PEER RELATIONSHIPS, GUT MICROBIOTA, AND HEALTH IN EMERGING ADULTHOOD

By

Erin Q. Austin

Presented to the Faculty of the Graduate School of

The University of Texas at Arlington in Partial Fulfillment

Of the Requirements

For the Degree of

DOCTOR OF PHILOSOPHY

THE UNIVERSITY OF TEXAS AT ARLINGTON

DECEMBER 2018

Acknowledgements

First and foremost, I want to thank the Jerry M. Lewis, M.D. Mental Health Research Foundation. Without their generous contribution, this dissertation would not have been possible. Additionally, I would like to extend my gratitude to students at the University of Texas at Arlington who volunteered their time to participate in this study.

To Team BAE, Ernesto, Michael, Sergio, Arteria, Rawan, and Amanda, thank you so very much. You were all outstanding undergraduate research assistants who made this project come alive. Thank you for your dedication and all the long hours you spent working to ensure the success of this dissertation. To my fellow graduate students, Allyson, Norma, Maria, and Sarah, thank you from the bottom of my heart, for your friendship, support, and for always having my back.

To my mentor, Lauri Jensen-Campbell, I want you to know how thankful I am for all the time and energy, patience and guidance, you have invested in me over these past six years. I cannot thank you enough for everything you have done for me. To my committee members, Angela Liegey Dougall, Jeff Gagne, Jason Kubinak, and Kelsey Medeiros, thank you for your advice and expertise throughout this process.

To Mom, Dad, and Ethan, I am eternally grateful for your unwavering love, encouragement, and support throughout my life. To Vicki, thank you for always picking up the phone and keeping me sane. To Jazzy and Dexter, thank you for keeping me company while I work and for all the cuddles breaks. Finally, to Joseph, you are my rock, my partner, and my best friend. Thank you for believing in me and cheering me on, for standing by my side and supporting me. You have my heart. I love you all.

November 26, 2018

Abstract

PEER RELATIONSHIPS, GUT MICROBIOTA, AND HEALTH IN EMERGING ADULTHOOD

Erin Q. Austin

The University of Texas at Arlington, 2018

Supervising Professor: Lauri A. Jensen-Campbell

Research has consistently shown that chronic stress has negative effects on overall physical and psychological wellbeing across the lifespan. The biological mechanisms through which stress exerts its effects on the body and the mind includes the recently discovered microbiome-gut-brain axis, the bidirectional communication between the brain and the enteric nervous system that is modulated by the microorganisms residing within the gastrointestinal tract. This dissertation examined the impact chronic psychosocial stress (e.g., peer victimization, daily hassles) had on gut diversity and the relative abundance specific bacterial groups in a diverse sample of emerging adults (N = 126, M_{age} = 20.07). Relationship between the gut microbiome, peer victimization, physical health including biological markers of inflammation (e.g., interleukin-6, C-reactive protein), and psychological symptoms (i.e., internalizing problems, depression, anxiety) were also evaluated. During two lab visits, participants completed self-report measures concerning peer victimization and physical and psychological health. Participants collected an at-home fecal sample between lab visits. 16S microbiome sequencing was performed on an Illumina MiSeq. Alpha and Beta diversity metrics were calculated and OTU tables were generated. Participant's blood was collected via antecubital venipuncture by a trained phlebotomist and ELISA assays were used to analyze IL-6 and CRP. Results showed that when peer victimization was treated as a continuous variable, it did not predict differences in alpha diversity (e.g., absolute OTU counts, Chao1 estimator, Shannon Index) and was not associated with differences at the phylum or genus

level for taxa associated with stress. Gut diversity was found to moderate the relationships between peer victimization and psychological health (i.e. internalizing problems, depression, anxiety), but not physical health. Beta diversity analyses revealed group differences between victims and non-victims driven by shifts in the relative abundance of taxa associated with social stress, depression, and anxiety. These findings highlight the importance of the microbiome-gut-brain axis within the relationship between peer victimization and poor health outcomes and represent a novel target for intervention to help alleviate the negative effects of psychosocial stress.

Table of Contents

List of Tables

List of Appendix A Tables

List of Figures

Chapter 1:

Introduction

Peer victimization is a chronic psychosocial stressor that affects individuals across the lifespan, from the playground to the workplace. Regardless of age, victimization is associated with a myriad of poor outcomes including classroom difficulties and adjustment issues in elementary school (Perren & Alsaker, 2006), physical health complaints in adolescence (Knack, Iyer, & Jensen-Campbell, 2012), and increased psychological distress in adulthood (Nielsen & Einarsen, 2013). Moreover, this chronic stressor has been shown to hasten cellular aging, increase low-grade systemic inflammation in victimized children, and lead to the hyposecretion of cortisol (Copeland, Wolke, Lereya, Shanahan, Worthman, & Costello, 2014; Guarneri-White, Arana, Boyd, & Jensen-Campbell, 2018; Shalev et al., 2013; Vaillancourt, Hymel, & McDougall, 2013).

Simply, peer victimization can disrupt homeostasis on the biological level resulting in the dysregulation of the immune, endocrine, and nervous systems. Furthermore, stress can also cause dysbiosis, a microbial imbalance among the naturally occurring microorganisms present in the gastro-intestinal tract. Indeed, in a sample of undergraduate students, Knowles, Nelson, and Palombo (2008) found that perceived stress during a period of high stress (i.e., exam week) was predictive of changes in microbial flora compared to low stress conditions. In rodents, psychosocial stressors including maternal separation, restraint stress, and social disruption are also associated with changes in gut microbiota composition (Bailey, Dowd, Galley, Hugnagle, Allen, & Lyte, 2011; Barouei, Moussavi, & Hodgson, 2012; O'Mahony et al., 2009). Further, exposure to a social stressor such as aggression has been shown to disrupt the gut microbiota of mice within a short two-hour period; this disruption is implicated in the pathogenesis of anxiety and depression (Bravo et al., 2011; Galley et al., 2014a; Galley et al., 2014b). Changes in gut

microbiota can affect social behavior, eating behavior, communication, cognition, and stress response (Archie & Tung, 2015; Cussotto, Sandu, Dinan, & Cryan, 2018; Dinan, Stilling, Stanton, & Cryan, 2015).

This dissertation examined the effect of chronic stress using a peer victimization framework on the human gut microbiome as well as explored the interplay between the gut-brain axis, inflammation, and health outcomes in emerging adults. Given that research has focused primarily on the role of the human gut microbiota in central nervous system related conditions (e.g., irritable bowel syndrome, multiple sclerosis, autism spectrum disorder; see Cryan & Dinan, 2012), this dissertation is one of the first to examine the impact of a general, though chronic, stressor (i.e., peer victimization) on the relative abundance of microbes and diversity of the human gut microbiota.

Peer Victimization

Peer victimization, or bullying, is characterized by repeatedly being the target of intentionally aggressive acts or behaviors of one's peers (Olweus, 1993). Victimization can be verbal, physical, or relational in nature (Crick, Casas, & Ku, 1999; Underwood, Beron, & Rosen, 2009). It is also observed electronically through cyberbullying, when individuals use mobile phones, video clips, photos and the internet to embarrass, threaten, or taunt the victim (Smith, Mahdavi, Carvalho, Fisher, Russell, & Tippett, 2008). Though predominately studied in children and adolescents, peer victimization persists into adulthood. College students, adults in the workplace, and retired elderly adults all report experiencing and witnessing peer victimization (Chapell, Casey, De la Cruz, Ferrell, Forman, & Lipkin, 2004; Nielsen, Matthiesen, & Einarsen, 2010; Rex-Lear, Knack, & Jensen-Campbell, 2011). Upwards of 30% of American youth report being repeated victims of their peers and 28% of adults in the workplace are frequently bullied

(Lutgen-Sandvik, Tracy, & Alberts, 2007; Nansel, Overpeck, Pilla, Ruan, Simons-Morton, & Scheidt, 2001).

As a form of chronic stress, peer victimization is associated with a variety of negative health outcomes. Persistent victimization can result in low psychological well-being (e.g., unhappiness, low self-esteem), social difficulties (e.g., loneliness, school absences), and increased psychological distress including anxiety and depression (see Rigby, 2003). Feelings of helplessness because of frequent bullying has also been shown to lead to increased suicidal ideation and suicide risk (Rivers & Noret, 2010; Van der Wal, De Wit, & Hirasing, 2003). This negative peer experience can cause general psychosocial maladjustment, dislike of school, and truancy issues which can also lead to lower academic performance (Nakamoto & Schwartz, 2010). For employees, workplace bullying can lead to decreased productivity, higher rates of turnover, and increased absenteeism in addition to lower job satisfaction and increased mental strain; each case of workplace bullying can cost companies thousands of dollars in lost revenue (Hoel, Sheehan, Cooper, & Einarsen, 2011).

Victims of bullying also experience poorer physical health outcomes and increased psychosomatic complaints such as abdominal pain, loss of appetite, headaches, sleep problems, and mouth sores (Biebl, DiLalla, Davis, Lynch, & Shinn, 2011; Fekkes, Pijpers, Fredriks, Vogels, & Verloove-Vanhorick, 2006). Victimized adults report decreased health satisfaction, shortness of breath, and report taking more sleep-inducing drugs and sedatives than non-victimized colleagues (Low, Radhakrishnan, Schneider, & Rounds, 2007; Vartia, 2001). Further, victims of workplace bullying had increased odds of being diagnosed with fibromyalgia over a two-year period (Kivimaki et al., 2004). These negative outcomes of peer victimization can engender more victimization experiences and have long lasting effects into adulthood (Hodges & Perry, 1999; Takizawa, Maughan, & Arseneault, 2014).

Stress in Emerging Adulthood

Although peer victimization can occur at any point in the life span and is typically studied in children and adolescents, the focus of this dissertation was the developmental period of emerging adulthood. From 18 to 25 years of age, emerging adulthood is characterized as a distinctly optimistic period in which individuals are focused on discovering who they are and what they want to become in all domains of life (i.e., career, education, relationships). As they gain independence from their parents, this time is rife with residential instability and feeling in-between; subjectively, individuals report feeling they have left adolescence behind but have yet to enter adulthood. Though emerging adults are in a transitory state, they are highly optimistic about their futures and the possibility of gaining a better position in life than that of their parents (Arnett, 2000). However, this developmental period is not without its own unique stressors.

Collectively, stress is the prime impediment to academic success in university students per the National College Health Assessment (American College Health Association, 2016). College students often experience strain regarding interpersonal (e.g., relationships), intrapersonal (e.g., finances, health behaviors), academic (e.g., course load, grades, degree choice), and environmental (e.g., living arrangements, cleanliness) stressors (Howard, Schiraldi, Pineda, & Campanella, 2006). Though these stressors are normative to this age group, how an individual perceives, responds to, and copes with this increased strain can influence their overall wellbeing (Brougham, Zail, Mendoza, & Miller, 2009). The importance of and negative emotion evoked by such hassles is predictive of the amount of stress experienced and reported (McIntyre, Korn, & Matsuo, 2008). Perceived academic stress and lack of social support from friends and family have been shown to predict poorer psychological (e.g., nervousness, depression) and physiological (e.g., nausea, loss of appetite) health symptoms in college students (Hefner & Eisenberg, 2009; Nonis, Hudson, Logan, & Ford, 1998). Further, the inability to cope

academically and meet study demands may decrease the odds of obtaining a college degree (Vaez & Laflamme, 2008).

Interpersonal conflict with roommates, coworkers, fellow students, and romantic partners as well as changes in one's social environment is another major source of stress for emerging adults (Ross, Niebling, & Heckert, 1999). In fact, peer victimization is a common experience in university environments; 25% of college students have experienced firsthand victimization, 61% have witnessed peers being bullied, and 22% reported being victims of cyberbullying (Chapell et al., 2004; MacDonald & Roberts-Pittman, 2010). Primarily, researchers have studied the prevalence rate of peer victimization within college environments specifically focusing on the occurrence and effects of cyberbullying (for review see: Lund & Ross, 2016; Watts, Wagner, Velasquez, & Behrens, 2017). Within this population, both cyber-victimization and traditional forms of victimization have been associated with lower self-esteem and increased loneliness as well as higher risk for depression, anxiety, and suicidal ideation (Giovazolias & Malikiosi-Loizos, 2015; Schenk & Fremouw, 2012). Further, peer harassment may elicit poor health habits and problematic alcohol and drug use such as binge drinking, cigarette use, and other harmful behaviors related to alcohol use (McGinley, Rospenda, Liu, & Richman, 2015). Consistently, chronic stress (i.e., peer victimization) can impact one's physical and mental health (Hawker & Boulton, 2000; Miller, Chen, & Zhou, 2007).

Stress Response Systems: A Biopsychosocial Approach

To fully understand the potential impact of chronic peer victimization on one's physical and psychological wellbeing, it is best to utilize Engel's (1980) biopsychosocial perspective that focuses on the biological (e.g., genetic predispositions, stress response, development, etc.), psychological (e.g., personality, mental health, emotions, etc.) and social (e.g., interpersonal relationships, environmental stressors, social support/isolation, etc.) factors that influence an

individual's experience of this chronic social stressor. Regarding health and illness, this holistic approach facilitates the exploration of interactions between central processes including biological, somatic, cognitive, and affective aspects and peripheral processes including the nervous, endocrine, and immune systems as well as how these interactions are modulated by social factors (Gatchel, 2004).

Simply, the biopsychosocial model allows researchers to examine the interplay between an individual's subjective (e.g., attributions, perceptions) and objective experiences (e.g., physiological changes). Indeed, studies of chronic pain show that nociception, the sensory nervous system's objective response to potentially harmful stimuli and tissue damage, interacts with pain, an individual's subjective response to this sensory information that is influenced by psychological and sociocultural factors (Gatchel, Peng, Peters, Fuchs, & Turk, 2007). Further, an individual's mindset towards stress regarding whether they perceive stress to be enhancing or debilitating can influence their physiological and behavioral responses to a stressor. Crum, Salovey, and Achor (2013) found that individuals who endorsed that stress was enhancing (i.e., increased productivity, learning, wellbeing) were more likely to desire feedback and have a lower cortisol response after completing the Trier Social Stress Test compared to individuals who viewed stress as debilitating (i.e., poorer health, performance, growth) which further shows how biological and psychosocial factors influence one another.

Of interest to this dissertation was the biological mechanisms through which chronic stress exerts its influence on health outcomes. Research on stress and health has focused on human body's ability to adapt to changes in the environment and maintain homeostasis. Allostasis, the ability to achieve stability through change, is achieved through the activation and subsequent inactivation of neural, neuroendocrine, and immune mechanisms such as the sympathetic–adrenal–medullary (SAM) system (e.g., fight-or-flight response) or the

hypothalamic-pituitary-adrenal (HPA) axis (McEwen, 1998a). When these systems become dysregulated and subsequently respond inadequately, fail to turn off, or are overworked due to chronic stress, allostatic load increases, and the body is weakened which may result in pathophysiology (McEwen, 1998b). Though the activation of stress response systems during fight-or-flight (i.e., acute stress) serves an adaptive purpose to promote survival and enhances innate and adaptive immune responses, it is during long periods of chronic stress when the stress response systems become overburdened and results in a dysregulation of the immune system including increases in pro-inflammatory molecules and suppression of immunoprotective cells. Generally, acute stress results in immuno-protection while chronic stress results in immune-suppression (Dhabhar, 2014; Marketon & Glaser, 2008). Additionally, not all stressors will affect these systems the same way; the type, duration, frequency, and severity of the stressor are all characteristics that will influence the impact the stressor has on an individual (Cohen, Kessler, & Gordon, 1997; Murali, Hanson, & Chen, 2007).

As part of the stress response, endocrine, nervous, and immune systems share a common biochemical language (e.g., hormones, cytokines, and neurotransmitters) and consequently, are interconnected. As such, the disruption of homeostasis in the endocrine system can modulate the functioning of the nervous and immune systems (Padgett & Glaser, 2003; Wilder, 1995). For instance, during an acute stress response, the HPA system is activated through a negative feedback loop. Simply, corticotrophin releasing hormone (CRH) is released by the hypothalamus, which stimulates the release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary. Interacting with the adrenal gland, ACTH stimulates the release of glucocorticoids like cortisol into the periphery. Cortisol helps prepare the body's energy stores to adequately cope with the stressor, but it also suppresses CRH production to return the body to pre-stress levels of circulating cortisol (Tsigos & Chrousos, 2002). Also, during a response to a

physical or psychosocial stressor, the SAM system releases catecholamines (e.g., adrenaline, noradrenaline) from the medulla and adrenal gland to help orchestrate the fight-or-flight response (Gunnar & Quevedo, 2007).

Together, cortisol and these catecholamines can influence the immune system by augmenting the production of pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor-necrosis factor (TNF; Glaser & Kiecolt-Glaser, 2005). IL-6 stimulates the production of C-reactive protein (CRP), an acute phase protein synthesized in the liver. CRP is a marker of inflammation and is commonly used in clinical settings for diagnostic purposes including rheumatoid arthritis and cardiovascular disease (Black, Kushner, and Samols, 2004). Further, chronic psychosocial stress (i.e., peer victimization) can alter the balance in the functioning of the endocrine system, which in turn can lead to greater immune dysregulation and increased inflammatory responses compared to acute stress responses (Cohen et al., 2012; for review, Herbert & Cohen, 1993). For example, Miller, Cohen, and Ritchey (2002) found that parents dealing with the chronic stress of caring for a child with cancer had a flatter diurnal cortisol pattern and impaired immune response preventing the suppression of pro-inflammatory cytokines compared to parents of healthy kids. In fact, experiencing peer victimization in elementary and middle school has been associated with an increased risk for low-grade systemic inflammation and increased levels of CRP in adulthood (Arana, Boyd, Guarneri-White, Iyer-Eimerbrink, Liegey-Dougall, & Jensen-Campbell, in press; Copeland et al., 2014; Takizawa, Damese, Maughan, & Arseneault, 2015).

Microbiome-gut-brain Axis

Recently, research has focused on another pathway between stress and health, the microbiome-gut-brain axis (Moloney, Desbonnet, Clarke, Dinan, & Cryan, 2014). The axis consists of the bidirectional communication between the gastrointestinal (GI) tract and the brain

through signaling molecules along the enteric nervous system (ENS) as well as all the microbes present in the human gut, collectively called the gut microbiota (Cryan & O'Mahony, 2011). The ENS is commonly referred to as the 'second brain' because it innervates the largest sensory organ within the human body, the GI tract. Though traditionally viewed as only a means to digest food, absorb nutrients, and excrete waste, current research shows that the gut plays an important and complex role (Dinan, Stilling, Stanton, & Cryan, 2015; Furness, Callaghan, Rivera & Cho, 2014).

The gut serves as the first line of defense against ingested containments. As such, more immune cells reside in the lining of the gut than anywhere else in the body including the bone marrow and blood. There are also more endocrine cells living in the wall of the gut than all other endocrine organs (e.g., adrenal, pituitary, and thyroid glands, etc.) combined (Mayer, 2016). Further, it is estimated that 95% of serotonin, a neurotransmitter implicated in depression and mood as well as partially responsible for gut contractions, appetite, and sleep, is found within the GI tract and ENS (Kim & Camilleri, 2000). Neurotransmitters, hormones, and inflammatory molecules from the gut send signals through the ENS to the brain and the brain sends signals to the endocrine, immune, and nervous cells of the gut to adjust their functions as needed; collectively this process is responsible for both gut sensations and gut reactions (Furness et al., 2014; Mayer, 2016).

This bidirectional communication is modulated by the approximately 39 trillion microbes present in the human microbiome (Sender, Fuchs, & Milo, 2016). Described as "a complex endocrine organism" or super organism, the gut microbiota consists of more than one thousand unique bacterial species primarily from the phyla Bacteroidetes, Firmicutes, and Proteobacteria and contains approximately 3.3 million genes (Clarke, Stilling, Kennedy, Stanton, Cryan, & Dinan, 2014; Spor, Koren, & Ley, 2011). These microbial genes are responsible for the

production of a variety of signaling molecules (e.g., hormones, cytokines, neurotransmitters) that can affect the functioning of the gut-brain axis (Moloney et al., 2014). Signals from the brain can also alter the gut microbiota through changes in the internal environment of the GI tract (e.g., secretion, motility, and permeability) and through direct signals to neurons and immune cells (Rhee, Pothoulakis, & Mayer, 2009). In other words, the human gut microbiota can also influence and be influenced by endocrine, nervous, and immune systems (Bengmark, 2013; Winter, Hart, Charlesworth, & Sharpley, 2018).

The gut microbiota is unique to everyone, like a fingerprint, and changes across the lifespan in response to genetics, diet, and immunological health (Spor et al., 2011; Van de Wiele, Van Praet, Marzorati, Drennan, & Elewaut, 2016). Anomalous, or atypical, patterns of the microbiota have been associated with disease states such as obesity (Ley, Turnbaugh, Klein, & Gordon, 2006), inflammatory bowel disease (Macfarlane, Blackett, Nakayama, Steed, & Macfarlane, 2009), and irritable bowel syndrome (Quigley, 2009). Gut microbiota has also been shown to influence depression (for review, Winter et al., 2018) and anxiety (Foster & Nuefeld, 2013; Malan-Muller et al., 2018), social interactions and stress responses (Dinan, Stilling, Stanton, & Cryan, 2015), and may even be related to the pathogenesis of autism spectrum disorders (Finegold et al., 2010) and neurodegenerative disorders such as Alzheimer's disease (Bhattacharjee & Lukiw, 2013). Figure 1 shows the general working model for this dissertation; specific focus was given to the pathway from peer victimization, gut microbiota, inflammatory markers, to poor health outcomes.

