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Low and Zero Calorie Sweeteners Possibly Mediating Mental Health Disorders Through the Gut Microbiome

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Abstract

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Mental health disorders, such as anxiety and depression, have been increasing substantially over the past 25 years, causing a public health burden all around the world. Additionally, low- and zero-calorie sweeteners used in place of traditional sweeteners to sweeten foods due to their potential health benefits have been increasing in consumption. Additionally, focus on the digestive system from mouth to anus known as the gastrointestinal (GI) tract, specifically the gut-brain axis, has been shifting in the scientific community as the GI tract's links to various biological processes have been emerging. It has been theorized that the GI tract may play a role in mental health disorders' prevalence and severity. Additionally, some research has analyzed the possible implications these low and zero calorie sweeteners may have on the GI microbiome. The goal of this literature review was to bridge the gap in this space to investigate if low and zero calorie sweeteners were mediating mental health disorders through the gut microbiome. This was done by analyzing 24 published studies on either low and zero calorie sweeteners' associations with mental health disorders, mental health disorders' associations with the GI tract, and low and zero calorie sweeteners' associations with the GI tract. Overall, it was concluded that low and zero calorie sweeteners are mediating mental health disorders and mental health disorders are altering the GI microbiome composition. Specifically, increased sucralose consumption and increased depression prevalence and severity of symptoms were associated with the GI microbiome through decreasing the abundance of the *Bacteroides* genus. Additional evidence supporting whether other L/Z calorie sweeteners are mediating mental health disorders through the GI microbiome is not robust. It is advised that additional research be conducted on whether the low and zero calorie sweeteners that are affecting mental health disorders are affecting similar GI bacteria that the mental health disorders are affecting. This additional research could be done via experiments analyzing the effects of varying pre-measured amounts of L/Z calorie sweeteners that are below the average daily intake limit in human subjects.

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1. Comprehensive Review of the Literature

Mental health disorders, such as anxiety and depression, have been increasing in both prevalence and severity substantially over the last 25 years, with them remaining a top 10 leading cause of burden (i.e., years lived with disability (YLDs), years of life lost (YLLs), and disability-adjusted life-years (DALYs)) worldwide since 1990 [1]. “A mental disorder is characterized by a clinically significant disturbance in an individual’s cognition, emotional regulation, or behavior” [2]. There are many kinds of mental health disorders, with anxiety and depression being among the most prominent [3]. Anxiety disorders are characterized by excessive fear and worry, with symptoms ranging from mild to significantly impairing. There are numerous different anxiety disorders but generalized anxiety disorder (GAD) is the most common. On the other hand, depression is characterized by feeling irritable, sad, empty, a loss of interest in previously enjoyed activities, lethargic, hopeless, and having poor concentration- to name a few. Both anxiety and depression disorders are more common in women than men, with the onset age being about 19. In 2019, approximately 970 million people worldwide (1 in every 8) suffered from a mental health disorder and in 2021, it was estimated that there was an additional 53.2 million cases of depression (27.6% increase) and 76.2 million cases of anxiety disorders (25.6% increase) globally due in part to the COVID-19 pandemic [1]. Along with being a top leading cause of YLLs and DALYs worldwide, these mental health disorders are a public health issue due to their high economic burden for societies. There is a need for more research in this area to increase the quality of life of almost a billion people world-wide and decrease YLDs and YLLs.

There has been a dire need in society to find the pathogenesis of these mood disorders. In past years, many researchers have investigated the etiology of these disorders such as “disturbances in peripheral and central metabolites” [4, 5], endocrine system dysfunction [6, 7],

altered neurotrophic activity such as levels of brain derived neurotrophic factor [8], inflammation [9], and Hypothalamic-Pituitary-Adrenal axis dysfunction [10], but there haven't been any universally accepted associations. Another possible association to mental health disorders' increasing severity and prevalence that has continued to circulate in the scientific community is low/zero (L/Z) calorie sweeteners consumption.

1.1 Aim 1: Low/Zero Calorie Sweeteners and Mental Health Disorders

L/Z calorie sweeteners are alternative sweeteners to traditional sucrose and fructose sweeteners that have increased in use world-wide due to the increasing consumption of sweet-tasting factory-made foods [11]. The reason for their rise in popularity is that they are lower in calories, associated with lower rise in blood sugar, and some have been found to not breakdown tooth enamel like traditional sweeteners do [11]. L/Z calorie sweeteners are comprised of both low- and zero- calorie sweeteners. Zero calorie sweeteners have a much higher sweetening intensity (200-20,000 times sweeter) and significantly less caloric density by weight compared to their traditional sucrose and glucose-based counterparts. Zero calorie sweeteners are broken down into synthetic, meaning man-made, and natural sweeteners. Natural zero-calorie sweeteners approved for use in food and drinks are monk fruit extract and steviol glycosides (commonly known as Stevia). Synthetic zero-calorie sweeteners are: acesulfame potassium (ace-K), advantame, aspartame, neotame, saccharin, and sucralose. Low calorie sweeteners, which are newer than many of the zero calorie sweeteners, are "low digestible carbohydrates derived from the hydrogenation of their sugar of syrup source" [11]. Low calorie sweeteners are sugar alcohols that are slightly decreased in calories and about 20 to 25% as sweet as glucose or sucrose [11]. Sugar alcohols that are commonly used in foods and drinks are: erythritol, isomalt,

lactitol, maltitol, mannitol, sorbitol, xylitol, and d-tagatose. For the sake of this literature review, both zero-calorie (synthetic and natural) and low-calorie sweeteners will be referred to as L/Z calorie sweeteners. Of all the L/Z calorie sweeteners, the most commonly used ones in foods and beverages to-date are ace-K, aspartame, saccharin, and sucralose and this is due to them being the oldest L/Z calorie sweeteners on the market earning approval from the Food and Drug Administration (FDA) in 1988, 1981, and 1958, and 1998 respectively [12]. They make up more than 50% of the L/Z calorie sweetener market [12]. Since their creation and approval, the rise in consumption of these L/Z calorie sweeteners and creation of new ones has grown exponentially [13]. Within the first decade that these L/Z calorie sweeteners began to gain popularity (1991-1999), 10% of the United States (US) population consumed them in their beverages and around 3-5% consumed them in their foods, with consumption rates jumping up by 37.7% and 14.2% for beverages and foods respectively from 1989 and 2004 [13]. They have continued to climb since then. Additionally, the consumption in weight has increased drastically as well. Dunford et al. [14] compared the types and amounts of L/Z calorie sweeteners purchased by US households and found that the consumption of these L/Z calorie sweeteners in foods and beverages combined jumped from 10.8 g/capita/day in 2002 to 36.2 grams/capita/day in 2018. Additionally, Dunford et al. [14] found that these L/Z calorie sweeteners are increasing in consumption rates, because they are appearing in more and more food and beverage products with the proportion of US households purchasing food and beverage products containing only these L/Z calorie sweeteners jumping up from 65.7 to 67.2% and from 46.7% to 74.1% for foods and beverages that contained both caloric sweeteners (sucrose and/or glucose) and L/Z calorie sweeteners combined [14]. Not only are their consumption by weight and the number of products they are appearing in increasing, so are the number of different L/Z calorie sweeteners available on the market. When

analyzing the estimated volume of food and beverage products containing specific kinds of L/Z calorie sweeteners purchased in US households in 2002, aspartame, sucralose, and "all other [L/Z calorie sweeteners]" were found to have the highest consumption rates at 94.7, 15.4, and 40.3 g/capita/day, respectively [14]. However, in 2018 that all shifted with aspartame decreasing to 80 g/capita/day and sucralose and "all other [L/Z calorie sweeteners]" increasing to 489.4 and 91.9 g/capita/day respectively. Additionally, Stevia increased from 0 g/capita/day in 2002 (due to it not being approved for use until 2008) to 7.6 g/capita/day in 2018 [14]. The massive increase in "all other [L/Z calorie sweeteners]" is due in part to the number of new L/Z calorie sweeteners created and approved for use between 2002 and 2018 such as: advantame (2014), neotame (2002), monk fruit extract (2010), steviol glycosides (2008), and all of the sugar alcohols [11]. This same trend of increasing L/Z calorie sweeteners consumption by weight/person, increasing availability by variety of L/Z calorie sweetener, and increasing percentage of products containing these sweeteners is seen not only in the US, but all over the world [15, 16]. North America, Europe, Central Asia, Latin America and the Caribbean had the highest non-nutritive sweetener sales by g/capita compared to North and Sub-Saharan Africa, Middle East, South and East Asia, and the Pacific and these sales are expected to see a +128.9%, +34.97%, and +15.6% growth respectively from 2007 to 2025 [16]. With L/Z calorie sweeteners appearing in a larger percentage of foods and beverages and increasing in consumption weight and rate around the world, it may be possible that the L/Z calorie sweeteners' increasing trends may be partially responsible for the increasing trends of prevalence and symptom severity seen in mental health disorders.

L/Z calorie sweeteners have seen an increase in consumption over the past 30 years [16] and given so has mental health disorder prevalence and severity [1]. I chose to compile published

works that studied whether these sweeteners may be associated with the prevalence and/or severity of mental health disorders-specifically anxiety and depression. I sought to investigate this through collecting human and animal studies that examined L/Z calorie sweeteners' exposure effect on mental health disorders' prevalence and severity and consolidating their findings. Scientists that used humas as subjects analyzed the association of L/Z calorie sweeteners on mental health disorders through diagnostic rates and symptom severity, whereas scientists that used animals as subjects analyzed L/Z calorie sweeteners' effects by measuring behavioral changes characteristic of anxiety and depression [17, 18].

1.2 Aim 2: Mental Health Disorders and The Gut Microbiome Composition

Alongside L/Z calorie sweetener intake, another possible attribute to mental health disorders' increasing severity and prevalence that has continued to circulate in the scientific community is the gastrointestinal tract (GI) microbiome [5]. Specifically, GI microbiome composition changes have been an area of increasing interest in relation to mental health disorders. There have been a few preliminary studies done on how the gut microbiome affects mood disorders through the gut-brain axis [5, 19]. The gut-brain axis is believed to be an elaborate pathway that consists of bidirectional communication between the intestines, the central nervous system (CNS), the autonomic nervous system, enteric nervous system, hypothalamic-pituitary-adrenal axis, and the microbiota [20]. It is believed to impact mood, cognition, and mental health [20]. The gut microbiome, an aspect of the gut-brain axis, is a "community of…bacteria, viruses, and fungi inhabiting the human gastrointestinal (GI) tract" [21]. The GI microbiome's bacteria are comprised of anaerobes, organisms that do not require oxygen to survive, and aerobes, organisms that require oxygen to survive [22]. Some of the most

common bacterial species that exist in the GI microbiome are members of the Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria phyla [22]. A majority of the bacteria that make up these phyla are anaerobes, because much of the GI microbiome has limited oxygen available. However, there are aerobes, just in less abundance and they are usually found in the upper GI tract where there is more oxygen availability [22]. The GI tract is responsible for many biological processes in the body such as: metabolism of nutrients, protecting the host through immune system development, hormone regulation, and food digestion, food absorption, and food elimination [22].

Within the last 20 years, there has been a major shift in focus on the gut microbiome's role in the gut-brain axis. One of the first major findings that lead to the belief that the gut microbiome impacted the brain was about 30 years ago when patients suffering from a neuropsychiatric disorder, known as hepatic encephalopathy, saw improvements after being administered oral antibiotics [23]. Hepatic encephalopathy is a neurological disorder caused by a dysfunction in filtration abilities of the liver [24]. The antibiotics were changing the composition of the gut and ultimately improving this disorder of the brain [23]. This finding suggested an association between the GI tract microbiome and brain disorders, thus making the scientific community shift their focus on to other brain disorders that the gut microbiome may mediate- such as mental health disorders. Specifically, the question was raised of whether there are gut microbiome composition differences between people who suffer from mental health conditions compared to those who do not. It is understood that the GI tract microbiota of each person has a unique distribution and abundance of various bacterial taxa; however, there are similarities among healthy people [22]. In the years following the study on hepatic encephalopathy, there have been associations found between mental health disorders, such as anxiety and depression,

and the gut microbiota composition [5, 25]. I sought to look further into this connection by collecting studies that analyzed this potential association and comparing their findings to see if there were particular microbes associated with the onset of these disorders and/or their fluctuating severity levels among each individual. If an association was found between L/Z calorie sweeteners and the GI microbiome and there was one found between L/Z calorie sweeteners and mental health disorders, then my next thought was to collect data on whether L/Z calorie sweeteners are associated with the gut microbiome composition. I was looking to see, if an association was found, whether L/Z calorie sweeteners were associated with similar GI bacterial taxa that mental health disorders were associated with.

1.3 Aim 3: Low/Zero Calorie Sweeteners and the Gut Microbiome Composition

Numerous aspects have been found to alter the gut microbiome such as: age, geographical location [26], maternal microbiota composition when in-utero [27], mode of delivery at birth [28], antibiotic usage [26], pre- and probiotic use [29], and diet [30]. Specifically, diet has been a large focal point in the gut microbiome research field [31]. Beginning as a newborn, diet has been seen to affect the gut microbiome composition of infants who were breastfed, seeing *Lactobacillus* and *Bifidobacterium* as dominate genera, and formula-fed babies having *Enterococcus*, *Streptococcus, Enterobacteria, Bacteroides*, and *Clostridia* as dominate species [32]. Diet has been seen to continue to modulate the GI microbiome composition beyond infancy through the study of the gut microbiomes of vegetarians vs non-vegetarians [30]. People who consume vegetarian diets have been seen to have a higher abundance of gut microbiome species that metabolize insoluble carbohydrates such as *Roseburia, Eubacterium*, and *Ruminococcus* [30], while subjects who consumed non-vegetarian

diets have possessed a lower abundance of the Firmicutes phylum that metabolizes dietary plant polysaccharides and an increase in the Bacteroides phylum, which is composed of bile-tolerant microorganisms [31]. Additionally, the length of time of exposure to a new diet has been shown to impact the GI tract microbiota composition, as well. Duration of exposure to macronutrient intake changes has shown an indirect relationship on the number of changes seen in the gut's composition [31]. Though many studies have focused on how diet, specifically vegetarian vs non vegetarian and western vs eastern diets can facilitate changes in the GI microbiomes of subjects, there has been limited research on how consuming foods manufactured with additives, such as low/zero (L/Z) calorie sweeteners may be impacting the gut microbiome.

With the rise of L/Z calorie sweetener use, and the connection of the brain-gut, I sought to see whether these L/Z calorie sweeteners were driving the change seen in the gut microbiome. L/Z calorie sweeteners have been found to influence the gut microbiome through glucose metabolism, amino acid metabolism, weight gain, and potentially metabolic byproducts of gut microbiota [33]. All of these physiologies are acted on by the gut microbiome. I sought to investigate how these L/Z calorie sweeteners specifically affected the gut microbiome composition by comparing and contrasting a select group of studies with comparable study parameters.

1.4 Overall Aim: Are L/Z Calorie Sweeteners Acting through the Gut Microbiome to Mediate Mental Health Disorders?

The goal was to examine whether L/Z calorie sweeteners were acting through the gut microbiome to mediate mental health disorders. There is a lack of research in the connection between L/Z calorie sweeteners and mood disorders. Researchers have identified the associations

with L/Z calorie sweeteners to explain many different morphometric measurement changes and metabolic disorders, but studies on mental health impacts are more limited. Of the few studies that have looked at the connection between L/Z calorie sweeteners and mental health disorders, very few have looked at the underlying mechanism through which these associations are being made. Considering the public health implications of the rise in mental health disorders' prevalence and severity, any associations found could provide beneficial leads to further research or advancement in treatments for these mental health disorders. Through these findings, the goal was to see if the rise in consumption of L/Z calorie sweeteners was a contributing factor to the rise in mood disorder prevalence and severity-specifically anxiety and depression. If L/Z calorie sweeteners are found to impact mental health disorders through altering the composition of the GI microbiome, it could prompt additional research to be done in the advancement in the treatment of these mental health disorders.

1.5. Problem Statement:

Mental health disorders are rising in prevalence and severity around the world which is causing increasing YLLs and DALYs worldwide. There are some data that looks at L/Z calorie sweeteners' effects on mood disorders [18, 34]. However, there is a lack of research looking at the mechanism by which L/Z calorie sweeteners may be mediating mood disorders. The literature on L/Z calorie sweeteners' impact on mental health disorders, mental health disorders' associations with the gut microbiome, and L/Z calorie sweeteners' associations with the gut microbiome that are available require careful cross examinations and comparisons to understand the robustness and consistency of the findings in order to make broader generalizations so that an

association between L/Z calorie sweeteners' impact on mental health disorders through the GI microbiome can be established or refuted.

