Summer 2020

Association Between Urinary Enterolignans as a Marker for Gut Microbiome Diversity and Depression in NHANES

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by

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Bachelor of Science
University of South Carolina, 2017

Submitted in Partial Fulfillment of the Requirements

For the Degree of Master of Science in Public Health in

Epidemiology

The Norman J. Arnold School of Public Health

University of South Carolina

2020

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ACKNOWLEDGEMENTS

I would like to thank my thesis advisor, Dr. Susan Steck and my thesis committee members, Dr. Matthew Lohman, Dr. Michael Wirth, and Dr. FeiFei Xiao, for their support and guidance.

ABSTRACT

Depression is a chronic disease which inflicts functional and emotional impairment that has been a growing issue in the United States (US). Depression is the most prevalent mental disorder in the US and is the leading cause of disability in the world. By 2030, It is projected that major depressive disorder will be the second largest contributor to global burden of disease. Causes of depression are still largely unknown and treatments are often expensive, time consuming, and only available to certain populations. Treatment often requires a combination of medicines and therapy to be effective. In recent years, the gut microbiome has emerged as an important contributor to a variety of cognitive functions. It has been demonstrated in many studies that a healthy gut supports normal central nervous system function through the gut-brain axis. Clinical studies have reported that the gut microbiota of depressed patients is significantly different from that of healthy controls while some research has found that both the microbiota diversity and richness was lower in depressed patients in comparison to healthy controls. The microbiome is an emerging field of research with many of its mechanisms and potential still unknown. The current study examined the associations between urinary enterolignans (enterolactone and enterodiol) as a marker for gut microbiome diversity and depressive symptoms in human subjects using the National Health and Nutrition Examination Survey. The Patient Health Questionnaire-9 was used for determining depression status of participants. We categorized urinary enterolignans into quartiles using the distribution among the healthy, non-depressed participants and

examined associations with depressive symptoms using survey logistic regression. The study found that participants in the highest enterolactone quartile were less likely to be experiencing depressive symptoms compared to those in the lowest quartile. While associations between depressive symptoms and urinary enterodiol were generally inverse, the effect estimates were weaker and none of the trend tests were statistically significant. The results provide support for the hypothesis that gut microbiome diversity is inversely related to depressive symptoms. These findings may lend support to intervention studies aimed at altering the gut microbiota composition for improving psychological symptoms.

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CHAPTER 1

INTRODUCTION

Statement of Problem

Mental health problems are a growing concern in the United States (US). Depression is the most prevalent mental disorder in the US with 17% of adults and 12% of adolescents experiencing a depressive episode at least once in their lives. In the years 2009 through 2012, it was estimated that depression affected more the 5% of adults in the US (Pratt et al., 2014). Depression is a chronic disease in which nearly all sufferers experience functional impairment and two thirds of sufferers experience severe impairment (Kessler et al., 2003). In fact, depression is the leading cause of disability in the world (Murray et al., 2002). It is projected that by 2030, major depressive disorder will be the second largest contributor to global burden of disease (Mathers, 2006). The World Health Organization predicts depression will be the top cause of death by 2030 (Miret et al, 2013).

Physical impairments are associated with depression and it is shown that those with depression have an elevated risk of morbidity and mortality. Depression can aggravate existing heart conditions and is associated with an increased risk of stroke, hypertension, and death after myocardial infarction (Lucas, 2018). Those affected by depression are 11 times more likely to attempt suicide with more than one out of every 10 depressed individuals attempting suicide at some point in their lives (Kessler 1999, Office of Applied Studies 2006).

Along with the emotional distress depression has on such a large population in our society, it also has created a significant burden on our economy. In 2000 alone, major depressive disorder cost $83.1 billion dollars (Greenberg et al, 2015). Of these 83.1 billion dollars, 31% were direct medical costs, 7% were suicide related mortality costs, and 62% were workplace costs (Greenberg et al, 2015). Many who are afflicted with depression are in greater need for primary and specialty care visits, utilize health services more frequently, and are more likely to be hospitalized (Mojitabai,2001). It is estimated that those with depression lose 5.6 hours of productivity per week due to their symptoms (Stewart et al, 2003). This loss of productivity is seen in the 225 million workdays that are missed and the equivocating $36.6 billion dollars in salary lost each year (Kessler, 2006). In addition, individuals with depression are twice as likely to be unemployed compared to those who do not suffer from this disease (Mojitabai, 2001).

There has been a 37% increase in disability-adjusted life years of depression from the year 1990 to 2010 (Murray et al, 2013). The percentage is likely to grow as the condition becomes more prevalent each year. Currently, the effective remission rate of antidepressants ranges from 60% to 80%, and the rate of favorable prognosis is approximately 30% (Wilson et al, 2015). It is crucial that new strategies for treatment and prevention are identified.

Risk Factors

Causes of depression are still largely unknown and treatments are often expensive, time consuming, and only available to certain populations (McLaughlin, 2011). Often a combination of treatments is needed to manage depression (McLaughlin, 2011).

Depression can be characterized as a neuroplastic, neurochemical, and organization dysfunction of the brain (Falowski et al, 2011). There is also clinical evidence that support the hypothesis that alterations in neuronal serotonergic and noradrenergic function in the central nervous system is a cause of depression. (Nemeroff, 2002).

Depression is different with each person and can be a combined result of genetic heredity and environmental influences (Sullivan et al, 2000). More recent studies have found inflammation to be a contributing factor in depression through the activation of the complement system and increased levels of inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-a) (Maes 1999, Berk et al, 2013).

Treatment

There is a wide range of treatment options for depression. Antidepressant drugs are a common option for those with depression. It should be noted that only one-third of depression patients exhibit beneficial results from drug treatment. While SSRIs (selective 5-HT reuptake inhibitors) and SNRIs (selective 5-HT norepinehphrine reuptake inhibitors) are newer more effective treatments, they are expensive and often have undesirable side effects that create new problems and difficulties for patients (Fournier et al, 2009).

Cognitive behavioral therapy is another form of treatment commonly used as a supplement for reducing depression-related symptoms. It is based on the premise that inaccurate beliefs that form a basis for repetitive negative beliefs have a role in the etiology of depression. Once the thinking is corrected, it is theorized that the symptoms will be reduced (Hollon et al, 2010). However, studies have shown that there are

disparities in the groups that benefit from cognitive behavioral therapy such as those with chronic illness, poor sociodemographic status, and education levels having a poorer response rate (Fournier et al, 2009). For this reason, naturopathic medicine has become a major area of research for treating depression (Peng et al, 2015).

Microbiome

The human body is not only a host to its own cells but also 100 trillion bacteria and 10 million microbiota genes known as the microbiome which is 10 times the number of human body cells. There are an estimated 1,000 species and 7,000 strains of bacteria residing on any given human (Kelly et al, 2016). The intestinal tract is one of the more populated areas of bacterial concentration with nearly 1kg of bacteria, viruses, protozoans, fungi, and arachaea inhabiting it (Latavola et al., 2017). The microbiome first develops through vertical transmission from the placenta, amniotic fluid, and the meconium (Gritz et al., 2015). The diversity of one's gut microbiome varies greatly due to differences in diet, environmental exposures, genetic diversity, and health status. Despite the large difference between gut microbiomes, it is possible to distinguish between them by examining the enterotypes populating them. Bacteroides, prevotella, and ruminococcus are three species that can be observed to determine the type of diet a person is largely consuming. By examining a person's gut microbiome, much information about their health and health practices can be determined (The Human Microbiome Project Consortium, 2012).

Stress, diet, and medications are all reasons why an individual's gut microbiome could be altered. When a microbiome is altered resulting in dysbiosis, a number of health outcomes can occur. When dysbiosis occurs, there is an increase in intestinal

permeability which allows bacteria and their metabolites to enter into the systemic circulation. This also is known as leaky gut syndrome which illustrates how the gut, when in dysbiosis can have major health implications to humans (Clapp et al., 2017). Research on the gut microbiome has exploded in recent years with the advent of the Human Microbiome Project which has assessed microbiota in 15-18 body sites (Human Microbiome Project Consortium, 2012).

Gut Brain Axis a Mechanism for Depression

It has been illustrated in many studies that a healthy gut supports normal central nervous system function (Daulatzai, 2015). The mechanism backing this claim is known as the gut-brain axis (GBA). The gut-brain axis is the bidirectional communication between the gut microbiota and the central nervous system (Clapp et al., 2017). The intestinal tract is controlled by both intrinsic and extrinsic nerves. With 200-600 million nerves, the gut is known as the "second brain" (Gershon, 1999). The vagus nerve is primarily responsible for conveying messages between the gut and the brain (Anglin et al., 2015). The microbiota can release multiple different neurotransmitters which are capable of regulating nerve signals. These nerve signals affect a variety of neuropsychiatric parameters such as mood and cognition (Kali et al, 2016). Inflammation of the gastrointestinal tract can cause dysbiosis as a result of the release of cytokines and neurotransmitters. When these molecules are able to enter the body systemically via the leaky gut, the blood brain barrier then exhibits permeability. Brain function is then altered which could lead to symptoms of anxiety and depression (Gadek et al. 2013, Biesman et al, 2015).

Lipopolysaccharides (LPS) are another example of a proinflammatory endotoxin released during an inflammatory response. There is usually only a small amount of LPS in the gut because of the intestinal barrier. However, when a leaky gut is present LPS can enter the blood stream resulting in its ability to regulate the neural system increasing the activity of the amygdala which in turn affects emotion. One study showed that humans given LPS had inflammatory cytokines released, and an increase in circulating norepinephrine occurred which is linked to high depression mortality (Magniola et al., 2016). Even a relatively small increase in LPS is associated with acute anxiety and depression (Bested et al., 2013). Thus, there is ample biologic evidence to suggest that the composition of the gut microbiota may impact depression. However, given the relatively few studies that have collected stool samples in human populations in which depression data also are available, there is a need for more human studies examining associations between gut microbiome diversity and depression.

Specific Aims and Proposal

The aim of this thesis was to examine the association between gut microbiome diversity and depression using the National Health and Nutrition Examination Survey (NHANES). We used urinary enterolignans (enterolactone and enterodiol) measured on a continuous scale as a marker for gut diversity. Severity of depressive symptoms as assessed by the Patient Health Questionnaire (PHQ-9) was used as an indicator for depression and was scored as a categorical variable.

Significance of Research

Depression is an extremely prevalent disease that has major negative impacts on not only an individual but also the nation's productivity. Current treatments do little to

help those suffering with depression cope with their illness and often have undesirable side effects. Drugs for depression are expensive and usually are not obtained until after several consultations and doctors' visits. Because a person's microbiome is largely dependent on what they eat, making dietary changes and fostering a healthy microbiome could be a nonpharmaceutical safer approach to treating symptoms of depression thus potentially cutting spending on expensive treatments and healthcare costs.