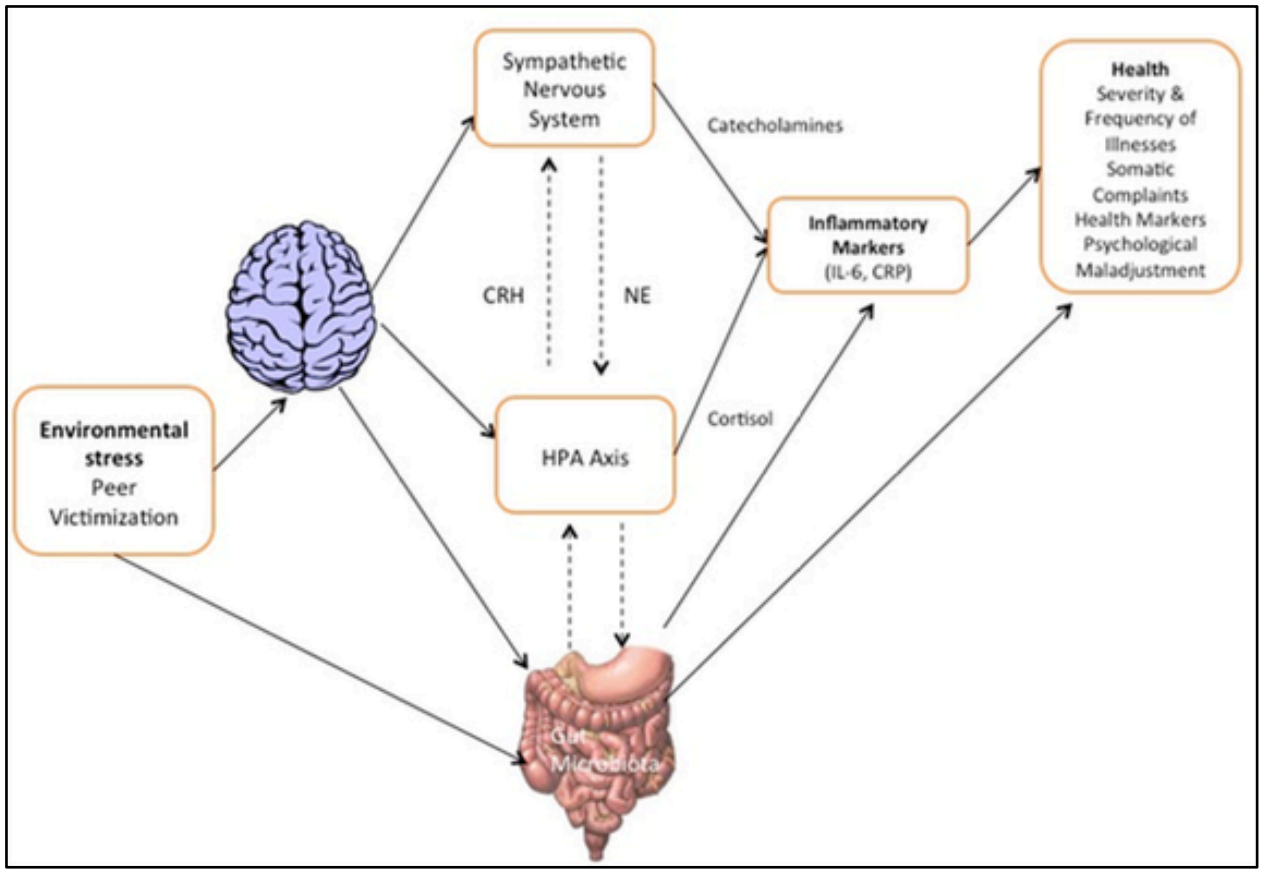


Figure 1. General theoretical model of the influence of peer victimization on health

Underlying Mechanisms in the Microbiome-gut-brain Axis

Though this dissertation focused on the broader associations between gut microbiota and health, it is important to understand the underlying mechanisms of bi-directional communication between the brain, gut, and microbiota in more detail. These mechanisms include neural pathways between the ENS and the brain, neurotransmitter synthesis and signaling, enteroendocrine signaling, immune regulation, and modulation of the intestinal barrier (for review see: Rhee, Pothoulakis, & Mayer, 2009; Cani & Knauf, 2016; Dinan, Stilling, Stanton, & Cryan, 2015; Liu, 2017). Though these pathways are not fully delineated yet, the underlying mechanisms help explain how gut microbiota exerts effects on the gut-brain axis and how the brain also influences microbial composition.

As previously mentioned, the gastrointestinal tract is innervated by the ENS which has regulatory control over basic GI functions including mucus secretion as well as localized blood flow and motility. However, communication with the brain occurs through the central nervous system (CNS) which exerts primary control of the GI tract through the vagus nerve (Furness et al., 2014; Liu, 2017). Indeed, this neural pathway may be the most important mechanism for bidirectional communication between the brain and gut microbiota with the vagus nerve playing a central role in the detection of pathogenic bacteria and subsequent anti-inflammatory responses (Forsythe, Bienenstock, & Kunze, 2014; Winter et al., 2018). Using an animal model of inflammatory bowel disease (IBD), Lyte and colleagues (2006) showed that eight hours after an initial infection with pathogenic bacteria (i.e., *Citrobacter rodentium*) there was no change in the number of circulating cytokines within the plasma indicating the infection had yet to reach the circulatory system. However, evidence of the infection was found in the vagus nerve via elevated levels of c-Fos protein, an activation marker within the vagal pathway. Further, behavioral changes and increased anxiety were observed in the animals eight hours post infection showing that the vagus nerve plays a critical role in the bidirectional relationship between gut microbes and the brain (Lyte, Li, Opitz, Gaykema, & Goehler, 2006). Research in rodents has also shown that an intact vagus nerve is necessary for probiotics, strains of beneficial bacterial species that help maintain a healthy gut commonly from the genus *Lactobacillus* and genus *Bifidobacterium*, to be effective (Bravo et al., 2011). In mice that have had their vagus nerve removed (i.e., vagotomy), the anxiety-reducing effects of the probiotic species *Bifidobacterium longum* were not observed (Berick et al., 2011). Probiotics' anxiolytic effects are thought to occur directly, by reducing the excitability of enteric neurons and indirectly, by altering gamma-Aminobutyric acid (GABA) receptor expression (Bravo et al., 2011; Berick et al., 2011).

Within the ENS and GI tract, the neurotransmitter serotonin, 5-hydroxytrytamine (5-HT), plays a critical role in regulating secretion, muscle contraction and relaxion, and pain perception; dysregulation of 5-HT signaling has also been implicated in gut disorders such as constipation, inflammatory bowel disease, and irritable bowel syndrome (Costedio, Hyman, & Mawe, 2007). Within the brain, serotonin can affect mood, behavior, memory, sexual desire, and sexual function (Young & Leyon, 2002). The wide-ranging effects of serotonin may explain the high comorbidity rate among gut disorders, anxiety, and depression (Graff, Walker, & Bernstein, 2009; Whitehead, Palsson, & Jones, 2002). Indeed, in meta-analytic reviews researchers have shown that antidepressants including tricyclics and selective serotonin reuptake inhibitors (SSRIs) were effective in treating and alleviating symptoms of gastrointestinal disorders (Ford, Talley, Schoenfeld, Quigley, & Moayyedi, 2009; Jackson, O'Malley, Tomkins, Balden, Santoro, & Kroenke, 2000). Gut microbiota also effects the synthesis of serotonin within the body. Specifically, indigenous spore-forming bacteria promote 5-HT synthesis in cells in the colon which in turn increases circulating levels of serotonin in the blood (Yano et al., 2015).

Microbes also regulate the metabolites, intermediate precursors needed for synthesis in metabolic pathways, of serotonin. Specifically, gut microbiota can regulate the metabolism of tryptophan, an amino acid that is one of the most important metabolites needed for serotonin synthesis. Certain bacterial strains utilize tryptophan to grow, which limits the availability of tryptophan for serotonin synthesis. However, other bacterial strains can produce serotonin directly as well as from tryptophan (Lyte, 2013; O'Mahony, Clarke, Borre, Dinan & Cryan, 2015; Tetel, de Vries, Melcangi, Ranzica, & O'Mahony, 2018). Further, probiotic species (e.g., *Bifidobacteria infantis*) have also been shown to increase plasma levels of tryptophan in animal studies (Clarke, Cryan, Dinan & Quigley, 2012; Desbonnet, Garrett, Clarke, Bienenstock, & Dinan, 2008; Yano et al., 2015). The initial postnatal colonization of the gut microbiome is

crucial in the development of these serotonergic pathways. In germ-free rodents, animals bred without microflora, CNS communication as well as levels of serotonin and tryptophan are drastically altered compared to control animals (Dinan, Stilling, Stanton, & Cryan, 2015). Moreover, recolonization of the germ-free animals can correct these disparities but only during early, not late, development (e.g., post weaning; Clarke et al., 2013). In addition to serotonin, intestinal microbiota also influences the synthesis, distribution, and consumption of other neurotrophins and neurotransmitters such as brain-derived neurotrophic factor (BDNF), noradrenalin, GABA, dopamine, and acetylcholine (Cryan & Dinan, 2012; De Palma, Collins, Bercik, & Verdu, 2014; Hyland & Cryan, 2010; Nuefeld, Kang, Bienenstock, & Foster, 2011; Strandwitz, 2018; Sudo et al., 2004).

Enteroendocrine signaling is another mechanism of communication between the brain, gut, and gut microbiota (for review, Cussotto, Sandhu, Dinan, & Cryan, 2018). Endocrine cells lining the GI tract produce and secrete peptides (i.e., gut hormones) that play a role in regulating gut motility and secretion, appetite, and satiation as well as in response to stress, mood, and emotional affect (for review, Latorre, Sternini, De Giorgio, & Greenwood-Van Meerveld, 2016). Further, gut microbes produce molecules that bind with enteroendocrine cells, stimulating the secretion of these peptides (Cani & Knauf, 2016). Peptides including ghrelin, peptide YY, and glucagon-like peptide-1 (GLP-1) regulate feelings of hunger and satisfaction. Changes in gut microbiota composition have been shown to modulate levels of these gastrointestinal peptides and even control the differentiation of specific enteroendocrine cells that contribute to higher production of these peptides (Cani, Hoste, Guiot, & Delzenne, 2007; Zhou et al., 2008). Dysregulation of these peptides contribute to the onset of obesity and diabetes (Parnell & Reimer, 2009).

Beyond the endocrine system and gut microbiota, gut hormones and peptides have far reaching effects and serve as information carrying signaling molecules throughout the entire microbiome-gut-brain axis including within the nervous and immune systems (Holzer & Farzi, 2014). Further, the relationship between hormones and gut microbiota is bi-directional (Tetel et al., 2018). Gender differences in microbial composition as well as gestation related changes in gut microbiota suggest that sex hormones (Dominianni et al., 2015) and pregnancy hormones such as estrogen and progesterone (Edwards, Cunningham, Dunlop, & Corwin, 2017) play a key role in modulating the gut microbiota. Further, evidence suggests that fecal transplants from male to female rodents increases androgen hormone levels in the females indicating that gut microbiota influences the endocrine system as well (Markle et al., 2013; Tetel et al., 2018).

Given that the GI tract is the biggest immune organ in the human body, the immune system is also an important pathway of communication in the microbiome-gut-brain axis (Macpherson & Harris, 2004). In fact, the gut microbiota is extremely important in the development of mucosal and systemic immunity. In germ-free rodents, the lining of the GI tract has a greatly reduced numbers of $CD4^+$ T cells and Immunoglobulin A (IgA), an antibody required for healthy immune function of mucosal membranes (Macpherson, Hunzler, McCoy, & Lamarre, 2001; Macpherson, Martinic, & Harris, 2002). It has been long understood that these immunological differences in germ free mice also extend to systemic immune abnormalities in the spleen and lymph nodes (Bauer, Horowitz, Levenson, & Popper, 1963). Although these alterations can be corrected by introducing bacteria into the GI tract of these germ-free animals, early life disturbances of typical gut microbiota can be long lasting. Animals who have experienced early life stress have been shown in adulthood to have increased immune responses and increases in pro-inflammatory cytokines (e.g., TNF-α, IFN-γ, IL-6), which led to changes in behavior and mood compared with control animals (O'Mahony et al., 2009). In humans,

colonization of the gut differs depending on whether a baby was born via vaginal or cesarean birth. Research shows that caesarean birth leads to short term alterations in immune responses and greater risk of developing immune diseases through adulthood compared to babies born vaginally (Bakhed et al., 2015; Cho & Norman, 2013). Further, Schwartz and colleagues (2012) have found that infants who were breast-fed, as opposed to formula-fed, had more diverse gut microbiota. When changes in gut microbiota composition lead to immune dysregulation, probiotics have been shown to restore B and T cell functioning, reduce HPA-axis reactivity, and increase the synthesis of IL-10, an anti-inflammatory cytokine (Dinan, Stanton, & Cryan, 2013; Forsythe, Sudo, Dinan, Taylor, & Bienenstock, 2010; Talham, Jiang, Bos, & Cebra, 1999).

As another means of protecting the body from contaminated food and from the microbes residing in our gut, the gastrointestinal tract is lined with epithelial cells that form tight junctions that maintain the intestinal barrier and permeability of gut membranes (Lee, 2015). The enteric nervous system, the vagal pathway, and interactions with immune cells modulate intestinal barrier functioning (Hyland, Quigley, & Brint, 2014). Further, disruption in the intestinal barrier is implicated in several gastrointestinal disorders including irritable bowel syndrome and inflammatory bowel disease (Soderholm, 2010; Teitelbaum, Gareau, Jury, Yang & Perdue, 2008) as well as responses to stress (Bailey et al., 2011). Specifically, when an individual experiences psychological or physiological stress the lining of the GI tract becomes compromised and results in increased intestinal permeability, colloquially referred to as a "leaky gut" (Kelly, Kennedy, Cryan, Dinan, Clarke, & Hyland, 2015). This stress-induced weakened barrier increases the likelihood of microbial translocation, meaning that microbes within the gut can more easily break through the intestinal lining to interact directly with immune cells and neural pathways (Foster & Nuefeld, 2013; Gareau, Silva, & Perdue, 2008).

Leaky gut is also associated increased activation of the immune response and is more prevalent among individuals with Major Depressive Disorder (MDD) compared to healthy controls (Foster, Rinaman, & Cryan, 2017). The translocation of microbes across the intestinal lining have been shown to influence the permeability of the blood-brain barrier which regulates CNS homeostasis (Braniste et al., 2014; Kelly et al., 2015). Further, weakened tight junction integrity, increased HPA activation and immune activity was observed in mice exposed to acute stress (Demaude, Salvador-Cartier, Fioramonti, Ferrier, & Bueno, 2006). Similar effects were also observed in adult mice that experienced early life trauma (Soderholm, Yates, Gaeau, Yang, MacQueen, & Perdue, 2002). Probiotic species, such as *Lactobacillus farciminis, Bifidobacteria longum,* and *Bifidobacteria longum*, have been shown to prevent stress-induced leaky gut, reduce HPA axis response, and suppress inflammation in rodents (Ait-Belgnaoui et al., 2012; Kelly et al., 2015; Savignac, Kiely, Dinan, & Cryan, 2014). Additionally, probiotic species *Lactobacillus helveticus* and *Bifidobactium longum* have been shown to reverse the negative effects (i.e., leaky gut, depression) of a myocardial infarction in rodents further illustrating the role the gut microbiota plays in maintain the integrity of intestinal lining (Arseneault-Bread et al. 2012).

Stress and the Microbiome-Gut-Brain Axis

In addition to the negative impact on intestinal permeability, recent evidence also shows that chronic stress affects all aspects of the microbiome-gut-brain axis primarily through the dysregulation of the HPA axis (De Palma, Collins, Bercik, & Verdu, 2014). Stress also plays a role in the composition of the gut microbiota by affecting gut diversity and the relative abundances of certain bacterial groups, as well as by inducing a dysbiotic state (Foster, Rinaman, & Cryan, 2017; Grenham, Clark, Cryan, & Dinan, 2011; Moloney et al., 2014; Tetel et al., 2018). Colonization of the gut microbiota is crucial for the development of the HPA axis. In a study comparing postnatal germ-free rodents and specific pathogen free (SPF) rodents (e.g.,

rodents with a normal microbial community but without certain pathogens), germ-free rodents had an elevated stress response, higher levels of ACTH, and lower levels of brain-derived neurotrophic factor (BDNF), an important protein that aids in neuronal growth and differentiation in addition to learning and memory. Interestingly, fecal transplant from the SPF rodents to the germ-free rodents attenuated the issue. However, fecal transplants were effective only in the early stages of development and not during later developmental stages indicating that there is a critical period for microbial exposure in order to have a fully functioning HPA axis (Sudo et al., 2004). Colonization of the gut microbiota is also important both pre- and post-natally for overall brain development, especially for stress-related cortices (for review, Al-Asmakh, Anuar, Zadjali, Rafter, & Petterson, 2012). Specifically, studies have shown that GF rodents have altered gene expression and neurogenesis within the hippocampus as well as altered structure and function of the amygdala (Foster, Rinaman, & Cryan, 2017).

Further, exposure to early life stress can induce dysbiosis of gut microbiota and have long term effects on the stress response. In animal models, maternal separation was associated with altered gut microbiota composition as well as increased depression and anxiety symptoms (O'Mahony et al., 2009; Winter et al., 2018). Rodents born to mothers who experienced high levels of prenatal stress had increased HPA axis activity in response to stress and long-lasting changes in gut microbiota composition including decreases in the genus *Lactobacillus* in adulthood (Golubeva et al., 2015). Additionally, animals exposed to chronic psychosocial stress had decreased diversity of the gut microbiota. This decrease was associated with behavioral changes including anxiety-like symptoms and social deficits as well as changes in immunoregulatory responses (e.g., elevated IL-6) and reduced metabolism of neurotransmitter precursors such as tryptophan (Bharwani, Mian, Foster, Surette, Bienenstock, & Forsythe, 2016).

In mice, exposure to prolonged stress resulted in drastically different composition of the gut microbiota compared to control mice, and moderate changes in the microbial community have even been observed in mice exposed to short term stressors (Bailey, Dowd, Parry, Galley, Schauer, & Lyte, 2010; Galley et al., 2014a; Galley et al., 2014b). Specifically, repeated social stress has been shown to decrease the relative abundance of bacteria in the genus *Bacteroides* and increase the relative abundance of bacteria in the genus *Clostridium,* which is linked to inflammation and illness (Bailey, et al., 2011). Galley and colleagues (2017) also found that exposure to prolonged social stress was associated with decreases in the phylum Firmicutes and increases the phylum Bacteroidetes; the effects of the stressor were not attenuated by the probiotic species *Lactobacillus reuteri*. The systemic release of catecholamines in response to a stressor has been shown to increase and change the behavior of microbes within the gut including *Escherichia coli* and *Salmonella* species (Freestone, Williams, Haigh, Maggs, Neal, & Lyte, 2002). Further, changes in bacterial genera (*Coprococcus*, *Pseudobutyrivibrio*, and *Dorea*) were significantly correlated with increased circulating pro-inflammatory cytokines (e.g., IL-6) showing that gut microbiota may play a role in the inflammation and immune response to stress (Bailey et al., 2010; Bailey et al., 2011). Indeed, researchers have shown that transplanting the microbiota from a rodent exposed to high levels of stress to a germ-free rodent induced an inflated inflammatory response and increased susceptibility to infection (Willing, Vacharaksa, Croxen, Thanachayanont, & Finlay, 2011). However, treatment with the probiotic species, *Lactobacillus rhamnosus*, has been shown to decrease stress-induced anxiety after a social defeat as well as increased the production of the anti-inflammatory cytokine, IL-10 (Bharwani, Mian, Surette, Bienenstock, & Forsythe, 2017).

Changes in gut microbiota composition are associated with other stress-related issues. For example, the relative abundance of certain bacteria groups in the gut microbiota can also lead to

obesity. In mice, when a normal size mouse receives a fecal transplant from an obese mouse, it too becomes overweight (Turnbaugh, Backhed, Fulton, & Gordon, 2008). In humans, low gut diversity is also associated with increased risk for obesity, insulin resistance, increased inflammatory response, and higher cholesterol compared to individuals with gut microbiota high in species richness (Le Chatelier et al., 2013). The gut microbiota is also implicated in the pathogenesis of depression and anxiety, a process mediated by alterations of the microbial community that lead to increased systemic inflammation and subsequent behavioral changes (Dinan & Cryan, 2013; Foster & Neufeld, 2013; Winter et al., 2018). Kelly and colleagues (2016) demonstrated the role gut microbiota plays in the development of behavioral and physiological symptoms of depression by transplanting fecal material from clinically depressed human patients to microbiota deficient rodents. After transplantation, rodents had increased anhedonia and anxiety-like behaviors as well as decreased richness and diversity of the gut microbiota. However, in rodents, probiotics have been shown to reduce the effects of acute stress (i.e., restraint, hyperthermia, suspension) differentially. Specifically, species *Bifidobacteria breve* reduced anxiety symptoms while *Bifidobacteria longum* reduced anxiety as well as stress and depressive symptoms. Further, these bacterial species were found to be more efficient with alleviating negative symptoms than an antidepressant drug (Savignac, Kiely, Dinan, & Cryan, 2014).

In humans, exposure to high levels of stress result in decreased levels of the genus *Lactobacillus,* a probiotic that can suppress inflammatory response, and subsequently result in increased susceptibility to illness (Jones & Versalovic, 2009; Knowles, Nelson, & Palombo, 2008). Infants born to mothers with high levels of prenatal stress and circulating cortisol have significantly higher relative abundances within the Proteobacteria phylum including the genera *Esherichia* and *Serratia*, and lower levels of probiotic genera *Lactobaccillus* and *Bifidobacteria*.

This pattern has been linked to increased allergic symptoms and poorer stress-related health outcomes, such as gastrointestinal complaints (Zijlmans, Korpela, Riksen-Walraven, de Vos, & de Weerth, 2015). Further, at the phyla level, individuals diagnosed with stress-related disorders such as MDD have increased levels of Bacteroidetes, Proteobacteria, and Actinobacteria and decreased levels of Firmicutes compared to healthy controls (Jiang et al., 2015). Additionally, in a sample of pregnant women, after exposure to an acute stressor (i.e., Trier Social Stress Test) cortisol response was positively associated with the genera *Ruminococcaceae*, *Prevotella*, and *Ruminococcus* and negatively associated with the genera *Bacteroides*, *Megasphaera*, and *Eubacterium* (Hantsoo et al., 2018).

In preclinical trials (for review see Table 1 in Foster, Rinaman, & Cryan, 2017), consuming probiotic species (e.g., *Lactobaccillus sp.*, *Bifidobacteria sp.*) have been shown to lower cortisol awakening response, increase positive attentional vigilance, and reduce cognitive reactivity to sad mood (Schmidt, Cowen, Harmer, Tzortzis, Errington, & Burnet, 2015; Steenbergen, Sellaro, van Hemert, Bosch, & Colzato, 2015). Additionally, preclinical trials have shown that probiotics have efficacy in mood improvement, reduction of anxiety symptoms, and the attenuation of psychological distress (Benton, Williams, & Brown, 2007; Messaoudi et al., 2011a; Messaoudi, Violle, Bisson, Desor, Javelot, & Rougeot, 2011b; Rao et al., 2009). Consumption of probiotics has also been shown to alter brain activity in response to negative cues. Specifically, Tillisch and colleagues (2013) gave participants a fermented milk product with probiotic species (e.g., *Bifidobacterium animalis, Streptococcus thermophilus, Lactobacillus bulgaricus, Lactococcus lactis*) twice a day over a four-week period. Compared to the control group, from pre- to post-treatment, consumption of the probiotic milk product was associated decreased activity in somatosensory and viscerosensory brain regions in an fMRI

emotional attention task designed to assess reactivity to negative cues suggesting that probiotics may help reduce emotional reactivity in negative situations (Tillisch et al, 2013).