1.6 Purpose Statement:

This systematic literature review will evaluate published studies to establish the consistency of the associations between L/Z calorie sweeteners and mental health disorders, associations between the gut microbiome composition and mental health disorders, and associations between L/Z calorie sweeteners and the gut microbiome composition. Published scientific literature on these topics that fit within the parameters of the inclusion and exclusion criteria will be compared. Additionally, descriptions of symptoms of these mental health disorders and diagnostic criteria used will be cross referenced with one another so that a fair comparison of results of each of the studies can be conducted. The overall purpose is to evaluate whether L/Z calorie sweeteners are acting through the gut microbiome to mediate mental health disorders.

1.7 Research Questions:

This thesis' overarching question is: Are L/Z calorie sweeteners acting through the gut microbiome to mediate anxiety and depression? Through the following three aims, I will examine this potential association.

Aim 1: Is there an association between L/Z calorie sweeteners and mental health disorders-anxiety and depression?

Aim 2: Is the gut microbiome composition associated with anxiety and/or depression?

Aim 3: Are L/Z calorie sweeteners affecting the gut microbiome composition?

1.8 Significance Statement:

Considering the public health implications of the rise in mental health disorders' prevalence and severity, any associations found could provide beneficial leads to further research or advancement in treatments for these mental health disorders. If associations between L/Z calorie sweeteners and mood disorders are found to be made through alterations in the gut microbiome, alternative or additional remedies to aid in the treatment of mental health disorders could be theorized and new regulations could be imposed on L/Z calorie sweeteners with the goal that less people around the world would be succumbing to these disorders.

2.0 Methods

2.1. Search Strategy

Systematic searches were conducted in early 2023 using the search engine Google Scholar and the following databases: PubMed, Scopus, Web of Science, Science Direct, PsycINFO, Nature, Wiley Online Library, Taylor and Francis Online, Elsevier ScienceDirect, Scopus, SpringerLink, JSTOR, and Proquest. Database searches were supplemented by searching through bibliographies of other relevant published articles that were relevant to the aim question being researched.

2.1a Aim 1: Is There an Association Between Mental Health Disorders and L/Z Calorie Sweeteners?

Phrases were searched by mental health disorder, L/Z calorie sweetener name or phrase. Initially, the following broad words for mental health disorders were searched: mental health disorder(s), mood disorder(s), or psychiatric disorder(s). Each mental health disorder had a different set of words. For “depression”: depression or depressive disorder or MDD or major depressive disorder or persistent depression disorder. For “anxiety”: Anxiety or generalized anxiety disorder or GAD or phobia disorder or panic disorder. Additional mental health disorders were searched for using the following words: schizophrenia, multiple personality disorder, and bipolar. Initially, I discovered the use of symptoms characteristic of these different mental health disorders were used in research in animals. Therefore, the following additional words were added in conjunction with the various mental health disorder names to broaden findings in the animal research space: emotional or locomotor behavior, immobilization, fear, and memory or learning retention. For L/Z calorie sweeteners the following phrases and their associated

acronyms were used: non-nutritive sweetener (NNS), low calorie sweeteners (LCS), non-caloric sweeteners (NCS), low/zero calorie sweeteners (L/Z). Additionally, each L/Z calorie sweetener approved for use by the FDA was searched for: acesulfame potassium (ace-K, ace K) or Sunett or Sweet One, advantame, aspartame or NutraSweet or Equal, neotame or Newtame, monk fruit extract, saccharin or Sweet'N Low, steviol glycosides or PureVia or Truiva or Stevia in the Raw, sucralose or Splenda, D-Tagatose, erythritol or Swerve or Zsweet, isomalt, lactitol, maltitol, mannitol, sorbitol, or xylitol or XyloSweet or Smart Sweet.

2.1b Aim 2: Is the Gut Microbiome Composition Associated with Mental Health Disorders?

Phrases used to search the databases were: mental health disorder and gut microbiome, mental health disorder and gastrointestinal tract, mental health disorder and gut, mental health disorder and microbial changes, and mental health disorder and gut microbiome. Additionally, key words used for specific mental health disorders were used in replacement of "mental health disorder" in the previous 5 phrases mentioned to create additional search phrases to broaden the search results. Keywords used for specific mental health disorders remained the same as for aim 1, mentioned in detail in section 2.1a.

2.1c Aim 3: Are L/Z Calorie Sweeteners Affecting the Gut Microbiome Composition?

This search method was very similar to the search method used for aim 2. However, instead of "mental health disorder," "L/Z sweetener" was used. Phrases used to search the databases were: L/Z calorie sweetener and gut microbiome, L/Z calorie sweetener and gastrointestinal tract, L/Z calorie sweetener and gut, L/Z calorie sweetener and microbial changes, and L/Z calorie sweetener and gut microbiome. To create additional search phrases,

keywords used for specific L/Z calorie sweeteners were used in place of “L/Z calorie sweetener”. The additional phrases/names of L/Z calorie sweeteners used were the same as in Aim 1 (detailed in section 2.1a) and the keywords used for gut microbiome were the same as in Aim 2 (detailed in section 2.1b).

2.2 Inclusion and Exclusion Criteria:

2.2a Aim 1: I**s There an Association Between Mental Health Disorders and L/Z Calorie Sweeteners?**

Inclusion criteria were both human and animal studies, studies performed within the last 30 years, and any gender. Only articles published in the last 30 years, between 1993 and 2023, were included. There were no study size restrictions. For animal studies, any species were included and for human studies there were no race, geographical location, gender, or age restrictions. Additionally, there were no specific diagnostic method restrictions for identifying mental health disorders. Self-reported, professionally diagnosed, and all diagnostic tests were appropriate. This was to diversify the findings so that the conclusions from this literature review could be applicable to many different people all over the world. Any tests used to identify symptoms of the various mental health disorders were permitted such as: open field tests, suspension tests, elevated maze, water maze, shuttle maze, etc... Exclusion criteria were any studies that looked at the effects of L/Z calorie sweeteners solely on neuro-chemical outcomes. This is due to the known aspect in the scientific and psychiatric/medical community that neuro-chemical levels are not directly proportional to mental health disorder severity or prevalence [35]. Studies that solely investigated the effects of L/Z calorie sweeteners on molecular or

cellular outcomes in specific brain regions, such as the impact of L/Z calorie sweeteners on apoptosis in the hippocampus, were also excluded.

2.2b Aim 2: Is the Gut Microbiome Composition Associated with Mental Health Disorders?

Both animal and human studies were included. The following methods to analyze the composition of the GI microbiome were included: shotgun sequencing, 16s rRNA sequencing, and comparative proteomics. Any diagnostic methods for identifying mental health disorders were permitted such as: self-reported, professionally diagnosed, and all diagnostic tests. All studies that did not look at the composition of the GI tract were excluded, along with studies that only sequenced a portion of the gut microbiome. For example, if they performed a selective analysis of the GI microbiome composition, it was excluded due to the limited ability to compare with other studies' findings. Time frame was restricted to the last 10 years-2013 to 2023. This restriction was due to the greater volume of studies found initially, which differed from aim 1, where any studies in the last 30 years were permitted. This was due to the more limited quantity of available data when searching for aim 1. Additionally, any studies with less than 40 subjects were excluded due to the vast number of studies completed with over 100 subjects. Regarding techniques used and data reported, any study that did not report any bacterial taxa below phyla were excluded, meaning they must have at least reported in one other category: family, genera, and/or species.

2.2c Aim 3: Are L/Z Calorie Sweeteners Affecting the Gut Microbiome Composition?

In vivo studies were included, meaning the L/Z calorie sweetener was ingested by the subjects and then the microbial samples from fecal matter were analyzed. Any *in vitro* and *ex vivo* studies were excluded. Any studies performed in the last 15 years, between 2008-2023, were included. Studies were excluded if they *only* reported gut microbial composition results after exposure to L/Z calorie sweetener(s) in conjunction with antibiotics. Additionally, if the subjects had been antibiotic free at least 6 months prior to the study beginning, then a study was permitted for use. Additionally, studies that *only* reported on L/Z calorie sweetener's effects on the GI microbiome in respect to food intake, metabolic and/or carbohydrate dysregulation, other GI functions, and/or weight gain were excluded.

2.3 Measurements

2.3a Mental Health Disorders' Assessments

Each study had a way of assessing the diagnosis and/or severity of the mental health disorders that were presented in each subject. All studies used more than 1 method as a means for evaluating these mental health disorders. The 4 main methods for assessing mental health disorders in humans were the Diagnostic and Statistical Manual of Mental Disorders (DSM) (both the 4th and 5th generations), Hamilton depression and/or anxiety scale, the Vandenberg Mental Test, and self-reporting scales.

The DSM is a medical book written by the American Psychiatric Association that is used by medical professionals to reference different mental health disorders [36]. The DSM highlights how medical professionals should use the book, specific diagnostic criteria and codes, and provides information on different diagnostic tools that can be used to diagnose the different mental health disorders [36]. The Hamilton Depression Scale and Hamilton Anxiety Scale are

both used to quantify the severity of depression or anxiety symptoms using a 17-item self-administered survey format [37]. The test has 17 different characteristics of depression and/or anxiety. Each question is given a scale anywhere from 0-4 where a patient can rank how they feel a particular action and/or feeling applies to them. For example, one question lists “Suicide” and then gives the following answer options: “0-Absent, 1-feels life is not worth living, 2-wishes he/she were dead or any thoughts of possible death to self, 3-ideas or gestures of suicide, or 4-attempts at suicide” [38]. A different test for measuring depression symptoms is the Vandenberg Mental Test (Vandenberg MRT) [39]. This evaluates spatial orientation, a component of working memory, which is used for storing and manipulating an environment. Decreased spatial orientation is a symptom of depression [39]. There were many different self-reporting scales such as: Zung’s Self-Reporting Depression and Irritability Scale and individualized evaluation surveys created by the researchers who performed the experiments. Zung’s Self Reporting Depression Scale is a 20-statement diagnostic test that can be answered with any of the following responses: a little of the time, some of the time, a good part of the time, and most of the time [40]. Some examples of the statements included are: “I feel hopeful about the future,” “I am more irritable than usual,” and “ I have crying spells or feel like it” [40]. Individualized evaluation surveys created by the researchers who performed the experiment often resembled the same model as Zung’s Self-Reporting Depression Scale and the Hamilton Depression/Anxiety Scale where multiple symptoms of depression and/or anxiety were given, and the patient was provided with fixed responses that had a number correlated to them for which they could respond [41, 42]. At the end, the responses were totaled according to their associated number and then their depression or anxiety was ranked on a severity scale.

In animal studies, evaluation of behaviors anthropomorphized to be representative of anxiety or depression were performed using behavioral tests such as: Morris water maze, tail suspension, forced swim tests, elevated plus maze test, and open field tests. The Morris water maze evaluated the animals' visual spatial memory (specifically hippocampus-dependent spatial reference memory) and memory recall, both of which are an evaluation of depression [43]. "Depressed individuals typically show poor memory for positive events, potentiated memory for negative events, and impaired recollection" [43]. Tail suspension evaluates depression by dangling the subjects by their tails and measuring the amount of time spent immobile [44]. Immobility increasing is representative of depression because it shows an inability to cope with a stressful situation. A characteristic of depression in individuals is a decreased ability to cope with stressful situations and the tail suspension test demonstrates this in animals by putting the animals in a stressful situation (suspending them by their tails) and their immobility represents giving up [44]. The forced swim test evaluates depression symptoms by measuring immobility as well [45]. Elevated plus mazes evaluate anxiety by measuring the amount of time spent in the open arms compared to the closed arms and the number of entries into each arm. An increase in the amount of time spent in the open arms and an increased number of entries into each arm is indicative of less anxiety, because it shows decreased fear [34, 45]. In open field tests, the animals were given free-range to an open field and the amount of time spent in the center of the field (25% of the field) was a measure of anxiety [46]. The more time spent along the walls of the maze, the more anxious the animal was. Rodents naturally have an aversion to open, light areas (the center of the maze) due to fear of the unknown because it is seen as dangerous-a characteristic of anxiety [46, 47].

2.3b Gastrointestinal Tract Microbiome Composition Assessments

Studies that investigated whether the GI tract microbiome composition is associated with mental health disorders [4, 25, 48-54] and those which investigated whether L/Z calorie sweeteners are affecting the GI microbiome composition [55-60], evaluated the composition of the GI microbiome using either 16S sequencing or shotgun sequencing, with the exception of one study, [49], that used comparative proteomics. 16S ribosomal RNA (rRNA) sequencing is a means to determine the composition of a microbial community and identify the bacterial species in a sample [61]. 16S sequencing uses a hypervariable portion of the 16S rRNA gene, so that its unique sequence can be amplified, sequenced, and identified. The 16S rRNA gene is amplified by polymerase chain reaction (PCR) and sequenced to determine the specific DNA sequence of the sampled region. Once sequenced, a database of reference sequences from known microbial organisms is used to provide the composition and diversity of the sample species present by matching the DNA sequences obtained to the reference sequences [61]. Shotgun sequencing, on the other hand, is a form of sequencing that differs from 16S in that it sequences all of the DNA in the entire community of the gut microbiome, such as bacteria, viruses, fungi, etc. [62]. The final product results in gene content, order, variation, and more [62].

Comparative proteomics was only used by one study included in this analysis [49]. Comparative proteomics differs from both 16S and shotgun sequencing in that it analyzes proteins expressed by the microbial community instead of the DNA sequences [49]. In this method, proteins are extracted from stool, separated, and sorted by differing physiological characteristics, such as: charge, hydrophobicity, size, and other various chemical and physical properties. From there, mass spectrometry is used to identify the proteins by mass-to-charge ratio and their abundances, and the result is compared to a database of microbial species by their

peptide sequences to identify the specific species of microbes. Comparative proteomics is most commonly used to identify proteins expressed by the gut microbiome to assess functional roles of the microbe, such as energy metabolism, immune regulation, and the breakdown of complex molecules [49]. However, the abundance and presence of certain protein markers identified can be used to identify the types of bacteria present compared to 16S sequencing or shotgun sequencing that sequence the 16S rRNA gene or all DNA fragments to identify bacterial composition [49].

3.0 Results

3.1 Aim 1: Is There an Association Between Mental Health Disorders and L/Z Calorie Sweeteners?

In past years, there has been evidence that points towards an association between using L/Z calorie sweeteners and the prevalence and severity of mental health disorders, such as depression and anxiety [63]. I sought to compile the data to determine the strength of the association between these two by performing a systematic literature review. Through my systematic literature review, I found that more than 90% of the comparable studies have shown that there is a positive association between anxiety and depression and the use of L/Z calorie sweeteners.

The methodologies, which are detailed in section 2.1, describe the parameters I used to search for and evaluate prior studies which examined associations between mental health disorders and L/Z calorie sweeteners. Using key terms and specific parameters, studies were narrowed down. There were only 2 human studies that specifically measured the effects of a fixed amount of L/Z calorie sweeteners consumed on a pre-diagnosed mental health disorder [39, 41]. The remaining human studies had unspecified amounts of L/Z calorie sweeteners [17, 42, 64]. All of the animal experiments had fixed L/Z calorie sweetener consumption rates per individual [18, 34, 47, 65]. Additionally, these experiments focused on behaviors that were characteristic of specific mental health disorders, since animals are not diagnosed with mental health disorders. Behaviors and emotions are the main criteria used to diagnose these disorders in people [36]. Therefore, these animal experiments that measured behavioral outcomes characteristic of mental health disorders were included. The result left me with 9 studies-5 of which were human studies and the remaining 4 were animal experiments. Each of these studies

are summarized in Table 1. Table 1 depicts the association between mental health disorders and sweeteners analyzed by each of the included studies. As all studies have, there were limitations that must be identified and taken into account when considering the results which are further discussed in section 4.3a. However, all these studies combined provided evidence that L/Z calorie sweetener intake correlates with mental health disorders' prevalence and severity of symptoms.