The microbiome is an emerging field of research with many of its mechanisms and potential still unknown. Over 90% of the more than 4,000 articles on microbiota were published in PubMed from 2010-2015 (Khanna et al 2014). We examined the associations between urinary enterolignans (enterolactone and enterodiol) as a marker for gut diversity and depression in human subjects.

If an increased microbial diversity is associated with a decreased risk of depression, it could indicate an area of research that needs to be more closely explored to identify the mechanism by which this is occurring and represents a new target for intervention to treat or prevent depression.

CHAPTER 2

LITERATURE REVIEW

Microbiome Diversity

Eighty percent of the 100 trillion bacteria, fungi, viruses and other microorganisms that exist as human microflora are found in the gut. The two bacteria phyla that account for 70-75% of the gut microbiome are Bacteroidetes and Firmicutes. Some of the other bacteria commonly found in the gut, though in smaller quantities, are *Proteobacteria*, *Actinomyces*, *Fusobacterium*, and *Verrucomicrobia* (Diamant et. al, 2011). The microbiota in our guts play multiple roles. The gut microbiome acts as an intestinal barrier, promotes the continuous existence of gut microbiota, stimulates intestinal epithelial cell regeneration, synthesizes vitamins, and produces mucus and nourishes mucosa by producing short-chain fatty acids (SCFAs) (Burger-van Paassen et al, 2009). At birth, humans have an innate immune system that the gut microbiome stimulates and helps mature large parts of the intestinal lymphoid tissue and helps develop acquired immunity by stimulating local and systemic immune responses (Nell et al., 2010). Under various physiologic conditions, the gut can continuously stimulate the immune system which creates low level inflammation in the body which is typically done to fight off pathogens (Rakoff-Nahoum, et al., 2004).

Healthy Gut Diversity

Clinical studies have reported that the gut microbiota of depressed patients is significantly different from that of healthy controls (Kelly et.al, 2016 Zheng et al, 2016).

Some research found that both the microbiota diversity and richness was lower among depressed patients in comparison to healthy controls. In a study where the fecal microbiota of depressed individuals was compared to control individuals, depressed individuals were shown to have a lower richness and diversity (Kelly et. al, 2016). The depressed group also had increased levels of the inflammatory cytokine IL-6. Fecal microbiome transplantations were performed from depressed humans to germfree rats. These rats where shown to develop behavioral and physiological features of depression post-transplant. These rats also experienced a reduction in richness and alpha diversity (Kelly et. al, 2016). Similar results were found in a study that transplanted the gut microbiome of a patient with major depressive disorder (MDD) to rats. Fecal samples collected from depressed and nondepressed individuals showed depressed individuals had less Bacteroidetes than those without depression (Zheng et al., 2016).

Human and animal studies alike have shown probiotics' ability to reduce symptoms of anxiety and depression. Probiotics are often used to increase gut microbiome diversity and foster a stable gut environment free from dysbiosis. One study provided human patients with chronic stress a three-week probiotic treatment that contained Bifidobacterial species. The subjects who scored as depressed in the elated/depressed scale showed improvement in mood with these supplements (Benton et al, 2007). Another double blinded clinical trial tested the effectiveness of probiotics on depression with both rats and humans. The human study gave participants either a placebo or a probiotic in a 30-day trial. Of the 66 participants that entered, 55 completed the study. Participants were Caucasian males and females ages 30-60 years. Depression was measured using the Hopkins Symptom Checklist (HSCL-90), the Hospital Anxiety

and Depression Scale (HADS), the Perceived Stress Scale, the Coping Checklist (CCL) and 24 h urinary free cortisol (UFC). The participants given probiotics had reduced urinary cortisol levels and improved psychological effects to a similar magnitude as those on the antidepressant. The 36 male rats were either given diazepam, probiotics, or a placebo. The reduction in stress/anxiety score were statistically significantly reduced for the probiotic and diazepam group compared to the placebo group (p-value 0.001) (Messaoudi et al., 2011). The results of these studies have public health relevance because probiotics are easily accessible to people through either capsules or those that naturally occur in foods like yogurt and kefir (Sehlub et al, 2014). A summary of the studies related to the gut microbiome and depression in humans is provided in Table 1.0.

Inflammation and Dysbiosis

When the gut microbiome undergoes stressors such as changes in diet, medications, or stress, the composition of the microbiome changes. Many diseases that are prevalent in Westernized countries are associated with dysbiosis and a loss of gut microbial diversity (Mosca et al, 2016). Loss of microbial diversity is a common feature in dysbiosis. Much of the Western lifestyle can explain why the US is shown to have lower gut diversity compared to other lesser developed countries (Mosca et al, 2016). Diets high in processed foods and low in fiber are unable to provide many of the healthy gut bacteria with enough nutrients to survive (Mosca et al, 2016). When the gut microbiome is in a dysbiotic state, there is an increase in intestinal permeability which allows bacterial metabolites and even bacteria themselves to leak into systemic circulation (Clapp et al.,2017). This contributes to chronic low-grade inflammation which can be observed in patients with depression. Chronic low-grade inflammation in the gut

has been associated with several subgroups of depressive disorders (Dantzer et al, 2008). Both internal and external factors can cause inflammation in the body. When inflammation appears in the gastrointestinal tract, cytokines and neurotransmitters are released. With the increase in intestinal permeability, the cytokines can travel systemically through the body. When the blood carries higher levels of cytokines such as TNF-a and monocyte chemoattractant protein, there is then a permeability in the blood brain barrier which allows these inflammatory cytokines to influence the brain (Gadek et al, 2013).

Gut-Brain Axis Pathways

The gut-brain axis is the bidirectional communication between the gut and the brain (Cryan et al., 2012). Gut bacteria can affect the cognitive functions of the brain (Jenkins et. al, 2016, Schmidt et al 2015). There is still much to be understood about the gut-brain axis but there are a few proposed pathways theorized. Some pathways from the gut microbiota include the neuroanatomical pathway of gut-brain axis, neuroendocrine hypothalamic–pituitary–adrenal axis (HPA axis) pathway, gut immune system, gut microbiota metabolism system, and intestinal mucosal barrier and blood brain barrier (Wang et al, 2016). Disruption in the gut microbiome/reduced diversity could offset any of these pathways (Wang et al, 2016).

Neuroanatomical Pathway of Gut-Brain Axis

Two neuroanatomical pathways the gut uses to interact with the brain are the autonomic system/vagus nerve and the enteric nervous system. The enteric nervous system is in the gut while the autonomic nervous system and vagus nerve are in the spine. The vagus nerve provides a direct link between the gut and the brain. Bacteria can

stimulate the afferent neurons in the enteric nervous system which signals the vagus nerve to induce either an anti-inflammatory or pro-inflammatory response. The most important effect of the dorsal motor nucleus of the vagus nerve is prominent in the upper gastrointestinal tract, and the cholinergic neurons on myenteron of upper gastrointestinal tract regulate vagal excitability effect (Chang et al, 2002).

Neuroendocrine HPA axis pathway

Gut bacteria are responsible for much of the hormone synthesis in the body. Dopamine and serotonin are synthesized in the gut which can directly influence the brain (Galland, 2014). Some bacterial enzymes in the gut are responsible for releasing neurotoxins as their byproducts which also can directly affect the brain (Galland, 2014).

Lacking gut microbiota and low/lack of expression of toll-like receptors (TLRs) contribute to producing a neuroendocrine response to the pathogens in the gut (O'hara et al., 2002). Some studies have shown that the HPA axis can be activated by the gut microbiome when there is increased permeability of the intestinal barrier due to an inflammatory environment. Stress induces endotoxemia and low-grade inflammation by increasing barrier permeability (de Punder et al., 2015). Gut microbiome development in early stages of life have been recognized to influence later brain function and behavior including neuroendocrine responses to stress (O'Mahony et al., 2017).

Gut and the immune system

Approximately 70–80% of the body's immune cells are contained within the gut-associated lymphoid tissue. Development of the immune system depends on gut microbiota (Mayer et al., 2015). While germ-free mice showed no immune activity, immune function could be produced when they were introduced to various microbes.

Lack of a gut immune system as a result of a germ-free environment has been shown to exemplify anxiety behaviors in multiple animal studies. Central nervous system neurotransmission can be disrupted through dysbiosis (Heijtz et al.,2011, Neufeld et al., 2010). Because the gut microbiome can induce an inflammatory response, it may also be involved in the regulation of emotion and behavior (Forsythe et al., 2010).

Gut microbes also metabolize bile acids and amino acids to produce other chemicals that affect the brain (Clarke et al., 2013). There is more than 100 times more genetic information in the human microbiome than the human genome. There are genes that encode proteins for metabolic functions that produce metabolites only found in the microbiome. These metabolites can affect the gastrointestinal environment and the gut wall and can reach the brain if they are absorbed into the systemic circulation (Clarke et al., 2014).

Urinary Enterolignans

Enterolignans (enterolactone and enterodiol) are metabolic byproducts of lignans, polyphenolic compounds found in plant foods such as grains, legumes and seeds. The gut has many microorganisms that are capable of digesting lignans which makes it the primary location for lignan metabolism. High urinary enterolignan concentrations can provide a marker of a microbial environment capable of biotransforming lignan precursors. In a cross-sectional study using human stool samples, gut microbiome diversity was positively related to urinary enterolactone levels but was not substantially associated with urinary enterodiol levels (Hullar et al., 2014). Both alpha- and beta-diversity were higher among subjects with high enterolactone excretion. Dietary intake is

also related to enterolignan excretion, with fiber intake being especially highly correlated with urinary enterolignan levels (Hullar et al. 2014).

Urinary enterolignan concentrations have been used as a marker for gut microbiome diversity in studies using NHANES data previously. One study reported that a more pro-inflammatory diet (as assessed by the Dietary Inflammatory Index) was associated with lower concentrations of urinary enterolignans, and hence, lower gut microbiome diversity (Shivappa et al, 2019). Urinary enterolignan data from NHANES also was used as a marker for gut microbiome diversity when examining the relationship between gut microbial environment and obesity (Frankenfeld et al., 2013). Currently, NHANES does not directly measure gut microbiota composition, such as by 16s rRNA sequencing methods or metagenomics, thus, urinary enterolignans are used in the current study as an indirect marker for gut microbiome diversity.