Bi-directional Communication. Given the bi-directional communication that occurs within the microbiome-gut-brain axis, a particular challenge to researchers is establishing causality. In their review of the relationship between the gut microbiota and depression, Winter and colleagues (2018) discuss the issue of cause and effect by exploring two working hypotheses within the literature. The first hypothesis is that depression modulates gut microbiota and the second hypothesis is that gut microbiota modulates depression; there is considerable evidence supporting both. For example, a stressful experience can induce a depressive or anxious state which can then lead to a reduction in gut diversity and richness (i.e., the first hypothesis). This process is thought to occur through depression-induced changes to the HPA axis. These changes subsequently alter the environment within the GI tract and leads to variations in gut microbial composition (Bailey et al., 2011; Bharwani et al., 2016; Winter et al., 2018). On the other hand, fecal transplant research has demonstrated that changes in gut microbiota composition can modulate behavior and mood (i.e., the second hypothesis) and that colonization of the microbiota is critical for normal brain development (Al-Asmakh et al., 2012; Sudo et al., 2004; Tetel et al., 2018; Winter et al., 2018). Evidence for both hypotheses suggest that the underlying mechanisms responsible for inducing these changes can occur independently of one another and may be sensitive to specific types of stress (Winter et al., 2018). Research continues to parse out the individual contributions of environmental influences as well as the underlying biological mechanisms responsible for changes within the microbiome-gut-brain axis (Cani & Knauf, 2016; Liu, 2017).

A Primer on Microbiome Data and Bioinformatics

There are several approaches to examining gut microbiota data, and each approach depends on the type of research question being asked. As with this dissertation, a common goal is to understand the composition of an individual's gut microbiota, how gut microbiota is influenced by environmental factors, and how the environment, both internal and external, influences the gut microbiota. This process begins with a biological sample (e.g., fecal matter) from which DNA is extracted, amplified, purified, and quantified (for specifics see 'Procedure for Processing Biological Data' section). From this procedure, millions of long sequences of genetic code are examined for similarity and grouped into Operational Taxonomic Units (OTU). Simply, OTUs refer to a grouping of similar DNA sequences that represent a specific microbial species and provide taxonomic classification (i.e., phylum, class, order, family, genus, and species) for that species. Thus, using OTUs, microbial composition can be analyzed at any taxonomic level (Kumar et al., 2014; Morgan & Huttenhower, 2012).

Alpha and beta diversity measures are often calculated using OTUs to examine microbial composition. Alpha diversity metrics (e.g., absolute OTU counts, Chao1 estimator, Shannon Index) examine the diversity of a microbial community composition within an individual sample and are useful in traditional statistical analyses. Alpha diversity is used to examine the richness (i.e., the number of unique species present) and the evenness (i.e., the distribution of species present) of the microbial community (Kumar et al., 2014). Absolute OTU counts and Chao1 estimator are measures of species richness. Absolute OTU counts is a simple and basic measure to examine how many different biological organisms are present in a sample (i.e., abundance). The Chao1 estimator (Chao, 1984) corrects the absolute OTU count by accounting for duplicate OTUs within a sample; a microbiota with more singleton OTUs is considered richer. Finally, the Shannon index examines both the richness and the evenness (i.e., relative abundance) of a

sample by considering the number of unique OTU's along with their abundance (Shannon, 2001). For a healthy adult, a Shannon index of 5.5 is considered normal, meaning that OTU's are evenly abundant.

Beta diversity examines the dissimilarity in microbial composition between groups and is useful in descriptive analyses. Common beta diversity metrics include Unweighted UniFrac, Weighted UniFrac, and Bray-Curtis estimates; each calculate beta diversity differently. Unweighted UniFrac is the most efficient in detecting differences in community membership by using the presence and absence of OTUs between groups along with phylogenetic distances, the degree to which specific OTUs are related to one another, in order to determine beta diversity (Lozupone & Knight, 2005). Weighted UniFrac is most efficient in detecting differences in OTU abundance between groups; it considers the relative abundance of OTUs and phylogenetic distances to calculate beta diversity (Lozupone & Knight, 2005; Lozupone, Hamady, Kelley, & Knight, 2007). Bray-Curtis does not consider phylogenetic relatedness between OTUs but accounts for the relative abundance as well as presence and absence of OTUs to determine beta diversity (Morgan & Huttenhower, 2012). Generally, Principal Coordinates Analysis (PCoA) plots are used to visualize beta diversity. PCoA plots are a graphical representation of the distance between groups using the distance matrix generated from Unweighted UniFrac, Weighted UniFrac, or Bray-Curtis estimates. The distance matrix is transformed and presented on a two- or three-dimensional plot where each axis represents a principal component (PC). The first axis, PC1, explains to greatest amount of variance followed by PC2 and so on (Kumar et al. 2014; Lozupone et al., 2007). Thus, alpha and beta diversity can be used to explore the relationship between the gut microbiota and the environment (i.e., stress) within the individual and between groups of interests.

Summary

Within the gut-microbiome-brain axis, exposure to acute and chronic stress can change the overall composition of the gut microbiota, by altering the richness or evenness of the microbial community (Kumar et al., 2014). Different patterns of gut microbiota have been linked to stressor-induced immune and endocrine functioning as well as inflammatory responses, physical, and psychological health (Bailey et al., 2010; Galley et al., 2017; Knowles, Nelson, & Palombo, 2008; Le Chatelier et al., 2013; Messaoudi et al., 2011b; Zijlmans et al., 2015). Thus, individuals who have been chronically victimized by their peers or who are under high levels of chronic stress may also have altered gut microbiota compared to non-victimized peers.

Current Study

This present study aimed to examine whether being victimized by one's peers in emerging adulthood may influence and lead to the development of aberrant, or atypical, colonization patterns of microbiota in the gut. Given that the microbiome-gut-brain axis is part of the normal stress response and chronic stress effects the relative abundance and diversity of the gut microbiota, it is expected that peer victimization, a chronic psychosocial stressor, would exert similar effects. This altered function, in turn, should be linked to obesity, higher blood pressure, and self-reported health complaints as well as anxiety, depression, and stressor-induced immune and endocrine functioning as assessed by elevated inflammation (i.e., higher levels of IL-6 and C-reactive protein). Given these associations (see Figure 1), it was predicted that this mechanism (e.g. the microbiome-brain-gut axis) will affect the relationship between poor interpersonal relationships and negative health outcomes.

Aims

Aim 1. First, the relationships between peer victimization and gut microbiota diversity were examined. It was anticipated that peer victimized individuals would have lower relative gut diversity (i.e., absolute OTU counts, Chao1 estimator, Shannon Index) compared to those who are not peer victimized. Whether peer victimization predicted differences in gut diversity over and beyond other forms of stress (i.e., non-social daily hassles) was also examined. It should be noted that low gut diversity has been associated with negative health outcomes related to stress such as obesity and inflammation. Additionally, exploratory analyses sought:

Aim 1a: To examine the mediating role of perceived stress within the relationship between peer victimization and gut diversity.

Aim 1b: To examine the moderating role of gender within the relationship between peer victimization and gut diversity.

Aim 2. Secondly, the relative abundance of microbiota that have been associated with stressors at both the phyla and genera level were also examined. More specifically, it was expected that peer victims will have higher levels from the Proteobacteria genera *Escherichia* and *Serratia* while also having lower levels of probiotic genera *Lactobaccillus* and *Bifidobacterium* compared to non-victimized peers. This pattern of microbiota has been linked to poorer stress-related health outcomes (Zijlmans et al., 2015). Additionally, it was also expected that peer victims will have higher levels of genus *Clostridium* and lower levels of genus *Bacteroides*, a pattern linked to inflammation and illness (Bailey et al., 2011). Table 1 shows the predicted associations regarding the relative abundance of microbial groups at the phylum and genus level within highly victimized individuals.

Table 1. *Expected Relative Abundance of Gut Microbiota Within Peer Victims*

Phylum	Genus
Bacteroidetes	*Bacteroides* ↓
Actinobacteria	*Bifidobacterium* ↓
Proteobacteria	*Escherichia* ↑
	Serratia ↑
Firmicutes	*Lactobacillus* ↓
	Clostridium ↑
	Coprococcus ↓
	Pseudobutyrivibrio ↓
	Dorea ↓

Aim 3. Whether or not peer victimization and gut microbiota diversity were associated with self-reported health complaints, inflammation (e.g. elevated plasma levels of CRP and IL-6), and physical markers of health (e.g., waste-to-hip ratio (WtHR), and blood pressure) was explored. It was expected that high levels of peer victimization would be associated with poorer mental and physical health. It was also expected that lower gut microbiota diversity would be associated with poorer psychological outcomes (e.g., depression, and anxiety) and physical outcomes such as waist-to-height ratio (WtHR; Le Chatelier et al., 2013*).* Additionally, exploratory analyses sought:

Aim 3a: To examine whether increased circulating pro-inflammatory cytokines (e.g., IL-6, CRP) would be associated with decreases in genera *Coprococcus*, and *Dorea* (Bailey et al., 2011).

Aim 3b: To examine whether individuals with high levels of probiotics reported fewer depressive symptoms (Kelly et al., 2016; Savignac et al., 2014).

Aim 3c: To examine whether probiotics buffered against the negative psychological effects of peer victimization.

Aim 4. Finally, beta diversity analyses were conducted to describe the differences in gut microbiota composition between groups. Using the upper and low quartile of peer victimization scores to form two groups, descriptive analyses were utilized to determine how individuals who reported experiencing high levels of psychosocial stress (i.e., peer victimization) differed from those who reported low levels of stress regarding the presence/absence of OTUs, the relative abundance of OTUs, and the relatedness of OTUs. Beta diversity estimates (i.e., Un-weighted UniFrac, Weighted UniFrac, Bray-Curtis distances) as well as which specific species differed in relative abundance between the two groups were reported. Further, differences in alpha diversity (e.g. absolute OTU counts, Chao1 estimator, Shannon Index) between the two groups were also examined. Given the exploratory and descriptive nature of this aim, no explicit predictions were made; however, broadly, it was expected that there would be dissimilarities between the two groups due to their differential experience with psychosocial stress (Galley et al., 2017; Foster, Rinaman, & Cryan, 2017; Knowles, Nelson, & Palombo, 2008).

Chapter 2:

Method

Participants

Participants were recruited though the Psychology Participant pool. A total of 126 young adult college students enrolled at the University of Texas at Arlington visited the Personality and Social Behavior lab for two sessions to participate in a larger study on peer relationships, daily hassles, and health in college students. The sample consisted of 57.1% females with an average age of 20.07 years. The sample was ethnically diverse and consistent with the university demographics: 38.9% White or Anglo American, 20.6% Black or African American, 17.5% Asian, 13.5% Hispanic or Latino, 3.2% Native American or Alaskan Native, 6.3% multiracial or other. 19.8% of participants reported being biracial. The sample size was large enough to provide power to detect pairwise and correlational effects (r = .30 to .50) (see La Rosa et al., 2015 for review; Biagi et al., 2010; Zijlmans et al., 2015). Indeed, Biagi et al. (2010) found moderate relations between IL-6 and gut microbiota in a sample of 88 adults (Range: .26 to .48 for M = .42). Significant small to moderate associations between social stress, cortisol levels, and gut microbiota have also been found in a sample of 56 infants (Zijlmans et al., 2015).

Prior to signing up, potential participants were informed about certain aspects of the study (i.e., fecal sample) so they could self-select out prior to the first session. In addition to receiving research credit for completing the surveys (i.e., 1 credit for Time 1; 2 credits for Time 2), individuals were paid an additional $20.00 for providing blood and fecal samples. Given that 25-30% of college students experience peer victimization, attempts were made to over-select victimized individuals. Individuals who indicated they are currently being victimized in the pre-screening of the Psychology Participant pool were solicited to participate in the study through an

email invitation. From this group, 4 individuals (3.2% of study population) participated in the study.

Materials

Peer Victimization and Stress Assessments

Direct and Indirect Aggression Scale—Victim Version (DIAS-VS). The victim version of the DIAS (Bjorkvist, Lagerspetz, & Osterman, 1992) assessed the frequency one experienced acts of aggression and/or victimization. Using a Likert scale with answers from 1 ("never") to 5 ("all the time"), the 24-item inventory assessed three different subscales: physical, verbal, and indirect aggression. The average of each subscale was computed as well as the total score for this measure. Cronbach's alpha was .89.

Children's Self-Experiences Questionnaire—Self-Report (CSEQ-SR). The CSEQ is a self-report measure that assessed the child's experience with peer relationships, specifically peer-related occurrences of victimization (Crick & Grotpeter, 1995). Items in the questionnaire were modified to better suit a young adult population (i.e., 'kids' was changed to 'peers'). The questionnaire consists of three subscales which responses are recorded on a Likert scale, which ranges from 1 ("never") to 5 ("all the time"). Each subscale consists of five questions that measure the frequency of behaviors received. The scales measure overt victimization, relational victimization, and the frequency of receiving or being the target of prosocial behaviors; an average of each subscale was created ($\alpha = .71$).

Cyberbullying Experiences Survey (CES). The CES is a self-report measure specifically designed to assess cyberbullying experiences in emerging adulthood (Doane, Kelley, Chiang, & Padilla, 2013). This 21-item scale examined experiences with electronic public humiliation and unwanted contact as well as the use of malice and deception in online victimization experiences. Items are answered using a 6-point Likert scale ranging from 0

("never") to 6 ("every day/almost every day"). A total cyberbullying experience score was calculated as well as average scores for the four subscales, humiliation, unwanted contact, malice and deception (α = .88).

College Daily Hassles Questionnaire (CDHQ). The CDHQ is a modification of the Inventory of College Student's Recent Life Experiences (ICSRLE; Kohn, Lafreniere, & Gurevich, 1990) and college stressors delineated by Insel and Roth (1985). Using a 4-point Likert scale ranging from 1 ("not at all part of my life") to 4 ("very much part of my life"), this questionnaire measured the degree to which one experiences academic and interpersonal stressors as well as financial concerns, time pressures, and worries about the future. A total score of daily hassles was computed (α = .93).

Perceived Stress Scale (PSS). The PSS is a 14-item questionnaire that measured the degree to which an individual appraises situations in their life as stressful (Cohen, Kamarck, & Mermelstein, 1983). Items include "In the last month, how often have you felt that things were going your way?" and "In the last month, how often have you felt nervous and "stressed"?" Items are scored on a 5-point Likert scale ranging from 0 ("never") to 4 ("very often"). All items were totaled to create an overall perceived stress score. Cronbach's alpha was .84.

Physical Health Assessments

Assessing Health Outcomes. This survey assessed the frequency and severity health symptoms linked with stress (Knack, Jensen-Campbell, & Baum, 2011). Using a Likert scale, 28 frequency items were measured from 0 ("not at all") to 4 ("all the time") and 28 severity items were rated from 0 ("does not hurt at all") to 4 ("unbearable pain"). Health problems assessed included extreme fatigue, sleep problems, stomach aches, nausea, muscle aches and pains, headaches or migraines, weight gain or loss, low energy, trips to the nurse or doctors, and chest pain. Overall health is also included in the measurement and assessed from 0 ("extremely poor")

to 5 (“excellent”). Self-report single-item general health measures have good reliability and validity and are also highly related to actual health (DeSalvo, Fisher, Tran, Bloser, Merrill, & Peabody, 2006). All frequency items were summed to create an overall frequency of health problems score (α = .92) and all severity items were summed to create an overall severity of health problems score (α = .90).

Abdominal Pain Index (API). The API (Walker, Smith, Garber, & Van Slyke, 1997) measured abdominal pain over the past two weeks, assessing the frequency, length, and intensity of abdominal pain experienced by participants. A total score was computed to indicate abdominal pain problems (α = .95).

Diet and Exercise. Items from the Health Behavior in School-aged Children (HBSC: Iannotti, 2005) were selected to assess behaviors related to diet and exercise. Selected questions measured the amount of time spent being sedentary and active as well as the intensity of any exercise throughout the week. Items that asked about the types of food consumed (e.g., junk food, fast food, fruits, vegetables, etc.) were also included. Information about current dieting efforts, past dieting attempts, and overall feelings and attitudes about one’s body were also collected. Hours a day spent sedentary, hours a day spent doing physical activities, amount of healthy food (e.g., fruits, vegetables) consumed per week, and amount of junk food (e.g., fast food, soft drinks) were scored; higher scores equal healthier behaviors. Therefore, an overall composite score for activity level (α = .77) was calculated where higher scores indicated a more active lifestyle and a composite score for diet (α = .62) was computed where higher scores equal healthier diet. This composite score was used as a control variable.

Psychological Health Assessments

Adult Self Report (ASR). The ASR (Achenbach, 2003) assessed one’s social competency, emotional issues, and behavior problems. Using a Likert scale from 0 (“not true”), 1

("somewhat or sometimes true"), and 2 ("very true or often true"), the 140-item inventory assessed several subscales: aggressive behavior, rule breaking behavior, attention problems, social problems, thought problems, somatic complaints, anxious/depressed, withdrawn/depressed, internalizing, externalizing, and total problems, and DSM-oriented scales. Specifically, internalizing problems, DSM depression, DSM anxiety, and DSM somatic complaints subscales were the focus of this dissertation.

Center for Epidemiological Studies Depression Scale (CES-D). The CES-D (Lewinsohn, Seeley, Roberts, & Allen, 1997) is a twenty-item questionnaire examining how someone has felt or acted in the past week concerning depression-like symptoms such as lack of appetite, trouble sleeping, and feeling sad. Responses were measured on a 4-point Likert scale from 0 ("not at all") to 3 ("a lot"). Items were totaled to create an overall depression score (α = .89).

Procedure

Participation of this study consisted of two sessions, approximately one week apart. Upon arrival to the lab for Session 1, a researcher informed the participant about all aspects of study, including at-home collection of fecal matter and an in-lab blood draw by a trained phlebotomist. Then the researcher directed the participant to read the consent form and to ask any questions they may have. Once the participant gave their consent to participate, they completed a series of surveys on their peer relationships and friendships. All measures can be found in Appendix B.

First, participants completed an eligibility checklist based off the one used in the National Institute of Heath's Human Microbiome Project – Core Microbiome Sampling Protocol A. Though this study was not involved in the NIH HMP in any way, this eligibility checklist is the gold standard for conducting research on the human gut microbiome. For example, exclusion criteria included if an individual has a chronic bowel disorder (e.g., Crohn's disease, Irritable

bowel syndrome), or has taken antibiotics in the last 6 months, or has drastically changed their diet in the last month. Participants who answered yes to any of the questions were thanked, given credit for the first session, and dismissed from the study. A total of three participants were found to be ineligible due to consuming antibiotics within the last 6 months, being outside the age range of interest (e.g., 18 to 25 years), or using a prescription cream. These individuals were thanked for their time, given research credit for Session 1 of the study, and dismissed.

Participants were then signed into Qualtrics with their unique, confidential identification code and directed to fill out the surveys on-line. During Session 1, participants completed measures of victimization, and daily hassles as well as non-diagnostic measures that assessed symptoms associated with depression. Using paper-and-pencil participants completed the Adult Self-Report (ASR; Achenbach, 2003), which contained measures of internalizing and externalizing problems. Further, participants completed other measures that were not part of this dissertation.

The last part of the first session involved teaching participants how to collect a fecal sample. To collect a fecal sample, participants were taught how to use the OMNIgene®•GUT kit for at home sample collection; participants watched a short animated video explaining the process in detail (https://www.youtube.com/watch?v=FnwG7D24_Uk), the researcher also verbally explained the collection using an example kit, and were also given detailed instructions to take home as a reminder (See Appendix C). A researcher provided the participant with a pre-labeled opaque bag to return their sample in at the start of Session 2. Session 1 lasted approximately 45 minutes to one hour for all participants.

Approximately one week after the first lab visit, participants came back to the lab for the second session to turn in their fecal sample and complete the remaining surveys. The participants placed the opaque bag with their fecal sample in a bin designated for the samples.

Participants then reported on their health and well-being as well as other measures not part of this dissertation. Specifically, overall frequency and severity of health symptoms including fever, stomachaches, sore throats, respiratory problems, coughing, cold sores, and fatigue were examined. Participants also completed the Abdominal Pain Index and the Pittsburg Sleep Quality Index (PSQI). Participants also provided information about their diet and exercise habits for control measures. A researcher then collected the following health markers: waist circumference (e.g., the soft, fleshy, torso area between the base of the rib cage and the top of the hip bone), hip circumference (e.g., the widest part of your hips, including the buttock), and neck circumference as well as height and weight. Waist-to-height ratio (WtHR) was calculated and used as a measure of abdominal obesity. WtHR has been shown to be a more reliable indicator of obesity compared to body-mass index (BMI) and is not subject to differences in sex or age (Savva et al., 2000). A researcher also took blood pressure measurements during this time.

After participants completed all questionnaires, a researcher directed them to the blood extraction laboratory, where a certified phlebotomist obtained a sample of the participant's blood via antecubital venipuncture. Once the sample was obtained, participants returned to the Personality and Social Behavior Lab for debriefing and payment ($20.00). Session 2 lasted 30 to 45 minutes for all participants.

Fecal matter collection and storage. Gut microbiota samples were collected when the participant had a bowel movement consisting of solid matter (at their house). Fecal samples were collected in a tube (OMR-200) with the assistance of a small spatula (see Appendix C). Participants were instructed to empty bladder before collection, and to collect the fecal sample free of urine or toilet water; tissue paper and non-latex gloves were provided in addition to the fecal sample collection kit. Participants were told the following instructions. Participants were instructed to then unscrew the purple cap of the tube, leaving the yellow tube top in place.

Researchers emphasized that it was important that the yellow tube top was not removed and that the stabilizing liquid inside the tube is not spilled. Researchers also explained that the spatula would be used to collect a small amount of fecal matter and to transfer the sample to the yellow tube top, continuing the process until the tube top was filled completely. Participants were then instructed to remove any external excess from the tube top, to screw the purple cap back on the tub, and shake vigorously for 30 seconds or until the sample was mostly dissolved. Then, participants were instructed to place the tube in the pre-labeled specimen bag and return with it upon their second lab visit. Samples could be stored at room temperature for up to 60 days. However, after the participant left the lab, the samples were moved and stored in a freezer in LS532 in a biohazardous container for future analysis.