Table 1: Articles Analyzing L/Z Calorie Sweeteners' Potential Associations with Mental Health Disorders

[1]	***Year***	***Country***	***Cohort size***	***Species***	***Matched variables***	***Population details***	***Mood disorder(s)***	***Sweetener analyzed***	***Exposure***	***Association and Outcomes?***
Lindseth, G. N., et al. (2014).	2014	USA	n=28	human	Group size	Healthy, Midwestern university students, aged 20-40yrs (mean age=20.8yrs) avg BMI= 24.1	Depression: -VMT -ZSRDS -ZSRIS	aspartame 1.) High = 25/mg/kg/day 2.) Low = 10 mg/kg/day	16 days (8 days/exposure)	Yes Impaired cognitive spatial orientation with high intake Increased depression and irritability with high intake
Walton, R. G., et al. (1993).	1993	USA	n=40	human	subjects=controls	subjects; over 24-60 5 men, 3 women, 5 on antidepressants controls: age-24-56 3 men, 2 women MDD under control ("doing well")	MDD: Brief Psychiatric Rating Scale after exposure	aspartame 30 mg/kg of body weight	20 days	Yes: * study was stopped early due to the severity of reactions to aspartame of what was collected: higher depression symptoms, and fatigue than before

[1] **Notes:** It is organized with First 5 studies human studies, last 4 are animal studies

Abbreviations: HC, healthy controls; US, unspecified amount; SR, self-reported; MDD, major depressive disorder; GAD, generalized anxiety disorder; VMT, The Vandenberg Mental Tests; ZSRDS, Zung's Self-Rating Depression Scale; ZSRIS, Zung's Self Rating Irritability Scale; DSM, Diagnostic and Statistical Manual for Mental Disorders; EPM, elevated plus maze test; FST, forced swim test; TS, tail suspension; OFT, open field test; SM, shuttle maze; WM, water maze

[1]	***Year***	***Country***	***Cohort size***	***Species***	***Matched variables***	***Population details***	***Mood disorder(s)***	***Sweetener analyzed***	***Exposure***	***Association and Outcomes?***
										consuming aspartame and then the controls after they consumed aspartame
Guo, X., et al. (2014)	2014	USA	n=263,923	human	N/A	participants of the NIH-AARP Diet and Health Study age: 50-71	Depression: -SR	US	1 year	yes: consumption was associated with a higher risk of depression
Yu, Z. M., et al. (2017)	2017	Canada	n=18838	human	N/A	18-39 years old (5854 men and 12984 women)	Depression: -SR	US	N/A	yes: higher consumption=more depressed

[1]	***Year***	***Country***	***Cohort size***	***Species***	***Matched variables***	***Population details***	***Mood disorder(s)***	***Sweetener analyzed***	***Exposure***	***Association and Outcomes?***
Perez-Ara, M. A., et al. (2020).	2020	The Netherlands, Spain, the United Kingdom, and Germany	n=941	human	age, sex, marital status, BMI, physical activity, smoking %, blood pressure, MoodFOOD diet score	overweight adults subsyndromal depressive symptoms aged 18 to 75 years bmi: 25–40 kg/m2	MDD: DSM-IV Inventory of Depressive Symptomatology-Self Report	US	12 months	Yes: drinking drinks more frequently with NNS showed an inverse relationship to past MDD diagnoses *only significant in Spain* drinking soft drinks sweetened with NNSs = lower rates of MDD diagnosis rates in the past in comparison with drinking soft drinks sweetened with sugar
Abu-Taweel, G. M., et al. (2014).	2014	Saudi Arabia	n=40	animal (male Swiss albino mice)	place of origin	male Swiss albino mice 8–10 weeks old bred and reared under controlled conditions	Depression: -WM -SM	aspartame 32 mg/kg of body weight	1 month	Yes: exposure showed decreases in learning and memory retention capabilities (were poor learners and had worse episodic memories)

[1]	***Year***	***Country***	***Cohort size***	***Species***	***Matched variables***	***Population details***	***Mood disorder(s)***	***Sweetener analyzed***	***Exposure***	***Association and Outcomes?***
Kumar, M. and M. Chail (2019).	2019	India	N/A	animal (Swiss albino mice)	type 2 diabetes weight (20 and 25 grams) age (6-8 weeks old)	N/A	depression: -TS -FST anxiety: -EPM	saccharin 10% sucrose in water	normal experimental group: 28 days withdrawal group: 35 days reinstatement group: 38 days	Yes: saccharin exposure in T2D mice decreased depression-like behavior compared to sucrose group (table sugar) and the water-only group withdrawal group: Withdrawal from sucrose or saccharin increased depression symptoms, but withdrawal from saccharin was associated with fewer symptoms than withdrawal from sucrose or water. reinstatement group: saccharin exposure decreased anxiety-like symptoms compared to withdrawal saccharin group

[1]	***Year***	***Country***	***Cohort size***	***Species***	***Matched variables***	***Population details***	***Mood disorder(s)***	***Sweetener analyzed***	***Exposure***	***Association and Outcomes?***
Ashok, I., et al. (2014)	2014	India	n=18	animal (Wistar strain male Albino rats)	N/A	200-220g weight	Anxiety: -EPM	aspartame 75 mg/kg of body weight	90 days	yes: -increased anxiety -decrease in the emotional and locomotor behavior was seen -increase in fear
Jones, S. K., et al. (2022)	2022	USA	N/A	animal (C57BL/6 mice)	weight (subjects and controls chose by preference)	N/A	Anxiety: -OFT -EPM	aspartame 0.015% of water	12 weeks	yes: aspartame produced more anxiety like behaviors and produced a shift in the excitation-inhibition balance in the amygdala towards excitation (more anxiety)

In each of the human studies, the association between sweetener use and mental health disorders was investigated in either a controlled experiment or an observational study [17, 39, 41, 42, 64]. Each of the 5 human studies that were included created one cohesive meta-cohort made up of 283,770 people. Of the 5 human studies, all looked at the outcome of L/Z calorie sweeteners on MDD [17, 39, 41, 42, 64]. MDD and depression were diagnosed via Zung's Self-Reporting Depression Scale and Irritability Subscale by 1 study [39], the DSM in 1 study [42], a self-reporting scale in 2 studies [17, 41], Vandenberg MRT in 1 study [39], and diagnosed by a medical doctor (although no diagnostic criteria were given) in 1 study [64]. In each experiment/study, sweetener use was determined using different mechanisms. In 2 experiments, the subjects were given a specific diet that consisted of a specified amount of the L/Z calorie sweetener being investigated relative to the subjects' body weight [39, 41]. The amount of L/Z calorie sweetener given to each subject, if a subject weighed 150 lbs., would have been about 2,000 mg of aspartame/day, which is about equal to one 12-oz diet soda/day [39]. There was then a specific wash-out time in between the control and experimental data collection periods as the subjects also served as the controls in each of these experiments. Three out of five of the human studies were observational studies where specific L/Z calorie sweetener amount was not specified, but instead was evaluated on a population level by broad frequency terms such as never, sometimes and frequently [17, 42, 64]. These terms were defined by broad measurements such as "0 cups a day", "more than or equal to X cups per day", or "X times per day/week"[17, 42, 64]. Guo et al. [64] and Yu et al. [17] both performed prospective studies that permitted participation as long as L/Z calorie sweeteners were in tea or coffee and were consumed within the last year. Perez-Ara et al.[42], on the other hand, measured L/Z calorie sweeteners in non-

alcoholic carbonated drinks. Specific kinds of L/Z calorie sweeteners were not indicated in any of those 3 observational studies [17, 42, 64].

In all 5 human studies, there was an association found between MDD/depression and/or their presenting symptoms and L/Z calorie sweetener consumption [17, 39, 41, 42, 64]. Four out of five studies showed a direct relationship between L/Z calorie sweetener consumption and MDD/depression, so as the amount and/or frequency of L/Z calorie sweetener consumption increased, so did MDD/depression's prevalence/presenting symptoms [17, 39, 41, 64]. Perez-Ara et al. [42] was the only human study analyzed that showed that consuming a greater amount of L/Z calorie sweeteners led to a "lower rate MDD history diagnoses" [42]. Using odds ratios (OR), Perez-Ara et al [42] found that drinking > or = 1 drink (200 mL)/day of soft drinks sweetened with L/Z calorie sweeteners was associated with lower rates of MDD diagnosis rates in the past (Odds Ratio (OR) = 0.55) in comparison with lower consumption frequencies of soft drinks sweetened with L/Z calorie sweeteners (1-6 times/week had an OR= 1.10 and < or =1/week had an OR =1) and drinking soft drinks sweetened with sugar > or = 1 time/day (OR= 2.41)]. This meaning that individuals who consumed ≥1 drink (200 mL)/day of L/Z calorie-sweetened soft drinks had 0.55 times lower odds of being diagnosed with MDD compared to individuals who consumed sugar-sweetened soft drinks at a similar frequency (≥1/ day). Additionally, individuals that consumed sugar-sweetened soft drinks ≥1 time/day had 2.41 times higher odds of being diagnosed with MDD compared to the reference group (individuals drinking ≥1 drink (200 mL)/day of NNS-sweetened soft drinks)." It is important to notate that this study was performed in multiple countries (The Netherlands, Spain, the United Kingdom, and Germany); however, the results reported are only representing subjects residing in Spain, because they were the only subjects who when exposed to unspecified L/Z calorie sweeteners,

displayed a significant change in MDD prevalence. Additionally, Perez-Ara et al. [42] was the only study that looked at the effects of L/Z calorie sweeteners on mental health disorders in overweight and obese subjects.

In each of the 4 animal studies, the association between sweetener use was investigated in an experimental study [18, 34, 47, 65]. Three of the four studies used mice as the subjects, either C57BL/6 mice [47] or male Swiss albino mice [18, 34], and one study used Wistar strain male Albino rats [65]. Of the 4 animal studies, 2 studies looked at the outcome of L/Z calorie sweeteners on anxiety [47, 65], 1 study looked at depression [18], and 1 looked at both anxiety and depression [34]. Anxiety was analyzed by 3 experiments- one used both open field tests and elevated maze tests [47] and two used just elevated maze tests [34, 47, 65]. On the other hand, depression was analyzed using a water maze and shuttle maze [18] or tail suspension and forced swim test [34]. Of the L/Z calorie sweeteners that were included, 3 of the 4 studies used aspartame [18, 47, 65] and 1 study looked at saccharin [34], both of which are zero-calorie sweeteners. Saccharin was used in the 1 study that looked at both anxiety and depression [34]. All 4 animal studies had an average exposure time of 60 days (2 months) to the chosen L/Z calorie sweetener [18, 34, 47, 65]. Additionally, sweetener use was experimentally provided by giving the subjects a specific diet that consisted of a fixed amount of the L/Z calorie sweetener being investigated, relative to their body weight [18, 34, 47, 65].

In all 4 animal experiments, there was an association found between MDD/depression or anxiety and L/Z calorie sweetener consumption [18, 34, 47, 65]. Three of the four animal experiments showed a positive correlation between L/Z calorie sweetener consumption and the mental health disorder, so as the amount and/or frequency of L/Z calorie sweetener consumption increased, so did the mental health disorders' prevalence/their presenting symptoms [18, 47, 65].

However, Kumar et al. [34] was the only study that looked at both depression and anxiety and it was the only animal study analyzed that showed that consuming a greater amount of L/Z calorie sweeteners led to a decrease in both anxiety and depression-like behaviors. However, Kumar et al. [34] only experimented on animals induced with Type 2 Diabetes. The fluctuating blood sugar levels may have impacted the prevalence of anxiety and/or depression in these subjects. Elevated blood sugar levels have been shown to increase inflammation and increased inflammation has been associated with increased mental health disorder severity [66]. This would not have been a factor that contributed to the results seen in the other 3 studies.

Combining the outcomes of all 9 studies, both the experimental animal models and human observational studies, suggest that there is an association between L/Z calorie sweeteners and mental health disorders [17, 18, 34, 39, 41, 42, 47, 64, 65]. Seven of the nine studies suggested that increased exposure to L/Z calorie sweeteners increased anxiety and/or depression diagnoses and/or severity of symptoms associated with each of these mental health disorders [17, 18, 39, 41, 47, 64, 65]. However, 2 of the 9 studies showed an inverse relationship, indicating exposure to L/Z calorie sweetener led to a decrease in the prevalence of behaviors of anxiety and depression and a decrease in the diagnosis of depression [34, 42]. It is important to note that Kumar and Chail [34] only evaluated depression and anxiety behaviors on animal subjects that were induced to have diabetes. The fluctuating blood sugar numbers could be influential in the inverse relationship seen between the L/Z calorie sweetener and mental health disorder behaviors. Additionally, Perez-Ara et al. [42] was a retrospective study that was only performed in overweight and obese individuals, and the findings were only significant in subjects residing in Spain, compared to the other European countries where this study was performed (The Netherlands, United Kingdom, and Germany). Diet may account for this geographical difference.

While a majority of the studies found a positive association between exposure to L/Z calorie sweeteners and anxiety and/or depression diagnoses [17, 18, 38, 40, 46, 63, 64], the mechanism by which these L/Z calorie sweeteners may be impacting the brain's functions to modulate MDD/depression, GAD/anxiety, or any other mental health disorders is largely unknown. Like all dietary inputs, L/Z sweeteners can impact the microorganisms residing in the GI tract [55]. Given the emerging evidence that the GI microbiome may be associated with neurological conditions [24], I next investigated whether specific compositions of the GI microbiome were associated with mental health disorders.

3.2 Aim 2: Is the Gut Microbiome Composition Associated with Mental Health Disorders?

Whether the GI microbiome is associated with mental health disorders is a newer topic of discussion [67]. It began circulating as cultures around the world started acknowledging the importance of these different mental health disorders. Experimental evidence has suggested that the GI microbiome contributes to mental health disorders' prevalence and severity [67]. In order to holistically and concretely assess the potential for this association, I performed a systematic literature review of comparable human studies completed over the past decade in an attempt to identify whether the GI microbes were altered in humans with different mental health disorders, specifically MDD/depression and GAD/anxiety.

I performed a search of the gut microbiome and mental health disorders using the following terms: gut microbiome and depression, gut microbiome and anxiety, microbe changes and mental health disorders, and many more highlighted in section 2.1. Studies were included if they were performed in the last 10 years (2013-2023), performed on humans, had at least 40 subjects, and were observational studies. Additionally, each study looked at global GI

microbiome composition in stool samples, without preferentially testing for only a select few bacterial taxa. With my results that fit the parameters, 9 studies were chosen [4, 25, 48-54]. Each of these studies are described in Table 2. Table 2 depicts the association between the gut microbiome and MDD/depression or GAD/anxiety. All of these studies combined provided evidence that certain gut microbiome compositions are associated with impacting mental health disorders' prevalence and severity of symptoms.

All 9 studies had cohorts that ranged from 40-121 subjects, which in their entirety created a meta-cohort that consisted of 619 human subjects, with their ages ranging from 18-65 years old [4, 25, 48-54]. The 9 studies each used one of 3 different techniques were utilized to identify the makeup of the GI microbiome: 16S sequencing [4, 25, 48, 50, 52-54], shotgun sequencing [51], or comparative proteomics [49]. Each technique's details are further detailed in section 3.2. While each study matched different variables between the controls and the subjects, all but 1 study matched BMI [52] and all but 1 study matched age [4]. All of the studies focused on either MDD/depression or anxiety as the mental health disorder of interest: 6/9 articles on MDD/depression [4, 25, 49, 51-53], 2/9 on anxiety [48, 50], and 1/9 on both depression and anxiety combined [54].