Potential Confounders

Antibiotics

The effects of antibiotics on microbial diversity has been heavily researched. Humans are exposed to antibiotics not only as medications but also in many of the foods consumed as antibiotics are often used in farming (Department of Agriculture, 2006). Broad spectrum antibiotics can affect the diversity of bacteria in a gut microbiome, reducing diversity by 30% (Dethlefesen et al., 2011). Currently, microbial resistance in human populations has led to concerns about overprescribing of antibiotics (Fleming-Dutra, 2016). One study used the National Ambulatory Medical Care Survey and National Hospital Ambulatory Medical Care Survey to assess antibiotic use trends (Fleming-Dutra, 2016). They defined an antibiotic prescription to be “unnecessary” based

on national guidelines and regional variation in prescribing. The diagnosis-specific prevalence and rates of total and appropriate antibiotic prescriptions were determined (Fleming-Dutra, 2016). Thirty percent of the 154 million antibiotic prescriptions filled each year were determined to be unnecessary (Fleming-Dutra, 2016) Forty-four percent of the outpatient antibiotic prescriptions were written to treat many respiratory conditions (Fleming-Dutra, 2016) with nearly half of these outpatient prescriptions determined to be unnecessary (Fleming-Dutra, 2016). The gut microbiome can recover from antibiotic exposure, but it often takes months or years (US Department of Agriculture, 2008).

There are theories that humans ingest antibiotics through the consumption of animal products. Because livestock are often raised in close quarters, safety measures must be taken to manage infections that can be spread from animal to animal. Antibiotics are used to treat many of the infections common among livestock. They are also used to increase animal growth (McEwen et al., 2002). Nearly 16% of lactating cows in the US receive antibiotics for acute infections. However, almost all dairy cows receive antibiotics following lactation to prevent the spread of infections (McCluskey, 2003). Almost 15% of calves receive antibiotics for acute infections while 10% of non-infected calves receive antibiotics for preventive measures (Dargatz, 2000). Around 88% of swine receive antibiotics as disease prevention and growth promotion (Dargatz, 2000).

Maurice et al. found in their laboratory study that antibiotics can alter the physiological state and activity of the gut resulting in an increased number of damaged membranes and a decrease in the number of active populations of bacteria. A number of genes involved with antibiotic resistance and stress response also were increased in expression as a result of antibiotic exposure (Maurice et al., 2012). When gut microbiome

diversity is inhibited, opportunistic bacteria have a greater chance at being able to flourish such as C. Difficile. In one study, early antibiotic exposure reduced the diversity of a child's microbiome and altered its composition (Tanaka et al., 2017).

Antibiotic use has been linked to depression in one study. A single antibiotic course of treatment was associated with a higher risk for depression [adjusted odds ratio (AOR) of 1.23 for penicillins (95% confidence interval (CI), 1.18-1.29) and 1.25 (95% CI, 1.15-1.35) for quinolones]. The risk increased with recurrent antibiotic exposures to 1.40 (95% CI, 1.35-1.46) and 1.56 (95% CI, 1.46-1.65) for 2-5 and > 5 courses of penicillin (Lurie et al., 2015). Thus, recent antibiotic use may be an important factor to consider in the relationship between gut diversity and depression.

Oral Steroid Use

While some steroids are use to treat gastrointestinal disorders such as Crohns, they also can increase the risk of other disorders such as ulcers due to the alteration in mucosal lining they can cause (Huang et al, 2015). When oral steroids are taken, gut bacteria has been shows to be responsible for keeping an anti-inflammatory environment. When the mucosal layer is damaged, inflammation increases and gut dysbiosis is more likely to occur (Huang et al, 2015).

Age

The composition of a microbiome varies greatly by age. An infant has a very different microbial composition compared to a young adult. Illness, medication use, and diet are some key components that explain why the gut microbiome changes over time. Langille et al. observed a statistically significant difference in taxonomic patterns in the gut microbiomes of young, middle, and old mice (Langille et al., 2014).

Similarly, gut flora clustering patterns were shown based on different age ranges, generally decreasing in diversity in samples of older individuals. Common to all age groups were the Bacteroidaceae, Lachnospiraceae, and Ruminococcaceae families. However, their abundance decreased as age increased (Yatsuneko et al., 2012).

Body Mass Index

Bacteroidetes and Firmicutes are two of the more dominating beneficial bacteria in the human gut microbiome. In a study done by Ley et al., the proportion of Bacteroidetes was lower in obese people compared to those who were lean. Weight loss and low-calorie diets were shown to reverse this disproportion (Ley et al.,2006). Turnbaugh et al. similarly found that the microbiome associated with a greater body mass index (BMI) had a greater capacity to harvest energy and that when lean mice were colonized with this microbiome, they gained a significantly greater body weight. Obese mice that were colonized with the lean microbiota lost weight (Turnbaugh et al., 2006).

Obesity is also one of the most common comorbidities of depression (De Wit et al., 2010). The temporality of the association is complex, as it has been reported that obesity can increase the risk of depression while depression can increase the risk of obesity (Luppino et al., 2010).

Gender

Women are nearly twice as likely to be depressed compared to men (American Psychiatric Association, 2013). This gap is apparent in all socioeconomic groups. Hormonal differences and differences in HPA axis activation levels are some proposed theories for this disparity. Women also experience different interpersonal environmental exposures than men (American Psychiatric Association, 2013, Kuehner, 2017).

Alcohol

When comparing alcoholics to a control group, chronic alcohol abuse has been shown to change the bacteria composition in the gut. This dysbiosis was correlated with high levels of serum endotoxin (Mutulu et al., 2012). Chronic alcohol abuse has been shown to lead to small intestinal bacterial overgrowth resulting in dysbiosis.

Some of the highest rates of depression are among those who abuse alcohol. Research has shown that anywhere between 30-50 percent of people who abuse alcohol experience depressive symptoms. One household survey found that 16% of those with depression had an alcohol diagnosis (Bode et al., 1984). Those with depression were found to have a 30% lifetime alcohol problem (Lynn et al., 2005).

Smoking

Smoking has been explored as an environmental factor that can alter the human gut microbiome community. In a clinical trial by Beidermann et al., gut microbiome composition was analyzed with smoking cessation. Diversity in those who quit smoking was significantly higher than their baseline measurements. After 8 weeks the trend continued, and microbiome diversity increased. Those in the control group who did not quit smoking showed the same levels of diversity throughout the study (Biedermann et al., 2013). In another human study, gut microbial diversity was assessed in Crohn's disease patients who smoked vs nonsmokers. Gut microbial gene richness, gene diversity, and species diversity was decreased in abundance among smoking participants along with other genera (Opstelleleten et al.,2016).

While it is unclear why smoking is more prevalent among those with mental health issues there is a clear association between depression and smoking. For every ten cigarettes smoked in the US, three are smoked by someone with a mental illness (CDC, 2013).

Socioeconomic Status

Socioeconomic status (SES) has been shown to be associated with risk for depression. Nationally representative data found a significant association between depression and SES (p-value <0.0001) across multiple countries. As SES increased, depression decreased (Freemen et al., 2016). An increasing amount of evidence shows that purchasing and consumption of unhealthy diets and also eating fewer fruits and vegetables is strongly associated with socioeconomic status. Those with lower SES have been shown to have a lower quality diet compared to those with a higher SES (Appelhans et al., 2012, Darmon and Drewnowski, 2008). Therefore, those with lower SES are less likely to have a varied and healthy diet that would foster a diverse gut microbiome.

Race

Depression rates have been reported to vary by race (Dunlop et al., 2013). Minorities usually present higher rates of depression compared to whites. Minorities more often have greater health burdens and a lack of health insurance (Dunlop et al., 2013). Using population based national data, one study found African Americans OR = 1.16, 95% CI= 0.93, 1.44) and Hispanics (OR = 1.44, 95% CI = 1.02, 2.04) had higher rates of major depression compared to whites (Dunlop et al., 2013). Studies have shown that gut microbiome diversity differs between races globally and across races in the US (Gupta et al.,2017, Brooks et al., 2018), likely related to ancestry of various races and

cultural and lifestyle differences (Gupta et al.,2017, Brooks et al., 2018). Western societies were shown to have the lowest gut diversity compared to foraging or agricultural societies (Gupta et al., 2017). Brooks et al. used data from the American Gut Project to analyze microbiome differences among Hispanics, Whites, Pacific Asians, and African Americans living in the US. Subtle but significant differences were found in taxonomic composition between four ethnicities (Brooks et al.,2018).

Table 2.1 Summary of Studies Related to Gut Microbiome Diversity and Depression

Author, yr	Study Design	Country	Sample Size	Outcome	Exposure	Confounders/ Effect modifiers	Results
M. Soledad Cepeda, 2016	Cross sectional	United States	18,090	Patient Health Questionnaire scores ≥10	Any probiotic food or supplement	age, gender race/ethnicity, overall health, lifestyle, and socioeconomic status	The overall prevalence of mild or more severe depression was 22.80% (PHQ-9 ≥5) and 7.59% for moderate or more severe depression (PHQ-9 ≥10). Subjects exposed to probiotics were less likely to have depression. After adjustment, the association between probiotic exposure and depression disappeared.
Benton, 2007	A double-blind placebo-controlled trial with random allocation of subjects.	United Kingdom	132	Profile of mood state questionnaire assessed mood	A probiotic-containing milk drink and a milk-based placebo		No general effects of taking the probiotic were found when daily mood ratings were considered.
Kelly, 2017	Clinical Trial	Ireland	34 depressed patients and 33 healthy patients	Alterations in depression using levels of SCFA	Alterations in the gut microbiota composition in patients	Education level, employment status, smoking and	Depression is associated with an altered gut microbiota composition, richness and diversity. Richness and diversity was lower in depressed patients.

					with depression	alcohol consumption.	
Zheng, 2016	Clinical Experiment	China	58 major depressive disorder patients and 63 healthy controls	Gut microbial communities from fecal samples	Depression	age and body mass index were controlled for	Compared with healthy controls, the relative abundances of Actinobacteria were increased in MDD subjects, while those of Bacteroidetes were decreased
Verdam, 2013	Clinical Experiment	Netherlands	28	Fecal microbiota compositio Fecal calprotectin and plasma C-reactive protein levels	Obesity	Exclusion if subjects received antibiotics in the last 6 months, used anti-inflammatory drugs, or reported alcohol consumption >63 g/week. Other exclusion criteria were acute and chronic inflammatory diseases	Obese participants showed reduced bacterial diversity, a decreased Bacteroidetes/Firmicutes ratio, and an increased abundance of potential proinflammatory Proteobacteria. Interestingly, fecal calprotectin was only detectable in subjects within the obese microbiota cluster. Plasma C-reactive protein was also increased in these subjects and correlated with the Bacteroidetes/Firmicutes ratio

Luries, 2015	Nested Case Control	United Kingdom	202,974	Risk for depression, anxiety, or psychosis	Therapy with 1 of 7 antibiotic classes > 1 year before index date	obesity, smoking history, alcohol consumption, SES, and number of previous urinary tract and upper and lower respiratory infections	Treatment with a single antibiotic course was associated with higher risk for depression with all antibiotic groups. The risk increased with recurrent antibiotic exposures to 1.40 (95% CI, 1.35-1.46) and 1.56 (95% CI, 1.46-1.65) for 2-5 and > 5 courses of penicillin. Recurrent antibiotic exposure is associated with increased risk for depression and anxiety but not for psychosis.