Blood collection and storage. Approximately 4ml of the participant's blood was collected by a certified phlebotomist. Blood was drawn from each participant between 1:00 and 4:00 p.m. to control for diurnal patterns. After the participant left the lab, the samples were centrifuged at 1100RPM at 18°C for 10 minutes in a timely manner (within four hours of the initial blood draw) to ensure the integrity of the sample. Approximately 3mL of plasma was extracted from each sample and then frozen at -78°C in the Andy Baum Memorial Bioassay Clinical Research Laboratory at the University of Texas at Arlington for future analyses that examined circulating levels of IL-6 and CRP.

Blood pressure measurement. Blood pressure was measured using Omron's Automatic Blood Pressure Monitor with Printer (Model HEM-705CP) twice during the participant's second visit to the lab after completion of all questionnaires. The first blood pressure measure was taken before physical measures were collected (e.g., weight, height, waist and neck circumference); both systolic and diastolic blood pressure was recorded. Participants were told to relax their arm, turn their palm upward and place it on the table. The cuff was placed on the participant's arm at

heart level. Research participants were told to not move their arm, place their feet flat on the ground, and to remain still to ensure an accurate blood pressure measurement. Five to ten minutes passed to allow the arteries to return to normal conditions and then blood pressure was measured and recorded for a second time. The two diastolic and two systolic blood pressure measures were averaged and used in subsequent analyses.

Procedure for Processing Biological Data

Gut Microbiome

Sequencing. Participants' fecal samples were sent to the University of Texas at Arlington's Genomics Core Facility to be analyzed; several bioinformatics were conducted. 16S Sequencing Microbiome analysis were conducted on the genetics of the microbiota of sample provided. This is considered a gold standard for assigning taxonomic names to bacteria in gut microbiome through analysis of ribosomal RNA sequencing to identify and compare the types of bacteria present within a given sample, quantifying several thousand microbial biomarker genes in a single analysis (Hamady & Knight, 2009; Kumar et al., 2014). Specifically, using 250 ml of homogenized fecal matter, the DNA was extracted using bead beating methods with the Qiagen Power Fecal DNA extraction kit and the V3 and V4 regions of the bacterial 16S rRNA gene was amplified using PCR. The amplification was purified, removing containments (i.e., salts, enzymes, primers, nucleotides) from the sample using the AxyPrep MagPCR Clean-up Protocol. Library pooling and quantification were processed via Qubit. Sequencing was conducted on an Illumina MiSeq with 300 cycles from each end.

Microbiome Data and Bioinformatics. Once samples were sequenced and in order to investigate higher-level taxonomic classification, Operational Taxonomic Unit (OTU) table generation was conducted using a closed-reference taxonomic classification and UCLUST algorithm in Quantitative Insights Into Microbial Ecology (QIIME) software (for methods see:

Caporaso et al., 2010; Quast et al, 2012) by Jason Kubinak at the University of South Carolina School of Medicine. For the purposes of this dissertation, the relative abundance of microbial groups was examined at the phyla and genera level. Furthermore, beta diversity analyses examined group differences at the species level. It was predicted that those who experience peer victimization would present with gut microbiota composition typifying a chronic stress environment.

Additionally, alpha diversity metrics (e.g., absolute OTU counts, Chao1 estimator, Shannon Index) were calculated. Again, it was expected that individuals with increased amounts of chronic stress (e.g., peer victimization, daily hassles) would have less diverse gut microbiota (Foster, Rinaman, & Cryan, 2017; Moloney et al., 2014; Tetel et al., 2018). Specifically, peer victims would have a lower absolute OTU count, Chao1 estimator, and Shannon diversity index. Further, exploratory analyses were conducted to examine beta diversity (i.e., group differences) between highly victimized individuals (i.e., top quartile of peer victimization scores) and individuals who experienced low levels of peer victimization (i.e., bottom quartile of peer victimization scores). Principal Coordinates Analysis (PCoA) plots and Community Variance boxplots were generated for each beta diversity metric.

Plasma Biomarker Assays

To examine participants' blood samples for circulating levels of IL-6 and CRP, samples were centrifuged at 1100RPM at 18°C for 10 minutes and subsequently frozen at -78° until ready for analysis. A capture (sandwich) enzyme-linked immunosorbent assay (ELISA) was used to analyze IL-6 and CRP. This ELISA is a very sensitive and robust analysis which ensures specificity and no cross-reactivity of secondary antigens. The Quantikine Human IL-6 (R&D Systems Product D6050) and CRP (R&D Systems Product DCRP00) kits were utilized due to

previous successful usage in the Personality and Social Behavior Lab as well as wide use in academic publications.

Using a 96-well microplate coated with the capture antibody specific to IL-6 or CRP, samples were pipetted into the wells along with standards in a buffered protein base. IL-6 and CRP then binded to the respective antibody present in the well during a two-hour incubation period at room temperature. Plates were then washed to remove unbound antigen (i.e., IL-6, CRP). A detection antibody conjugated with an enzyme specific to IL-6 (a polyclonal antibody conjugated against alkaline phosphatase) or CRP (a monoclonal antibody conjugated against horseradish peroxidase) was added to the well and allowed to incubate at room temperature. The wells will then be washed to remove unbound antibody-enzyme conjugates. A substrate specific for each enzyme was then added to the plates; plates were covered and placed away from light to incubate. Afterwards, for IL-6 only, an amplifier solution was added and then a stop solution consisting of sulfuric acid was added to IL-6 and CRP plates. This allowed the color to develop in proportion to the amount of antigen (IL-6 or CRP) in each sample. Using an ELISA plate reader for quantitative analysis, the color was measured at 450nm with wavelength correction set to 540nm or 570nm. Each sample was run twice for accuracy.

Analytical plan

Aim 1: Diversity of Gut Microbiota. The first aim examined whether peer victimization predicted differences in gut diversity and whether peer victimization predicted differences over and beyond non-social daily hassles (e.g., financial burdens, academic struggles, long commute). Additionally, Aim 1 explored the roles of perceived stress and gender within the relationship between peer victimization and gut diversity.

Specifically, to examine that highly victimized individuals had lower relative gut diversity compared to those who were not peer victimized, a series of hierarchical regressions

were conducted with alpha diversity metrics (i.e., absolute OTU count, Chao1 estimator, and Shannon diversity index) as the dependent variable, respectively. Using items from the HBSC (Iannotti, 2005), composite scores for healthy behaviors were calculated where higher scores indicate a more active lifestyle (i.e., time spent being active) and healthier diet (i.e., more fruits and vegetables, less junk and fast food). Age, gender, activity level, diet, and non-social daily hassles were entered in the first block, and peer victimization measures (i.e., traditional and cyber-victimization) in the second block (Aim 1). Additionally, the role of perceived stress within the relationship between peer victimization and gut diversity was examined (Aim 1a). It was expected that individuals who perceived higher levels of stress would have lower gut diversity compared to individuals who perceived low levels of stress; it was predicted that this relationship would be stronger for those who experienced high levels of victimization. To examine the indirect effect of victimization on gut diversity measures via perceived stress, a series of simple mediations (i.e., Model 4) were conducted utilizing Hayes' (2013) PROCESS macro in SPSS.

To examine how gender moderates the relationship between peer victimization and gut diversity (Aim 1b), a series of moderated multiple regressions (i.e., Model 3) were conducted utilizing Hayes' (2013) PROCESS macro in SPSS with peer victimization measures, gender, and their interaction was entered into the model; measures of gut diversity (e.g. absolute OTU count, Chao1 estimator, and Shannon diversity index) were treated as dependent variables, respectively. Though there were no specific predictions concerning the differential effects of gender on the relationship between peer victimization and gut diversity, women who are victimized generally have poorer health outcomes compared to men (Gruber & Fineran, 2008). Therefore, it is expected that women who are victimized will have lower diversity compared to men.

Aim 2: Relative Abundance of Gut Microbiota. To examine that highly victimized individuals will have lower levels of Proteobacterial groups compared to those who are not peer victimized a series of regressions will be run with gender, activity level, and diet as covariates, victimization as the predictor, and the number of OTUs assigned to the phyla of interest (i.e., the abundance of Proteobacteria; Table 1) as the dependent variable. Exploratory analyses will examine the effect of peer victimization on relative abundance of gut microbiota belonging to the phyla Firmicutes, Bacteroidetes, Actinobacteria. Additionally, a series of multiple regressions will be run to examine the effect of peer victimization on the relative abundance of gut microbiota belonging to the genera *Escherichia, Serratia, Lactobaccillus, Clostridium, Bacteroides,* and *Bifidobacterium*. It is expected that peer victims will have higher levels of *Escherichia, Serratia,* and *Clostridium* and lower levels of *Bacteroides*, *Lactobaccillus*, and *Bifidobacteriium* compared to non-victimized peers.

Aim 3: Gut Microbiota and Health Outcomes. Aim 3 sought to examine the role of gut microbiota diversity within the relationship between peer victimization and self-reported health complaints, inflammation, and physical markers of health. It should be noted that Aim 3 was initially proposed as a mediation. However, given that this was the first study to examine the role of gut diversity within the relationship between psychosocial stress and health outcomes, it was important to consider whether gut diversity is a mediator or a moderator of this relationship. For example, Shannon diversity could help explain (i.e., mediation) the relationship between peer victimization and poor health outcomes. Specifically, peer victimization could lead to decreases in gut diversity and in turn, decreased gut diversity could lead to poorer health outcomes. Alternatively, gut diversity could moderate the relationship between stress and health outcomes. It was expected that increases in peer victimization would predict poorer health outcomes, this relationship could be stronger for those with lower gut diversity.

Indeed, deciding which approach is more appropriate is a common research problem. Baron and Kenny (1986) have described the difficulty of distinguishing between mediators and moderators pointing out that a variable could be both depending the method of measurement (i.e., experimental manipulation, self-report questionnaire). Other researchers have argued that moderation is a form of mediation, highlighting the fact that the linear model used in the two analyses is the same when the independent variable is categorical (Kraemer, Wilson, Fairburn, & Agras, 2002). Further, a moderation analyses could provide insight into the underlying mechanisms (i.e., mediators) involved and a mediation analyses could be used to identify targets (i.e., moderators) for intervention (Baron & Kenny, 1986).

To examine whether a variable mediates or moderates a relationship, most researchers will test the moderation model first in order to precede to a mediation analysis. Judd and Kenny (1981) outlined the evidence needed for mediation using three conclusions: (1) the predictor must affect the outcome variable, (2) the predictor must affect the mediator, and (3) the mediator must affect the outcome variable while controlling for the predictor. Additionally, the researchers recommended checking if there is a significant interaction between the predictor and the mediator which would result in changes in the outcome variable (i.e., moderation). Though mediation represents a causal inference, changes in the outcome variable may be caused by the predictor altering the underlying mechanism (i.e. the mediator) involved which would result in a mediation-moderation process (Judd & Kenny, 1981). More recently, other researchers have argued that in order for a mediation to be considered causal and a true mediation, the interaction between the predictor and the mediator should not be present (Lachowicz, Preacher, & Kelley, 2018). Simply, the first step of a mediation analyses should be to see if the mediator moderates the relationship between the predictor and outcome variables.

Thus, Aim 3 sought to examine if Shannon diversity moderated the relationship between peer victimization and poor health outcomes. This approach would help provide the evidence needed to claim mediation such as whether peer victimization predicted differences in gut diversity (i.e., conclusion 2 from Judd & Kenny, 1981) and whether peer victimization and gut diversity interact to predict health outcomes (Lachowicz, Preacher, & Kelley, 2018). To examine how gut diversity influences the strength of the relationship between peer victimization and psychological and physical health outcomes, a series of moderated multiple regressions were conducted (see Figure 2). Victimization variables and gut diversity measures (i.e., Shannon diversity index) were standardized and gender, age, activity level, and diet were used as covariates. Reiteratively, diastolic blood pressure, systolic blood pressure, IL-6, CRP, WtHR, frequency and severity of health complaints, and overall self-reported health as well as CES-depression, internalizing problems, DSM depression, DSM anxiety, and DSM somatic complaints measures were entered as dependent variables. If evidence for mediation (i.e., peer victimization predicts differences in diversity, no interaction between victimization and diversity) was present, mediation analyses were conducted.

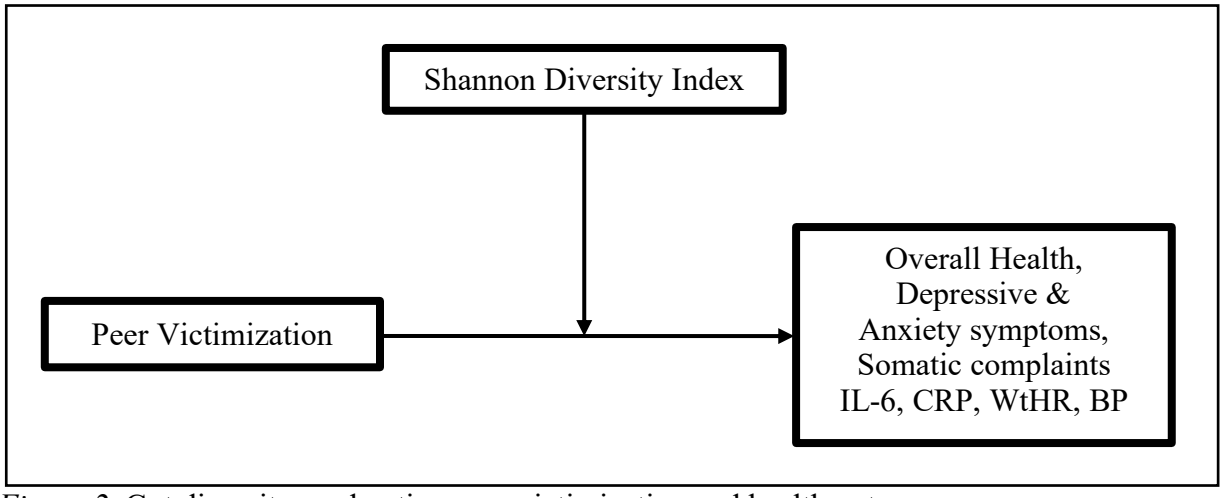


Figure 2. Gut diversity moderating peer victimization and health outcomes.

It was also expected that increased circulating pro-inflammatory cytokines (e.g., IL-6, CRP) would be associated with decreases in the genera *Coprococcus* and *Dorea* (Bailey et al., 2011). As such, a bivariate correlation was conducted to examine the associations between inflammatory markers and bacterial species abundance (Aim 3a). It was expected that these associations would be stronger for victimized compared to non-victimized individuals.

Given the established link between the gut microbiota and depression (Dinan & Cryan, 2013; Foster & Neufeld, 2013), an exploratory regression (Aim 3b) was conducted to examine whether individuals with high levels of probiotics (e.g., genus *Bifidobacteria*) reported fewer depression symptoms as measured by the CES-Depression as well as the DSM depression and DSM anxiety subscales of the ASR (Achenback, 2003; Lewinsohn et al., 1997). Further, a moderated multiple regression (i.e., Model #1 in PROCESS) was conducted to examine whether high amounts of probiotics within an individual's gut microbiota buffered against the negative outcomes of peer victimization (Aim 3c). Specifically, peer victimization and the relative abundance of the *Bifidobacteria* genus was entered into the model along with gender, age, activity level, and diet as controls. Reiteratively, CES-D depression, DSM depression, and DSM anxiety subscales were entered as dependent variables. It was expected that individuals with high levels of peer victimization and low levels of probiotics will have poorer health outcomes.

Aim 4: Group Differences in Gut Microbiota Composition. Beta diversity metrics were used to explore the dissimilarity in gut microbiota composition between individuals in the top quartile compared to individuals in the bottom quartile of peer victimization scores. Beta diversity metrics (e.g., Unweighted UniFrac, Weighted UniFrac, and Bray-Curtis distances) were calculated and statistical differences between groups were estimated using multivariate PERMANOVA. A visual representation of beta diversity was generated in the form of Principal Coordinates Analysis (PCoA) plots for all three metrics (Kumar et al. 2014; Lozupone et al.,

2007). Additionally, Community Variance boxplots were also generated for each beta diversity metric which allows for within group variance to be compared between groups. To examine differences in relative abundance of OTUs between the two groups, a goodness-of-fit test, which maximized the number of differences observed, was utilized. False discovery rate (FDR) adjusted p-values were used to account for multiple testing and to reduce the probability of Type 1 errors. Further, independent samples t-tests were conducted in order to make pairwise comparisons of alpha diversity estimates (e.g. absolute OTU counts, Chao1 estimator, Shannon Index) between the two groups. Though no specific predictions were made, it was expected that the gut microbiota of those under high levels of stress would differ regarding the presence/absence of OTUs, the relative abundance of OTUs, and the relatedness of OTUs from individuals who do not experience that degrees of stress (Galley et al., 2017; Foster, Rinaman, & Cryan, 2017; Knowles, Nelson, & Palombo, 2008).

Preliminary Analyses

Biological Materials.

Fecal Samples. Given the nature of the study, participants were told several times that an at home fecal sample was required for participation in this study. Researchers provided reminders to participants upon them signing up for the study, through an email reminder, and once again, during the informed consent process. These efforts yielded 99.2% (126 out of 127 participants) return rate for at home fecal sample collection. One participant declined to return for Session 2 of the study.

Quality of sequencing. After extraction and sequencing, the quality of the data was examined. Overall, base calling accuracy was high, %≥Q30 = 87.02%. Q30 logarithmically represents that there is a 99.9% probability that a base is correctly called during the sequencing process; Q30 75% or higher is the benchmark for sequencing quality (Ewing, Hillier, Wendi, &

Green, 1998.) Additionally, the percent aligned for the data set was high (48.98%) meaning that out of 23.9 million initial reads, 11.3 million pass quality filtering and are considered good reads. After the data were processed down further and an additional quality filter step to remove extraneous sequences from the data, 4.2 million reads remained. Additionally, three samples were removed due to low sequence yields (i.e., < 10,000 paired reads) in order to maximize sample depth.

Further, OTU tables were generated to examine the relative abundance of groups at the phyla and genera level. It was noted that genera of interest (see Table 1), *Escherichia* and *Pseudobutyrivibrio*, were not found among any participants within the sample. Moreover, ~90% of the sample did not have *Serratia* and *Lactobacillus* genera within their gut microbiota. These particular genera were removed from further analyses.

Blood Samples. Overall, out of the 126 participants to complete the study, there was an 86.5% success rate for venipuncture, 11.9% of attempts were unsuccessful, and 1.6% of participants declined to provide a blood sample.

Missing Value Analysis

Out of the 126 participants in the study, 53 cases had at least one variable item missing; however, this equated to less than .5% of all data values. Using the list-wise or pair-wise case deletion method to address the missing data would reduce the sample size by 42% and significantly lower statistical power. Therefore, data imputation was utilized to estimate the missing data. First, item nonresponses were examined to determine if there was a pattern to the missing data. Specifically, data were analyzed to determine if the data was missing completely at random (MCAR) meaning that the missingness did not depend on or was caused by the observed values (Rubin, 1976; Graham, 2009). To test this assumption, Little's MCAR test was conducted and was not significant, χ^2 (12,329) = 303.86, p = 1.00, which indicates that there is no

identifiable pattern within the missing data. Of the missing data, 3 participants (2.4%) failed to answer the majority of the dieting questionnaire and two participants (1.6%) did not answer twelve out of the 21 items on the Cyberbullying Experiences Survey. All other items had 1.6% or fewer missing data.

Given that missing data was sparse and evenly spread across participants without any observable patterns, missing data was imputed using the expectation-maximization (EM) method in the Missing Value Analysis program in SPSS 24. This method utilizes an iterative two-step process that begins with the 'E' step which examines the current estimates of the parameters around the observed scores to develop conditional expectations (e.g., log-likelihood) that will be used to replace the missing data. In the second step, the 'M' step, computes maximum likelihood estimates using the expected values found in the 'E' step. This process repeats until convergence. Thus, data were imputed, and the completed dataset was used in all analysis.

Creating Victimization Score

A principal components analysis with oblique rotation (e.g., direct oblimin) was conducted on the three victimization measurements to determine whether traditional forms of victimization and cyber-victimization consist of a single factor or are two separate factors. The subscales from the Direct and Indirect Aggression Scale (physical, verbal, and indirect), Children's Self-Experiences Questionnaire (overt and relational), and Cyberbullying Experiences Survey (humiliate, malice, unwanted contact, and deception) were used as individual items for the factor analysis. Two unique factors (eigenvalues > 1) emerged, traditional victimization (e.g., DIAS and CSEQ subscales) and cyber-victimization (e.g., CES subscales), and accounted for 65.81% of the total variance. It should be noted that there is some overlap between these two factors; indirect and verbal subscales loaded on both factors but factor loading scores were higher for traditional victimization. See Table 2 for factor loading scores. The two factors were

moderately correlated with one another (r = .43). Thus, factor scores were saved, and cyber-victimization and traditional victimization scores were treated separately in all analyses.

Group differences. In beta diversity analyses, the dissimilarity in microbial composition between groups was examimed (Aim 4). Of interest to this dissertation, was the effect peer victimization had on gut microbiota composition. Thus, two groups were created using the total composite score for traditional victimization only. The first group consisted of individuals (N = 31) from the top quartile of peer victimization scores (i.e., those who scored in the top 25%) and the second group consisted of individuals (N = 33) from the lowest quartile of peer victimization scores (i.e., those who scored in the bottom 25%). This approach allowed for the exploration of potential differences in gut microbiota composition between the two groups. Specifically, examining beta diversity provided a preliminary understanding of how these two groups differ in the presence/absence of OTUs, the relative abundance of OTUs, and/or the relatedness of OTUs.

Table 2. *Principal Component Analysis Factor Loadings for Victimization Measures*

Subscale	Traditional	Cyber
Physical	**.92**	-.10
Overt	**.96**	-.13
Relational	**.65**	.21
Indirect	**.59**	**.42**
Verbal	**.56**	**.39**
Humiliate	.22	**.57**
Deception	.19	**.60**
Malice	.06	**.81**
Unwanted contact	-.23	**.89**
Variance	51.50%	14.31%

Descriptive Statistics

Descriptive statistics for victimization, daily hassles, and perceived stress can be observed in Table 3. Descriptive statistics for outcome variables including overall health,

internalizing problems, depression, and anxiety can be found in Table 4. Anthropometric variables (e.g., blood pressure, WtHR) and biological data (e.g., Il-6, CRP) can be observed in Table 5. Descriptive statistics for gut microbiota diversity measures and groups of interests are available in Table 6.