Table 2: Studies Analyzing the Potential Association Between Mental Health Disorders and the Gut Microbiome Composition

2	Year	Country	Cohort size	Subjects	Matched variables	Population details	Mood disorder(s) and how they are identified	Method used	Association and Outcomes?
Chen, Z., et al. (2018).	2018	China	n=40	human	sex age BMI	MDD group: age: 18-56 50%men, 50% women Healthy Controls: age: 24–65 50%men, 50% women	MDD: DSM 4th edition Hamilton's Depression Scale	comparative protenomics	phyla breakdown: richness of MDD patients was higher for 2, lower for 2 Family breakdown: richness of MDD patients was higher in 9 families richness of MDD patients was lower compared to HC in 6 families genus breakdown: richness of MDD patients was higher in 5 genera, lower in 5 genera species level breakdown: richness of MDD patients was higher in 7 species, lower in 11 species

[2] **Notes:** First 6 studies are analyzing MDD/depression, last 3 are analyzing GAD/anxiety
Abbreviations: HC, healthy controls; MDD, major depressive disorder; GAD, generalized anxiety disorder; BMI, body mass index

2	Year	Country	Cohort size	Subjects	Matched variables	Population details	Mood disorder(s) and how they are identified	Method used	Association and Outcomes?
Huang, Y., et al. (2018).	2018	China	n=54	human	age height weight BMI	Ethnicity: Han Chinese residents living in Beijing for a long time BMI: 18 to 30 kg/m2	MDD: diagnostic criteria of ICD-10 MDD	16 S Sequencing and PCR	lower gut microbiota diversity in MDD patients -the percentage of firmicutes phylum is significantly higher in healthy controls than MDD samples.
Jiang, H., et al. (2015).	2013	China	n=76	human	age	age 18-40	MDD: DSM, 4th edition Hamilton's Depression Scale (HAMDS) Montgomery–Asberg Depression Rating Scale (MADRS)	16S Sequencing	more bacterial diversity in MDD patients than the controls Phyla differences: 3 more abundant in active MDD group than the controls, 2 less abundant Family differences: -5 more abundant in active MDD group than the controls, 6 less abundant genus level: 10 more abundant in the active MDD patients than the healthy controls, 5 less abundant

2	Year	Country	Cohort size	Subjects	Matched variables	Population details	Mood disorder(s) and how they are identified	Method used	Association and Outcomes?
Kelly, J. R., et al. (2016).	2016	Ireland	n=67	human	gender age BMI Metabolic Equivalent Task Units Hours sitting/day	n/a	Hamilton depression scale	16S sequencing	depressed group had decreased richness and phylogenetic diversity family differences: 2 increased in depressed group, 1 decreased Genus differences: -6 increased in depressed group, 2 decreased
Lai, W. T., et al. (2021).	2019	China	n=55	human	age gender BMI	MDD patients: age range: 32–52 HCs: age range: 28–51	MDD: DSM5, Mini-International Neuropsychiatric Interview Hamilton's Depression Scale-17 Hamilton's Anxiety Scale Hypomania Checklist (mania symptoms)	shotgun metagenomic sequencing	Actinobacteria increased in MDD patients compared to HC at phyla level 7 increased in MDD patients compared to HC at genus level 5 increased in MDD patients compared to HC at species level 1 decreased in MDD patients compared to HC: Bacteroidetes (phyla)

2	Year	Country	Cohort size	Subjects	Matched variables	Population details	Mood disorder(s) and how they are identified	Method used	Association and Outcomes?
Zheng, P., et al. (2016).	2016	China	n=121	human	gender BMI household composition to community composition controls	N/A	MDD: DSM-IV-TR (the Structured Psychiatric Interview) 17-item Hamilton Depression Rating Scale	16S RNA sequencing	In MDD people Actinobacteria in abundance, Bacteroidetes was decreased -In MDD individuals the 29/54 OTU's were over expressed healthy individuals 25/54 OTU's were over expressed
Chen, Y. H., et al. (2019).	2019	China	n=60	human	Sex age BMI smoking intake alcohol intake	age: 18-65 Ethnicity: Han Chinese	GAD: DSM, 5th edition Hamilton Anxiety Rating Scale Self-rating Anxiety Scale Self-rating Depression Scale	16S sequencing	community richness was lower in people with active GAD compared to the healthy controls -no significant community diversity differences between people with active GAD and healthy controls 9 richer in patients with GAD 22 Richer in healthy controls than GAD patients:

2	Year	Country	Cohort size	Subjects	Matched variables	Population details	Mood disorder(s) and how they are identified	Method used	Association and Outcomes?
Jiang, H. Y., et al. (2018).	2016	China	n=76	human	age sex BMI smoking status	age 21-55	GAD: DSM, 4th edition (the Mini-International Neuropsychiatric Interview) Hamilton Anxiety Rating Scale (HAMA)	16S sequencing	Yes: gut microbiota richness (measured by the number of OTUs) was lower in most individuals with GAD (40) compared with the healthy controls -Compositional differences at the phylum level: 1 lower in GAD patients, 2 were overrepresented -compositional differences at the genus level: 3 decreased in GAD patients, 5 enriched 5 more abundant in the HCs compared with treatment-naive GAD patients, 5 more abundant

2	Year	Country	Cohort size	Subjects	Matched variables	Population details	Mood disorder(s) and how they are identified	Method used	Association and Outcomes?
Mason, B. L., et al. (2020).	2019	USA	n=70	human	age BMI gender ethnicity (Hispanic vs non) on meds (on them or not)	Female:80% white: 75.5%, the rest non-white	MDD w/ anxiety, Depression w/o anxiety, and anxiety only Quick Inventory of Depressive Symptoms-Self-Rated (QIDS-SR)	16S sequencing	Depression and anxiety together: Clostridium leptum group was lower, Bacteroides group was significantly lower anxiety (independent of the presence of depression): reduced Bacteroides

Table 2 summarized each of the 9 articles analyzed [4, 25, 48-54]. All 9 articles identified an association between the GI microbiome and the studied mental health disorder(s) [4, 25, 48-54]. Additionally, all 9 studies showed microbial differences between healthy controls and individuals with differing mental health disorders. To evaluate the mental health disorders, the DSM and the Hamilton anxiety/depression rating scales (8/9 studies) [4, 25, 48-53] or a self-reported symptoms inventory questionnaire (1/9 studies) [54] were utilized. Notably, the only study that looked at microbiome alterations with both anxiety and depression combined was also the only study that used a self-reported inventory of symptoms to identify the mental health disorders in the subjects as opposed to the DSM and/or the Hamilton anxiety/depression symptomatic scales [54]. Six of the nine studies controlled for categorical variables such as sex, BMI, age, antidepressant use, smoking status, and/or drinking status [4, 47-50, 53]. No significant associations between microbial diversity and/or richness and the analyzed categorical variables (sex, BMI, age, antidepressant use, smoking status, or drinking status) were identified in any of the 6 studies [4, 48-51, 54]. Therefore, the alterations in the microbiomes seen cannot be attributed to those factors, thus making it more likely that any changes seen are more strongly associated with anxiety and depression. Huang et al. [25] and Kelly et al. [53] did not control for these factors to see if they had an influence on their results. Jiang et al. [52] did look at responded-MDD (medicated) and active-MDD (not medicated). Although, for the purposes of this literature review, the results from the responded-MDD patients were not included as it was a separate experiment with differing controls and subjects. Additionally, due to the fact that they were considered “responded,” they did not actively have a mental health disorder as defined by the methodology requirements for this literature review. Chen et al. [50] supported other published findings that the confounding factors of sex, smoking, and alcohol intake did all

significantly influence GI microbiome composition in individuals without a mental health disorder, in this case GAD [68, 69]. However, when these confounding factors (sex, smoking, and alcohol intake) were analyzed in individuals alongside a mental health disorder (GAD), these factors were no longer significantly impacting the GI microbial diversity or richness [50].

To strengthen this relationship, some studies not only analyzed changes in the abundance of phyla and family levels of bacteria but went as far as to look at the genus and species level. This is depicted in Tables 3, 4, 5, and 6. Which all summarize the differing abundances shown in subjects with these mental health disorders vs without in terms of bacterial kingdoms from broadest to most specific, respectively: phyla, families, genus, and species.

Table 3: Significant Phyla Alterations in Subjects with Mental Health Disorders

[3]	**Chen, Z., et al. (2018)**	**Huang, Y., et al. (2018)**	**Jiang, H., et al. (2015)**	**Kelly, J. R., et al. (2016)**	**Lai, W. T., et al. (2021)**	**Zheng, P., et al. (2016)**	**Chen, Y. H., et al. (2019)**	**Jiang, H. Y., et al. (2018)**	**Mason, B. L., et al. (2020)**
Actinobacteria	⬆		⬇		⬆	⬆			
Bacteroidetes and Fusobacteria	⬇		⬆		⬇	⬇	⬆	⬆	
Firmicutes (aka Bacillota)	⬆	⬇	⬇				⬇	⬇	
Proteobacteria (aka Pseudomonadota)	⬇		⬆						
Tenericutes (aka Mycoplasmata)							⬇		

Table 3: In the 6 articles focused on MDD/depression, at the phyla levels, 4 of the 6 studies found statistically significant changes at the Actinobacteria phylum level [4, 25, 49, 51-53]. Three of the 6 MDD studies found Actinobacteria to increase in individuals with MDD

[3] **Notes:** First 6 studies are analyzing MDD/depression, last 3 are analyzing GAD/anxiety, Fusobaceteria was placed with Bacteroidetes, because they were formally in the same group
Key: ⬇, decreasing in abundance; ⬆, increasing in abundance

compared to the healthy controls [4, 49, 51], while one, Jiang et al [52], found it to decrease. Those same 4 studies found changes in the abundance at the Bacteroidetes phylum: Jiang et al. [52] was the only study to find an increase and the remaining three [4, 49, 51] found a decrease in Bacteroidetes abundance. Jiang et al. [52] differed from the other 3 studies in that it was the only study to look at both active MDD (untreated with medication) and responded MDD (managed at the time of the study with medication). Per the parameters used for this literary analysis, only the active MDD participants' data was included. The other studies did not separate out people who were well-responded to medication vs those not. Firmicutes was found to have statistically significant differences in 3 MDD/depression studies; Chen et al. [49] found an increase in Firmicutes abundance in MDD individuals when compared to the healthy controls, while Huang et al. [25] and Jiang et al. [52] found Firmicutes to decrease. Chen et al. [49] differed from Huang et al. [25] and Jiang et al. [52] in that it had 20 participants, while Huang et al. [25] and Jiang et al. [52] had 54 and 76 participants respectively. Additionally, Chen et al. [49] used comparative proteomics to analyze the GI microbiota and Huang et al. [25] and Jiang et al. [52] both used 16S sequencing. Statistically significant changes in the Proteobacteria phylum were only notated in 2 out of all 9 studies and both of the studies were evaluating MDD; Jiang et al. [52]and Chen et al. [49] both found significant changes at the Proteobacteria phylum with Jiang et al. [52] finding an increase and Chen et al. [49] finding a decrease. Kelly et al. [53] found no significant differences at any of the phyla levels.

Jiang et al., Chen et al., and Mason et al. [48, 50, 54] focused on GAD/anxiety. Mason et al. [54] found no significant changes at the phyla levels, while Jiang et al. [48] and Chen et al. [50] both found Bacteroidetes to increase and Firmicutes to decrease. Furthermore, Chen et al. [50] was the only study to find a significant change in the Tenericutes phylum; they found

Tenericutes microbes to decrease in abundance in GAD patients compared to the healthy controls. When comparing the p-values that show significant changes at the phyla levels for Chen et al. [50], the Tenericutes phylum has the smallest p-value. at less than 0.001.

When combining all 9 MDD and anxiety studies, at the Actinobacteria phylum, 3 increased and 1 decreased, at the Bacteroidetes level: 3 decreased and 3 increased, at the Firmicutes level: 4 decreased and 1 increased, at the Proteobacteria level: 1 increased and 1 decreased, and finally at the Tenericutes level: 1 decreased [4, 25, 48-54]. Additionally, there were differences of abundance changes at the phyla level when comparing anxiety and depression. At the Actinobacteria phylum for depression, abundance increased overall [4, 49, 51], but for anxiety, there were no significant changes seen at this phylum [48, 50, 54]. For the Bacteroidetes phylum, a majority of the depression studies saw a significant decrease [4, 49, 51], but all of the anxiety studies that found a significant change at this phylum found that Bacteroidetes increased in abundance [48, 50]. One similarity at the phyla level was for the Firmicutes phylum, where a majority of both the anxiety and depression studies found Firmicutes to decrease in abundance [25, 48, 50, 52]. Higher taxonomic ranks often are not different, due to the numerous levels of taxa that file under higher ranks [70]. Therefore, changes can be lost when they are all grouped together. For this reason, I also documented data down to the lowest possible taxonomic rank based on the data provided by each of the studies. Tables 4-6 sought to look deeper into these other possible gut microbiome alterations.

Table 4: Significant Family Alterations in Subjects with Mental Health Disorders

[4]	Chen, Z., et al. (2018)	Huang, Y., et al. (2018)	Jiang, H., et al. (2015)	Kelly, J. R., et al. (2016)	Lai, W. T., et al. (2021)	Zheng, P., et al. (2016)	Chen, Y. H., et al. (2019)	Jiang, H. Y., et al. (2018)	Mason, B. L., et al. (2020)
Actinomycetaceae (A)	⬆					⬆			
Corynebacteriaceae (A)						⬆			
Coriobacteriaceae (A)					⬆	⬆			
Chitinophagaceae (A)	⬇								
Micrococcaceae (A)					⬆				
Atopobiaceae (A)					⬆				
Streptomycetaceae (A)	⬆								
Nocardiaceae (A)	⬆								
Bifidobacteriaceae(A)	⬆				⬆				
Cytophagaceae (B)					⬇				
Sphingobacteriaceae (B)					⬇				
Flavobacteriaceae (B)					⬇				
Bacteroidaceae (B)			⬇		⬇	⬇	⬆		
Rikenellaceae (B)			⬆			⬇			
Prevotellaceae (B)	⬇		⬇	⬇			⬆		
Muribaculaceae (B)							⬇		
Porphyromonadaceae (B)			⬆						
Mariniabiliaceae (B)	⬇								
Unclassified otu0496 (F)						⬆			
Unclaffified otu0144 (F)						⬆			
Eubacteriaceae (F)	⬆								
Lachnospiraceae (F)	⬆	⬇	⬇			⬇			
Heliobacteriaceae (F)					⬆				
Thermoanaerobacteriaceae (F)				⬆					
Eubactereriaceae (F)						⬆			
Ruminococcaceae (F)	⬆	⬇	⬇			⬆			
Clostridiaceae (F)	⬆	⬇				⬆			
Tannerellaceae (F)									
Oscillospiraceae (F)	⬇				⬆				
Erysipelotrichaceae (F)	⬆		⬇			⬆			
Peptococcaceae (F)					⬆				

[4] **Notes:** First 6 studies are analyzing MDD/depression, last 3 are analyzing GAD/anxiety
Abbreviations/Key: ⬇, decreasing in abundance; ⬆, increasing in abundance; (A), Actinobacteria Phylum; (B), Bacteroidetes Phylum; (F), Firmicutes Phylum; (P), Proteobacteria Phylum

[4]	Chen, Z., et al. (2018)	Huang, Y., et al. (2018)	Jiang, H., et al. (2015)	Kelly, J. R., et al. (2016)	Lai, W. T., et al. (2021)	Zheng, P., et al. (2016)	Chen, Y. H., et al. (2019)	Jiang, H. Y., et al. (2018)	Mason, B. L., et al. (2020)
Eggerthellaceae (F)					↑				
Acidaminococcaceae (F)			↑		↑				
Lactobacillaceae (F)					↑				
Veillonellaceae (F)			↓		↑				
Enterococcaceae(F)					↑				
Fusobacteriaceae (F)			↑						
Streptococcaceae (F)									
Sutterellaceae (P)	↓								
Enterobacteriaceae (P)	↓		↑				↑		
Succinivibrionaceae (P)							↓		
Burkholderiaceae (P)							↑		

Table 4 documented changes seen at the family level. For the 6 MDD/depression studies [4, 25, 49, 51-53], all 9 family levels under the Actinobacteria phylum increased, except for 1 family, *Chitinophagaceae* [49]. In the Bacteroidetes families for the 6 MDD/depression studies, 7/9 families decreased in abundance and 2/9 increased. Jiang et al. [52] was the only study to have families decrease in abundance under the Bacteroidetes phylum level. Huang et al. [25] found no significant changes at the any of the family levels under the Bacteroidetes phylum. The 6 MDD/depression studies found the greatest changes at the family levels under the Firmicutes phylum [4, 25, 49, 51-53]. There were 31 total family changes notated under the Firmicutes level, 9 decreasing and 22 increasing, and they were all notated for the MDD/depression studies. At the *Lachnospiraceae* family level, 3 studies found decreases [4, 25, 52], while one [49] found an increase at this family level. At the *Ruminococcaceae, Oscilliospiraceae,* and *Veillonellacace* family levels, 50% of the studies that found significant changes, found the value to be higher in MDD patients than their healthy controls and 50% found them to be lower. Only 4/6 of the MDD/depression articles found significant changes under the Proteobacteria phylum's families

[4, 25, 51, 53] and the changes were only found in 2 of the 4 family levels: *Sutterellaceae* and *Enterbacteriaceae*. Two of the changes found higher abundances and 1 found lower abundances among patients with MDD/depression compared to their healthy controls. Chen et al. [49] only found the abundance to be lower and Jiang et al. [52] only found it to be higher.

For all 3 GAD/anxiety studies, there were no significant changes found at any of the family levels under the Actinobacteria phylum [48, 50, 54]. In fact, ⅔ of the GAD articles found no changes at any of the family levels [48, 54]. Chen et al. [50] was the only GAD study that found changes at the family level. Chen et al.[50] found 3 changes at the Bacteroidetes family levels: 2 increased and 1 decreased. There were no Firmicutes family level changes reported for any of the GAD articles. A total of 2/4 families under the Proteobacteria phylum saw significant changes for MDD/depression studies. Only one GAD article found any changes at the family level under the Proteobacteria phylum [50]. Chen et al. [50] discovered changes at 3 family levels under the Proteobacteria phylum: 2 increased and 1 decreased.