CHAPTER 3

RESEARCH METHODS

To accomplish the specific aims, we utilized cross-sectional data from NHANES, including multiple waves (2005-2006, 2007-2008, and 2009-2010). The data are de-identified and freely available to the public and thus deemed exempt from IRB approval. NHANES is a cross-sectional survey with multiple stages that is produced by the National Center for Health Statistics. The goal of the NHANES survey is to measure the health and nutrition status of both children and adults in the US. The NHANES survey is administered every two years to collect a nationally representative depiction of the health of the United States. The study population for the project was restricted to 2005-2006, 2007-2008, and 2009-2010 NHANES survey data with no missing data on any covariates. We excluded subjects who reported oral antibiotic or oral steroid use within the past 30 days. Years were chosen based on when urinary enterolignans were measured and PHQ-9 was used in NHANES along with the other covariate measures.

Main Exposure: Urinary Enterolignans as a Marker for Gut Diversity

Enterolignans (enterolactone and enterodiol) were measured in spot urine samples. Urine samples were collected the morning after a fast by agreeing participants and the samples were stored at -20C until they were processed and shipped to the Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention to be analyzed. High performance liquid chromatography–atmospheric pressure photoionization–tandem mass spectrometry

(HPLC–APPI–MS/MS) was used for the quantitative detection of enterolactone and enterodiol. Human urine samples were processed using enzymatic deconjugation of the glucuronidated phytoestrogens followed by size-exclusion filtration. Phytoestrogens were then separated from other urine components by reversed-phase HPLC, detected by APPI–MS/MS, and quantified by isotope dilution. Assay precision was improved by incorporating carbon-13 labeled internal standards for each of the analytes, as well as a 4-methylumbelliferyl glucuronide and 4-methylumbelliferyl sulfate standards to monitor deconjugation efficiency (Geinstein, 2010). Creatinine values were used to account for urine dilution. To do this, each individual's enterolignan concentration value (ng/ml) was divided by the corresponding urinary creatinine value (mg/dL) and standardized to ug/g units (Shippava et al., 2019). We categorized urinary enterolignans into quartiles using the distribution among the healthy, non-depressed participants.

Primary outcome

Depression was measured in NHANES with the Patient Health Questionnaire (PHQ-9). The PHQ-9 is a 9-item depression screening instrument that asks participants to choose one of four responses about frequency of depressive symptoms during the previous 2 weeks. NHANES has been using the PHQ-9 since 2006 and it can be used to assess major depression and depression severity. There are nine items on the examination with a final follow up question regarding overall impairment of the depressive symptoms. The responses for the nine questions range from "not at all", "several days", "more than half the days" and "nearly every day." These were scored 0-3. The test is administered by trained interviewers using a Computer-Assisted Personal Interviewing System as part of the private interview in English or Spanish. No proxies or interpreters are allowed for this

examination. The exam allows for a maximum score of 27 and a minimum score of 0 (CDC, 2013). Two different methods were used to create the dichotomous outcome. The Pfizer method classified a participant as depressed if a participant marks 2-4 questions as a level 2 or 3 response (one of which corresponds to the first or second question in the survey). Those not meeting this criterion were categorized as not having depressive symptoms. In the Kroenke method, subjects are classified as having depressive symptoms if PHQ-9 scores are greater than or equal to 10. In their study, a cut point of 10 was shown to be 88% sensitive in identifying participants with a depressive disorder (Kroenke et al., 2001). In exploratory analyses, we also used the method by Pfizer which creates a three-level outcome. A total score of 0-4 is minimal depression, a score of 5-14 is moderate depression, and a score greater than 15 is severe depression (Cepeda et al., 2017).

Confounders

Antibiotic and Steroid Use

NHANES collects data on prescription medications used in the past 30 days. This is done through direct abstraction from prescription medication containers. During the household interview, respondents aged ≥16 years were asked: 'In the past 30 days, have you used or taken medication for which a prescription is needed?' For respondents under the age of 16 or those that could not respond to the question, a proxy responded. Those who answered affirmatively were asked to give their prescription medication containers to the interviewer and report details of its use. The participant verbally reports medications used if the container is not available.

The drug names reported were converted into a generic drug name for the data release and a therapeutic drug class was assigned based on the Multum Lexicon Drug Database. A dichotomous variable was created to indicate whether a participant had taken an antibiotic in the past 30 days by examining the first level category 'anti-infectives'. Subjects who reported using an antibiotic within the past 30 days were excluded from analyses. Topical antibiotics were not excluded (Kantor et al, 2015). Anyone who had taken prednisone, prednisolone, methylprednisolone, or methylprednisolone acetate was marked as positive for taking an oral steroid in the past 30 days and was then excluded (Sversky et al., 2011,).

Laxatives/Stomach Illness

Any participant who had reported taking a laxative before their laboratory testing or reported having a stomach illness in the past 30 days were excluded.

Alcohol Use

Alcohol was measured by the self-reported average number of alcoholic drinks consumed each day over the previous year (continuous variable).

Smoking

Smoking data were self-reported by the subjects in response to the following questions: Have you smoked at least 100 cigarettes during your entire life?; Do you smoke cigarettes now?; On the average, how many cigarettes a day do you smoke?; How long has it been since you smoked cigarettes fairly regularly?; During the period when you were smoking the most, about how many cigarettes a day did you actually smoke?; and About how old were you when you first started smoking cigarettes fairly regularly? Nonsmokers were defined as: never smoked 100 cigarettes and does not smoke cigarettes

currently. Former smokers included recent former smokers (quit smoking cigarettes during the year prior to interview), and longer-term former smokers (quit smoking cigarettes more than one year prior to interview. Categories include never smoker, former smoker, and current smoker. Current cigarette smokers were categorized by pack years; 0-10, 11-20, and >20. Former smokers were categorized by how long ago they quit smoking; <10 years or >10 years.

Gender

The variable gender in the NHANES dataset is a self-reported measure having the value male or female.

Age

Age is a self-reported measures and continuous variable.

Body mass index (BMI)

BMI values are calculated for NHANES participants using measured height and weight values as follows: weight (kilograms)/height (meters)^2. BMI criteria were used to screen for weight categories: underweight (BMI values < 18.5), normal or desirable weight (BMI values 18.5-24.9), overweight (BMI values 25.0-29.9), obese (BMI values greater than or equal to 30) (National Institutes of Health, 1998).

Race

Races is a self-reported measure and is a categorical variable: Mexican American, Non-Hispanic White, Non-Hispanic Black, or Other Race.

Poverty Index Ratio (PIR)

Poverty level index (INDFMMPI) was then grouped into three categories (i.e., INDFMMPI ≤1, 2 < INDFMMPI ≤ 4; INDFMMPI >5.

Marital Status

Marital status is a self-reported measure and a categorical variable: single, married, widowed, or separated/divorced.

Education

Education is a self-reported measure and a categorical variable: Less than 9^{th}, 9-11 grade, high school, some college, or greater than college.

Missing Data

Any participants missing outcome, covariate, or exposure data were not included in the final analyses.

Statistical Analyses

NHANES uses a complex survey design which requires the use of sample weights to create a representative US population. Environmental 2-year weights provided for the phytoestrogen data were utilized. Using the estimation procedure guidelines provided by NHANES, we multiplied the weight variable by 1/3 because we included three survey periods. (National Center for Health Statistics 2007). All statistical analyses were completed using SAS 9.4.

A complex survey design was used to analyze the association between urinary enterolignans and depression. We first ran a simple survey logistic regression model for the main exposure and outcome variables. We then used survey logistic regression models to calculate the OR and 95% CI for the association between urinary enterolignan levels and depression while controlling for potential confounders listed above. Covariate selection was chosen based on the literature supporting their association with both gut

diversity and depression. The p for trend was calculated using the continuous urinary enterolignan variable in each of the models.

We hypothesized that higher urinary enterolignan levels would be associated with lower odds of depressive symptoms. We ran the analysis with urinary enterolignan levels being categorical variables (quartiles) using cut points based on the control (non-depressed) population. We used the lowest urinary enterolignan quartile as the referent group in logistic regression analyses.

Depression was coded as a dichotomous outcome with two different scoring methods. Using the Kroenke method, subjects are classified as having depressive symptoms if PHQ-9 scores were greater than or equal to 10. Those less than 10 was classified as not having depressive symptoms. Using the Pfizer method, a participant who marks 2-4 questions as a level 2 or 3 response (one of which corresponds to question 1 or 2) was categorized as having depressive symptoms. Those not meeting this criterion were categorized as not having depressive symptoms.

In exploratory analyses, we also ran a survey logistic regression model utilizing a three-level outcome variable where a total score of 0-4 is minimal depression, a score of 5-14 is moderate depression, and a score greater than 15 is severe depression. Three separate survey logistic regression models were run; minimal depression vs severe depression, minimal depression vs moderate depression, and moderate depression vs severe depression. (Cepeda et al., 2017). To examine whether the association between urinary enterolignans and depression is modified by certain covariates (specifically age, gender, smoking status, BMI, and race), we stratified on the *a priori* chosen categorical covariates.

CHAPTER 4

RESULTS

Tables 4.1-4.3 Descriptive Statistics

Participants missing data on enterolactone concentration, enterodiol concentration, PHQ-9 scoring, and/or any of the covariates were excluded. The final sample consisted of 1,933 subjects (Figure 4.1). These tables represent raw sample sizes. When conducting the analysis, weighted means and frequencies were utilized for descriptive purposes. When using the Pfizer dichotomous outcome scoring method, 1,712 participants were not depressed and 221 had depressive symptoms. When using the Kroenke dichotomous outcome scoring method, 1,739 participants were not depressed, and 194 participants had depressive symptoms. When categorizing depression as a three-level outcome, 1,314 had minimal depression, 554 had moderate depression, and 65 had severe depression. In all three scoring methods, average enterolactone and enterodiol concentrations were lower in the depressed groups when compared to the non-depressed counterparts. In each scoring method, a greater percentage of females where in the depressed categories.

Table 4.4 Reference Ranges

Enterolignan quartile cutpoint values were determined by using the distribution of urinary enterolignan concentrations of the non-depressed group in each scoring method. The ranges for the quartile cutpoints are provided in Table 4.4.

Table 4.5 Main Effects in Dichotomous Outcome – Pfizer

When using the Pfizer method for categorizing the dichotomous outcome variable, an inverse association between urinary enterolactone and depressive symptoms was observed in the simple model (OR_{Q4vsQ1}: 0.39, 95% CI: 0.19-1.02) which was slightly attenuated after adjustment for multiple covariates (OR_{Q4vsQ1}: 0.52, 95% CI: 0.24-1.13). No association was observed between urinary enterodiol concentration and depressive symptoms (OR_{Q4vsQ1}: 0.97, 95% CI: 0.50-1.90 in the multivariable adjusted model).