A series of bivariate correlations were conducted to examine the relationships between stress variables (i.e, victimization, non-social daily hassles, perceived stress) and physical and psychological health outcomes as well as measures of gut diversity and relative abundance of phyla and genera of interest. Broadly, inter-correlations between victimization, daily hassles, perceived stress, and health outcomes were significant. Il-6 was significantly correlated with daily hassles, overall health, frequency of health problems, WtHR, somatic complaints, and depressive symptoms; CRP was associated with abdominal pain, WtHR, diastolic blood pressure, and Il-6 (Table 7). Victimization, daily hassles, perceived stress were not correlated with gut diversity measures or with the relative abundance at the Phyla level. At the genus level, traditional victimization, daily hassles, and perceived stress were significantly associated with the genus *Dorea* (Table 8). Shannon Diversity index was significantly correlated with internalizing problems, CRP, and WtHR (Table 9).

Table 3. *Descriptive Statistics for Victimization and Stress Measures*

Measure	Range	Min.	Max.	*Mean*	*SD*	Skewness	Kurtosis
Victimization							
DIAS	7.27	3.00	10.27	4.41	1.20	1.48	3.81
CSEQ	4.89	2.00	6.80	2.68	.82	2.08	5.91
Total traditional	12.07	5.00	17.07	7.09	1.91	1.81	5.571
CES	9.28	4.00	13.28	5.91	2.02	1.69	2.71
DIAS subscales							
Physical	1.98	1.00	2.97	1.17	.27	3.15	15.30
Verbal	2.80	1.00	3.80	1.67	.62	1.06	.62
Indirect	2.50	1.00	3.50	1.57	.50	1.26	3.03
CSEQ subscales							
Overt	2.20	1.00	3.20	1.21	.36	2.92	11.06
Relational	2.60	1.00	3.60	1.47	.58	1.56	2.39
CES subscales							
Humiliate	1.34	1.00	2.33	1.17	.23	1.95	4.74
Malice	5.00	1.00	6.00	1.80	.98	1.78	3.46
Unwanted contact	3.00	1.00	4.00	1.55	.80	1.70	2.17
Deception	2.33	1.00	3.33	1.41	.54	1.52	1.78
Stressors							
N.S. Daily Hassles	78.00	47.00	125.00	80.68	17.62	.25	-.43
Perceived Stress	38.00	21.00	59.00	42.24	7.91	-.19	.03

Table 4. *Descriptive Statistics for Psychological Outcomes*

Measure	Range	Min.	Max.	*Mean*	*SD*	Skewness	Kurtosis
CES-Depression	41.03	20.00	61.03	34.15	9.20	.87	.25
Internalizing problems	58.00	0	58.00	18.22	12.12	.87	.59
DSM-Oriented scales							
Depressive problems	19.00	0	19	6.37	4.39	.77	-.20
Anxiety problems	14.00	0	14	6.33	3.36	.19	-.83
Somatic problems	12.00	0	12	1.96	2.57	1.8	3.09

Table 5. *Descriptive Statistics for Physical Health-Related Outcomes*

Measure	Range	Min.	Max.	*Mean*	*SD*	Skewness	Kurtosis
Overall Health	3.00	2.00	5.00	3.58	.89	.034	-.74
Frequency	74.00	27.00	101.00	40.64	10.10	2.05	9.40
Severity	52.00	27.00	79.00	34.08	7.39	2.45	10.52
Abdominal Pain	33.00	5.00	38.00	8.75	6.82	2.05	4.13
Age	11.00	18.00	29.00	20.07	2.23	1.53	2.28
Diet	3.40	3.00	6.40	4.77	.76	-.09	-.69
Activity level	38.00	13.00	51.00	31.31	6.88	-.04	.41
WtHR	1.49	.57	2.06	1.28	.23	.10	2.02
Systolic BP	54.00	97.00	151.00	121.46	12.53	.26	-.39
Diastolic BP	39.00	54.00	93.00	70.59	8.20	.42	.33
IL-6 (log)	2.10	-.81	1.30	0.40	.32	.16	1.73
CRP (log)	2.55	1.40	3.94	2.81	.61	-.11	-.91

Table 6. *Descriptive Statistics for Gut Diversity and Microbiota*

Measure	Range	Min.	Max.	*Mean*	*SD*	Skewness	Kurtosis
Gut Diversity							
Shannon	4.68	2.51	7.19	5.73	.97	-1.3	1.79
Chao 1	829.2	348.83	1178.03	780.14	174.41	-0.16	-0.31
Absolute OTUs	505	230	735	517.43	122.05	-0.23	-0.59
Phylum							
Actinobacteria	7573	1	7574	677.29	1176.35	3.37	13.59
Bacteroidetes	8710	41	8751	2738.02	2131.13	0.77	-0.22
Firmicutes	9603	1120	10723	7163.52	2263.26	-0.70	-0.12
Proteobacteria	1333	0	1333	269.33	259.06	1.87	4.07
Genus							
Bacteroides	6632	14	6646	1842.39	1687.78	0.94	-0.07
Bifidobacterium	7574	0	7574	646.48	1177.84	3.39	13.71
Serratia	128	0	128	1.35	11.70	10.68	116.18
Lactobacillus	5	0	5	0.19	0.81	4.93	24.83
Clostridium	101	0	101	6.31	12.03	5.15	34.00
Coprococcus	1181	9	1190	153.4	193.46	3.02	10.68
Dorea	248	0	248	62.82	55.15	1.45	1.52

Table 7. *Correlations Between Stress Variables and Health Outcomes*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. Traditional Victimization	-																
2. Cyber Victimization	.43**	-															
3. N.S. Daily Hassles	.39**	.41**	-														
4. Perceived Stress	.31**	.37**	.61**	-													
5. Overall Health	.00	-.17	-.15	-.26**	-												
6. Frequency	.21*	.48**	.50**	.37**	-.31**	-											
7. Severity	.24**	.36**	.37**	.32**	-.30**	.80**	-										
8. Abdominal Pain	.072	.21*	.27**	.22*	-.06	.43**	.44**	-									
9. CES-Depression	.28**	.38**	.51**	.61**	-.22*	.48**	.42**	.26**	-								
10. Internalizing Problems	.43**	.53**	.61**	.65**	-.38**	.57**	.50**	.33**	.69**	-							
11. DSM Depressive	.35**	.46**	.63**	.63**	-.40**	.52**	.41**	.29**	.64**	.86**	-						
12. DSM Anxiety	.31**	.39**	.57**	.63**	-.42**	.49**	.40**	.26**	.63**	.88**	.75**	-					
13. DSM Somatic	.21*	.38**	.42**	.40**	-.25**	.61**	.54**	.48**	.42**	.66**	.51**	.57**	-				
14. IL-6	.11	0.02	.25**	.13	-.20*	.22*	.18	.14	.13	.16	.26**	.19	.25**	-			
15. CRP	.11	-.04	.01	-.08	-.01	.07	.03	.20*	-.07	-.01	.05	-.05	.01	.43**	-		
16. WtHR	-.04	-.01	.10	.02	-.33**	.08	.02	-.05	.03	.14	.16	.09	.08	.27**	.40**	-	
17. Systolic BP	-.06	.28**	.07	-.09	-.05	.05	-.08	.02	.01	.11	.09	.06	.13	-.17	.12	.26**	-
18. Diastolic BP	-.13	0.15	.05	-.01	-.25**	.06	.02	.05	-.05	.08	.07	.06	.17	.19*	.42**	.38**	.53**

Note. * = $p < .05$; ** = $p < .01$

Table 8. *Correlations Between Stress Variables, Gut Diversity and Microbiota*

	Traditional Victimization	Cyber-Victimization	Non-social Daily Hassles	Perceived Stress
Gut Diversity				
Shannon Diversity	.015	-.047	-.051	-.091
Chao 1 Estimator	-.061	-.124	-.056	-.050
Absolute OTU	-.064	-.085	-.035	-.081
Phylum				
Actinobacteria	-.056	-.040	-.051	.112
Bacteroidetes	-.033	-.073	.004	-.169^{+}
Firmicutes	.026	.098	-.002	.099
Proteobacteria	.099	-.110	.021	-.104
Genus				
Bacteroides	-.016	-.057	-.058	-.102
Bifidobacterium	-.066	-.052	-.058	.110
Serratia	-.049	-.095	.002	.017
Lactobacillus	.096	-.126	.054	.042
Clostridium	-.063	-.078	.01	.021
Coprococcus	-.045	-.077	-.084	-.169^{+}
Dorea	.137	.036	.248**	.187*

Note. $^{+} = p < .10$; * = $p < .05$; ** = $p < .01$

Table 9. *Correlations Between Gut Diversity and Health Outcomes*

	Shannon Diversity	Chao 1 Estimator	Absolute OTU
Overall Health	.12	.077	.051
Frequency	.009	-.093	-.079
Severity	.036	-.081	-.064
Abdominal Pain	.133	.074	.072
CES-Depression	-.058	-.091	-.065
Internalizing Problems	-.178*	-.156^{+}	-.149
DSM Depressive	-.096	-.055	-.039
DSM Anxiety	-.129	-.110	-.078
DSM Somatic	.086	.019	.043
IL-6	.055	.042	.051
CRP	.165^{+}	.102	.094
WtHR	-.187*	-.130	-.105
Systolic BP	-.099	-.067	-.074
Diastolic BP	-.042	.014	.021

Note. $^{+} = p < .10$; * = $p < .05$

Chapter 3:

Results

Aim 1. Does peer victimization predict lower gut diversity?

To examine whether peer victimization predicted lower gut diversity, three hierarchical regressions were conducted. Age, gender, activity level, and diet were used as covariates and entered into the first block along with non-social daily hassles. Cyber-victimization and traditional peer victimization were entered in the second block. Absolute OTU count, Chao1 estimator, and Shannon diversity index were used as dependent variables, respectively. Results showed[1] that traditional peer victimization and cyber-victimization did not predict lower gut diversity even after controlling for non-social daily hassles and covariates (p = .367 - .754; Table 10). Further, supplementary analyses were conducted to examine the relationship between subtypes of peer victimization and gut diversity measures. Overall, subtypes of traditional victimization (i.e., physical, verbal, indirect, overt, and relational) and cyber-victimization (i.e., malice, unwanted contact, and deception) did not predict differences in gut diversity (p = .094 - .938). However, public humiliation, a subtype of cyber-victimization significantly predicted difference in absolute OTU count, Chao1 estimator, and Shannon diversity index (Table 11).

[1] All analyses were performed with and without transformations of key variables. Transformations did not alter the results of the analyses. Thus, results using non-transformed variables were reported.

Table 10. *Regression Results for Victimization Predicting Gut Diversity*

Outcome	Predictor	β	*t*	*p*	95% CI	sr^2
Shannon Diversity						
R = .27	Age	-.10	-1.02	.311	-.28, .09	.01
	Gender	-.25	-2.38	.019	-.44, -.04	.05
	Diet	-.08	-0.81	.419	-.28, .12	.01
	Activity Level	.08	0.76	.447	-.13, .29	.01
	N.S. Daily Hassles	-.13	-1.18	.242	-.33, .09	.01
	Traditional Victimization	.08	0.71	.478	-.14, .29	.00
	Cyber-Victimization	-.05	-0.42	.673	-.25, .16	.00
Chao 1 Estimator						
R = .31	Age	-.08	-0.79	.430	-46.74, 20.04	.01
	Gender	-.17	-1.70	.093	-65.78, 5.10	.02
	Diet	-.25	-2.45	.016	-80.03, 8.44	.05
	Activity Level	.00	0.04	.965	-35.89, 37.51	.00
	N.S. Daily Hassles	-.07	-0.67	.506	-49.40, 24.50	.00
	Traditional Victimization	-.01	-0.10	.921	-39.35, 35.58	.00
	Cyber-Victimization	-.15	-1.34	.182	-61.86, 11.87	.02
Absolute OTU Count						
R = .26	Age	-.08	-0.84	.403	-33.53, 13.58	.01
	Gender	-.17	-1.60	.112	-45.22, 4.79	.02
	Diet	-.19	-1.88	.063	-49.23, 1.28	.03
	Activity Level	.03	0.27	.785	-22.31, 29.47	.00
	N.S. Daily Hassles	-.06	-0.55	.584	-33.30, 18.85	.00
	Traditional Victimization	-.04	-0.37	.712	-31.37, 21.50	.00
	Cyber-Victimization	-.09	-0.84	.403	-37.02, 15.00	.01

Note. Models were not significant, *p* = .367 - .754. Values shown are from the second block of analyses.

Table 11. *Regression Results for Victimization Subtypes Predicting Gut Diversity*

Predictor	*R*	β	*t*	*p*	95% CI	sr^2
Outcome						
Physical						
Shannon Diversity	.24	.04	.44	.663	-.14, .22	.00
Chao 1 Estimator	.25	-.04	-.37	.712	-38.10, 26.12	.01
Absolute OTU count	.22	-.04	-.42	.677	-27.50, 17.93	.00
Verbal						
Shannon Diversity	.24	.04	.42	.677	-.15, .23	.00
Chao 1 Estimator	.27	.11	1.17	.245	-53.53, 13.83	.01
Absolute OTU count	.23	-.09	-.93	.353	-35.12, 12.64	.01
Indirect						
Shannon Diversity	.24	-.05	-.51	.611	-.23, .14	.00
Chao 1 Estimator	.28	-.12	-1.28	.204	-53.48, 11.57	.01
Absolute OTU count	.23	-.10	-1.06	.294	-35.34, 10.79	.01
Overt						
Shannon Diversity	.26	.10	1.03	.307	-.09, .283	.01
Chao 1 Estimator	.25	-.05	-.47	.637	-41.23, 25.32	.00
Absolute OTU count	.22	-.04	-.44	.660	-28.79, 18.30	.00
Relational						
Shannon Diversity	.25	-.09	-.97	.336	-.28, .10	.01
Chao 1 Estimator	.27	-.12	-1.22	.226	-53.23, 12.70	.01
Absolute OTU count	.25	-.14	-1.41	.161	-39.86, 6.691	.02
Public Humiliation						
Shannon Diversity	.34*	-.27	-2.74	.007	-.44, -.07	.06
Chao 1 Estimator	.36*	-.39	-2.99	.003	-81.14, -16.43	.07
Absolute OTU count	.32*	-.25	-2.58	.011	-53.24, -7.01	.05
Malice						
Shannon Diversity	.24	.04	.387	.700	-.14, .21	.00
Chao 1 Estimator	.25	-.04	-.43	.671	-38.50, 24.88	.00
Absolute OTU count	.21	-.01	-.06	.956	-23.07, 21.81	.00
Unwanted Contact						
Shannon Diversity	.23	.02	.17	.868	-.17, .20	.00
Chao 1 Estimator	.29	-.16	1.70	.094	-59.63, 4.76	.02
Absolute OTU count	.23	-.10	-1.03	.308	-34.48, 10.98	.01
Deception						
Shannon Diversity	.23	-.01	-.08	.938	-.19, .18	.00
Chao 1 Estimator	.26	-.08	-.85	.397	-47.29, 18.88	.01
Absolute OTU count	.22	-.05	-.51	.609	-29.23, 17.21	.00

Note. Overall model was significant at: * = $p < .05$. Covariates, gender, age, time spent active, and diet, are not shown in the table.

Aim 1a. Is the relationship between peer victimization and gut diversity mediated by perceived stress?

To examine whether young adults who are victimized have lower gut diversity via perceived stress, a mediation analyses was conducted using ordinary least squares regression-based path analysis. Gender was used as a covariate; given that age, diet, and activity level were consistently unrelated to gut diversity measures in Aim 1, they were dropped from subsequent analyses. The data were entered into SPSS via Hayes' (2013) PROCESS macro using Model #4. Results showed (see Table A1 in Appendix A) that there is not an indirect effect of victimization on gut diversity via perceived stress. Specifically, though victimization significantly predicted perceived stress, $b = .35$, $SE_b = .08$, $t(120) = 4.20$, $p < .001$, 95% CI [.18, .51], perceived stress did not significantly predict Shannon diversity index ($p = .127$), Chao 1 Estimator ($p = .550$), and absolute OTU count ($p = .388$). Consistent with previous findings (Aim 1), there was no total or direct effects for traditional peer victimization on gut diversity measures ($p = .146 – .910$). Similar results were observed for cyber-victimization (see Table A2 in Appendix A). Cyber-victimization significantly predicted perceived stress, $b = .41$, $SE_b = .08$, $t(120) = 5.10$, $p < .001$, 95% CI [.25, .57], but perceived stress did not significantly predict Shannon diversity index ($p = .215$), Chao 1 Estimator ($p = .816$), and absolute OUT count ($p = .471$). Consistent with previous findings (Aim 1), there was no total or direct effects for cyber-victimization on gut diversity measures ($p = .163– .800$).

Aim 1b. Is the relationship between peer victimization and gut diversity moderated by gender?

Three moderated multiple regressions were conducted to examine whether the relationship between peer victimization and gut diversity measures (e.g. absolute OTU counts, Chao1 estimator, Shannon Index) were moderated by gender (Aiken & West, 1991).

Victimization variables were standardized, and gender was coded using unweighted effects codes. Males were coded as 1 and females were coded as -1. The data were entered into SPSS via Hayes' (2013) PROCESS macro using Model #3, which allowed for the moderating effect of gender to be tested for both the traditional and cyber-victimization as well as the three-way interaction between victimization measures and gender. Gender did not moderate the relationship between peer victimization and gut diversity measures (p = .829 – .948; see Table A3 in Appendix A). Similar results were found for cyber-victimization (p = .609 – .978). Further, there were no significant interactions between traditional and cyber victimization (p = .285 – .533.) and there were no significant three-way interactions (p = .675– .879).

Aim 2. Does peer victimization predict differences in the relative abundance of microbiota at the phyla and genera level?

To examine whether traditional or cyber- victimization predicted differences in the relative abundance of microbiota at the phylum and genus level, a series of iterative regressions were conducted with traditional and cyber-victimization as predictors and gender as a covariate. Again, genera of interest (see Table 1) *Escherichia, Pseudobutyrivibrio*, *Serratia,* and *Lactobacillus* were either not found or found at very low levels within this sample and subsequently were removed from analyses. The relative abundance of each phylum (i.e., Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria) and of genera of interest (*Clostridium, Bacteroides, Bifidobacterium, Dorea, Coprococcus*) were treated as dependent variables, respectively. Results showed[2] that traditional peer victimization (p = .119– .866) and cyber-victimization (p = .052– .838) did not predict the relative abundance at the phylum level. Additionally, traditional peer victimization (p = .140 – .981) and cyber-victimization (p = .527–

[2] Analyses were performed with and without transformations of relative abundance of OTUs. Transformations did not alter the results of the analyses. Therefore, results using non-transformed variables were reported.

.797) did not predict the relative abundance at the general level (see Table A4 and A5 in Appendix A).

Aim 3. Is the relationship peer victimization and poor physical and psychological health outcomes moderated by gut diversity?

Aim 3 sought to examine the role of gut microbiota diversity within the relationship between peer victimization and health outcomes. This aim was initially proposed as a mediation. The first step of a mediation analyses should be to see if the mediator moderates the relationship between the predictor and outcome variables and if the predictor affects the mediator (Judd & Kenny, 1981; Lachowicz, Preacher, & Kelley, 2018). Indeed, gut diversity appeared to moderate the relationship between peer victimization and health. Therefore, a series of moderated multiple regressions were tested to examine whether the relationship between peer victimization and poor health outcomes were moderated by gut diversity (i.e., Shannon Diversity Index). Victimization and gut diversity measures were entered into SPSS via Hayes' (2013) PROCESS macro using Model #1 and gender, age, activity level, and diet were used as covariates. For physical health outcomes, results showed that traditional victimization significantly predicted increased frequency and severity of health outcomes (Table 12). Additionally, after controlling for traditional victimization, gender, activity level and diet, Shannon Diversity Index significantly predicted waist-to-hip ratio; Specifically, as gut diversity increased the ratio decreased, though the overall model was not significant (p = .196). Traditional victimization did not interact with gut diversity to predict differences in physical health (Table 12).

For psychological health outcomes (see Table 13), traditional victimization significantly predicted internalizing problems (i.e., withdrawn/depressed, anxious/depressed, somatic complaints), DSM Depression, and DSM Anxiety. Additionally, after controlling for victimization and covariates, Shannon Diversity Index significantly predicted internalizing

problems. Specifically, as gut diversity increased, internalizing problems decreased. A similar effect was observed for DSM anxiety, though it was not significant (p =.064). Further, the interaction between traditional victimization and Shannon Diversity index significantly predicted internalizing problems, DSM Depression, and DSM Anxiety (Table 13).

Table 12. *MMR Results for Traditional Victimization, Gut Diversity, and Physical Health*

Predictor	*b*	*t*	*p*	95% CI	sr^2
Outcome					
Traditional Victimization					
Overall Health	-.07	-.83	.410	-.24, .10	.00**
Frequency	1.92	1.94	.055	-.04, 3.88	.03*
Severity	1.63	2.30	.024	.22, 3.03	.04*
Abdominal	.59	.91	.364	-.70, 1.88	.01
IL-6	.04	1.25	.214	-.02, .10	.02
CRP	.09	1.51	.134	-.03, .21	.02
WtHR	-.00	-.06	.955	-.04, .04	.00
Systolic BP	-1.23	-1.01	.312	-3.64, 1.18	.01**
Diastolic BP	-.76	-.93	.353	-2.39, .86	.01
Shannon Diversity Index					
Overall Health	.12	1.45	.149	-.04, .28	.02
Frequency	-.82	-.85	.395	-2.74, 1.09	.01
Severity	-.33	-.48	.630	-1.70, 1.04	.00
Abdominal	.32	.50	.615	-.94, 1.58	.00
IL-6	.01	.38	.706	-.05, .08	.00
CRP	.04	.71	.482	-.08, .17	.00
WtHR	-.06	-2.67	.009	-.10, -.01	.06
Systolic BP	-.76	-.63	.531	-3.14, 1.63	.00
Diastolic BP	-.95	-1.18	.242	-2.56, .653	.01
Victimization X Shannon					
Overall Health	.16	1.52	.132	-.05, .36	.02
Frequency	-1.43	-1.18	.240	-3.81, .96	.01
Severity	-.08	-.09	.927	-1.79, 1.63	.00
Abdominal	-.46	-.58	.561	-2.03, 1.11	.00
IL-6	-.04	-1.02	.311	-.12, .04	.01
CRP	-.11	-1.49	.139	-.26, .04	.02
WtHR	-.01	-.462	.645	-.06, .04	.00
Systolic BP	-1.60	-1.06	.292	-4.59, 1.39	.01
Diastolic BP	-.69	-.68	.497	-2.71, 1.32	.00

Note. Overall model was significant at: * = $p < .05$; ** = $p < .01$. Covariates, gender, age, time spent active, and diet, are not shown in the table.