Between all 9 studies, there were 42 found families with statistically significant changes [4, 25, 48-54]. Table 4 highlights this in more detail. There were 9 different families that had significant changes at the Actinobacteria phylum, 9 families under the Bacteroidetes phylum, 20 families under the Firmicutes phylum, and 4 families under the Proteobacteria phylum that had significant changes. There were slightly more than double the number of significant changes found at different family levels under the Firmicutes phylum than the Bacteroidetes and Actinobacteria phyla and 5 times more than the Proteobacteria phylum. When combining all MDD/depression and GAD studies, there were 40 findings of higher abundances at the family level in subjects with a mental health disorder compared to their healthy controls and 25 findings of lower abundances at the family level among 42 different families [4, 25, 48-54]. When

comparing anxiety and depression studies, there were a couple of differences. At the *Bacteroidaceae* and Prevotellaceae families (both under the Bacteroidetes phylum), 50% of the depression studies found both of these families to decrease in abundance [4, 25, 49, 51-53], but only one anxiety study [50] found a significant change for these families and it was the opposite of what was seen with the depression studies' findings-an increase in abundance

Table 5: Significant Genera Alterations in Subjects with Mental Health Disorders

[5]	Chen, Z., et al. (2018)	Huang, Y., et al. (2018)	Jiang, H., et al. (2015)	Kelly, J. R., et al. (2016)	Lai, W. T., et al. (2021)	Zheng, P., et al. (2016)	Chen, Y. H., et al. (2019)	Jiang, H. Y., et al. (2018)	Mason, B. L., et al. (2020)
Atopobium (A)					⬆				
Bifidobacterium (A)					⬆				
Collinsella (A)						⬆			
Coriobacterium (A)					⬆				
Eggerthella (A)				⬆	⬆				
Olsenella (A)					⬆	⬆			
Rothia (A)					⬆				
Slackia (A)					⬆				
Bacteroides (B)	⬇		⬇		⬇			⬆	⬇
Prevotella 9 (B)				⬇			⬇		
Alistipes (B)			⬆			⬇			
Fusobacterium (B)								⬆	
Paraprevotella (B)				⬆					
Prevotella (B)	⬇		⬇						
Prevotellaceae NK3B31 group (B)							⬇		
Prevotellaceae UCG-001 (B)							⬇		
Sphingobacterium (B)					⬇				
Lactobacillus (F)					⬆			⬆	
Ruminoccus (F)	⬆								
Acidaminococcus (F)					⬆				

[5] **Notes:** First 6 studies are analyzing MDD/depression, last 3 are analyzing GAD/anxiety
Abbreviations/Key: ⬇, decreasing in abundance; ⬆, increasing in abundance; (A), Actinobacteria Phylum; (B), Bacteroidetes Phylum; (F), Firmicutes Phylum; (P), Proteobacteria Phylum; (S), Spirochetes Phylum

5	Chen, Z., et al. (2018)	Huang, Y., et al. (2018)	Jiang, H., et al. (2015)	Kelly, J. R., et al. (2016)	Lai, W. T., et al. (2021)	Zheng, P., et al. (2016)	Chen, Y. H., et al. (2019)	Jiang, H. Y., et al. (2018)	Mason, B. L., et al. (2020)
Agathobacter (F)							↓		
Anaerofilum (F)				↑					
Anaerostipes (F)						↑			
Blautia (F)	↑	↓	↑			↑			
Bulleidia (F)		↑							
Butyricicoccus (F)								↓	
Clostridium Innocuum Group (F)							↓		
Clostridium leptum group (F)									↓
Clostridium XIX (F)			↑						
Clostridium XlVa (F)						↓			
Coprococcus (F)	↑	↓							
Coprococcus_3 (F)		↓					↓		
Desulfitobacterium (F)					↑				
Dialister (F)			↓	↓			↓		
Dorea (F)		↓							
Enterococcus (F)					↑				
Erysipelotrichaceae Incertae Sedis (F)						↑			
Eubacterium coprostanoligenes group (F)	↑		↑				↓		
Eubacterium Rectale Group (F)								↓	
Faecalibacterium (F)	↑	↓	↓			↓		↓	
Gelria (F)				↑					
Gemella (F)		↑							
Heliobacterium (F)					↑				
Holdemanella (F)							↓		
Holdemania (F)				↑					
Lachnoclostridium (F)					↑				
Lachnospira (F)								↓	
Lachnospiracea incertae sedis (F)			↑			↑			
Lachnospiracea incertae sedis (F)						↓			
Lachnospiraceae NK4A136 group (F)							↓		
Megamonas (F)			↑			↓	↓		
Megasphaera (F)					↑				
Mitsuokella (F)							↓		

[5]	Chen, Z., et al. (2018)	Huang, Y., et al. (2018)	Jiang, H., et al. (2015)	Kelly, J. R., et al. (2016)	Lai, W. T., et al. (2021)	Zheng, P., et al. (2016)	Chen, Y. H., et al. (2019)	Jiang, H. Y., et al. (2018)	Mason, B. L., et al. (2020)
Oscillibacter (F)	↓		↑		↑				
Parvimonas (F)		↑				↑			
Peptostreptococcus (F)		↑							
Phascolarctobacterium (F)			↑			↓			
Roseburia (F)			↑			↓		↓	
Ruminococcaceae NK4A214 group (F)							↓		
Ruminococcaceae UCG-014 (F)							↓		
Ruminococcus (F)			↓						
Streptococcus(F)					↑				
Subdoligranulum (F)	↓						↓	↓	
Turicibacter (F)				↑					
Tyzzerella 3 (F)							↑		
Raoultella (P)							↑		
Acinetobacterv(P)							↓		
Buchnera (P)							↓		
Escherichia–Shigella (P)							↑	↑	
Oxalobacter (P)		↑							
Parasutterella (P)			↑						
Pseudomonas (P)		↑							
Succinivibrio (P)							↓		
Sutterella (P)								↓	
Sphaerochaeta (S)					↑				

Table 5 sought to get even more specific by documenting changes at the genus level. Among the 6 MDD/depression studies, there were changes at all 8 of the genera under the Actinobacteria phylum [4, 25, 49, 51-53]. However, ½ of the MDD/depression articles showed no significant changes at all at this level under this phylum [25, 49, 52]. Of the remaining ½ of MDD/depression studies that did show changes at the genera level of the Actinobacteria phylum, all of them showed an increase in abundance among patients with MDD compared to their healthy controls [4, 51, 53]. For MDD/depression studies, there were differences seen in 6 of the

9 genera under the Bacteroidetes phylum when comparing people with MDD and healthy individuals [4, 25, 49, 51-53]. There were no significant differences seen in the *Fusobacterium*, *Prevotellaceae N3B31 Group*, or *Prevotellaceae UCG-001* genera (under the Bacteroidetes phylum) among MDD and healthy participants. There were 10 changes seen at this genera level under the Bacteroidetes group: 8/10 were decreased abundances, while the remaining 2/10 were increases in abundance for MDD participants [4, 25, 49, 51-53]. Jiang et al. and Kelly et al. [52, 53] were the only two studies that showed the increases. Additionally, Huang et al. [25] showed no changes at all at the genera level under the Bacteroidetes phylum. For the MDD/depression studies, there were differences seen in 36/48 genera at the Firmicutes phylum with a total of 52 significant difference found: 35/52 were higher among MDD patients than healthy controls, while the remaining 17 found lower abundances in MDD patients [4, 25, 49, 51-53]. The *Blautia* genera, under the Firmicutes phylum, was found to have significant differences in 4/6 MDD studies, with all showing an increase in abundance in MDD patients [4, 49, 52], except for one [25]. For the 6 MDD/depression studies, only 2/6 found significant differences in the genera in the Proteobacteria phylum. There were 3 differences detected in 3/9 of all the genera in the Proteobacteria phylum. All 3 differences showed an increase in abundance. Lastly, one genus under the Spirochaete phylum was detected to have a significant difference: *Sphaerochaeta [51]*. This genus was detected to be in higher abundance among MDD patients when compared to healthy people in [51].

For the 3 GAD studies, there were no significant changes seen at the genera level of the Actinobacteria phylum [48, 50, 54]. Looking at the GAD studies, there were 6 significant differences seen at 5/9 genera at the Bacteroidetes level: 4 lower and 2 higher in the GAD patients when compared to the healthy controls [48, 50, 54]. Jiang et al. [48] was the only GAD

study that found an increase in the genera that make up the Bacteroidetes phylum. For the 3 GAD studies, there were 21 differences spotted among 20 genera under the Firmicutes phylum: 19 lower abundances and 2 higher when comparing GAD patients' microbiota to healthy individuals [48, 50, 54]. For the 3 GAD studies, there were 7 changes detected among 6/9 genera under the Proteobacteria phylum: 4 lower abundances and 3 higher. Mason et al. [54] showed no significant differences at the genera under the Proteobacteria phylum. Furthermore, at the Escherichia–Shigella genera, both findings showed an increased in abundance [48, 50].

Overall, among the 9 studies, there were 75 different genera where significant differences were found when comparing people with mental health disorders to their healthy controls [4, 25, 48-54]. There were 8 genera under the Actinobacteria phylum, 9 under the Bacteroidetes phylum, 48 under the Firmicutes phylum, 9 under the Proteobacteria phylum, and 1 under the Spirochaete phylum [4, 25, 48-54]. There were 69 significant differences found among all mental health disorders in the genera under the Firmicutes phylum with a majority of the differences being increases found for MDD/depression [4, 25, 49, 51-53], and decreases among the GAD studies [48, 50, 54]. Additionally, when looking at the results of all the mental health disorders combined [4, 25, 48-54], the *Faecalibacterium* genera in the Firmicutes phylum was found to be in lower abundance in 4 different studies but increased in only one [49]. Overall, the GAD articles found increases and decreases in abundances at the genera level of the Proteobacteria phylum [48, 50, 54], while the MDD/depression articles only found increases [4, 25, 49, 51-53]. Additonally, the *Bacteroides* genus decreased in abundance for 50% of depression studies [4, 25, 49, 51-53], but there were not any consistencies in the trends for the *Bacteroides* genus for anxiety studies [48, 50, 54]. Under the *Blautia* genus, a majority of the depression studies that found significant differences for this genus found *Blautia* to increase in abundance [4, 25, 49, 51-

53], while no anxiety studies found any significant changes at this genus [48, 50, 54]. Escherichia-Shigella increased in abundance for 2/3 of anxiety studies [48, 50], but none of the studies that analyzed depression found any significant changes under this genus. On the other hand, one similarity is 50% of depression studies found *Faecalibacterium* to decrease in abundance [4, 25, 52] and the one anxiety study [48] that found *Faecalibacterium* to significantly change also found it to decrease in abundance.

Table 6: Significant Species Alterations in Subjects with Mental Health Disorders

[6]	Chen, Z., et al. (2018)	Huang, Y., et al. (2018)	Jiang, H., et al. (2015)	Kelly, J. R., et al. (2016)	Lai, W. T., et al. (2021)	Zheng, P., et al. (2016)	Chen, Y. H., et al. (2019)	Jiang, H. Y., et al. (2018)	Mason, B. L., et al. (2020)
bifidobacterium adolescentis (A)					⬆				
rothia mucilaginosa (A)					⬆				
olsenella uli (A)					⬆				
slackia heliotrinireducens (A)					⬆				
eggerthella lenta (A)					⬆				
atopobium parvulum (A)					⬆				
coriobacterium plomerans (A)					⬆				
bifidobacterium longum (A)					⬆				
bifidobacterium dentium (A)					⬆				
bifidobacterium bifidum (A)					⬆				
bifidobacterium brev (A)					⬆				
bacteroides helcogenes (B)					⬇				
prevotella stetcorea (B)	⬇								
bacteroides plebeius (B)	⬆								
bacteroides thetaiotaomicron (B)	⬆								
bacteroides dorei (B)	⬇								
bacteroides eggerthii (B)	⬇								
bacteroides fragilis (B)	⬇								

[6] **Notes:** First 6 studies are analyzing MDD/depression, last 3 are analyzing GAD/anxiety
Abbreviations/Key: ⬇, decreasing in abundance; ⬆, increasing in abundance; (A), Actinobacteria Phylum; (B), Bacteroidetes Phylum; (F), Firmicutes Phylum; (P), Proteobacteria Phylum; (S), Spirochetes Phylum; (T), Thermodesulfobacteria Phylum

[6]	Chen, Z., et al. (2018)	Huang, Y., et al. (2018)	Jiang, H., et al. (2015)	Kelly, J. R., et al. (2016)	Lai, W. T., et al. (2021)	Zheng, P., et al. (2016)	Chen, Y. H., et al. (2019)	Jiang, H. Y., et al. (2018)	Mason, B. L., et al. (2020)
bacteroides massiliensis (B)	↓								
bacteroides uniformis (B)	↓								
bacteroides vulgatus (B)	↓								
bacteroides xylanisolvens (B)	↓								
prevotella buccae (B)	↓								
prevotella copri (B)	↓								
eubacterium ruminantium group (F)							↓		
acidaminococcus fermentans (F)					↑				
acidaminococcus intestini (F)					↑				
enterococcus faecium (F)					↑				
blautia hansenii (F)	↑								
coprococcus catus (F)	↑								
faecalibacterium prausnitzii (F)	↑								
oscillibacter valericigenes (F)					↑				
streptococcus pyogenes (F)					↑				
mollicutes_rF39_norank (F)							↓		
heliobacterium modesticaldum (F)					↑				
eubacterium limosum (F)					↑				
clostridium saccharolyticum (F)					↑				
megasphaera elsdenii (F)					↑				
streptococcus parasanguinis (F)					↑				
lactobacillus crispatus (F)					↑				
ruminococcus gnavus (F)								↑	
ruminoccus bicirculans (F)	↑								
ruminococcus bromii (F)	↑								
subdoligranulum variabile (F)	↓								
escherichia-shigella (P)								↑	
treponema brennaborense (S)					↑				
desulfovibrio vulgaris (T)					↑				

Table 6: This table arranged what species had significant changes among individuals with mental health disorders and those without. It is a more finite group, and many studies did not report taxa this detailed in their results. This is due in part to a majority of the studies using 16S

sequencing as a technique to identify the GI microbial composition as 16S sequencing has restrictions in species-level resolution due to the limitations on the resolution needed to distinguish species [71]. Additionally, after a sequencing technique is used, whether it is 16S or shotgun, there is sometimes limited species level representation in the reference database used to identify the taxonomic species. Due to this, there are less data points to compare than higher taxonomic rankings. 5/9 studies did not report any species data [4, 25, 48, 52-54]. Out of the 6 MDD/depression studies, only 2/6 studies found significant changes at the species level [49, 51]. Chen et al. [49] found that of the 20 differences discovered: 14 were at the species level under Bacteroidetes phylum (12 lower, 2 higher) and the remaining 6 significant differences were found in species under the Firmicutes phylum (1 lower, 5 higher). Lai et al. [51] found a majority of the significant differences at the species level under the Actinobacteria and Firmicutes phyla. They found that under both of these phyla, the species levels all increased for participants with MDD/depression compared to their healthy controls [51].

For the 3 GAD/anxiety studies, ⅔ reported significant changes at the species level [48, 50, 54]. Chen et al. [50] only found significant differences at the species level under the Firmicutes phylum; *mollicutes_RF39_norank* and *eubacterium ruminantium group*, which both showed a lower abundance in GAD individuals. Additionally, Jiang et al. [48] showed only 2 species that had significant differences, *ruminoccus gnauvus* and *escherichia-shigella*-both being in higher abundance for GAD individuals than healthy controls. When comparing anxiety and depression studies analyzed, there is not a single species that shows significant changes for both depression and anxiety; there is no overlap.

3.3 Aim 3: Are L/Z Calorie Sweeteners Effecting the Gut Microbiome Composition?

Since there had been an association found between L/Z calorie sweeteners and mental health disorders and between the gut microbiome and mental health disorders, I sought to see if there was an association between L/Z calorie sweeteners and the gut microbiome.

