate of the recovery (Borgo *et al*., 2017). Probiotics may therefore be considered as a part of recovery and renourishment.

2.1 Conclusion

This thesis summarizes the findings from studies of the intestinal microbiota in eating disorders and revealed significant dysbiosis in individuals with anorexia nervosa and binge-eating disorder. In binge-eating disorder, the dysbiosis might be correlated to abnormal eating patterns in form of episodes of binge-eating with a higher sugar- and sugar alcohol consumption, resulting in low-grade inflammation. The two key microbes are *Roseburia* and *Akkermansia,* affecting dysbiosis. An increased intake of isomalt and other sugar alcohols together with the increase in certain bacteria might result in increased flatulence and gastrointestinal abnormalities. In the case of bulimia nervosa, more research is required before any conclusion can be drawn. However, numerous published research on the microbiota in anorexia nervosa found dysbiosis as either the cause or consequence of malnutrition and fasting. In AN, *M. smithii* stands for the optimal energy extraction out of low-calorie food. Moreover, the psychopathology behind depression and reduced serotonin levels are connected to *Alistipes.* Further research is required to elucidate if there is any connection between a higher aspartame intake and increased levels of *Enterobacteriaceae*. Furthermore, more research in the area of microbiota is needed, particularly in binge-eating disorder and bulimia nervosa. The other kinds of eating disorders diagnoses and the connection between psychological symptoms should be investigated further. More research into the impact of sugar and sweetener consumption on the intestinal microbiota in individuals with eating disorders is warranted in the future.

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