Table 13. *MMR Results for Traditional Victimization, Gut Diversity, and Mental Health*

Predictor	*b*	*t*	*p*	95% CI	sr^2
Outcome					
Traditional Victimization					
CES-Depression	2.67	2.89	.005	.84, 4.50	.07
Internalizing Problems	5.19	4.96	<.001	3.12, 7.26	.17**
DSM Depression	1.56	4.03	<.001	.79, 2.34	.11**
DSM Anxiety	1.10	3.58	<.001	.49, 1.70	.10**
DSM Somatic	.45	1.97	.051	-.00, .90	.03
Shannon Diversity Index					
CES-Depression	-.86	-.97	.335	-2.62, .900	.01
Internalizing Problems	-2.75	-2.69	.008	-4.77, -.72	.05
DSM Depression	-.54	1.41	.160	-1.29, .22	.01
DSM Anxiety	-.56	-1.87	.064	-1.15, .03	.03
DSM Somatic	.01	.04	.968	-.43, .45	.00
Victimization X Shannon					
CES-Depression	-.74	-.67	.502	-2.94, 1.45	.00
Internalizing Problems	-2.64	-2.07	.041	-5.16, -.11	.03
DSM Depression	-1.11	-2.35	.021	-2.05, -.17	.04
DSM Anxiety	-.89	-2.39	.019	-1.63, -.15	.04
DSM Somatic	-.36	-1.31	.193	-.92, .19	.01

Note. Overall model was significant at: * = $p < .05$; ** = $p < .01$. Covariates, gender, age, time spent active, and diet, are not shown in the table.

To probe these interactions, the effect of traditional peer victimization on internalizing problems, DSM Depression, and DSM Anxiety was examined at low (-1 SD) and high (+1 SD) levels of gut diversity. For internalizing problems (Figure 3), at low levels of gut diversity, peer victimization was a significant predictor, $b = 7.83$, $SE = 2.77$, $t(109) = 4.43$, $p < .001$, $sr^2 = .13$, such that as peer victimization increased, internalizing problems also increased. At high levels of gut diversity, peer victimization did not significantly predict internalizing problems, $b = 2.56$, $SE = 1.52$, $t(107) = 1.683$, $p = .095$, $sr^2 = .02$, suggesting that gut diversity may help buffer against the negative outcomes of peer victimization.

Similar results were observed for DSM depression (Figure 4) and DSM anxiety (Figure 5). At low levels of gut diversity, peer victimization was a significant predictor of depressive symptoms, $b = 2.68$, $SE = .66$, $t(107) = 4.072$, $p < .001$, $sr^2 = .11$, such that as peer victimization increased, depressive symptoms also increased. At high levels of gut diversity, peer victimization did not significantly predict depressive symptoms, $b = .45$, $SE = .57$, $t(107) = .800$, $p = .425$, $sr^2 = .00$. Additionally, at low levels of gut diversity, peer victimization was a significant predictor of anxiety symptoms, $b = 1.99$, $SE = .52$, $t(107) = 3.837$, $p < .001$, $sr^2 = .11$, such that as peer victimization increased, anxiety symptoms also increased. At high levels of gut diversity, peer victimization did not significantly predict anxiety symptoms, $b = .21$, $SE = .44$, $t(107) = .464$, $p = .644$, $sr^2 = .001$.

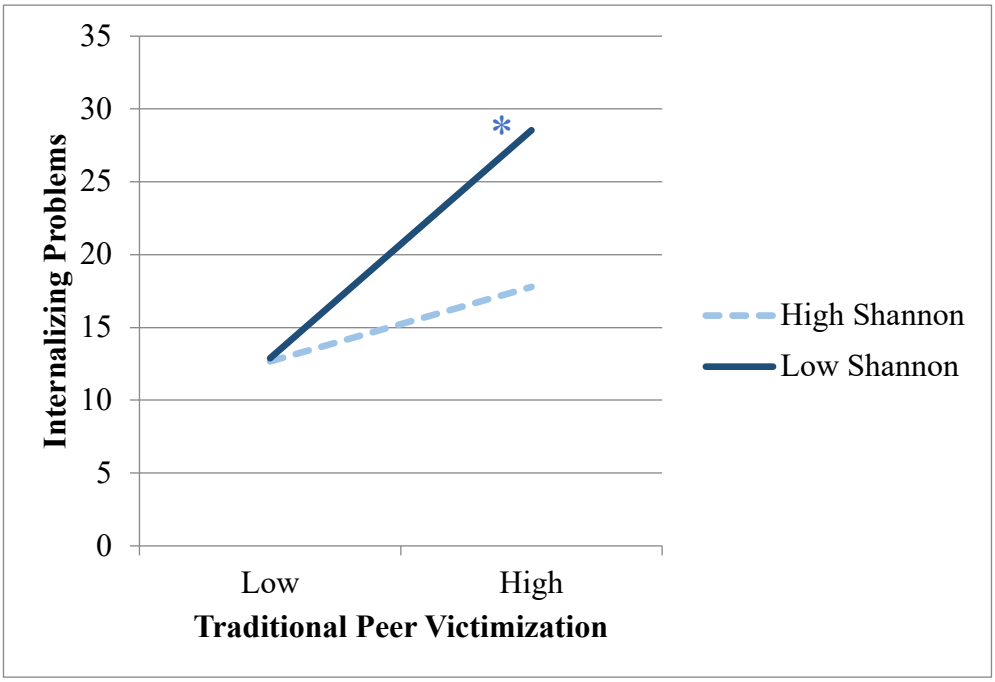


Figure 3. Gut diversity as a moderator for peer victimization and internalizing problems.

Note. * = $p < .001$.

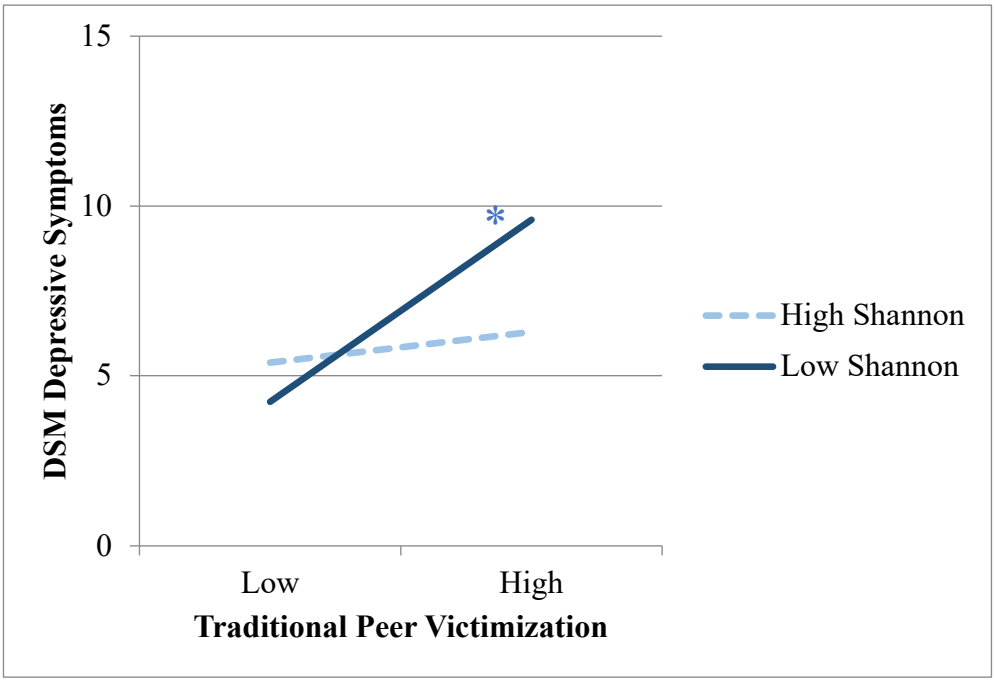


Figure 4. Gut diversity as a moderator for peer victimization and DSM depressive.

Note. * = $p < .001$.

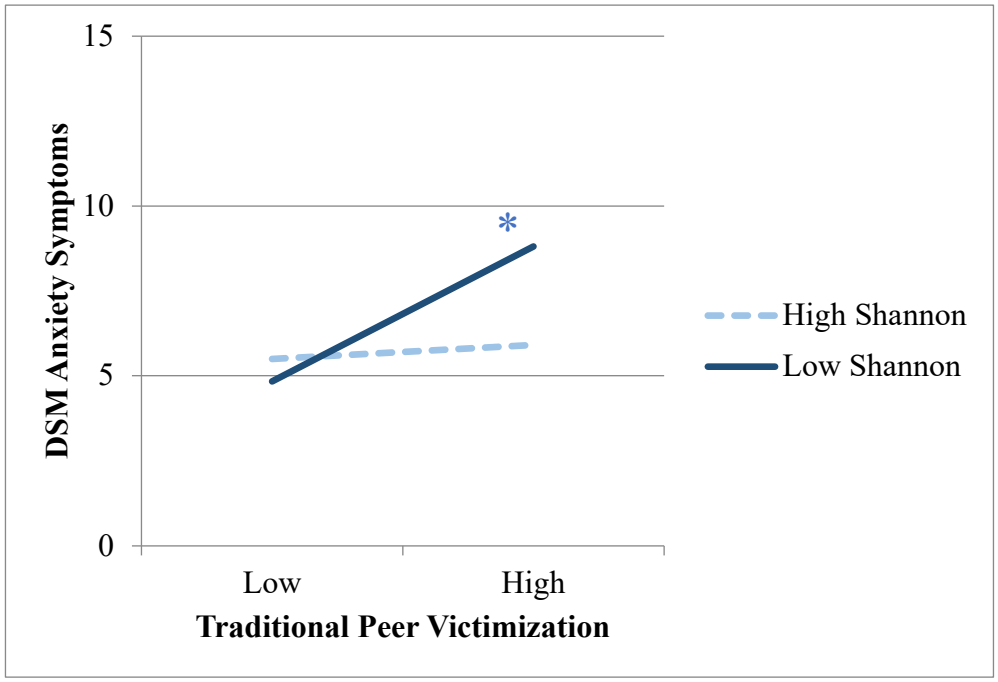


Figure 5. Gut diversity as a moderator for peer victimization and DSM anxiety symptoms.

Note. * = $p < .001$.

Similar results were observed for cyber-victimization. For physical health outcomes, results also showed that cyber-victimization significantly predicted increased frequency and severity of health outcomes as well as increased systolic blood pressure (Table 14). Additionally, after controlling for cyber-victimization, gender, activity level and diet, Shannon Diversity Index significantly predicted internalizing waist-to-hip ratio, though the overall model was not significant (p = .192). Cyber-victimization and gut diversity did not interact to predict difference in physical health measures (Table 14).

For mental health outcomes, cyber-victimization significantly predicted CES-depression scores, internalizing problems, DSM depression, DSM anxiety, and DSM somatic complaints (Table 15). Additionally, after controlling for victimization and covariates, Shannon Diversity Index significantly predicted internalizing problems. Specifically, as gut diversity increased, internalizing problems decreased. Further, the interaction between cyber-victimization and Shannon Diversity index significantly predicted internalizing problems and DSM Anxiety; the interaction was not significant for DSM depression (p =.081; Table 14).

Table 14. *MMR Results for Cyber-Victimization, Gut Diversity, and Physical Health*

Predictor	*b*	*t*	*p*	95% CI	sr^2
Outcome					
Cyber Victimization					
Overall Health	-.19	-2.32	.022	-.35, -.03	.04**
Frequency	4.91	5.80	< .001	3.23, 6.59	.22**
Severity	2.63	4.05	<.001	1.34, 3.92	.12**
Abdominal	1.52	2.49	.014	.31, 2.73	.05
IL-6	.01	.33	.746	-.05, .07	.00
CRP	-.01	-.17	.869	-.13, .11	.00
WtHR	.00	.13	.893	-.04, .04	.00
Systolic BP	3.59	3.20	.002	1.36, 5.82	.08**
Diastolic BP	1.50	1.94	.056	-.037, 3.04	.03
Shannon Diversity Index					
Overall Health	.10	1.17	.245	-.07, .26	.01
Frequency	-.37	-.44	.664	-2.07, 1.32	.00
Severity	-.14	-.22	.830	-1.44, 1.16	.00
Abdominal	.48	.77	.442	-.75, 1.70	.00
IL-6	.02	.55	.583	-.05, .08	.00
CRP	.05	.85	.397	-.07, .18	.01
WtHR	-.06	-2.64	.010	-.10, -.01	.06
Systolic BP	-.42	-.36	.716	-2.73, 1.88	.00
Diastolic BP	-.81	-1.01	.317	-2.40, .79	.01
Cyber X Shannon					
Overall Health	.04	.36	.718	-.16, .24	.00
Frequency	-.93	-.87	.387	-3.05, 1.19	.00
Severity	-.56	-.69	.493	-2.19, 1.06	.00
Abdominal	.30	.39	.700	-1.23, 1.83	.00
IL-6	.00	.06	.949	-.08, .08	.00
CRP	-.04	-.52	.603	-.20, .12	.00
WtHR	-.01	-.54	.592	-.07, .03	.00
Systolic BP	-1.01	-.71	.477	-3.82, 1.80	.00
Diastolic BP	-.30	-.30	.763	-2.24, 1.65	.00

Note. Overall model was significant at: * = $p < .05$; ** = $p < .01$. See Table A6 in Appendix A for covariates results: gender, age, time spent active, and diet.

Table 15. *MMR Results for Cyber-Victimization, Gut Diversity, and Mental Health*

Predictor	*b*	*t*	*p*	95% CI	sr^2
Outcome					
Cyber Victimization					
CES-Depression	3.69	4.20	<.001	1.95, 5.42	.14**
Internalizing Problems	6.10	6.50	<.001	4.24, 7.95	.25**
DSM Depression	1.87	5.20	<.001	1.16, 2.59	.17**
DSM Anxiety	1.28	4.48	<.001	.71, 1.85	.14**
DSM Somatic	1.00	4.97	<.001	.60, 1.40	.18**
Shannon Diversity Index					
CES-Depression	-.51	-.60	.551	-2.20, 1.18	.00
Internalizing Problems	-2.14	-2.25	.026	-4.02, -.26	.03
DSM Depression	-.33	-.90	.372	-1.05, .40	.01
DSM Anxiety	-.41	-1.42	.158	-.98, .16	.01
DSM Somatic	.11	.53	.600	-.30, .51	.00
Cyber X Shannon					
CES-Depression	-.15	-.16	.885	-2.26, 1.95	.00
Internalizing Problems	-2.77	-2.34	.021	-5.11, -.42	.03
DSM Depression	-.80	-1.76	.081	-1.70, .10	.02
DSM Anxiety	-.75	-2.08	.040	-1.47, -.03	.03
DSM Somatic	-.22	-.85	.398	-.72, .29	.01

Note. Overall model was significant at: * = $p < .05$; ** = $p < .01$. See Table A7 in Appendix A for covariates results: gender, age, time spent active, and diet.

To probe these interactions, the effect of cyber-victimization on internalizing problems, and DSM Anxiety was examined at low (-1 SD) and high (+1 SD) levels of gut diversity. For internalizing problems (Figure 6), at low levels of gut diversity, peer victimization was a significant predictor, $b = 8.86$, $SE = 1.53$, $t(107) = 5.796$, $p < .001$, $sr^2 = .20$, such that as cyber-victimization increased, internalizing problems also increased. At high levels of gut diversity, cyber-victimization also significantly predicted internalizing problems, $b = 3.33$, $SE = 2.49$, $t(107) = 2.233$, $p = .028$, $sr^2 = .03$. This suggests that though the relationship between cyber-victimization and internalizing problems is strong, this relationship is weaker for individuals with high gut diversity. Similar results were observed for DSM Anxiety (Figure 7). At low levels of gut diversity, cyber-victimization was a significant predictor of DSM Anxiety, $b = 2.03$, $SE = .47$, $t(107) = 4.359$, $p < .001$, $sr^2 = .14$, such that as cyber-victimization increased, anxious symptoms also increased. At high levels of gut diversity, cyber-victimization did not significantly predict DSM Anxiety, $b = .53$, $SE = .46$, $t(107) = 1.170$, $p = .245$, $sr^2 = .01$.

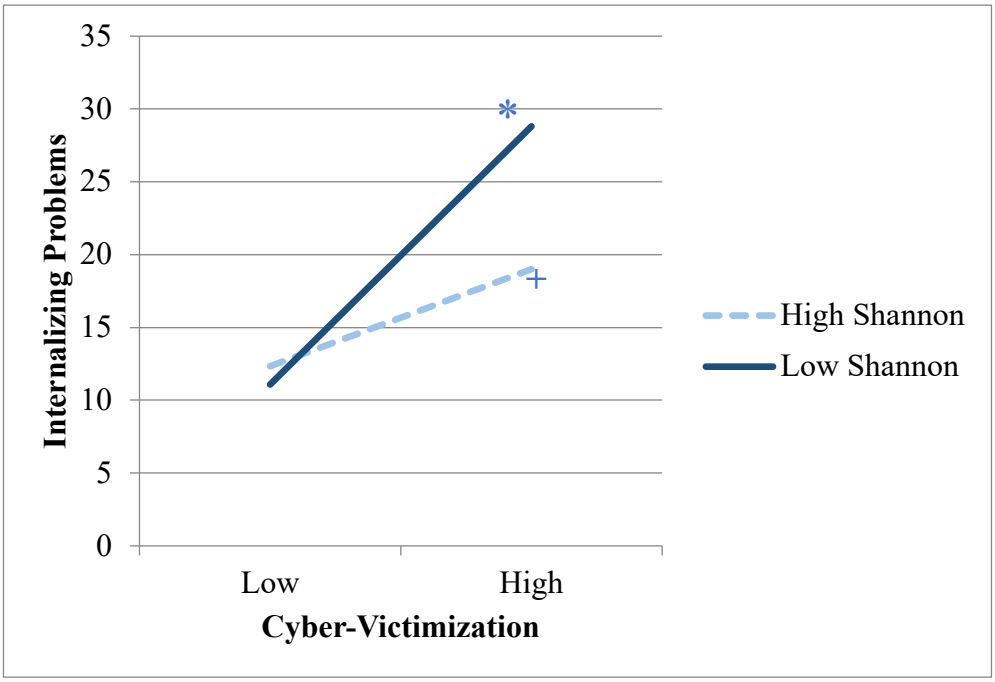


Figure 6. Gut diversity as a moderator for cyber-victimization and internalizing problems.

Note. $^{+}$ = $p < .05$;* = $p < .001$.

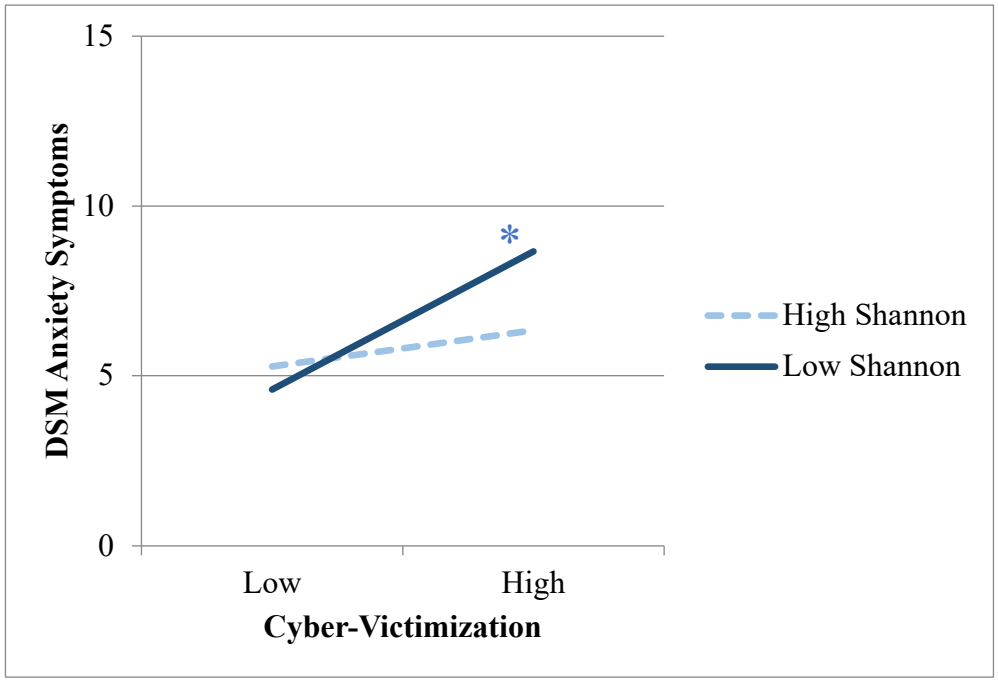


Figure 7. Gut diversity as a moderator for cyber-victimization and DSM anxiety symptoms.

Note. * = $p < .001$.

Aim3a. Are pro-inflammatory cytokines associated with lower levels of in genera *Coprococcus*, and *Dorea?*

A series of bivariate correlations were conducted to examine the associations between inflammatory markers IL-6 and CRP with genera *Coprococcus* and *Dorea* abundance (Bailey et al., 2011). Il-6 was not associated with the genus *Coprococcus* (p = .975) or the genus *Dorea* (p = .780). Similar results were found for CRP (p = .350 – .622). Further, these associations did not differ at high (+1 SD) or low (-1 SD) levels of traditional or cyber-victimization.

Aim3b. Does probiotic bacterial species predict fewer depressive symptoms?

To examine whether probiotics (i.e., genus *Bifidobacteria*) predicted fewer depressive symptoms, a series of iterative regressions were conducted with the relative abundance of the genus *Bifidobacteria* as predictor variable, age, gender, diet, and activity level as covariates, and CES-D depression, DSM Anxiety, and DSM Depression subscales were entered as dependent variables, respectively. Results showed[3] (Table A8) that the genus *Bifidobacteria* did not predict fewer depressive symptoms for CES-Depression (p = .229), DSM Anxiety (p = .863), or DSM Depression (p = .652).

Aim3c. Is the relationship between peer victimization and depressive symptoms moderated by probiotics?

A series of moderated multiple regressions (i.e., Model #1 in PROCESS) were tested to examine whether the relationship between peer victimization and depressive symptoms were moderated by probiotics (i.e., genus *Bifidobacteria*). The relative abundance of the genus *Bifidobacteria* were centered and gender, age, activity level, and diet were used as covariates. Results showed (Table A9; Table A10) that the relative abundance of the genus *Bifidobacteria*

[3] Analyses were performed with and without transformations of *Bifidobacteria*. Transformations did not alter the results of the analyses. Thus, results using non-transformed variables were reported.

did not interact with either peer victimization measures to predict fewer depressive symptoms (p = .089 – .804).