Table 7: Summaries of Studies Analyzing Low/Zero Calorie Sweeteners' Potential Associations with Gut Microbiome Composition

[7]	Year	Country	Cohort size	Species	Matched variables	Population details	Sweetener analyzed	Exposure	Methods	Association and Outcome?
Suez, J., et al. (2014)	2014	USA	N/A	animal (C57Bl/6 mice)	N/A	lean, 10-week-old mice	saccharin 0.1 mg ml−1	1 week	shotgun sequencing 16S sequencing	Yes: over-representation of Bacteroides and under-representation of Clostridiales" Many of the taxa that increased in relative abundance belonged to the Bacteroides genus and Clostridiales order (underrepresented)
Frankenfeld, C. L., et al. (2015).	2015	USA	n=31	human	bmi, energy intake (double check this)	at least 18, F, could not be pregnant, could not have a digestive disorder, 65% white, 81% never smoked, average bmi was 24.3	aspartame US (average intake was 5.3mg/day to 112 mg/day) acesulfame k, US- (average intake was 1.7 mg/day to 33.2 mg/day)	4 days	16s sequencing	No: aspartame: no significant differences at the class or order level, Bacteroidetes and firmicutes has the highest median abundances -bacterial diversity was different between aspartame and ace-k consumers and non-consumers but there were no significant differences in relative abundance of gene function however bacterial diversity differences were significantly different across consumers of either sweetener or non-consumers of both sweeteners

[7] **Abbreviations:** HFD, high fat diet; LFD, low fat diet; US, unspecified amount; M, male; F, female; BMI, body mass index

[7]	Year	Country	Cohort size	Species	Matched variables	Population details	Sweetener analyzed	Exposure	Methods	Association and Outcome?
Wang, Q. P., et al. (2018)	2018	Australia	n=32 (8 mice in each group)	animal (C57BL/6 mice)	N/A	5 weeks old	Sucralose ~3.3 mg/kg/d bodyweight in the normal chow group, and ~1.5 mg/kg/d in the HFD group	8 weeks	16 rDNA sequencing	Yes: (important to note for both control groups (high fat and normal with no L/Z calorie sweetener) There was no significant difference in alpha diversity between control and sucralose in normal chow or HFD-fed mice -sucralose fed mice +chow: -had a significant increase in abundance of firmicutes (however this dipped after the first 2 weeks of exposure and then was = to the chow only mice. -Bifidobacterium was significantly ($p<0.05$) increased in the context of chow + sucralose compared to just chow (just not in the HFD an sucralose group) , -sucralose + high fat diet fed mice: - show a significant and long-lasting increase in Firmicutes when compared to HFD controls -while Bacteroidetes species were reduced in both groups(hfd and hfd +sucralose - a significant increase in Clostridium and reduction in Bifidobacterium

[7]	Year	Country	Cohort size	Species	Matched variables	Population details	Sweetener analyzed	Exposure	Methods	Association and Outcome?
										overall: sucralose changes firmicutes abundance ad HFD further accentuates those changes

[7]	Year	Country	Cohort size	Species	Matched variables	Population details	Sweetener analyzed	Exposure	Methods	Association and Outcome?
Uebanso, T., et al. (2017)	2017	Japan	n=41 (16 controls, 8 for low dose sucralose, 8 for high dose sucralose, and 9 for acesulfame-K group	animal (mice) C57Bl/6J mice	the environment (temp, humidity, lighting)	M and F 4 weeks old	sucralose (0) acesulfame-K (0) different doses for different groups: high dose sucralose group: 15 mg/kg body weight low-dose sucralose group: 1.5 mg/kg body weight per day (n = 8) acesulfame-K group: 15 mg/kg body weight per day	8 weeks	16S sequencing	Yes: Sucralose: -decreased the amount of Clostridium cluster XIVa (genera) in feces in a dose-dependent manner (Clostridium cluster XIVa in control was higher than the high sucralose group and slightly less high than the low sucralose group in week 1) ace-k-no significant differences
Abou-Donia, M. B., et al. (2008)	2012	USA	n=50	animal (Sprague-Dawley rats)	n/a	M only	sucralose 1 ml/kg in water by. Group 2, Splenda: 100 mg/kg/d in water (1.1 mg/kg/d sucralose). Group 3, Splenda: 300	12 weeks	16S rRNA sequencing	Yes: the bacterial counts of Bifidobacteria, lactobacilli, and Bacteroides were reduced. As the amount of Splenda increased, the 5 anaerobes that decreased grew larger. recovery period: Bifidobacteria at 300 and 500 mg/kg continued to be reduced. Neither lactobacilli, Bacteroides,

[7]	Year	Country	Cohort size	Species	Matched variables	Population details	Sweetener analyzed	Exposure	Methods	Association and Outcome?
							mg/kg/d in water (3.3 mg/kg/d sucralose). Group 4, Splenda: 500 mg/kg/d in water (5.5 mg/kg/d sucralose) Group 5, Splenda: 1000 mg/kg/d in water (11 mg/kg/d sucralose).			clostridia, total aerobes, nor enterobacteria were significantly different from controls during the recov-recovery period (meaning they recovered to normal levels after Splenda was no longer consumed)
Bian, X., et al. (2017)	2017	USA	n=20	animal (CD-1 mice (~7 weeks old)	gender, weight	10 F, 10 M 8 weeks old	ace-K (0) 37.5 mg/kg body weight/day.	4 weeks	16S rRNA sequencing	Yes, gender specific changes: in male: Bacteroides, anaerostripes, and sutterella are higher in abundance than the controls in female: lactobacillus, clostridium, unassigned genus in ruminococcaceae, and unassigned genus in oxalobacteraceae have lower abundances in the ace-k group than the control group and mucispirillum has a higher abundance in ace-k females than the female controls

[7]	Year	Country	Cohort size	Species	Matched variables	Population details	Sweetener analyzed	Exposure	Methods	Association and Outcome?
										both: ace-k administered animals' gut microbial metabolomes profiles differed from the controls. for females: most metabolites were down regulated for males: most metabolites were upregulated

Table 7 summarizes 6 articles that were gathered that analyzed the relationship between the GI microbiome and L/Z calorie sweeteners [55-60]. Six studies were included, due to the available studies applicable to this literature review after exclusion criteria was applied. Additional inclusion and exclusion criteria for this aim is elaborated on in section 3.1c. Five articles were animal experiments and 1 was a human study [55-60]. Four out of five animal studies used mice as their subjects, either C57Bl/6 mice [55] CD-1 mice [60], and 1 study used Sprague-Dawley rats [59]. Some of the articles analyzed more than one L/Z calorie sweetener. Three articles analyzed ace-K [56, 58, 60], 1 article analyzed Saccharin [55] and 3 analyzed Sucralose [57-59].

In studies summarized in Table 7, ace-K, a synthetic, zero calorie sweeteners, was shown to evoke no changes in the composition of the gut microbiome in 2/3 studies [56, 58] and it was shown to have sex-specific gut microbiome composition changes in one article [60]. Specifically, Bian et al. [60] found that male subjects were found to have higher abundances of *Bacteroides, Anaerostripes*, and *Sutterella,* and in females, *Lactobacillus*, *Clostridium*, and unassigned genus in the *Ruminococcaceae* and Oxalobacteraceae families had a lower abundance. Opposite of the genera in females that saw a decrease*, Muscispirillium* appeared in higher abundance in females than in the controls that received no ace-K [60]. While Bian et al. [60] saw changes in gut microbiota composition and the other two studies analyzing ace-K consumption saw no significant microbiota composition changes, Bian et al. [60] had their subjects consume a significantly larger amount of ace-K compared to the other two studies. Uebanso et al. [58] had their subjects consume 15 mg/kg of body weight/day and Frankenfeld et al. [56] allowed subjects to self-graze so ace-K consumption ranged from 1.7mg total/day to 33.2 mg/day. Both of these studies' [56, 58] animal subjects consumed significantly less ace-k than Bian et al. [60], which

allowed their animal subjects to consume 37.5 mg/kg of weight/day of ace-K. Using the scale Bian et al. [60] used for their rodent subjects, a 150 lb. person would consume 2,510.68 mg of ace-K per day and it is not recommended for humans to consume more than 15 mg/kg of body weight/day of ace-K or 1,020.6 mg per day [63]. This means that Bian et al. was administering a little more than double the recommended maximum amount of ace-K per day to their rodent subjects. An average diet soda that uses ace-K as a sweetener uses about 41 mg of ace-K per one 12 oz can of soda [60]. In other words, using Bian et al's [60] dosage administered to their rodent models would equate to about 25 12-oz sodas/day for a 150 lb person. Also, the length of time ace-K was administered ranged in each of these studies; Bian et al. [60] did 4 weeks of exposure, Uebanso et al. [58] did 8 weeks of exposure, and Frankenfeld et al. [56] did only 4 days of exposure. Changes to the GI microbiome require time to manifest after diet changes, and then after a certain length of time of exposure regresses back to its normal state [59]. It has also been demonstrated that sometimes, short-term exposure to diet changes invokes immediate changes in the GI microbiome [31]. Therefore, it is possible that any of these differences may explain, in part, why significant changes were seen in Bian et al. [60], but not in the others. Overall, associations point towards ace-K not producing any alterations in the gut microbiome composition of animals when consumed in an amount below the average consumption of humans [56, 58]. However, if ace-K consumption is far above the recommended maximum intake (15 mg/kg of body weight/day), then there may be a sex-specific effect in GI microbiome composition changes with a sex-specific effect [60].

Another synthetic, zero-calorie sweetener, sucralose, was analyzed by 3 different studies [57-59]. Table 7 highlights the studies that showed sucralose to produce various different gut-microbiome changes. Overall, *Bacteroides* was shown to decrease significantly in 2/3 studies

[57, 59] but no changes were seen at this level in Uebanso et al.'s [58] findings. While Wang et al. [57] was the only study to find sucralose exposure to cause the Firmicutes phylum to significantly increase in abundance, Uebanso et al. [58] also found Firmicutes to increase in abundance in the sucralose groups compared to the controls-just not significantly. Other than that, there were no consistencies seen among the different studies. Wang et al. [57] found that sucralose can elicit different GI composition changes based on whether it was consumed alongside a regular diet or a high fat diet. This was seen with *Bifidobacterium* significantly increasing in animals who consumed a regular diet along with sucralose, but it was not shown to significantly change at all in rodents who consumed a high fat diet alongside sucralose. Abou-Donia et al. [59] saw the opposite with *Bifidobacterium* decreasing by 36.9% in animals who consumed sucralose. Additionally, Abou-Donia et al. [59] was the only study that saw *Lactobacilli* decrease in abundance with sucralose consumption. One other major difference seen among all these studies that analyzed sucralose was Uebanso et al. [58] saw the *Clostridium cluster* decrease significantly, whereas Wang et al. [57] saw an increase (however, it was not significant). Overall, there are associations seen where *Bacteroides* may decrease when sucralose is consumed, but overall sucralose is shown to have very minimal effects on the GI microbiome of rodents. Additional studies should be done to further evaluate the potential association of *Bacteroides* and sucralose exposure by analyzing both exposure to surcralose with different caloric diets (i.e. high fat diet vs normal diet vs low fat diet) and at different times (i.e. analyzing impacts before exposure, after beginning, and then after a recovery period with sucralose discontinued) in the same experiment.

Table 7 also summaries studies that analyzed aspartame and saccharin, both of which are synthetic, zero-calorie sweeteners [14, 65]. They are both commonly used as they were some of

the first L/Z calorie sweeteners used in commercially consumed foods all over the world [12]. Frankenfeld et al. [56] analyzed aspartame's effects on the GI microbiome and found that there were no significant GI microbiome composition differences seen among subjects and controls. It is important to note that exposure was only done for 4 days, which may have played a part in no changes seen [56]. Suez et al. [55] analyzed saccharin's effects on the GI microbiome and found that the *Bacteroides* genus increased in abundance and lower OTU members of the *Clostridiales* both increased and decreased, depending on whether saccharin was ingested purely or in conjunction with other nutritive sweeteners such as sucrose and/or glucose. For example, *Lactobaacillus OTU 259372* increased in abundance, but the *Lactobacillus reuteri OTU 355305* and *OTU 354911* decreased in abundance [55]. While this literature review did not analyze any other studies that assessed aspartame or saccharin's effects on the GI microbiome, due to no other studies being published that fell within the parameters of the inclusion and exclusion criteria, the 2 studies that were included can be compared to other L/Z calorie sweeteners' effects on the GI microbiome in order to assess the differences one L/Z calorie sweetener may have on the GI microbiome composition in comparison to a different L/Z calorie sweetener. When comparing the sucralose findings with saccharin's, there were a few taxa that showed inverse reactions [55, 57-59]. The *Bacteroides* association seen with saccharin was the inverse of what was seen with sucralose [55, 57-59]. Additionally, *Clostridales* was not reported in overall findings for any of the studies analyzing sucralose, because the amounts were not significantly different, but *Clostridium* was shown to increase in 2 of the sucralose studies showing another inverse result from saccharin's findings [55, 57-59]. Additionally, aspartame showed no significant changes in gut microbiota, much like a majority of the ace-K studies.

When comparing all of the different L/Z calorie sweeteners assessed, the only similar taxa that showed changes in abundance when exposure to different L/Z calorie sweeteners were *Bacteroidetes* and *Clostridales. Bacteroidetes* showed altered abundance in sucralose [57, 59], ace-K [60], and saccharin [55]. *Bacteroidetes* was shown to significantly decrease in 2/3 sucralose studies [57, 59], increase in abundance (only in men) in 1/3 ace-K studies [60], and decrease in abundance in 1/1 saccharin study [55]. *Clostridales* showed altered abundances in saccharin [55] and sucralose [57, 58] with a decrease in abundance in both saccharin and sucralose studies. However, *Clostridales* decreasing in abundance in 2/3 of the sucralose studies was not a significant decrease [57, 58]. Outside of *Bacteroidetes* and *Clostridales,* there were no additional taxa that showed consistent changes among multiple different L/Z calorie sweeteners. Overall, while there did not appear to be one or more bacteria species whose abundance was altered in the same way for all L/Z calorie sweeteners, there seems to be an association between specific lower-level bacterial taxa and specific L/Z calorie sweeteners (i.e., sucralose and saccharin associated with *Bacteroidetes* and *Clostridiates*).

3.4 Overall: Are L/Z Calorie Sweeteners Acting Through the Gut Microbiome to Mediate Mental Health Disorders?

Overall, there was an association seen between some L/Z calorie sweeteners and mental health disorders, and a few L/Z calorie sweeteners and the GI microbiome. Aspartame consumption was associated with increased MDD/depression and GAD/anxiety symptoms and severity, but no significant alterations to the GI microbiome [18, 39, 41, 47, 56, 65]. Saccharin's consumption effects on mental health disorders and the GI microbiome is unclear [34, 55]. Saccharin was shown to have an inverse effect on depression and anxiety symptoms and severity, and a significant effect on the GI microbiome (*Bacteroides* genus increasing and

Clostridales order decreasing) [34, 55]. However, there was only one study that analyzed saccharin for mental health disorders and one that analyzed its effects on GI microbiome composition [34, 55]. Sucralose and ace-K's effect on mental health disorders is unknown (because no studies in this literature review explicitly analyzed their effects on mental health disorders). However, sucralose did show a minimal effect on the gut microbiome in rodent models [57-59], but ace-K showed no significant changes to the GI microbiome when consumed in an amount that was not well above the recommended maximum consumption limit [56, 58, 60]. Unspecified L/Z calorie sweeteners overall showed an increase in depression [17, 64], with one study [42] showing a decrease that may be due to to the unique population studied further explained in section 4.3a. The impact of unspecified L/Z calorie sweeteners on the GI microbiome could not be reported, due to the specific L/Z calorie sweeteners consumed being unknown.

The gut microbiome was seen to be associated with depression and anxiety through shifting composition in the Actinobacteria, Bacteroidetes, and Firmicutes phyla and decreased abundance of the *Bacteroides* genus was associated with increased depression prevalence and severity. However, specific species level bacterial taxa have still not been associated with specific mental health disorders. Increased abundance of the Actinobacteria phylum was associated increased depression.[4, 49, 51]. Decreased abundance of the Bacteroidetes phyla was associated with increased depression, but increased abundance of Bacteroidetes phyla was associated with increased anxiety prevalence and/or severity. Decreased abundance of the Firmicutes phyla was associated with increased depression prevalence and severity. However, there were no consistencies between family and/or species outside of the finding that people

suffering from anxiety and/or depression had differences in bacterial diversity compared to healthy controls [4, 25, 47-53].

Overall, there was an association seen between some L/Z calorie sweeteners and anxiety and depression, anxiety and depression and the GI microbiome, and some L/Z calorie sweeteners and the GI microbiome; however, the *Bacteroides* genus was the only GI bacteria shifting in the same direction (decreasing) with consumption of a specific L/Z calorie sweetener (sucralose only) as depression was. Concluding that L/Z calorie sweeteners are associated with anxiety and depression, and anxiety and depression are associated with the gut microbiome's composition. Additionally, sucralose is shown to be associated with the same genus (*Bacteroides* genera abundance decreasing) as depression. However, whether sucralose is associated with MDD/depression must be further analyzed as this literature review analysis contained no studies that specifically analyzed sucralose's association with any mental health disorders. Additionally, whether other L/Z calorie sweeteners (aspartame, saccharin, and/or ace-K) are associated with same GI bacteria taxa as anxiety and/or depression has not been demonstrated. Other L/Z calorie sweeteners may act through individualized shifts in abundances of gut microbiota taxa that vary from person to person based on their individualized, unique starting gut microbiome composition or potentially through another avenue. This requires additional research that will investigate whether these L/Z calorie sweeteners that are affecting mental health disorders are affecting similar GI bacteria taxa that the mental health disorders are affecting.