Table 4.6 Main Effects in Dichotomous Outcome - Kroenke

For enterolactone, a statistically significant reduced odds of depression was observed among those in the highest quartile in the simple model. The association remained statistically significant in the adjusted model for both quartile 3 and quartile 4 as compared to quartile 1 (OR_{Q3vsQ1}: 0.53, 95% CI: 0.30-0.95; OR_{Q4vsQ1}: 0.29, 95% CI: 0.14-0.60).

While not statistically significant in either the simple or adjusted model, a modest inverse association was observed between enterodiol concentration and depression (OR_{Q4vsQ1}: 0.79, 95% CI: 0.41-1.54 in the multivariable-adjusted model).

Table 4.7-4.9 Main Effects in Three Level Outcome

When comparing those in the minimal depression group to those in the severe depression group, a statistically significant inverse association was observed in the simple model for urinary enterolactone (OR_{Q4vsQ1}: 0.31, 95% CI: 0.10-0.93) which was attenuated and no longer statistical significant in the multivariable-adjusted model (OR_{Q4vsQ1}: 0.46, 95% CI: 0.15-1.42). While not statistically significant in either the

simple of adjusted model, an inverse association was observed between urinary enterodiol concentration and severe depression (OR_{Q4vsQ1}: 0.56, 95% CI: 0.15-2.12 in the multivariable adjusted model).

When comparing those in the no depression group to those in the moderate depression group, a statistically significant inverse association was observed in the simple model comparing quartile 4 of urinary enterolactone to quartile 1 (OR_{Q4vsQ1}: 0.60, 95% CI: 0.41-0.87) which was attenuated and no longer statistically significant in the adjusted model (OR_{Q4vsQ1}: 0.74, 95% CI: 0.48-1.13). For enterodiol, no association was observed with depressive symptoms (OR_{Q4vsQ1}: 1.09, 95% CI: 0.69-1.74 in the multivariable adjusted model).

When comparing those in the moderate depression group to those in the severe depression group, inverse associations were observed for both urinary enterolactone and enterodiol, though CIs were imprecise (enterolactone: OR_{Q4vsQ1}: 0.67, 95% CI: 0.23-1.92; enterodiol: OR_{Q4vsQ1}: 0.66, 95% CI: 0.19-2.26).

Table 4.10-4.14 Effect Modification by Gender, Smoking Status, BMI, Age, Race

For all stratified analyses, we utilized the dichotomous outcome variable created using the Kroenke method because the strongest main effects were observed for that outcome. When stratified by gender, inverse associations between urinary enterolactone and depressive symptoms were observed in both males and females (males: OR_{Q4vsQ1}: 0.24, 95% CI: 0.07-0.80; females: OR_{Q4vsQ1}: 0.27, 95% CI: 0.13-0.59). No substantial associations were observed for urinary enterodiol in males or females.

Inverse associations between urinary enterolactone and depressive symptoms were observed across all strata of smoking status, though associations were strongest

among former and current smokers (p-trend 0.07 for non-smokers, 0.03 for former smokers, and 0.02 for current smokers). For urinary enterodiol, associations were strongest among former smokers, though confidence intervals were wide and there was no clear trend among any category of smoking status.

When stratifying by BMI, an inverse association between urinary enterolactone concentration and depressive symptoms was observed across all categories of obesity status, though strongest associations were observed among overweight participants (OR_{Q4vsQ1} 0.09, 95% CI: 0.03-0.31). For enterodiol, no clear trends in associations were observed among strata of obesity status, and confidence intervals tended to be wide, indicating imprecision of the estimates.

Age group did not substantially modify the inverse association of enterolactone concentration and depressive symptoms. For those who were 65 and older the inverse association of enterodiol concentration and depressive symptoms is present in all three upper quartiles (OR_{Q2vsQ1} 0.13, 95% CI: 0.02-1.06; OR_{Q3vsQ1} 0.31, 95% CI: 0.06-1.60; and OR_{Q4vsQ1} 0.19, 95% CI: 0.03-1.19).

When stratifying by race, an inverse association between urinary enterolactone concentration and depressive symptoms was observed among all race/ethnicity groups across all quartiles except for Quartile 3 in the Other race group (OR_{Q3vsQ1} 1.40, 95% CI: 0.23-8.43). The confidence interval was wide likely due to the small sample size. An inverse association between urinary enterodiol concentration and depressive symptoms for Black and Mexican American race groups was observed but no clear trends in associations were observed among White or Other race participants and confidence intervals tended to be wide, indicating imprecision of the estimates.

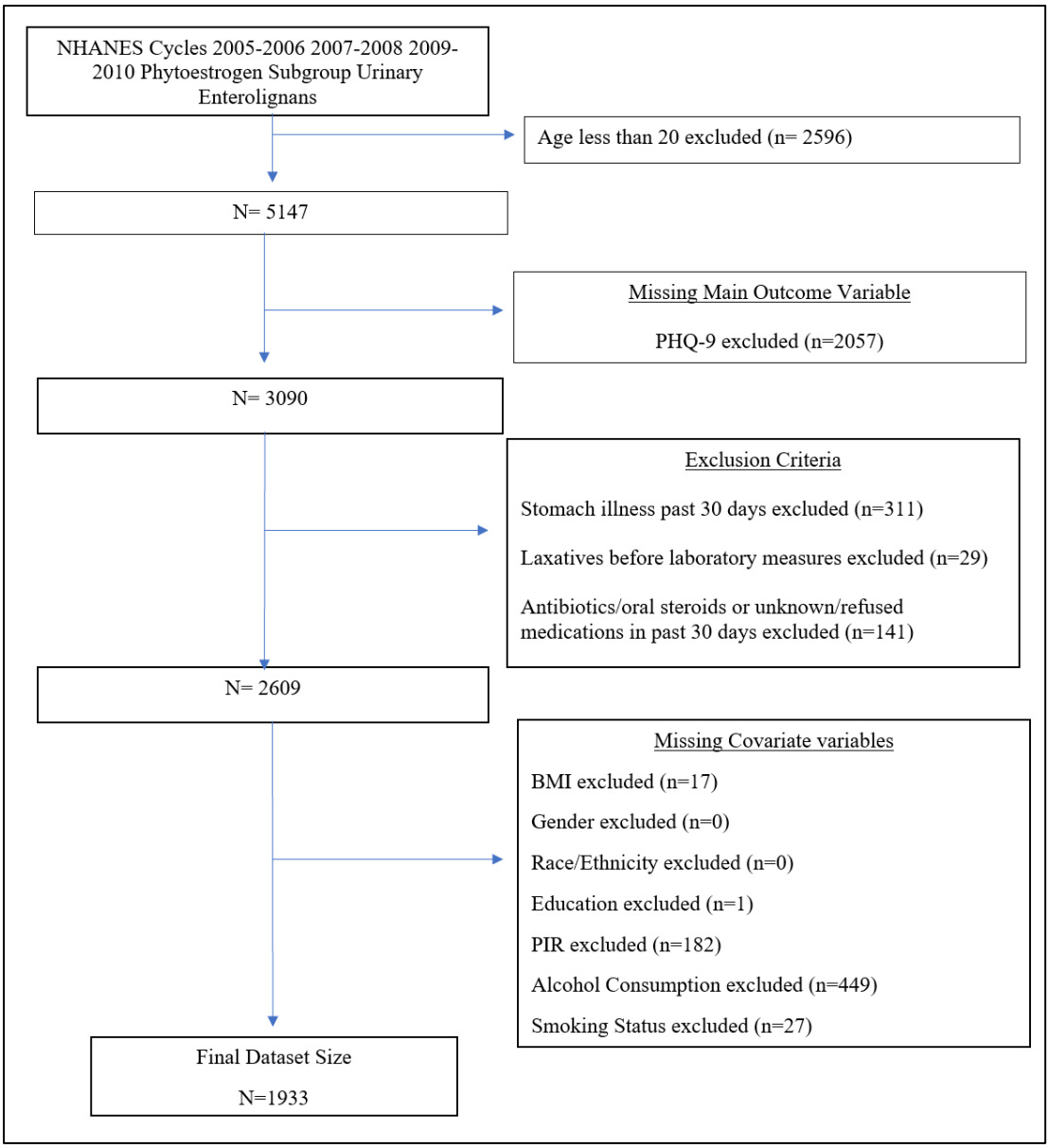

Figure 4.1 Exclusion Flowchart

Table 4.1 Creatinine Adjusted Urinary Enterolignan and Demographic Characteristics of NHANES Participants by PHQ-9 (3 Level Outcome Variable)

	Overall N=1933	Minimal N=1314	Mild/Moderate N=554	Moderately Severe/Severe N=65
Enterolignans, mean (SD) (creatinine adjusted)				
Enterolactone (µg/g)	880.19 (79.79)	973.71 (98.25)	662.50 (97.93)	396.46 (35.43)
Enterodiol (µg/g)	174.50 (21.20)	188.30 (27.57)	145.74 (19.26)	71.12 (12.18)
Gender, n (%)				
Male	882 (46)	643 (49)	221 (40)	18 (28)
Female	1051 (54)	671 (51)	333 (60)	47 (72)
Age, mean (SD)	44.26 (0.63)	44.82 (0.75)	42.66 (1.09)	44.34 (1.42)
BMI, n (%)				
Underweight	27 (1)	17 (1)	9 (2)	1 (1)
Normal	563 (29)	391 (30)	156 (28)	16 (25)
Overweight	620 (32)	435 (33)	167 (30)	18 (28)
Obese	723 (38)	471 (36)	222 (40)	30 (46)
Race/Ethnicity, n (%)				
White	986 (51)	702 (53)	253 (46)	31 (48)
Black	360 (19)	245 (19)	99 (18)	16 (25)
Mexican American	354 (18)	220 (17)	122 (22)	12 (18)
Other	233 (12)	147 (11)	80 (14)	6 (9)
Education, n (%)				
Less than 9^{th} grade	178 (9)	103 (8)	62 (11)	13 (20)
9-11 grade	293 (15)	172 (13)	113 (21)	8 (12)

Highschool	477 (25)	321 (24)	140 (25)	16 (25)
Some college	568 (29)	380 (29)	166 (30)	22 (34)
College +	417 (22)	338 (26)	73 (13)	6 (9)
Marital Status, n (%)				
Single	368 (19)	242 (18)	115 (21)	11 (17)
Married	1171 (60)	839 (64)	307 (55)	25 (38)
Widowed	128 (7)	83 (7)	40 (7)	5 (8)
Separated/Divorced	266 (14)	150 (11)	92 (17)	24 (37)
PIR Category, n (%)				
< 1	399 (21)	229 (18)	144 (26)	26 (40)
2-4	1150 (59)	779 (59)	336 (61)	35 (54)
5+	384 (20)	306 (23)	74 (13)	4 (6)
Smoking Status, n (%)	1037 (54)	727 (55)	285 (51)	25 (38)
Never Smoker				
Ex Smoker	199 (10)	137 (10)	51 (9)	11 (17)
<10 yrs quit	258 (14)	196 (15)	58 (11)	4 (6)
>10 yrs quit				
Current Smoker				
Pack years	217 (11)	121 (9)	86 (16)	10 (15)
0-10	81 (4)	45 (4)	33 (6)	3 (5)
11-20	141 (7)	88 (7)	41 (7)	12 (19)
>20				
Alcohol				
Avg drinks per day, n (%)	1717 (89)	1177 (90)	479 (86)	61 (94)
0-5	216 (11)	137 (10)	75 (14)	4 (6)
6+				