Aim 4. Are there differences in gut microbiota composition between the top and bottom quartile of peer victimization scores?

Diversity analyses sought to examine group differences between individuals from the top quartile of peer victimization scores (i.e., high victimization) and individuals from the lowest quartile of peer victimization scores (i.e., low victimization). Specifically, differences between the two groups in alpha and beta diversity as well as relative abundance of specific OTUs were explored.

Alpha Diversity. A series of independent samples t-tests were conducted to explore whether there were differences in alpha diversity between individuals in the top and bottom quartile of peer victimization scores. Results showed that groups did not differ in alpha diversity. For Shannon diversity (p = .593), individuals in the non-victim group (M = 5.58, SE = .19) did not differ from individuals in the victim group (M = 5.72, SE = .18). Similar results were observed for Chao 1 estimator (p = .747; Lower: M = 774.07, SE = 33.61; Upper: M = 758.51, SE = 34.17) and for absolute OTU counts (p = .593; Lower: M = 504.91, SE = 23.22; Upper: M = 504.80, SE = 23.36).

Beta Diversity. Beta diversity estimates were calculated using Unweighted UniFrac, Weighted UniFrac, and Bray-Curtis distance matrices which allowed for differences between groups to be explored regarding the presence/absence of OTUs, the relative abundance of OTUs, and the relatedness of OTUs. Results show that there were no differences in community composition based on Unweighted UniFrac distances (p = .21), which accounts for the presence/absence of OTUs as well as phylogenetic relatedness. There was also no difference between groups based on Weighted UniFrac distances (p = .21), which accounts for relative

abundances of OTUs as well as phylogenetic distance. These findings indicated that the microbial communities between high and low victimizations are not dissimilar in phylogenetic relatedness (i.e., the microbial community of each group consists of similar bacterial species). However, microbial community composition was significantly different between individuals in the victims and non-victims based on Bray-Curtis distances ($p = .01$), which accounts for relative abundance and presence/absence of OTUs (not phylogenetic relatedness). This finding suggests that differences in community diversity between the two groups is driven by a shift in the relative abundance of species shared between the two microbial communities. A visual representation of these findings (i.e., PCoA plots) for each metric can be observed in Figure 8. Additionally, in order to examine whether peer victimization influenced variance in community composition between groups, Community Variance boxplots were also generated to compare differences in within-group beta diversity estimates. Results showed that there is significantly more ($p < .0001$) variance in community composition within individuals in the victim group compared to those in non-vicitm group based on Unweighted UniFrac and Bray-Curtis estimates (see Figure 9). Simply, community composition is more variable within the victim group compared to the non-victim group; this difference is variance is likely due to the presence/absences of OTUs.

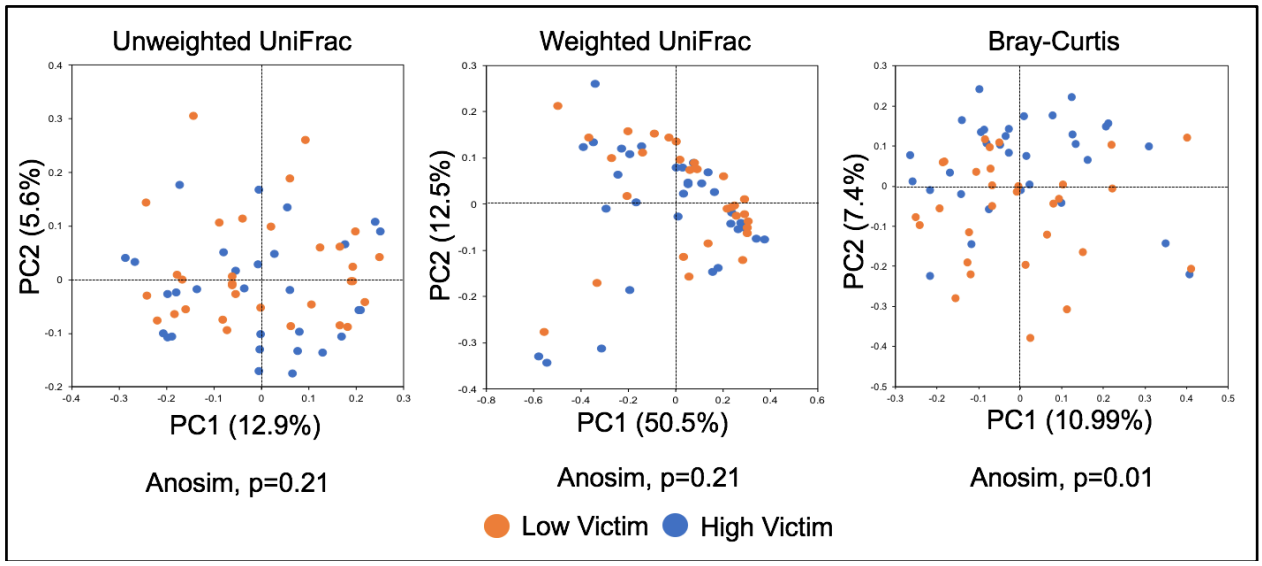


Figure 8. Beta diversity PCoA plots between victimization groups.

Note. Analysis of Similarity (ANOSIM) *p*-value is based on 999 permutations.

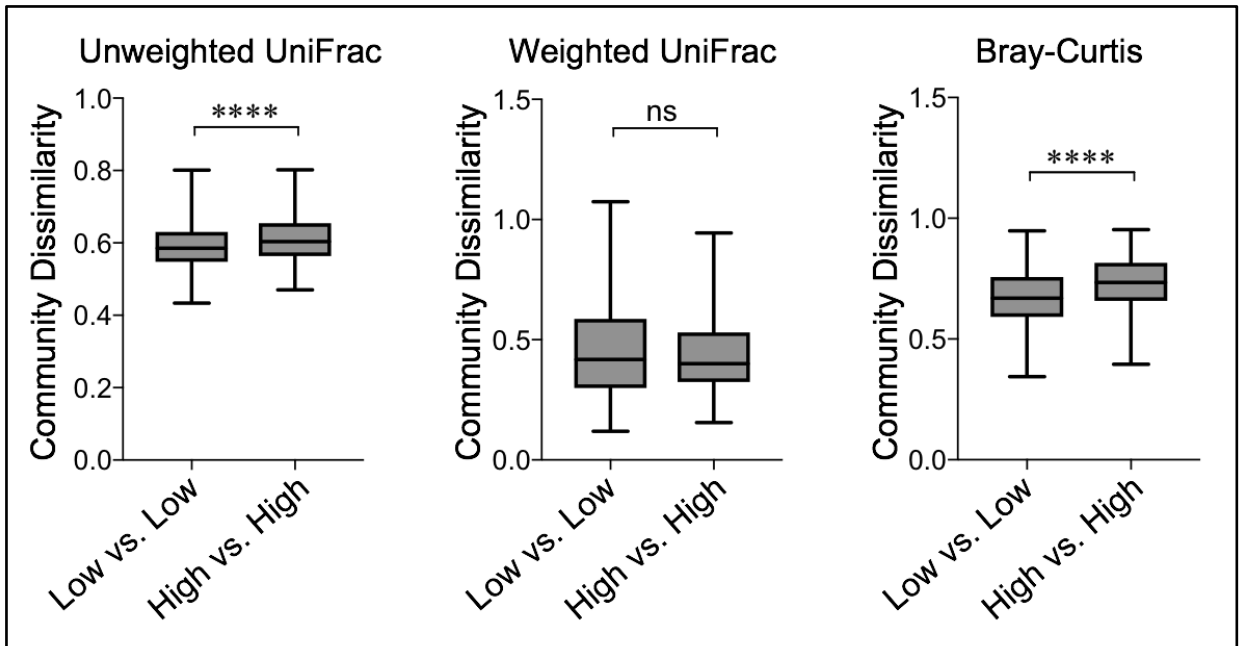


Figure 9. Community variance boxplots comparing variance within victimization groups.

Note. **** = $p < .0001$.

Differences in Relative Abundance. Given that high and low victimization groups differed based on Bray-Curtis distances and that evidence suggested that groups differences were due to shifts in the relative abundance of OTUs, exploratory analyses sought to identify which specific OTUs contributed to these differences. To examine differences in relative abundance of OTUs between the two groups, a goodness-of-fit test, which maximized the number of differences observed, was utilized and FDR adjusted *p*-values were used to account for multiple testing. Table 16 shows the OTUs that were significantly different regarding relative abundance between non-victim and victim groups. Broadly, the victim group was associated with increased levels of species belonging to the families Lachnospiraceae, Ruminococcaceae, and Enterobacteriaceae as well as increased levels of species belonging to the order Clostridiales. Additionally, the victim group was associated with decreased levels of the species *Bifidobacterium adolescentis*, *Faecalibacterium prausnitzii*, *Bacteroides ovatus*, and *Prevotella copri* as well as decreases in other unidentified species belonging to the genus *Bifidobacterium*, which was predicted in Aim 3c (Table 16).

Table 16. *Shifts in OTU Relative Abundance Between Low and High Victim Groups*

Level of Classification	OTU Relative Abundance (100%) Low Victimization	High Victimization
Species		
Bifidobacterium adolescentis	3.76	1.73
Faecalibacterium prausnitzii	22.00	11.86
Bacteroides ovatus	0.65	0.23
Parabacteroides distasonis	0.60	1.17
Prevotella copri	11.91	6.98
Genus (Unidentified species)		
Blautia sp.	3.20	6.44
Lachnospira sp.	1.17	0.84
Roseburia sp.	1.52	2.51
Bifidobacterium sp.	4.44	1.24
Oscillospira sp.	1.68	2.88
Ruminococcus sp.	4.07	3.61
Sutterella sp.	1.76	1.64
Family		
Lachnospiraceae	21.23	26.47
Ruminococcaceae	18.08	26.69
Enterobacteriaceae	0.14	0.67
Order		
Clostridiales	3.78	5.04

Note. Taxa are significantly different based on FDR-adjusted *p*-values.

Chapter 4:

Discussion

Peer victimization is a chronic psychosocial stressor that is associated with variety of poor outcomes such as psychological distress including depression, anxiety, and low self-esteem as well as somatic complaints including headaches, sleep problems, and stomach aches (Biebl et al., 2011; Low et al., 2007; Rigby, 2003). An important pathway between stress and health is the microbiome-gut-brain axis, a bidirectional communication system that connects the nervous, endocrine, and immune systems with the gastrointestinal track and the gut microbiome (Moloney et al., 2014). Chronic psychosocial stress has been shown to induce dysbiosis of the gut microbiota, leading to altered microbial composition and lower diversity. This is associated with poorer immune functioning, HPA axis dysregulation, and changes in mood (i.e., depression, anxiety) and behavior (Bakhed et al., 2015; Demaude et al., 2006; Malan-Muller et al., 2018; Winter et al., 2018). The purpose of this dissertation was to examine the influence of peer victimization on the relative abundance and diversity of the gut microbiota and to explore whether changes in microbial composition associated with peer victimization are also associated with poor mental and physical outcomes.

Peer Victimization and Gut Diversity

This dissertation was one of the first to examine the effects of psychosocial stress on gut diversity in emerging adults. Specifically, the first aim of this dissertation was to examine if self-reported differences in peer victimization (i.e., traditional and cyber-bullying) predicted differences in gut diversity. Results showed that neither traditional or cyber-victimization predicted changes in alpha diversity measures (i.e., absolute OTU count, Chao1 estimator, and Shannon diversity); similar results were also observed after controlling for daily hassles (Aim 1a). Analyses also revealed that the relationship between peer victimization and gut diversity was

not mediated by perceptions of stress (Aim 1b) or moderated by gender (Aim 1c). Additionally, gut diversity was not significantly correlated (see Table 8) with any stress-related variable (i.e., traditional victimization, cyber-bullying, daily hassles, perceived stress), which may suggest that the participants in this study were not experiencing a high degree of stress. Therefore, the results of Aim 1 failed to conceptually replicate previous findings within the literature when examining alpha diversity.

The influence of stress on the gut microbiota has been well documented in animal studies. Early-life stress such as maternal separation has been shown to predispose rodents to increased intestinal permeability (Demaued et al., 2006; Söderholm et al., 2002) and altered microbial composition (O'Mahony et al., 2009)**.** Further, psychosocial stress (i.e., social disruption) in rodents significantly altered diversity and richness of the gut microbiota (Bharwani et al., 2016; Bailey et al., 2011; Galley et al., 2014a). Stress has also been shown to have prolonged effects on microbial composition (Galley et al., 2017). In humans, the majority of studies on the gut microbiome have focused on clinical populations focusing on gut diversity and richness in groups with gastrointestinal disorders (Costedio, Hyman, & Mawe, 2007; Quigley, 2009), neurodegenerative disorders (Bhattacharjee & Lukiw, 2013; Cryan & Dinan, 2012), autism spectrum disorder (Finegold et al., 2013) metabolic issues (Le Chatelier et al., 2013; Ley et al., 2006; Parnell & Reimer, 2009), and mood disorders, such as anxiety and depression (Malan-Muller et al., 2018; Winter et al., 2018).

Very few studies have explored the effects of generalized stress on gut diversity in healthy subjects (Moloney et al., 2018). Among these studies, gut microbiota composition is typically examined either at the beginning (i.e., colonization, early life experiences) or at the end of the lifespan (i.e., maintenance, regulation of loss). Regarding gut diversity, large variation in gut microbiota composition was observed in infants exposed to maternal prenatal stress

(Zijlmans et al., 2015) and infants born prematurely (Barrett et al., 2013). In the elderly, adults living in a long-term care facility had less diverse microbial communities compared to older healthy adults (Cleasson et al., 2012). Indeed, Knowles, Nelson, and Palombo (2008) conducted one of the only studies to explore the effects of acute stress (i.e., college exams) on the gut microbiota in healthy young adults. However, rather than focusing on broad changes in gut diversity, the study focused on changes in the abundance of lactic acid bacteria, a common probiotic.

In this present study, psychosocial stress did not influence gut diversity, an overall positive outcome for victims of cyber- and traditional bullying. However, it is important to note that the majority of the participants in this sample fell within the normal range of victimization scores (i.e., +/- 1 SD) and thus, this group may be relatively unvictimized. Indeed, though attempts were made to recruit self-reported peer victims into this study, only four individuals from this group participated. These findings do not suggest that peer victimization is unrelated to gut diversity, but, due to a restriction of range, there is not enough variation within this sample to detect the effects peer victimization may have on gut diversity and microbial composition.

Additionally, the relationship between peer victimization and gut diversity was not mediated by perceptions of stress (Aim 1b). Though both forms of peer victimization significantly predicted increases in perceived stress, perceptions of stress did not subsequently predict changes in gut diversity. However, given the established link between stress and altered microbial composition, factors unique to emerging adulthood may have also influenced these findings. Characterized as a period of exploration and experimentation, it is common for emerging adults to travel to new places, frequently move households and change roommates, meet and date multiple people (Arnett, 2000). Further, moving out of childhood homes is also associated with changes in diet and lifestyle. On the microscopic level, these new sights and

smells create more diverse but more variable gut microbiota during this developmental period. Simply, due to the changes emerging adulthood brings, gut microbiota are less stable in young adulthood compared to microbiota in middle adulthood (Spor, Koren, & Ley, 2011). The lack of stability in emerging adults and within their gut microbiota makes it difficult to parse out specific factors that may influence microbial composition.

Additionally, exploratory analyses found that the relationship between peer victimization and gut diversity was not moderated by gender (Aim 1c). Traditional and cyber-victimization did not significantly predict changes in gut diversity for both males and females. Though there were no specific predictions regarding this aim, it was thought that females would be more adversely affected by peer victimization than males. Consistently, gender differences have been observed regarding perceptions of stress, coping and responding to stress, and stress-induced outcomes. While both college-aged males and females report being victims of bullying at similar rates, women generally report higher levels of overall stress as well as stress-related to daily hassles, finances, and family and social relationships (Brougham et al., 2009; Lund & Ross, 2015; MacDonald & Roberts-Pittman, 2010; Watts et al., 2017). Further, experiencing stress such as peer victimization may put women at greater risk for psychological distress, somatic complaints, suicide ideation, depression, and anxiety as well as lead to maladaptive coping patterns including avoidance (Chaplin, Hong, Bergquist, & Sinha, 2008; Matud, 2004; Van der Wal, De Wit, & Hirasing, 2003).

Broadly, studies examining gender differences in human gut microbiota composition are rare and findings are often inconsistent (Cong et al., 2016). In humans, gender differences in microbial composition (i.e., diversity, relative abundance of specific taxa) have been observed in preterm infants (Cong et al., 2016), young adults (Ding & Schloss, 2014), and middle-aged adults (Dominianni et al, 2015; Haro et al., 2016). In rodents, Kovacs and colleagues (2011)

examined eight genetically different groups of mice and found that genotype was a stronger predictor of microbial composition than gender. Though gender differences in microbial composition are not fully delineated, gut microbiota may help explain why women are more at risk for gastrointestinal problems and for developing autoimmune and inflammatory diseases (Gomez, Luckey, & Taneja, 2015; Sankaran-Walters et al., 2013).

Peer Victimization and Relative Abundance

The second aim of this dissertation sought to examine the effect of peer victimization on the relative abundance of microbial groups at the phylum and genus level. Based on previous research (Bailey et al., 2010; Bailey et al., 2011; Zijlmans et al., 2015), gut microbiota associated with elevated stress response and with poorer stress-related health outcomes were examined (see Table 1). Results showed that traditional and cyber-victimization did not predict changes at the phylum level (i.e., Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria) or at the genus level (i.e., *Clostridium, Bacteroides, Bifidobacterium, Dorea, Coprococcus*). Further, peer victimization was not significantly correlated with any taxa of interest (Table 8). It should be noted that some genera of interest were either not found within this sample (i.e., *Escherichia*, *Pseudobutyrivibrio*) or were too rare to be analyzed (i.e., *Serratia*, *Lactobacillus*). Though it is encouraging that peer victimization did not predict changes in the relative abundance of these microbial groups, these findings highlight some methodological issues of gut microbiota research.

First, the profusion of gut microbiota research over the past decade has primarily utilized mouse models to examine all aspects of the microbiome-gut-brain axis. The use of mouse models allows for increased experimental control while helping to establish causality through either the direct manipulation of microbial composition, the exploration of treatment effects (i.e., antibiotics, dietary changes, fecal transplants), or the alteration of the rodent's genetic material.

However, innate differences between humans and mice including anatomy, physiology, and genetics sometimes make it difficult to apply pre-clinical research to human subjects (Nguyen, Vierira-Silva, Liston, & Raes, 2015). For example, comparisons of the gut microbiota composition in healthy human participants to healthy rodent samples has shown that 85% of OTUs found in rodent gut microbiota at the genus level are not present in human subjects (Ley, Backhed, Turnbaugh, Lozupone, Knight, & Gordon, 2005). Nguyen and colleagues (2015) compared mouse and human gut microbiomes using available data (i.e., five mice and four human studies) and found 79 common genera, though there were differences in the relative abundance of the most common genera between humans and mice. For example, the genus *Lactobacillus* is much more abundant in the gut microbiota of mice and is found at low levels in human gut microbiota. To circumvent this disparity between rodent and human microbiomes, some researchers are now using humanized gnotobiotic mice (i.e., germ-free rodents colonized with human fecal microbiota) which allow for a level of environmental and genetic control beyond what is possible in human studies (Faith et al., 2010; Turnbaugh, Ridaura, Faith, Rey, Knight, & Gordon, 2009).

Secondly, interindividual variability in gut microbiota composition is another issue in microbiome-gut-brain axis research. From the onset of the Human Microbiome Project, researchers have sought to define the parameters of the core microbiota, a collection of bacterial species common to all healthy adults (Eckburg et al., 2005; Qin et al., 2010; Tap et al., 2009). However, cross-cultural and culture-independent studies have shown that there is a large degree of variation at the species level between healthy adults due to age, genetics, diet, and environment (Yatsunenko et al., 2012). Though a typical gut microbiota is characterized by the phylum Bacteroidetes and the phylum Firmicutes, the species within that phylum are quite variable between healthy adults suggesting that a core microbial composition may not exist

(Lozupone, Stombaugh, Gordon, Jansson, & Knight, 2012; Spor, Koren, & Ley, 2011). Evidence is building for a functional core microbiome; indeed, though specific species may differ from person to person, the genetic function (i.e., carbohydrate, amino acid metabolism) is similar between varying microbial compositions (Lozupone et al., 2012; Hamady & Knight, 2009; Huttenhower et al., 2012; Qin et al., 2010). In the present study, given that no associations were found between peer victimization and stress-related taxa of interest, it may be useful to explore the effect of peer victimization on other functionally related microbial groups, a difficult task beyond the scope of the current study.

Further, interindividual variability makes it difficult to identify consistent and meaningful trends in microbial composition (Moloney et al., 2014). There are several examples of this issue within the literature. For instance, researchers have observed phenotypic differences in germ-free rodents, where some display reduced anxious behaviors (Luczynski et al. 2016) while other have increased anxiety-like behaviors (Crumeyroll-Arias et al., 2014). Further, research regarding the gut microbiota of autistic children has shown that in one study autism was associated with increases in *Bacteroidetes* (Finegold et al., 2010) but another study found the disorder was associated with decreases in that genus (Williams et al., 2011). Contradictory findings have also been observed in IBD research (i.e., Bostrom et al., 2012; Morgan et al., 2012) and in patients with major depressive disorder (Jiang et al., 2015; Naseribafrouei et al., 2014). Additionally, in pre-clinical probiotic research Bravo and colleagues (2011) found that *Bifidobacterium longum* reduced anxiety like behaviors while other researchers found that it did not (Bercik et al., 2010). These inconsistencies emphasize the importance of reporting and publishing complete, not just significant, findings. Though this present study did not find any significant relationships between psychosocial stress and taxa of interest, the results are important nevertheless.