4. Discussion and Conclusions

4.1 Key Findings

This literature review was conducted in 3 parts, all coming together to answer the main question- Are L/Z calorie sweeteners affecting the gut microbiome to mediate mental health disorders? Aim 1, which consisted of 9 articles that analyzed whether there was an association between mental health disorders and L/Z calorie sweeteners, concluded that there is an association between MDD/depression and GAD/anxiety and the use of L/Z calorie sweeteners. To explain this finding, I hypothesize that L/Z calorie sweeteners are associated with mental health disorders, specifically depression and anxiety, because of changes seen in behaviors and/or feelings of animal models and humans after consuming L/Z calorie sweeteners that are characteristic of depression and anxiety. This finding is explained by the studies that found the use of aspartame and unspecified L/Z calorie sweeteners increased MDD/depression and GAD/anxiety prevalence and severity [17, 18, 39, 41, 47, 64, 65]. However, one unspecified L/Z calorie sweetener study found an inverse relationship between MDD prevalence and L/Z calorie intake [42]. This was also the only study that used subjects who were overweight or obese, which may explain why the association found was the opposite from the association all of the other unspecified sweetener studies concluded. My hypothesis is further explained by findings on saccharin use, which was found to decrease MDD/depression and GAD/anxiety as use increased [34]. Kumar et al.[34] was the only saccharin study included in this literature review. Therefore, there were limited comparisons. Additionally, there was a major limitation of induced Type 2 Diabetes in all animal subjects that possibly could have resulted in the inverse results seen [34]. This limitation, along with Perez-Ara et al's [42] overweight/obese limitation, is further discussed in detail in section 4.3a. Overall, it was demonstrated that L/Z calorie sweeteners are

associated with anxiety and depression. Additionally, an overall increase in anxiety and depression symptoms were seen with increased consumption of a majority of L/Z calorie sweeteners.

Aim 2 analyzed studies to investigate whether the GI microbiome is associated with mental health disorders. It was concluded that certain gut microbiome bacteria are associated with anxiety and/or depression. To explain this finding, I hypothesize that the GI microbiome is affecting mental health disorders through the communication channel the GI tract has with the brain, known as the gut-brain axis [21]. This is supported by shifting composition in the Actinobacteria, Bacteroidetes, and Firmicutes phyla seen in people with anxiety and/or depression and in animals displaying symptoms characteristic of anxiety and/or depression [4, 25, 48-54]. Specifically, Actinobacteria's abundance was shown to increase overall in depressed subjects, Bacteroidetes was shown to decrease in abundance in depressed subjects and increase in abundance for subjects with anxiety, and Firmicutes' abundance was shown to decrease overall in patients with depression [4, 25, 48-54]. Additionally, the *Bacteroides* and *Faecalibacterium* genera were shown to decrease in abundance and the *Blautia* genus was shown to increase in depressed individuals [4, 25, 49, 51-53]. However, specific bacterial species increasing/decreasing in abundance was not associated with depression and/or anxiety due to the inconsistencies seen in fluctuating abundances [50, 54]. Overall, the GI microbiome is associated with mental health disorders; however, the specific families and/or species that are shifting in abundance are not definitively known.

Aim 3, which analyzed articles that investigated whether L/Z calorie sweeteners are affecting the gut microbiome composition, concluded that some L/Z calorie sweeteners affected the gut microbiome composition significantly in rodent models, while others did not at all. To

explain this finding, I hypothesize that some L/Z calorie sweeteners are affecting gut microbiome composition through shifting the relative abundances of certain bacterial species in the GI tract. This can be explained by the findings on sucralose, ace-K, aspartame, and saccharin. Sucralose was found to have very minimal effects on the GI microbiome in rodent models [57-59]. Sucralose caused a decrease in *Bacteroides* [57, 59] in animal models, but there were no other significant consistencies found as other bacterial taxa fluctuated differently within each study. Findings on ace-K showed no changes in any taxa when consumed at an amount below the maximum daily limit suggested [56, 58]. Aspartame showed no significant alterations to the GI microbiome in the studies analyzed by this literature review [56]. However, Frankenfeld et al. [56] was the only article included that analyzed gut microbiota changes with aspartame exposure. Potential weaknesses and strengths of this study are evaluated in section 4.3a. Frankenfeld et al.'s [56] findings differed from other studies, which found that microbial changes were seen in rodents that were exposed to aspartame-specifically, Enterobacteriaceae and *Clostridium leptum* increased [72, 73]. However, these studies had to be excluded from this literature review due to their experimental methods not analyzing the entire gut microbiome. It is important to note that aspartame is hydrolyzed rapidly in the small intestine, so almost no aspartame is found in circulating blood because it is broken down so fast [74]. Therefore, a majority of any aspartame consumed would never reach the large intestine, where most GI bacteria are located, thus not giving aspartame the opportunity to directly affect the majority of GI bacteria. Further research is needed to analyze gut microbiota composition after aspartame exposure. Finally, saccharin showed *Bacteroides* to increase and *Clostridales* to decrease [55]. Concluding that some L/Z calorie sweeteners do affect the gut microbiome composition through shifting the relative abundances of certain bacterial species in the GI tract.

All of the previous findings fed into the main question, which analyzed whether L/Z calorie sweeteners were mediating mental health disorders through the gut microbiome. It was concluded that L/Z calorie sweeteners are mediating mental health disorders, and mental health disorders are shown to alter the gut microbiome's composition. Specifically, sucralose was associated with mediating depression through decreasing the *Bacteroides* genus' abundance. However, whether other L/Z calorie sweeteners are mediating mental health disorders through the GI microbiome was not shown in the studies analyzed. To explain this finding, there was an association seen between L/Z calorie sweeteners and mental health disorders, L/Z calorie sweeteners and the gut microbiome, and the gut microbiome and mental health disorders, but besides the association between *Bacteroides* genus and sucralose found, it was not shown that the same GI bacteria that are changing with L/Z calorie sweetener exposure are the same bacteria associated with the mental health disorders analyzed. Other L/Z calorie sweeteners and mental health disorders may be associated with individualized shifts in abundances of gut microbiota taxa that vary from person to person based on their individualized, unique starting gut microbiome composition or potentially through another avenue such as metabolism disruption, increasing inflammation, and/or altering oxidative stress that are further discussed in the limitation and recommendation sections below.

4.2 Strengths

4.2.a Strengths of the Studies' Approaches

One strength of the studies' design was cohort organization and size. In aim 1, which looked at the association between mental health disorders and L/Z calorie sweeteners (irrespective of mechanism), many of the human studies had large cohorts, with the largest

cohort being up to 263,923 people [64]. This gave the researchers more data and decreased the odds that cofounding variables controlled for the outcomes seen.

Another strength of the studies were the subjects chosen to participate in the studies. The studies that used animals as subjects when analyzing L/Z calorie sweeteners possible association with mental health disorders strengthened their findings, because animal studies looked at disorders based off symptoms seen instead of diagnostic tools created by humans that can give false positives [18, 34, 47, 65]. Animals are not able to fabricate behaviors to produce a desired outcome like humans are able to. The studies that used humans as subjects strengthened their findings, because the main question was looking for findings to be applied to humans and humans are the best subjects to use to mirror results seen in the real world [75]. Humans are each much more complex than animals and only so many external factors can be replicated in experimental conditions created for animal models. Therefore, results from studies that use humans as subjects are more applicable to humans, due to the diversity seen in the cohorts.

Another strength of the studies was their specific experimental design. In aim 1, all 4 of the animal studies controlled for L/Z calorie sweetener by weight and they had all the animals on the same diet outside of the L/Z calorie sweeteners [18, 34, 47, 65]. This allowed researchers to see whether L/Z calorie sweeteners only contributed to mental health disorders at a certain intake threshold, and it limited the odds that additional additives were contributing to the mental health disorder symptoms seen. Additionally, all of the studies in aim 2 controlled for various confounding factors such as BMI, age, sex, and smoking status to see if these factors added to the mental health disorder symptoms and prevalence seen or not [4, 25, 48-54]. This was a strength, because it limited the ability for their findings to be discredited if the study was repeated with a different cohort. Another strength of some of the studies' design was varying the

amount of L/Z calorie sweetener consumption given to the subjects, so whether the amount of L/Z calorie sweetener impacted either the associations with mental health disorders or changes in the GI microbiota could be analyzed. Studies that analyzed both higher and lower rates of the L/Z calorie sweetener were able to demonstrate whether there was or was not an association between the amount of L/Z calorie sweetener consumed and mental health disorders or changes in the GI microbiota composition. In aim 3, which looked at whether L/Z calorie sweeteners were associated with the gut microbiome through microbiome composition changes, 50% of the studies analyzed looked at both high intake and lower intake of L/Z calorie sweeteners and whether the different levels had different effects on the gut microbiome. This added to their findings, because it was able to pin-point whether a particular threshold caused gut microbiome changes [57-59].

4.2.b Strengths of This Literature Review's Approach

Alongside of the strengths of the methods used by the individual studies were the strengths I used to conduct this literature review that added to the validity of the associations found. Strengths of this literature review centered around article selection based on inclusion and exclusion criteria. For the 3 aims, I put a limitation on the years that each aim could reach back to find studies to include in this literature. For aim 1, it was the last 30 years, for aim 2, it was the last 10 years, and for Aim 3 studies must have been published in the last 15 years to be included. This allowed the associations I found to be the most up-to-date findings. It was advantageous to use recent research, so that if any new research counteracted the old findings, it would be included.

Study selection was another strength of this literature review. For aim 1, the number of human studies and the number of animal studies were almost equivalent (5 and 4 respectively), which allowed for better comparison to be made between the findings of each. Rodent models are not always indicative of what happens to the human gut microbiome [76]. Having almost equal numbers of both animal and human studies in this aim allowed for the association between L/Z calorie sweeteners and mental health disorders to be investigated for further research recommendations to be made accordingly.

Another strength of the exclusion criteria was excluding any studies that included methods that selectively assessed targeted gut microbes, such as quantitative polymerase chain reaction (qPCR), rather than global evaluation. QPCR is a targeted molecular techniques that analyzes and identifies the abundance of specific bacterial taxa, which provides limited taxonomic resolution [77]. The whole microbiome composition needed to be analyzed to create a holistic picture of what was impacted. If studies had been included that employed techniques that did not analyze the whole microbiome, then I would not have been able to conclude that a particular L/Z calorie sweetener or mental health disorder did or did not affect certain microbes, because they may not have been analyzed. It would have allowed for the possibility that GI microbiome changes may have occurred with exposure to certain L/Z calorie sweeteners or possessing certain mental health disorders, but not identified due to selectivity of the method used. All of the articles included used a technique that analyzed the whole gut microbiome to eliminate this possibility.

Alongside the advantages of excluding studies that employed sequencing techniques that would have limited findings, excluding any studies that employed *in vitro* or *ex vitro* methods to conduct the experiment was also a strength of this literature review. *In vivo* experiments mean

the subject consumed the L/Z calorie sweetener or possessed the mental health disorder being studied and then the microbial changes were measured [78]. In *ex vivo* experiments, cells or tissues are taken from an organism and then manipulated in a laboratory. In *in vitro* experiments, there is no living organism involved and an artificial environment such as a culture dish or test tube is used to study an exposure. Only *in vivo* studies were included because they allow for the examination of the complex interaction between L/Z calorie sweeteners and the GI tract and mental health disorders and the GI tract, while the host was intact [78]. While including this exclusion criteria in this literature review did greatly limit the number of available studies, particularly for aim 3, it allowed for conclusions to be more representative of what actually happens in humans.

4.3 Weaknesses and Limitations

4.3.a Weaknesses and Limitations of the Studies' Approaches

All studies have their weaknesses/limitations that must be considered when assessing the validity of this literature review's findings. Experimental design was one limitation that affected the results seen. For aim 1's human studies, 3 of the 5 human studies were observational, so they did not control for the amount or variety of L/Z calorie sweetener consumed [17, 42, 64]. While these studies did classify L/Z calorie sweetener intake into categories, such as frequency of consumption per week, they were broad descriptions with no specific measurements. Therefore, mental health disorder outcomes only carried so much weight because results may have been due to only consuming a particular kind of L/Z calorie sweetener, the unique combination of L/Z calorie sweeteners, or the amount of each one. Another experimental design limitation was all studies not possessing different experimental groups that were administered different doses (high

vs low) of each L/Z calorie sweetener. While most of the human articles compared L/Z calorie intake groups (subjects) to the subjects who received no L/Z calorie sweetener (controls), many did not have differing intake groups so different experimental groups could be compared. Lindseth et al. [39] was the only study whose results showed the differences in the group who received low aspartame to the group who contained high aspartame. All of the other studies not possessing experimental groups that were administered different doses of L/Z calorie sweeteners is a weakness. While the results of a majority of these studies did reflect that L/Z calorie intake increases prevalence and severity of MDD/depression, it may be possible that there is no difference between people who consume no L/Z calorie intake and low L/Z calorie intake. However, the authors did not report these findings.

It is imperative that studies create specific parameters around subjects' characteristics, so that confounding factors can be limited. However, there were a few studies that had confounding factors that could have a major impact on the results seen. In all of the human studies in aim 1, none controlled for diet or matched for diet, so that may have been a confounding variable affecting the results notated. An association has been found in rodents where L/Z calorie sweeteners affected gut microbiome changes and in conjunction with certain diets (high fat diets), these changes in GI bacterial abundances were further accentuated [57]. This could be the same for L/Z calorie intake in humans and severity/prevalence of mental health disorder(s), where differing diets accentuate findings seen. However, that cannot be analyzed when diet was not controlled for. For aim 2, which analyzed whether the gut microbiome is associated with mental health disorders, all but one experiment [48], did not separate out medicated and unmedicated subjects. For the 8 experiments that did not separate out medicated vs unmedicated subjects or exclude medicated subjects, their findings are weak due to the possibility that the gut

microbiome compositions of these individuals were affected by their mental health disorder(s), but it did not appear because they were altered by their medication(s). Moreover, there were a few studies that individually stuck out for their unique confounding factors. Peres-Ara et al. [42] was the only human study that demonstrated as L/Z calorie sweetener intake increased, mental health severity and prevalence decreased and a potential cause of this may have been due to all of the subjects included being overweight or obese. The possible increased inflammation and stress due to the weight of the subjects may have been a confounding variable that effected mood. Additionally, Perez-Ara et al. [42] was performed in multiple countries and the results were only found to be significant in subjects that originated and resided in Spain, which points towards cultural diet practices being a potential confounding variable. Kumar and Chail [34] found as saccharin increased, anxiety and depression symptoms decreased, and it was the only study to have subjects that were induced to have Type 2 Diabetes. The fluctuating blood sugars, and increased body mass index may have contributed to the results seen, thus making their results less influential on the overall findings that L/Z calorie sweeteners do impact mental health disorders.

Additionally, diagnostic methods used by some individual studies limited the impact of the findings of this literature review. In the human studies in aim 1, how the subjects were diagnosed varied significantly with MDD a Zung's Self-Reporting Depression Scale and Irritability Subscale being used by 1 study [39], the DSM in 1 study [42], a self-reporting scale in 2 studies [17, 41], Vandenberg MRT in 1 study [39], and diagnosed by a medical doctor (although no diagnostic criteria were given) in 1 study [64]. These variations in diagnostic methods used could have caused more people to be able to report being "more/less depressed" due to the different scales used. This could have interfered with the degree to which these L/Z

calorie sweeteners were credited for causing these changes. An additional weakness in the diagnostic methods of some studies in aims 2 and 3 is that some studies did not report lower-level bacterial taxa differences. Sometimes it is seen where statistically significant differences in abundances at the phyla level may not be detected, but there may be relative differences at the family, genera, and/or species level. Specifically, Frankenfeld et al. [56] found no significant differences at the phyla level. However, overall bacterial diversity was different for consumers of ace-K vs non-consumers according to comparisons of fecal collections. There is a possibility that the differences in bacterial diversity was seen due to lower-level taxa abundances differing, but without the reporting, it remains unknown.