Table 4.2 Creatinine Adjusted Urinary Enterolignan and Demographic Characteristics of NHANES Participants by PHQ-9 (2 Level Outcome Using Pfizer Method)

	Overall N=1933	Not Depressed N=1712	Depressed N=221
Enterolignans, mean (SD) (creatinine adjusted)			
Enterolactone (µg/g)	880.19 (79.79)	918.93 (85.14)	468.47 (50.43)
Enterodiol (µg/g)	174.50 (21.20)	175.04 (22.18)	168.71 (65.01)
Gender, n (%)			
Male	882 (46)	794 (46)	88 (40)
Female	1051 (54)	918 (54)	133 (60)
Age, mean (SD)	44.26 (0.63)	44.23 (0.70)	44.10 (0.91)
BMI, n (%)			
Underweight	27 (1)	23 (1)	4 (2)
Normal	563 (29)	510 (30)	53 (24)
Overweight	620 (32)	549 (32)	71 (32)
Obese	723 (38)	630 (37)	93 (42)
Race/Ethnicity, n (%)			
White	986 (51)	909 (53)	77 (35)
Black	360 (19)	302 (18)	58 (26)
Mexican American	354 (18)	299 (17)	55 (25)
Other	233 (12)	202 (12)	31 (14)
Education, n (%)			
Less than 9^{th} grade	178 (9)	139 (8)	39 (18)
9-11 grade	293 (15)	248 (14)	45 (20)
Highschool	477 (25)	423 (25)	54 (24)
Some college	568 (29)	506 (30)	62 (28)
College +	417 (22)	396 (23)	21 (10)

Marital Status, n (%)			
Single	368 (19)	320 (19)	48 (22)
Married	1171 (60)	1063 (62)	108 (49)
Widowed	128 (7)	112 (6)	16 (7)
Separated/Divorced	266 (14)	217 (13)	49 (22)
PIR Category, n (%)			
< 1	399 (21)	329 (19)	70 (32)
2-4	1150 (59)	1023 (60)	127 (57)
5+	384 (20)	360 (21)	24 (11)
Smoking Status, n (%)			
Never Smoker	1037 (54)	927 (54)	110 (50)
Ex Smoker			
<10 yrs quit	199 (10)	177 (10)	22 (10)
>10 yrs quit	258 (14)	235 (14)	23 (11)
Current Smoker			
Pack years			
0-10	217 (11)	185 (11)	32 (14)
11-20	81 (4)	70 (4)	11 (5)
>20	141 (7)	118 (7)	23 (10)
Alcohol			
Avg drinks per day, n			
(%)	1717 (89)	1523 (89)	194 (88)
0-5	216 (11)	189 (11)	27 (12)
6+			

Table 4.3 Creatinine Adjusted Urinary Enterolignan and Demographic Characteristics of NHANES Participants by PHQ-9 (2 Level Outcome Using Kroenke Method)

	Overall N=1933	Not Depressed N=1739	Depressed N=194
Enterolignans, mean (SD) (creatinine adjusted) Enterolactone (µg/g) Enterodiol (µg/g)	 880.19 (79.79) 174.50 (21.20)	 921.80 (83.17) 182.59 (22.36)	 365.52 (41.86) 74.42 (8.20)
Gender, n (%) Male Female	 882 (46) 1051 (54)	 832 (48) 907 (52)	 50 (26) 144 (74)
Age, mean (SD)	 44.26 (0.63)	 44.30 (0.68)	 44.30 (0.68)
BMI, n (%) Underweight Normal Overweight Obese	 27 (1) 563 (29) 620 (32) 723 (38)	 24 (1) 506 (29) 563 (33) 646 (37)	 3 (2) 57 (29) 57 (29) 77 (40)
Race/Ethnicity, n (%) White Black Mexican American Other	 986 (51) 360 (19) 354 (18) 233 (12)	 909 (52) 318 (18) 310 (18) 202 (12)	 77 (40) 42 (21) 44 (23) 31 (16)
Education, n (%) Less than 9^{th} grade 9-11 grade Highschool Some college College +	 178 (9) 293 (15) 477 (25) 568 (29) 417 (22)	 150 (9) 250 (14) 429 (25) 510 (29) 400 (23)	 28 (14) 43 (22) 48 (25) 58 (30) 17 (9)

Marital Status, n (%)			
Single	368 (19)	330 (19)	38 (20)
Married	1171 (60)	1081 (62)	90 (46)
Widow	128 (7)	112 (7)	16 (8)
Separated/Divorced	266 (14)	216 (12)	50 (26)
PIR Category, n (%)			
< 1	399 (21)	324 (19)	75 (39)
2-4	1150 (59)	1043 (60)	107 (55)
5+	384 (20)	372 (21)	12 (6)
Smoking Status, n (%)			
Never Smoker	1037 (54)	950 (55)	87 (45)
Ex Smoker			
<10 yrs quit	199 (10)	178 (10)	21 (11)
>10 yrs quit	258 (14)	244 (14)	14 (7)
Current Smoker			
Pack years			
0-10	217 (11)	183 (10)	34 (18)
11-20	81 (4)	69 (4)	12 (6)
>20	141 (7)	115 (7)	26 (13)
Alcohol			
Avg drinks per day, n (%)			
0-5	1717 (89)	1546 (89)	171 (88)
6+	216 (11)	193 (11)	23 (12)

Table 4.4 Creatinine Adjusted Urinary Enterolignan Cut-points Based on Non- or Minimally-Depressed Population

		1st Quartile	2nd Quartile	3rd Quartile	4th Quartile
Overall	Enterolactone	<89.43	89.43-336.38	336.39-903.39	>903.39
	Enterodiol	<17.18	17.18-46.45	46.46-115.18	>117.18
Minimal Depression (three level)	Enterolactone	<105.12	105.12-355.96	355.97-972.76	>972.76
	Enterodiol	<17.50	17.50-47.36	47.37-119.94	>119.94
No Depression (dichotomous Pfizer)	Enterolactone	<95.88	95.88-344.43	344.44-928.19	>928.19
	Enterodiol	<17.26	17.26-46.91	46.92-117.98	>117.98
No depression (dichotomous Kroenke)	Enterolactone	<98.32	98.32-349.29	349.30-941.76	>941.76
	Enterodiol	<17.30	17.30-46.77	46.78-118.05	>118.05

Table 4.5 Association between Creatinine Adjusted Urinary Concentrations of Enterolactone and Enterodiol (Quartiles) and Depressive Symptoms (2 Level Outcome Using Pfizer Method)

		Simple Model[a]		Adjusted Model[b]	
	N, depressed/non depressed cases	**OR, 95% CI**	**P_{trend}**	**OR, 95% CI**	**P_{trend}**
Enterolactone (µg/g) creatinine quartiles			0.003		0.02
≤ 95.87	78/404	Ref		Ref	
95.88-344.43	51/429	0.65 (0.36-1.19)		0.69 (0.36-1.32)	
344.44-928.19	56/450	0.60 (0.32-1.12)		0.70 (0.37-1.33)	
> 928.19	36/429	0.39 (0.19-1.02)		0.52 (0.24-1.13)	
Enterodiol (µg/g) creatinine quartiles			0.90		0.66
≤ 17.25	68/440	Ref		Ref	
17.26-46.91	65/443	0.94 (0.55-1.58)		1.06 (0.59-1.91)	
46.92-117.98	47/424	0.84 (0.42-1.59)		1.04 (0.51-2.12)	
> 117.98	41/405	0.70 (0.40-1.22)		0.97 (0.50-1.90)	

[a]Adjusted for age
[b]Additionally adjusted for BMI, alcohol intake, PIR, marital status, race, education, smoking status, gender

Table 4.6 Association between Creatinine Adjusted Urinary Concentrations of Enterolactone and Enterodiol (Quartiles) and Depressive Symptoms (2 Level Outcome Using Kroenke Method)

		Simple Model[a]		Adjusted Model[b]	
	N, depressed/non depressed cases	**OR 95% CI**	**P_{trend}**	**OR 95% CI**	**P_{trend}**
Enterolactone (µg/g) creatinine quartiles			0.0003		0.001
≤ 98.31	70/418	Ref		Ref	
98.32-349.29	48/431	0.55 (0.31-0.99)		0.55 (0.29-1.04)	
349.30-941.76	52/454	0.48 (0.28-0.84)		0.53 (0.30-0.95)	
> 941.76	24/436	0.23 (0.11-0.47)		0.29 (0.14-0.60)	
Enterodiol (µg/g) creatinine quartiles			0.004		0.06
≤ 17.29	62/447	Ref		Ref	
17.30-46.77	50/453	0.78 (0.45-1.37)		0.87 (0.47-1.57)	
46.78-118.05	45/431	0.81 (0.40-1.63)		0.93 (0.43-2.01)	
> 118.05	37/408	0.62 (0.36-1.05)		0.79 (0.41-1.54)	

[a]Adjusted for age
[b]Additionally adjusted for BMI, alcohol intake, PIR, marital status, race, education, smoking status, gender

Table 4.7 Association between Creatinine Adjusted Urinary Concentrations of Enterolactone and Enterodiol (Quartiles) and Depressive Symptoms (3 Level Outcome – No Depression vs Severe)

		Simple Model[a]		Adjusted Model[b]	
	N, severe/none	**OR, 95% CI**	**P_{trend}**	**OR, 95% CI**	**P_{trend}**
Enterolactone (µg/g) creatinine quartiles			0.01		0.03
≤ 105.11	23/313	Ref		Ref	
105.12-355.96	16/328	0.50 (0.20-1.26)		0.61 (0.22-1.72)	
355.97-972.76	17/346	0.46 (0.20-1.10)		0.61 (0.25-1.45)	
> 972.76	9/327	0.31 (0.10-0.93)		0.46 (0.15-1.42)	
Enterodiol (µg/g) creatinine quartiles			0.15		0.31
≤ 17.49	18/334	Ref		Ref	
17.50-47.36	22/340	1.10 (0.43-2.79)		1.35 (0.46-3.99)	
47.37-119.94	15/326	0.73 (0.26-2.05)		0.94 (0.34-2.65)	
> 119.94	10/314	0.49 (0.98-1.02)		0.56 (0.15-2.12)	

[a]Adjusted for age
[b]Additionally adjusted for BMI, alcohol intake, PIR, marital status, race, education, smoking status, gender

Table 4.8 Association between Creatinine Adjusted Urinary Concentrations of Enterolactone and Enterodiol (Quartiles) and Depressive Symptoms (3 Level Outcome – No Depression vs Moderate)

		Simple Model[a]		Adjusted Model[b]	
	N, moderate/none	**OR, 95% CI**	**P_{trend}**	**OR, 95% CI**	**P_{trend}**
Enterolactone (µg/g) creatinine quartiles			0.17		0.40
≤ 105.11	172/313	Ref		Ref	
105.12-355.96	125/328	0.71 (0.46-1.10)		0.75 (0.49-1.15)	
355.97-972.76	151/346	0.83 (0.59-1.16)		0.95 (0.67-1.35)	
> 972.76	106/327	0.60 (0.41-0.87)		0.74 (0.48-1.13)	
Enterodiol (µg/g) creatinine quartiles			0.40		0.83
≤ 17.49	163/334	Ref		Ref	
17.50-47.36	146/340	1.07 (0.77-1.49)		1.02 (0.73-1.42)	
47.37-119.94	130/326	1.07 (0.77-1.49)		1.06 (0.73-1.54)	
> 119.94	115/314	1.12 (0.72-1.72)		1.09 (0.69-1.74)	

[a]Adjusted for age
[b]Additionally adjusted for BMI, alcohol intake, PIR, marital status, race, education, smoking status, gender

Table 4.9 Association between Creatinine Adjusted Urinary Concentrations of Enterolactone and Enterodiol (Quartiles) and Depressive Symptoms (3 Level Outcome– Moderate vs. Severe)

		Simple Model[a]		Adjusted Model[b]	
	N, severe/moderate	**OR, 95% CI**	**P_{trend}**	**OR, 95% CI**	**P_{trend}**
Enterolactone (µg/g) creatinine quartiles			0.08		0.08
≤ 105.11	23/172	Ref		Ref	
105.12-355.96	16/125	0.85 (0.31-2.33)		0.78 (0.30-2.04)	
355.97-972.76	17/151	0.60 (0.25-1.41)		0.64 (0.24-1.65)	
> 972.76	9/106	0.52 (0.16-1.74)		0.67 (0.23-1.92)	
Enterodiol (µg/g) creatinine quartiles			0.15		0.44
≤ 17.49	18/163	Ref		Ref	
17.50-47.36	22/146	1.19 (0.49-2.89)		1.21 (0.50-2.89)	
47.37-119.94	15/130	0.86 (0.31-2.38)		0.93 (0.34-2.61)	
> 119.94	10/115	0.52 (0.19-1.43)		0.66 (0.19-2.26)	

[a]Adjusted for age
[b]Additionally adjusted for BMI, alcohol intake, PIR, marital status, race, education, smoking status, gender

Table 4.10 Association[a] between Creatinine Adjusted Urinary Concentrations of Enterolactone and Enterodiol (Quartiles) and Depressive Symptoms (2 Level Outcome Using Kroenke Method) Stratified by Gender

		Male			**Female**	
	N, depressed/non depressed cases	**OR, 95% CI**	**P_{trend}**	**N, depressed/non depressed cases**	**OR, 95% CI**	**P_{trend}**
Enterolactone (µg/g) creatinine quartiles			0.04			0.01
≤ 105.11	21/242	Ref		49/176	Ref	
105.12-355.96	11/216	0.60 (0.17-2.05)		37/215	0.53 (0.29-0.94)	
355.97-972.76	15/205	0.86 (0.31-2.37)		37/249	0.46 (0.24-0.90)	
> 972.76	3/169	0.24 (0.07-0.80)		21/267	0.27 (0.13-0.59)	
Enterodiol (µg/g) creatinine quartiles			0.36			0.09
≤ 17.49	18/264	Ref		44/183	Ref	
17.50-47.36	15/229	0.96 (0.35-2.60)		35/224	0.85 (0.44-1.66)	
47.37-119.94	10/201	0.92 (0.27-3.17)		35/230	0.89 (0.42-1.88)	
> 119.94	7/138	1.05 (0.28-3.93)		30/270	0.79 (0.38-1.65)	
[a] Adjusted for age, BMI, alcohol intake, PIR, marital status, race, education, smoking status						

Table 4.11 Association[a] between Creatinine Adjusted Urinary Concentrations of Enterolactone and Enterodiol (Quartiles) and Depressive Symptoms (2 Level Outcome Using Kroenke Method) Stratified by Smoking Status

		Never			Former			Current	
	N, depressed /non depressed cases	**OR 95% CI**	**P_{trend}**	**N, depressed /non depressed cases**	**OR 95% CI**	**P_{trend}**	**N, depressed /non depressed cases**	**OR 95% CI**	**P_{trend}**
Enterolactone (µg/g) creatinine quartiles			0.07			0.03			0.02
≤ 98.31	25/193	1 Ref		12/91	1 Ref		33/134	1 Ref	
98.32-349.29	21/243	0.63 0.28-1.43		10/83	0.68 0.24-1.87		17/105	0.43 0.17-1.12	
349.30-941.76	28/255	0.67 0.29-1.53		7/120	0.14 0.05-0.44		17/79	0.79 0.33-1.87	
> 941.76	13/259	0.47 0.16-1.35		6/128	0.13 0.03-0.46		5/49	0.16 0.04-0.68	
Enterodiol (µg/g) creatinine quartiles			0.12			0.23			0.34
≤ 17.29	24/232	1 Ref		9/83	1 Ref		29/132	1 Ref	
17.30-46.77	24/239	0.81 0.35-1.89		8/115	0.39 0.08-1.83		18/99	1.20 0.48-3.00	
46.78-118.05	19/234	0.64 0.28-1.46		8/110	1.19 0.25-5.75		18/87	0.95 0.28-3.25	
> 118.05	20/245	0.94 0.41-2.19		10/114	0.33 0.08-1.38		7/49	0.87 0.21-3.61	

[a] Adjusted for age, BMI, alcohol intake, PIR, marital status, race, education, gender

Table 4.12 Association[a] between Creatinine Adjusted Urinary Concentrations of Enterolactone and Enterodiol (Quartiles) and Depressive Symptoms (2 Level Outcome Using Kroenke Method) Stratified by BMI

		Normal			Overweight			Obese	
	N, depressed /non depressed cases	**OR. 95% CI**	**P_{trend}**	**N, depressed /non depressed cases**	**OR, 95% CI**	**P_{trend}**	**N, depressed /non depressed cases**	**OR 95% CI**	**P_{trend}**
Enterolactone (µg/g) creatinine quartiles			0.05			0.02			0.23
≤ 98.31	20/108	Ref		22/115	Ref		27/192	Ref	
98.32-349.29	12/112	0.53 (0.20-1.40)		14/139	0.42 (0.17-1.03)		21/174	0.78 0.28-2.17	
349.30-941.76	16/140	0.62 (0.28-1.37)		13/147	0.35 (0.10-1.26)		23/160	0.66 0.24-1.78	
> 941.76	9/146	0.39 (0.13-1.15)		8/162	0.09 (0.03-0.31)		6/120	0.41 0.10-1.74	
Enterodiol (µg/g) creatinine quartiles			0.07			0.12			0.52
≤ 17.29	15/127	Ref		17/143	Ref		28/172	Ref	
17.30-46.77	118/99	1.44 (0.50-4.12)		12/147	0.45 (0.15-1.34)		19/202	0.72 (0.29-1.77)	
46.78-118.05	15/131	1.02 (0.32-3.32)		14/134	1.35 (0.48-3.79)		16/159	0.69 (0.21-2.29)	
> 118.05	9/149	0.38 (0.14-1.09)		14/139	0.73 (0.30-1.76)		14/113	1.53 (0.43-5.47)	

[a] Adjusted for age, alcohol intake, PIR, marital status, race, education, gender, smoking status

Table 4.13 Association[a] between Creatinine Adjusted Urinary Concentrations of Enterolactone and Enterodiol (Quartiles) and Depressive Symptoms (2 Level Outcome Using Kroenke Method) Stratified by Age

		20-45			46-65			65+	
	N, depressed /non depressed cases	**OR 95% CI**	**P_{trend}**	**N, depressed /non depressed cases**	**OR 95% CI**	**P_{trend}**	**N, depressed /non depressed cases**	**OR 95% CI**	**P_{trend}**
Enterolactone (µg/g) creatinine quartiles			0.13			0.01			0.04
≤ 98.31	44/248	Ref		22/128	Ref		4/42	Ref	
98.32-349.29	28/267	0.54 (0.25-1.19)		16/121	0.49 (0.18-1.34)		4/43	0.76 (0.12-4.69)	
349.30-941.76	24/227	0.57 (0.28-1.19)		21/135	0.42 (0.17-1.03)		7/92	0.47 (0.06-3.49)	
> 941.76	6/164	0.23 (0.05-1.02)		13/156	0.31 (0.11-0.84)		5/116	0.17 (0.03-1.05)	
Enterodiol (µg/g) creatinine quartiles			0.32			0.16			0.30
≤ 17.29	37/272	Ref		19/122	Ref		6/53	Ref	
17.30-46.77	29/236	1.03 (0.54-1.96)		18/147	0.65 (0.23-1.84)		3/70	0.13 (0.02-1.06)	
46.78-118.05	24/225	1.23 (0.47-3.19)		15/132	0.57 (0.20-1.64)		6/74	0.31 (0.06-1.60)	
> 118.05	12/173	0.80 (0.28-2.30)		20/139	0.87 (0.28-2.70)		5/96	0.19 (0.03-1.19)	

[a] Adjusted for BMI, alcohol intake, PIR, marital status, race, education, gender, smoking status

Table 4.14a Association[a] between Creatinine Adjusted Urinary Concentrations of Enterolactone and Enterodiol (Quartiles) and Depressive Symptoms (2 Level Outcome Using Kroenke Method) Stratified by Race- White/Black

		White			Black	
	N, depressed/non depressed cases	**OR, 95% CI**	**P_{trend}**	**N, depressed/non depressed cases**	**OR, 95% CI**	**P_{trend}**
Enterolactone (µg/g) creatinine quartiles			0.02			0.36
≤ 98.31	33/210	Ref		18/87	Ref	
98.32-349.29	17/204	0.45 (0.16-1.24)		10/91	0.48 (0.17-1.33)	
349.30-941.76	19/233	0.42 1.18-0.98		10/91	0.64 (0.26-1.57)	
> 941.76	8/262	0.19 (0.06-0.57)		4/49	0.30 (0.07-1.27)	
Enterodiol (µg/g) creatinine quartiles			0.26			0.57
≤ 17.29	21/191	Ref		16/110	Ref	
17.30-46.77	21/225	0.73 (0.17-3.19)		13/96	0.78 (0.28-2.18)	
46.78-118.05	23/242	1.56 (0.35-7.08)		6/65	0.64 (0.20-2.05)	
> 118.05	12/251	1.07 (0.32-3.57)		7/47	0.65 (0.18-2.31)	