Another weakness of these studies was that some of them did not employ methods that replicated what is seen in real life-one being the time of exposure to L/Z calorie sweeteners. In the animal studies analyzed for aim 1, the average exposure time of animals to these L/Z calorie sweeteners was 60 days. However, the results are supposed to reflect a trend seen in humans who have been consuming these sweeteners for a majority of their lives. Jones et al. [47], found that aspartame exposure for 6-8 weeks was needed for anxiety-like behaviors to occur. Therefore, it is a possibility that studies that analyzed L/Z calorie sweetener ingestion in connection with mental health disorders and did not find an association that was as strong may be due in part to the short length of exposure-time. Another condition that was used in many articles in aim 1 and 3 that did not represent what is seen in humans in the world pertains to the amounts of L/Z calorie sweeteners they exposed their subjects to. Many studies exposed the subjects to amounts of L/Z calorie sweeteners that are very close to the maximum acceptable daily intake (ADI) established for humans or beyond that limit. Each L/Z calorie sweetener has an ADI that is established by the joint Food and Agriculture Organization (FAO) of the United Nations (UN)

and the World Health Organization's (WHO) Expert Committee on Food Additives, known as JECFA [79, 80]. The ADI is "'the amount of a substance that can be consumed each day, even over a lifetime, without risk' [that] is usually expressed as milligrams of the substance per kilogram of body weight per day" [80]. The ADI for aspartame ranges from 40 to 50 mg/kg of body weight/day ($1/6^{th}$ of one 12-oz can of soda/kg of body weight), 5 mg/kg of body weight/day for saccharin (about 3 12-oz sodas/day for a 150 lb. person), 15 mg/kg of body weight/day for ace-K (about 25 12-oz sodas/day for a 150 lb. person) and 15 mg/kg of body weight/day for sucralose (about 34 12-oz sodas/day for a 150 lb. person) [12, 81]. Ashok and Wankhar [65] exposed their animal subjects to 75 mg of aspartame/kg of body weight/ day for 90 days. Frankenfeld et al. [59] had higher exposure groups that were exposed to 5.5 and 11/mg of sucralose/kg of body weight and Bian et al. [60] exposed their subjects to 37.5 mg of ace-K/kg of body weight, which are all more than the ADI allowed for either of any of those L/Z calorie sweeteners. Suez at el. [55] exposed the animal subjects to 0.1 mg of saccharin/mL of water and Uebanso et al. [58] had a high-dose sucralose group and ace-K exposure group that were each exposed to 15 mg/kg of body weight, which was exactly equal to the ADI for each of these L/Z calorie sweeteners. Additionally, many other studies exposed subjects to amounts of L/Z calorie sweeteners that were in the upper 75% of the ADI: [41] (30 mg of aspartame /kg) and [18] (32 mg of aspartame/kg). While Frankenfeld et al. [56] did not control the amount of L/Z calorie sweeteners or the kinds used, they did have very specific inclusion and exclusion criteria for their population and then analyzed their intake and the associated outcomes on the gut microbiome. The portion of their population that ingested aspartame consumed an average intake of 5.3 mg/day to 112 mg/day and for ace-K it was 1.7 to 33.2 mg/day, with the averages for both groups being way below the average amount of each of these L/Z calorie sweeteners consumed.

Their population was not representative of the public. These weaknesses make it so that results are hard to relate to humans due to it being above the average amount recommended and/or way above /below the average amount most consumed by humans. Additionally, administering amounts to subjects that is way above the maximum ADI advisory may be the reason for changes seen (or not seen). Specifically, Bian et al. [60] administered an amount of ace-K that was more than double the ADI and more than double the amount studied in the other two ace-K and gut microbiome studies [56, 58] and it was the only one of those three studies that found significant gut microbiome composition changes in connection with ace-K ingestion. The goal of these studies is to see if L/Z calorie sweetener consumption is the reason for mental health disorder prevalence or severity and/or gut microbiome changes, but that is not a possible outcome to draw if the exposure conditions do not replicate what is commonly seen in humans.

4.3.b Weaknesses and Limitations of This Literature Review's Approach

Alongside the weaknesses/limitations of the experiments, there were also a few short comings within the approach used in this literature review that must be considered when considering the findings overall. One weakness of this literature review was variation in study selection. For aim 1, due to the exclusion criteria used, the only human studies that were included resulted in all MDD/depression studies and no anxiety ones. This could have limited the findings, because there are many mental health disorders out there, however they were not represented in this data, so no findings were revealed as to whether L/Z calorie sweeteners impacted these other disorders. Another weakness of study selection is that only studies on aspartame, saccharin, and unspecified L/Z calorie sweeteners were included in aim 1 studies and only ace-K, sucralose, aspartame, and saccharin were included for aim 2. This is due to those

being the only L/Z calorie sweeteners included in studies that also followed the inclusion and exclusion criteria set forth by this literature review. This was a weakness, because one of the hopes of this literature review was to relate the increase in mental health disorders seen over the past 30 years in the world to the increase in L/Z calorie sweetener use seen within this time. However, this conclusion is limited due to the amount of missing data on some of the most common L/Z calorie sweeteners that have been consumed over the last few years such as: advantame, neotame, monk fruit extract, isomalt, lactitol, maltitol, mannitol, sorbitol, xylitol, and d-tagatose.

Alongside variation of study selection, another weakness of this literature review was not including more exclusion criteria. Studies included could have been limited to only experiments that controlled for the amount of L/Z calorie sweeteners combined. As previously stated, there were only 2 human experiments in aim 1 that controlled for the amount of L/Z calorie sweeteners [39, 41] used and the other 3 were observational [17, 42, 64]. Of all 5 human studies, only one showed an inverse reaction where increased L/Z calorie sweetener consumption increased, mental health disorder symptoms decreased [42]. One reasoning for this could have been due to L/Z calorie sweetener consumption not being finitely measured and controlled for. Including only articles that controlled for the amount of L/Z calorie sweetener consumed and the kind could have eliminated this limitation. Additionally, not excluding animal studies from being included was a weakness for all 3 aims-particularly aim 3 that used all animal studies, except for one. It has been discovered that what happens in rodent models is not as indicative as to what happens to humans as it was once believed to be [82]. This is due to humans being much more complex than rodents and the large number of external, uncontrolled for factors impacting both mental health disorders and gut composition in humans. The additional factors impacting

humans' mental health disorders and gut composition, alongside what is ingested (in this case, L/Z calorie sweeteners), makes humans a unique species that is often hard to find an equivalent subject to study when findings will then be attributed to humans. It is worth noting that had that exclusion criteria been applied, it would have limited the number of studies that were included. Acknowledging each of these weaknesses and limitations allows for these studies to be altered and replicated or for completely new hypothesis to be analyzed.

4.4 Implementation and Recommendations

4.4a Further Research

The findings of this literature review opened several new possibilities for additional research to strengthen the association surrounding how L/Z calorie sweeteners are affecting the gut microbiome and how the gut microbiome is connected to mental health disorders. Mental health disorders and L/Z calorie sweeteners are associated with shifts seen in gut microbiome composition but have not been connected to the same bacterial taxa. Instead of researching whether particular bacterial taxa are increasing/decreasing in all individuals with a particular mental health disorder, further research could investigate whether L/Z calorie sweeteners are responsible for disrupting the unique gut microbiota composition in each person with a mental health disorder diagnosis. This could be done through longitudinal studies where subjects are followed from birth onwards. They could potentially even begin the study prior to birth to see whether parents' consumptions of these L/Z calorie sweeteners while the baby is *in utero* is causing these mental health disorders as Jones et al. [47] found as a possibility. The aim of these studies would be to evaluate exposure to L/Z calorie sweeteners effects before individuals have

been exposed and before they have been diagnosed with mental health disorders to compare the changes before and after in the same individuals.

Another recommendation for further research is to focus on exposing subjects to pre-measured L/Z calorie sweeteners that are below the ADI for humans. Current research that evaluates L/Z calorie sweetener exposure that is above the ADI and/or above the average amount consumed by humans is not a fair explanation as to what is occurring in humans after exposure. Jones et al. [47] showed a dose-dependent reaction where mental health disorder symptoms increased as L/Z calorie sweetener amount consumed increased. Therefore, dose does matter and should be taken into effect when conducting further research. Currently, aspartame, saccharin, sucralose, and ace-K are the most widely studied L/Z calorie sweeteners, which has much to do with them being the oldest L/Z calorie sweeteners on the market. Since their approval for usage in 1981, 1958, 1998, and 1988, respectively, there have been 6 more L/Z calorie sweeteners approved [12]. For further research, it is recommended that these newer L/Z calorie sweeteners are used to see if their creation has led to the increase in mental health disorder prevalence and severity that has been seen over the past 10 years. Additionally, further research should analyze L/Z calorie sweeteners effects in the group that is most affected by mental health disorders-women aged 18 + [1].

There were a few unintended findings revealed while performing this literature review that led to recommendations for further research. Ashok and Wankhar [65] discovered that aspartame released a toxic substance, methanol, as a metabolite when it was consumed and the amount of methanol produced was 32 times the Environmental Protection Agency's (EPA) limit. Further research should investigate whether toxic metabolites released by these L/Z calorie sweeteners are a potential mediator of mental health disorders. While research on L/Z calorie

sweetener's toxic metabolites has been researched some, the findings have not been related to mental health disorders-mainly metabolic and inflammatory diseases [65]. Additionally, L/Z calorie sweeteners were found to impact glucose metabolism thus leading to inflammation and oxidative stress [55]. It has been shown that increased oxidative stress and inflammation have been associated with anxiety and depression [83, 84]. Additional research should be conducted to analyze whether this inflammation and oxidative stress caused by L/Z calorie sweeteners created through alterations to glucose metabolism is causing an increase in anxiety and depression's prevalence and severity. With additional studies investigating alternative possibilities as to how L/Z calorie sweeteners are affecting mental health disorders through altering functions of the GI tract, the hope is that the etiology of these mental health disorders will be uncovered, and treatments will be able to be improved accordingly.

4.4.b Public Health Implications: Current Policies and How They Should Change Regarding L/Z Calorie Sweeteners

Currently, there are very limited policies in any countries limiting and/or restricting L/Z calorie sweeteners. WHO released a statement in 2023 advising people not to use L/Z calorie sweeteners for "control of body weight or reduce the risk of noncommunicable diseases" [85]. A recommended change is for WHO to add "potential worsening of mental health disorders' symptoms" as a reason to limit and/or restrict L/Z calorie sweeteners, since both anxiety and depression are public health burdens worldwide. While there is just an association and not causation between L/Z calorie sweeteners and mental health disorders, there is currently only an association between anthropometric measurements and L/Z calorie sweeteners, and they released a statement suggesting people limit their consumption of them. If WHO is able to put out a

warning to limit L/Z calorie sweeteners based off existing evidence that points towards a possible causation for some disorders, then a warning to limit L/Z calorie sweeteners can be made for mental health disorders as well. Additionally, there are no policies requiring products to stay below the RDI for each L/Z calorie sweetener, only suggestions. It is recommended that policies be enacted in countries where L/Z calorie sweeteners are widely consumed that limit the total percentage of the daily RDI of each L/Z calorie sweetener that can be included in one serving of a food product.

Alongside releasing statements and policies limiting these L/Z calorie sweeteners, proper labeling should be required for each of these L/Z calorie sweeteners. Currently in each of the countries where L/Z calorie sweeteners are widely consumed, there are no requirements to include L/Z calorie sweeteners on the nutrition label of foods. Specifically in the US, the FDA requires that L/Z calorie sweeteners are included in the ingredients list (which is in order by weight), but it is not required to be listed on the label. Currently, the nutrition label on foods has "carbohydrates" as a category with "added sugars" as a required subcategory. It is recommended that "L/Z calorie sweeteners" is added under "added sugars" as an additional subcategory, so that more people can make informed decisions about their L/Z calorie sweetener intake. At the very least, food companies should be required to include the total amount of L/Z calorie sweetener used, even if it is not in plain sight on the nutrition label. Currently in the US, L/Z calorie sweeteners are only required to be included on the ingredients list, which puts the ingredients in order by weight. However, that gives very limited knowledge. For example, many L/Z calorie sweeteners are included in Greek yogurts on the shelf, and they often are listed on the ingredients label under "less than 1% of the total weight." To the average person, this seems negligible. However, a common single serve yogurt is 5.3 oz (150,252 mg), so less than 1% could be any

weight up to 1,502 mg of L/Z calorie sweetener. Depending on the L/Z calorie sweetener being used, this could be up to over 15% of the RDI in just one snack. When units and wording are changed, the amount no longer seems negligible. Diction is also important when labeling foods with L/Z calorie sweeteners and current policies surrounding word choice used with L/Z calorie sweetener labels need to be changed. Often, labels will say "no artificial sweeteners," when they include L/Z calorie sweeteners. While many people think that means no L/Z calorie sweeteners are included in their sweet-tasting foods, it actually is just companies finding loopholes and using L/Z calorie sweeteners that are derived from natural sources such as monk fruit extract and Stevia. From the findings, it is clear that naturally derived L/Z calorie sweeteners can have just as much of an effect on gut microbiome composition and mental health disorders as synthetic sweeteners. Additionally, health conscience words are often paired with these L/Z calorie sweeteners such as "healthier" and "better for you." Currently in the US, it is illegal to make positive health claims on alcoholic beverages and nicotine containing products, due to their overall negative health outcomes [86]. With the findings from this literature review, it is advised that policies and laws be drafted to make the same limitations on products containing L/Z calorie sweeteners. Considering 1 in every 5 US adult is depressed or about 7% of the total depression cases in the world reside in the US and about 31.1% of the US adult population or about 29% of the total anxiety cases in the world are in the USA [1], drastic measures such as those made for alcohol and nicotine products, should be made for L/Z calorie sweeteners. Knowing that L/Z calorie sweeteners are associated with increased mental health disorders and such a large percentage of the total world's cases of mental health disorders reside in the US, it would be beneficial for more people in the US to know how much L/Z calorie sweeteners they are consuming in an attempt to limit them.

4.4.c Public Health Implications: Nutritional Recommendations as Supplemental Treatment for Mental Health Disorders

Currently there are many treatments listed for mental health disorders such as: prescription medicines, lifestyle changes, psychotherapy or counselling, brain stimulation therapy, hospital and/or residential treatment programs [87]. However, many of these treatments are not sufficient on their own, come with many side effects and/or at an exorbitant cost that is not attainable by most. There is a dire need to create alternative and collaborative treatments that can be used in conjunction with other therapies. One recommendation is to limit or abstain from consuming L/Z calorie sweeteners if someone suffers from mental health disorders, specifically if someone with mental health disorders is using prescription medications to treat their disorder(s). Alongside the findings that L/Z calorie sweeteners worsen mental health disorder symptoms and potentially disrupt gut microbiota, it was discovered that they may disrupt medication efficacy through increased expression of CYP isozymes. Specifically, Abou-Donia et al. [59] found that sucralose increased expression of P-Glycoprotein and Cytochrome P-450 (CYP3A4 and CYP2D1 respectively), both of which limit the bioavailability of drugs by accelerating drug metabolism ultimately reducing their efficacy. This is just like grapefruit and many medications have warning to not consume grapefruit while taking the medication [88]. The same warning could be made for L/Z calorie sweeteners. While this finding needs additional studies to support the association, while further research is being done, it is not harmful to limit consumption or avoid L/Z calorie sweeteners all together. Another recommendation is creating a probiotic supplementation to offset gut microbiota changes in people who consume L/Z calorie sweeteners. Probiotic supplementation was found to restore gut microbiota composition in people treated with antibiotics and in babies who were delivered via caesarean section-both of

which have been shown to alter gut microbiota composition negatively [89]. This potential treatment would have to be created in conjunction with further research done on which microbes are associated with mental health disorders and/or L/Z calorie sweeteners. Potentially compounding unique pre- and pro-biotic blends for each individual based on their specific gut microbiota composition changes could be a futuristic treatment.

4.5 Summary and Restated Findings

L/Z calorie sweeteners are mediating mental health disorders, mental health disorders are associated with the GI microbiome, and some L/Z calorie sweeteners are associated with alterations in the GI microbiome. Sucralose may be mediating depression through altering the abundance of the *Bacteroides* genus. Additionally, other L/Z calorie sweeteners may be mediating depression and/or anxiety through altering individualized, unique balances of gut microbiota in the Firmicutes, Bacteroidetes, and Actinobacteria phyla. However, the specific species that they may be acting through and that are associated with anxiety and/or depression do not appear the same in the current datasets. It is recommended that current policies regarding food labeling and L/Z calorie sweetener consumption limitations be set forth by governing bodies and avoiding/limiting L/Z calorie sweetener intake be used as a supplemental treatment alongside other mental health disorder treatments.

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