[a] Adjusted for age, BMI, alcohol intake, PIR, marital status, education, gender, smoking status

Table 4.14b Association[a] between Creatinine Adjusted Urinary Concentrations of Enterolactone and Enterodiol (Quartiles) and Depressive Symptoms (2 Level Outcome Using Kroenke Method) Stratified by Race- Mexican American/Other

		Mexican American			**Other**	
	N, depressed/non depressed cases	**OR, 95% CI**	**P_{trend}**	**N, depressed/non depressed cases**	**OR, 95% CI**	**P_{trend}**
Enterolactone (µg/g) creatinine quartiles			0.01			0.38
≤ 98.31	10/63	Ref		9/58	Ref	
98.32-349.29	14/83	1.32 0.52-3.35		7/53	0.67 0.13-3.48	
349.30-941.76	15/85	0.81 0.26-2.61		8/45	1.40 0.23-8.43	
> 941.76	5/79	0.20 0.04-1.15		7/46	0.62 0.12-3.20	
Enterodiol (µg/g) creatinine quartiles			0.39			0.10
≤ 17.29	17/87	Ref		8/59	Ref	
17.30-46.77	12/89	0.68 0.29-1.58		4/43	0.73 0.17-3.19	
46.78-118.05	9/78	0.58 0.17-1.92		7/46	1.56 0.34-7.08	
> 118.05	6/56	0.48 0.13-1.83		12/54	1.07 0.32-3.57	

[a] Adjusted for age, BMI, alcohol intake, PIR, marital status, education, gender, smoking status

CHAPTER 5

DISCUSSION

Summary and Significance of Results

This study examined the association between urinary enterolignans as a marker for gut microbiome diversity and depressive symptoms using NHANES data. The study found that participants in the highest enterolactone quartile were less likely to be experiencing depressive symptoms compared to those in the lowest quartile. While associations between depressive symptoms and urinary enterodiol were also generally inverse, the effect estimates were weaker and none of the trend tests were statistically significant. Given that urinary enterolactone, but not urinary enterodiol, has been associated with gut microbiota diversity (Hullar et al., 2014), our results support the hypothesis that higher gut microbiota diversity is linked to reduced odds of depressive symptoms.

The outcome variable, depressive symptoms, was scored using three different methods. Using the Pfizer scoring method, a two-level and a three-level outcome variable were produced. Using the Kroenke method, another two-level outcome was created. Exposure variables were categorized by using quartiles determined by distribution of enterolignan concentration for the non-depressed groups. While the dichotomous outcome using the Kroenke method was the only outcome that resulted in statistically significant reduced odds of depressive symptoms among those in the higher enterolignan quartiles, all outcome variable methods produced odds ratios that reflected an inverse

relationship. In our three-level outcome analyses the association could be attenuated as a result of dividing the already small depressed population into multiple groups. For the Pfizer method, the extra criteria of having to mark either question 1 or 2 as "more than half the days", while an important marker of depression, may have categorized those with high PHQ-9 scores as not depressed incorrectly because they did not meet that criteria. In contrast, there are less participants classified as depressed in the Kroenke method (n=194) compare to the Pfizer method (n=221). This could be due to an increased number of false positives in the Pfizer method as the overall score needed to be considered depressed (overall score as low as 4) is less than the score Kroenke uses (score of 10 or more). Misclassification of the outcome may have attenuated the results.

Urinary enterolactone showed the most notable inverse relationship to depressive symptoms as associations were stronger and more results were statistically significant as compared to enterodiol. Differences in the effects of the two metabolites could be related to differences in location along the metabolic pathway. Enterolactone is one step further in the lignan metabolism pathway than enterodiol which suggests that the later steps may be more involved with a depressed-associated microbial environment (Frankenfeld et al., 2013). Enterodiol has a mean elimination half-life and mean residence time much smaller than enterolactone (Kujsten et al, 2005). It is also worth noting that on average, urine samples consisted of a smaller quantity of enterodiol compared to enterolactone. This observation was corroborated in another study looking at the absorption and excretion of enterolignans which found that the majority of enterolignans excreted via urine was enterolactone at 58% (Kujsten et al., 2005). Smaller variability in urinary enterodiol concentrations (as compared to urinary enterolactone) may have inhibited our ability to

detect a significant difference between depressive categories, and as noted previously, urinary enterodiol was not associated with gut microbiome diversity in a previous study (Hullar et al., 2014).

The associations between enterolignans and depressive symptoms using the Kroenke outcome method for classifying depressive symptoms were not modified substantially by gender, smoking status, obesity status, age, or race with the exception that strong inverse associations were observed for urinary enterodiol among participants aged 65 or older which were not observed in other age groups. Confidence intervals were wide given the small number of individuals with depressive symptoms among the older study population, and we cannot rule out the role of chance in this finding. Furthermore, when stratified by race, an inverse association between urinary enterodiol concentration and depressive symptoms was observed for Black and Mexican American participants but not for White or Other race participants. Confidence intervals tended to be wide in these associations as well, indicating imprecision of the estimates. It is difficult to draw any major conclusions from the results given that the population size for those categorized as having depressive symptoms is small and stratifying even further often resulted in unstable effect estimates.

Overall, our results align with our hypothesis that increased urinary enterolactone (signifying an increased gut microbiome diversity) is inversely associated with depressive symptoms. Circulating enterolignan levels have been positively associated with dietary fiber, diets low in fat and rich in fruits and vegetables (Lampe et al., 1999, Stumpf et al, 2000). These findings align with previous studies that have been conducted using both human and rodent models showing that the gut microbiome of depressed patients is

significantly different from that of healthy controls (Kelly et.al, 2016 Zheng et al, 2016). In one study, microbiota diversity and richness was lower in depressed patients in comparison to healthy controls using fecal microbiota as a marker (Kelly et. al, 2016). In another study, fecal samples collected from depressed and nondepressed individuals showed depressed individuals had less Bacteroidetes than those without depression (Zheng et al., 2016). Through colonic fermentation, Bacteroidetes in the gut produce butyrate which can have antineoplastic properties and could play a role in maintaining a healthy gut (Kim and Milner, 2007). Other studies show that a reduced abundance of Bacteroidetes is a characteristic of obesity in both human and animal models (Petriz et al., 2014, Duca et al., 2014).

Microbiota are believed to affect depressive symptoms through the gut-brain axis. Among 655 adults aged 18 and older, fecal samples and questionnaire data revealed that higher anxiety and stress scores were linked to a decreased diversity of gut bacteria (Johnson, 2019). Research has shown that a healthy gut supports normal central nervous system function (Daulatzai, 2015). The gut-brain axis is the bidirectional communication between the gut microbiota and the central nervous system (Clapp et al., 2017). When there is dysbiosis, a common result of reduced gut microbiome diversity, there is more intestinal permeability, which allows inflammatory cytokines to travel systemically through the body. A higher of concentration of these cytokines in the blood creates a permeability in the blood brain barrier which allows these inflammatory cytokines to influence the brain (Gadek et al, 2013). Brain function is then altered which could result in symptoms of anxiety and depression (Gadek et al. 2013, Biesman et al, 2015). Our research study provides support for the hypothesis that gut microbiome dysbiosis could

contribute to depressive symptoms. Our findings support other studies that have suggested that reduced diversity or depletion of gut microbiome bacteria can induce inflammatory responses that can be associated with various mental health disorders such as depression (Berk et al., 2013). Prior research has suggested that mental illnesses are not just caused by psychological stressors but also inflammatory conditions that could be rooted in our gut (Berk et al., 2013).

If corroborated in additional studies, our findings may lend support to intervention studies aimed at altering the gut microbiota composition, such as through probiotics, as one option for improving psychological symptoms. One study found that administering probiotic bacteria can modulate brain activity in humans confirming the link between the gut microbiota and brain (Tillisch et al., 2013). In a rodent study, mice who were administered certain strains of probiotics demonstrated a reduced number of anxiety-related behaviors compared to mice not given the probiotics (Matthews et al., 2013). Thus, altering the gut microbiota through probiotics or other dietary modifications to beneficially affect depressive symptoms is an area in need of future research.

Strengths and Limitations

NHANES collects data from a nationally representative population which provides a diverse group of participants, a large sample size, and external validity. The data collected from NHANES questionnaires and laboratory data were comprehensive which allowed for a variety of covariates to be included to adjust for potential confounding. As one of the few studies in humans on gut microbiome diversity and depressive symptoms, the study advances the field and provides more evidence for potential therapeutic approaches related to gut microbiota manipulation.

A few limitations should be noted. While NHANES provided a large comprehensive sample of participants, it is a cross sectional design so temporality of the exposure-outcome relationship cannot be established, and reverse causality is a possibility. We utilized a one-time measurement of urinary enterolignans and previous studies have shown dose-dependent increases in urinary enterolignans in as little as 24 hours after consuming the precursor lignans (Kuijsten et al., 2005) with large variability between individuals which may be explained by inter-individual differences in microbiota diversity (Hutchins et al., 2000). Thus, more research is needed to determine is averaging multiple urinary measurements over time to reduce the day-to-day variability would provide more accurate estimates of gut microbiota diversity. While enterolignans are an established marker of gut microbiome diversity, it is still a limitation that this study is not using a more direct measurement of gut microbiome diversity such as through metagenomics of fecal samples (Hullar et al. 2014, Shivappa et al, 2019, Frankenfeld et al., 2013). The PHQ-9 is a widely used instrument for measuring depressive symptoms, but it is not a clinical diagnosis. Using PHQ-9 could result in either an over report of depressive symptoms or under report of depressive symptoms as a licensed professional does not verify the diagnosis and the questionnaire reflects participants symptoms within the past two weeks only. To include all the covariates of interest in the analysis, our age group consisted of those 20 or older. These findings can therefore not be generalizable to the youth of America. While we were able to control for several medications and medical conditions, we may have missed some unknown or unprovided information that could affect gut diversity or mental health and could bias our results.

Recommendations for Future Research

This study found inverse associations between urinary enterolactone concentration and the odds of elevated depressive symptoms scoring. Urinary enterolactone is a marker for gut microbiome diversity. The results of the study provide support for the hypothesis that gut microbiome diversity is inversely related to depressive symptoms and provides justification to conduct future studies using more direct methods of measuring gut microbiome diversity and its association with depression. If feasible, using a more accurate or clinically verified method for diagnosing depression would be recommended for future studies instead of using PHQ-9. A prospective study design would be recommended in order to establish the temporal relationship between gut microbiome diversity and depressive symptoms. Clinical relevance could be found by identifying a diet that supports gut health which could be used as a template for those trying to manage depressive symptoms. Also identifying specific strains of bacteria associated with improved depressive scoring could help further advance the clinical relevance of this area of research.

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