The discrepancies observed in these studies also highlight another source of variability, the methods used from sample acquisition to data analysis (Kumar et al. 2014). Once samples are ready for processing, researchers must decide which primers to use, what equipment to use, and how to account for errors while sequencing the DNA; researchers must also choose from the variety OTU clustering and classification methods available as well as which software to use when analyzing the data (Kuczynski et al., 2012; Kumar et al., 2014). As Hamady and Knight (2009) point out, these choices are important but have the potential to create more variability than the research realizes. This variability can make it difficult to compare findings from different studies and from different research labs. For example, the different algorithms (e.g., nearest neighbor, de novo, etc.) for grouping genetic sequences together can produce drastically different results and the methods used to assign taxonomy to each OTU (e.g., Blast, RDP classifier, UCLUST) also can also influence findings (Goodrich et al., 2014; Hamady & Knight, 2009). It is also important to acknowledge that the influx of microbiome-brain-gut axis research is equally matched by the advancements in data-driven, analytical methodologies. Indeed, new computational techniques are frequently emerging to assist researchers in studying the intricacies of the microbiome (Goodrich et al., 2014; Mayer, Knight, Mazmanian, Cryan & Tillisch, 2014; Xia & Sun, 2017). Without a foundation in computational science and a thorough knowledge of the nature of metagenomic data, researchers have the potential to misunderstand and misreport their own findings. Thus, research on the microbiome often requires interdisciplinary collaboration between data and computers scientists and those who work in the life sciences (Wooley, Godzik, & Friedberg, 2010).

A final methodological issue worth mentioning, is there is an on-going debate concerning which level of taxonomic classification (i.e., phylum, class, order, family, genus, and species) provides the most meaning for researchers to analyze. Researchers have argued that since there is

variability between samples and individuals, it is best to take a broad approach and analyze differences in composition at the phyla level first (Hamady & Knight, 2009). However, taking a broad approach overlooks important details. Lozupine and colleagues (2013) argue that differences at the phyla level are likely caused by subset of species within that phylum. Other researchers advise against collapsing OTUs into lower-level taxonomic classifications like genus and species. Goodrich and colleagues (2014) point out that the genus *Clostridium* is found in several different families meaning that one could incorrectly assume that all OTUs classified in the genus Clostridium are phylogenetically related when in fact it is an insignificant distinction. To get around this issue, some studies examine and report the relative abundance of OTUs at each level of taxonomic classification (Bruce-Keller et al., 2015).

Gut Microbiota as a Moderator

The third aim of this dissertation examined the role of gut diversity within the relationship between peer victimization and health outcomes. Though this aim was initially proposed as a mediation, gut diversity was first examined as a moderator of this relationship to evaluate if it met the criteria needed to claim mediation (i.e., the mediator should not interact with the predictor; Judd & Kenny, 1981). Further, it should be noted that gut diversity could be both a mediator and a moderator depending on the experimental context (Baron & Kenny, 1986). For example, research has shown that both physical and psychological stress can reduce the diversity of the gut microbiota resulting in poor outcomes (i.e., mediation; Bailey et al., 2011; Bharwani et al, 2016; Galley et al., 2014a; Turnbaugh et al., 2008). Conversely, targeted interventions such as fecal transplants and probiotic treatments can increase gut diversity and subsequently buffer against the negative effects of stress (i.e., moderation; Berick et al., 2011; Dinan, Stanton, & Cryan, 2013; Forsythe et al., 2010; Savingnac et al., 2014; Tillisch et al., 2013). Given that this is the first study to examine the relationships between peer victimization,

gut diversity, and health outcomes, it was important to test the two competing theories. Simply, gut diversity may moderate, not mediate, the relationship between stress (i.e., peer victimization) and health outcomes.

Broadly, peer victimization predicted significantly poorer physical health outcomes (i.e., overall health, frequency and severity of health symptoms, systolic blood pressure) and psychological symptoms (i.e., internalizing problems, DSM anxiety, DSM depression). These findings replicate previous research about the negative effects of peer victimization (Guarneri-White, Arana, Boyd, & Jensen-Campbell, 2018; Knack, Iyer, & Jensen-Campbell, 2012). However, unlike Arana and colleagues (in press), peer victimization was not directly associated with low-grade systemic inflammation in this sample (i.e., Il-6, CRP) although it is indirectly related to inflammation via perceived health and WTHR, especially for Cyber-victimization (Austin & Jensen-Campbell, 2018). Additionally, men who reported being traditionally victimized within the sample had higher levels of IL-6 and CRP (r's = .30, and .33, p's < .03).

Regarding gut diversity, results showed that Shannon diversity did not mediate the relationship between peer victimization and poor health outcomes; both victimization measures did not predict changes in gut diversity (Aim 1), a critical piece of evidence required for mediation (Judd & Kenny, 1981; Lachowicz, Preacher, & Kelley, 2018). Further, results showed evidence for moderation. Consistently, both traditional and cyber-victimization interacted with Shannon diversity to predict differences in psychological health outcomes. Specifically, Shannon diversity moderated the relationship between peer victimization and internalizing problems as well as DSM-oriented anxiety problems. Traditional victimization also interacted with gut diversity to predict changes in DSM-oriented depressive problems, though this relationship was not significant for cyber-victimization (p = .081). As peer victimization increased, psychological outcomes increased; this relationship weaker for individuals with high levels of gut diversity.

Simply, gut diversity buffered against the negative effects of peer victimization. Further, Shannon diversity significantly predicted decreases in internalizing problems after controlling for peer victimization.

These findings are consistent with previous research suggesting that gut diversity does influence psychological health. Broadly studies have examined the connection between depression, anxiety, and gut microbiota, focusing both on the overall gut diversity as well as specific taxa of interest (Malan-Muller et al., 2018; Winter et al., 2018). Consistently, stress-induced-depression is associated with changes in microbial composition in human subjects and rodent models (Bailey et al., 2011; Bharwani et al., 2016; Jiang et al., 2015). For example, transplanting a fecal sample from a patient with major depressive disorder to a healthy rodent has been shown to decrease gut diversity and induce depressive symptoms in the rodent (Kelly et al. 2016). Bercik and colleagues (2011) conducted a similar study examining anxiety in rodents. However, given the bidirectional communication of the microbiome-brain-gut axis, it is still unclear whether changes in gut diversity are an antecedent or a result of depression and anxiety (Winter et al., 2018).

Additionally, Shannon diversity did not influence the relationship between peer victimization and physical health outcomes in any meaningful way. Within the literature, the relationship between gut diversity and physical health is well established in obesity research (Turnbaugh et al., 2009). In animal studies, obesity is associated with decreases in overall diversity as well as decreases in the phyla Bacteroidetes and increases in Firmicutes (Ley et al., 2005; Ley et al. 2006). Rodents who received a fecal transplant characteristic of a high-fat diet had lower gut diversity compare to healthy controls. Moreover, the inherited microbiota was associated with behavioral changes on the social and cognitive level absent of weight gain (Bruce-Keller et al., 2015). Le Chatelier and colleagues (2013) have shown that low diversity in

humans is associated with higher fat composition, insulin resistance, and other metabolic issues such as inflammation. In animal studies, chronic psychosocial stress has been found to be associated with changes in gut diversity and elevated inflammatory markers (i.e., Il-6; Bailey et al., 2010; Bailey et al., 2011; Bharwani et al., 2016). Exploratory analyses (Aim 3a) sought to replicate previous findings showing that increases in pro-inflammatory cytokines were associated with decreases in *Coprococcus*, and *Dorea* (Bailey et al., 2011) but failed to do. Though the present findings did not find associations between traditional and cyber-victimization, physical health, and gut diversity, Shannon diversity was significantly associated with waist-to-hip ratio (WtHR) and the public humiliation subscale of the Cyberbully Experience Survey significantly predicted differences in gut diversity. Furthermore, there were significant associations between WtHR, blood pressure, and inflammatory markers; future research should examine these relationships more closely.

Though more research needs to be conducted to examine whether gut diversity is best conceptualized as a mediator or moderator, these findings present a potential target of intervention. Indeed, it is possible through specific dietary changes to increase (or decrease) the diversity of one's own gut microbiota. Sonnenburg and colleagues (2016) showed that diet can alter the overall gut diversity in rodents. Specifically, rodents were fed a diet of carbohydrates that were inaccessible to gut microbes resulting in decreased gut diversity; however, when rodents were fed carbohydrates microbes could eat, gut diversity increased. Diet-induced changes in gut bacteria have also been associated with improved working and reference memory as well as reduced anxiety in rodents (Li, Dowd, Scurlock, Acosta-Martinez, & Lyte, 2009). In humans, it well established that diet is the predominant influence on gut diversity and composition (Conlon & Bird et al., 2014; Spor, Koren, & Ley, 2011; Turnbaugh et al., 2009). Dietary changes may help individuals alter their microbiota to be more diverse which could lead

to reductions in stress-related problems. For an exhaustive review of dietary changes aimed at optimizing your gut microbiota and wellbeing see Anderson, Cryan, and Dinan's (2018) book, *The Psychobiotic Revolution: Mood, Food, and the New Science of the Gut-brain Connection*.

Exploratory analyses also examined probiotics as a possible target of intervention for victimization individuals. Specifically, Aim 3b sought to evaluate whether individuals with high levels of probiotics (i.e., genus *Bifidobacteria*) reported fewer depressive symptoms and Aim 3c examined whether probiotics buffered against the negative psychological effects of peer victimization. Results showed that the probiotic *Bifidobacteria* did not predict differences in depressive symptoms (i.e., CES-D depression, DSM Anxiety, and DSM Depression) nor did it moderate the relationship between peer victimization and depressive symptoms. Within the literature, the significance of probiotics is currently being debated and results are mixed regarding their benefit (Kelly et al., 2017). In one systematic review of randomized controlled trials, researchers found that probiotic treatments (i.e., pill, milk, yogurt, or powder) reduced depression and/or anxiety symptoms in the majority of studies with healthy adults, individuals with chronic fatigue syndrome, and patients with major depressive disorder (Pirbaglou, Katz, de Souza, Sterans, Motamed, & Ritvo, 2016). However, another systematic review found that probiotic treatments did not have any effects on the richness or evenness of microbial composition (Kristensen, Bryup, Allin, Nielsen, Hansen, & Pedersen, 2016). Other reviews of preclinical and clinical research show an inconsistent picture of the benefit of probiotics (Foster & Nuefeld, 2013; Foster, Rinaman, & Cryan; Kelly et al., 2015). Further, research is emerging that suggest it is quite difficult for probiotics to colonize the gut microbiota (Zmora et al., 2018) and probiotic may delay the restoration of a healthy gut microbiota after a course of antibiotics (Suez et al., 2018). More research is needed to determine the contexts where probiotics are most beneficial but broadly, probiotic tend to be the most helpful in clinical populations.

Group Differences in Microbial Composition

The final aim (Aim 4) of this dissertation used beta diversity metrics to explore the dissimilarity in gut microbiota composition between individuals in the top quartile compared to individuals in the bottom quartile of traditional peer victimization scores. Further, differences in alpha diversity (e.g. absolute OTU counts, Chao1 estimator, Shannon Index) between the two groups were also examined. Consistent with the results observed in Aim 1, the two groups did not differ in alpha diversity; the gut microbiota of individuals in the victim group were as even, rich, and diversity as the microbiota of individuals in the non-victim group. Further, beta diversity results also showed that the two groups were phylogenetically related to one another.

However, based on Bray-Curtis distances the two groups significantly differed (see Figure 8) regarding the relative abundance and/or presence and absence of OTUs. These differences between victim and non-victims is likely being driven by the relative abundance of OTUs. Specifically, exploratory analyses also examined shifts in the relative abundance of OTUs between the two groups in sixteen different taxa (Table 16). These findings are purely descriptive, but it is interesting that the victimization group had lower levels of probiotics compared to non-victims (i.e., *Bifidobacterium adolescentis,* unidentified *Bifidobacterium* species). Though the relationship was not significant, in Aim 3c it was predicted the genus *Bifidobacteria* may play a role in the relationship between peer victimization and poor psychological outcomes. Further, the victim group had lower levels of *Faecalibacterium prausnitzii*, a microbe that has been shown to have anti-inflammatory properties (Sokol et al., 2008) and be reduced in patients suffering from depression (Jiang et al., 2015; Naseribafrouei et al., 2014). Victims also have lower levels of *Prevotella copri*, a microbe thought to help maintain the integrity of the intestinal lining (Scheperjans et al., 2015), as well as increased levels of the *Parabacteroides distasonis*, which has been associated with increased social stress in rodents

(Bailey et al., 2011). However, other observed differences between victims and non-vicitms, such as in the family Lachnospiraceae, the family Ruminococcaceae and the genus Ruminococcus, add to the inconsistencies present within the literature; researchers have observed that stress and depressive symptoms are associated with increases or decreases in these microbial groups depending on the particular study (Hantsoo et al. 2018; Jiang et al., 2015; Naseribafrouei et al., 2014; Winter et al., 2018).

Additionally, the victimization group (i.e., top quartile) also had significantly more variance within individuals belonging to that group compared to the non-victim group. This suggests that those who are experiencing a chronic stress would have more variability in behaviors and responses to that stress that translates to more variable microbiota from individual to individual within that group. Though these findings are preliminary, they will serve as a guide for future research. Replication and additional research into peer victimization and microbial composition is needed before any conclusions can be reached about the differences observed between these two groups.

Limitations and Future Directions

The findings reported in this dissertation are the first of its kind. However, like many firsts, this study has limitations to consider. To begin, this study attempted to examine a bidirectional pathway using cross-sectional data; any assumptions about causality should be tempered. The relationships presented here should be tested longitudinally in order to evaluate whether or not gut diversity is a moderator of the relationship between peer victimization and poor health outcomes. Further, a longitudinal analysis would help clarify if gut microbiota modulates psychological symptoms or if psychological symptoms modulate gut microbiota in response to psychosocial stress (Winter et al., 2018).

Though attempts were made to have a high degree of both methodological and statistical control, the measurements used also have limitations. For example, this study adapted the Children's Self-Experience Questionnaire (Crick & Grotpeter, 1995) and the Direct and Indirect Aggression Scale (Bjorkvist, Lagerspetz, & Osterman, 1992) to assess peer victimization in a young adult sample. Some of the questions include "How often does another peer kick you or pull your hair?" and "How often does another peer yell at you and call you mean names?" Though these questions work well in adolescents and children, the may be too simplistic and may not adequately captured peer victimization in emerging adulthood. Additional assessments of stress (i.e., workplace bullying, adult daily hassles) should be included in future research in order to better understand the effects of stress on the gut microbiota.

Further, better control measures should be utilized. Questions from the Health Behavior in School-Aged Children scale (Iannotti, 2005) were used for control measures include diet and activity level. However, this measurement is a poor indicator of a healthy diet. For instance, participants were asked how many times a week you drink "low fat milk" and how many times you drink "whole milk." Depending on the point of view, a person can consider either to be the healthier or unhealthier choice. For future research, participants should be asked to keep a food diary over the week between visits to obtain a more realistic picture of their dietary habits. Additionally, due to the lack of stability in the gut microbiota of emerging adults, it is important to record data concerning recent life events such as a recent move, roommate change, or new romantic partner to control for the interindividual variability.

Another limitation of this dissertation that future research should address is the fact that a convenience sample was used. Participants in this study were recruited from UTA's Psychology Participant Pool, which consists of ubiquitous college students. Convenience sampling can lead to a more homogenous sample and subsequently decrease the generalizability of research

findings sample. For the present study, there may have been a restriction of range problem within traditional and cyber-vitimization variables. Simply, the majority of participants were non-victims. The lack of variation within this sample may have prevented the effects of peer victimization on the gut diversity to fully be examined. Thus, future research should focus on recruiting a more diverse, more victimized sample to examine potential relationships.

Conclusion

This dissertation was the first to examine the role the microbiome-gut-brain axis plays in the relationship between psychosocial stress and health in emerging adults. Though peer victimization was not directly related to gut diversity and specific stress-related taxa when examining alpha diversity, it was evident that gut diversity influenced the relationship between victimization and psychological health. Further, beta diversity analyses revealed that victims and non-victims significantly differed in the relative abundance of taxa associated with psychosocial stress, anxiety, and depression. Given the complexities of this pathway future research should focus on establishing causality within the relationships reported in this study. As this field continues to develop, it is important to recognize the various methods and computational tools available to analyze metagenomic data; a thorough knowledge is needed to understand findings. The discrepancies from study to study also highlight the need for clear and concise reporting of every step of the research process. Finally, these findings underscore the importance of the microbiome-gut-brain axis and the role psychosocial stress plays within this bi-directional pathway. Given that social stress influences and is influenced by the gut microbiota, these novel results represent significant opportunities for researchers to develop targeted interventions to reduce the negative effects of stress and to improve psychological and physical health outcomes.

Appendix A:

Additional Tables

Table A1. *Aim 1a: Perceived Stressed, Traditional Victimization, and Gut Diversity*

Outcome	*R*	*b*	*t*	*p*	95% CI
Predictor					
Perceived Stress	.42**				
Traditional Victimization		.35	4.20	<.001	-.18, .51
Gender		-.29	-3.41	<.001	-.45, -.12
Shannon Diversity	.23				
Traditional Victimization		.10	1.05	.296	-.09, .29
Gender		-.22	-2.35	.021	-.40, -.03
Perceived Stress		-.15	-1.54	.127	-.24, .04
Chao 1 Estimator	.15				
Traditional Victimization		-2.86	-.17	.869	-37.13, 31.40
Gender		-24.62	-1.43	.155	-58.65, 24.86
Perceived Stress		-10.80	-.60	.550	-46.45, 24.86
Absolute OTU Count	.15				
Traditional Victimization		-1.36	-.11	.910	-25.09, 22.38
Gender		-16.89	-1.42	.158	-40.46, 6.68
Perceived Stress		-10.81	-.87	.388	-35.51, 13.88

Note. ** = $p < .01$.

Table A2. *Aim 1a: Perceived Stressed, Cyber-Victimization, and Gut Diversity*

Outcome	*R*	*b*	*t*	*p*	95% CI
Predictor					
Perceived Stress	.47**				
Cyber-Victimization		.41	5.10	<.001	.25, .57
Gender		-.28	-3.45	<.001	-.44, -.12
Shannon Diversity	.21				
Cyber-Victimization		.03	.30	.763	-.16, .22
Gender		-.20	-2.14	.034	-.38, -.02
Perceived Stress		-.13	-1.25	.215	-.33, .07
Chao 1 Estimator	.17				
Cyber-Victimization		-16.99	-.96	.339	-52.01, 18.03
Gender		-21.33	-1.25	.212	-55.01, 12.35
Perceived Stress		-4.30	-.23	.816	-40.90, 32.30
Absolute OTU Count	.15				
Cyber-Victimization		-4.58	-.37	.710	-28.92, 19.75
Gender		-16.16	-1.37	.174	-39.56, 7.25
Perceived Stress		-9.28	-.72	.471	-34.71, 16.15

Note. ** = $p < .01$.

Table A3. *Aim 1b: Victimization, Gut Diversity, and Gender*

Outcome	*R*	*b*	*t*	*p*	95% CI
Predictor					
Shannon Diversity	.19				
Traditional Victimization		.08	.73	.467	-.14, .31
Cyber-Victimization		-.02	-.18	.855	-.24, .20
Gender		-.18	-1.86	.066	-.37, .01
Traditional X Cyber		-.05	-.63	.533	-.23, .12
Traditional X Gender		-.01	-.06	.949	-.23, .22
Cyber X Gender		.00	.03	.978	-.22, .23
Traditional X Cyber X Gender		.01	.15	.880	-.16, .18
Chao 1 Estimator	.21				
Traditional Victimization		8.22	.40	.690	-32.57, 49.02
Cyber-Victimization		-13.27	-.65	.516	-53.67, 27.11
Gender		-21.72	-1.24	.218	-56.49, 13.05
Traditional X Cyber		-14.92	-.95	.342	-45.90, 16.06
Traditional X Gender		-4.46	-.22	.829	-45.26, 36.33
Cyber X Gender		-10.46	-.51	.609	-50.85, 29.94
Traditional X Cyber X Gender		2.90	.19	.853	-28.08, 33.87
Absolute OTU Count	.18				
Traditional Victimization		.70	.05	.961	-27.72, 29.12
Cyber-Victimization		-1.07	-.08	.940	-29.22, 27.07
Gender		-15.41	-1.26	.210	-39.63, 8.82
Traditional X Cyber		11.72	-1.08	.285	-33.30, 9.87
Traditional X Gender		-1.54	-.11	.915	-29.96, 26.89
Cyber X Gender		-3.70	-.26	.795	-31.85, 24.44
Traditional X Cyber X Gender		4.59	.42	.675	-17.00, 26.17

Table A4. *Aim 2: Peer Victimization and Relative Abundance at the Phyla Level*

Outcome	*R*	β	*t*	*p*	95% CI
Predictor					
Actinobacteria	.06				
Traditional Victimization		-.05	-.47	.639	-296.79, 182.76
Cyber-Victimization		-.02	-.21	.838	-263.37, 213.95
Gender		.01	.12	.908	-209.32, 235.21
Bacteroidetes	.23				
Traditional Victimization		-.04	-.35	.725	-496.62, 346.42
Cyber-Victimization		-.09	-.88	.380	-606.32, 232.82
Gender		.22	2.39	.018	81.06, 862.54
Firmicutes	.25				
Traditional Victimization		.02	.17	.866	-404.99, 480.69
Cyber-Victimization		.12	1.25	.216	-163.78, 717.80
Gender		-.24	-2.61	.010	-950.59, -129.58
Proteobacteria	.24				
Traditional Victimization		.16	1.57	.119	-10.60, 92.05
Cyber-Victimization		-.20	-1.96	.052	-101.65, 0.53
Gender		.13	1.44	.152	-12.91, 82.24

Table A5. *Aim 2: Peer Victimization and Relative Abundance at the Genera Level*

Outcome	*R*	β	*t*	*p*	95% CI
Predictor					
Bacteroides	.07				
Traditional Victimization		.00	.02	.981	-338.41, 346.71
Cyber-Victimization		-.07	-.63	.531	-449.18, 232.76
Gender		.05	.50	.616	-236.90, 398.19
Bifidobacterium	.07				
Traditional Victimization		-.01	-.08	.936	-4.33, 3.99
Cyber-Victimization		-.06	-.62	.537	-5.43, 2.84
Gender		.04	.41	.682	-3.05, 4.65
Clostridium	.16				
Traditional Victimization		-.01	-.14	.888	-2.57, 2.23
Cyber-Victimization		-.05	-.51	.613	-3.00, 1.78
Gender		-.14	-1.53	.128	-3.94, 0.50
Coprococcus	.11				
Traditional Victimization		.00	-.04	.971	-38.24, 36.85
Cyber-Victimization		-.07	-.64	.527	-49.36, 25.39
Gender		-.07	-.78	.437	-48.51,21.10
Dorea	.14				
Traditional Victimization		.15	1.48	.140	-2.74, 19.11
Cyber-Victimization		-.03	-.26	.797	-12.29, 9.45
Gender		-.02	-.22	.825	-11.26, 8.99

Table A6. *Aim 3: MMR Results for Covariates and Physical Health*

Predictor	β	*t*	*p*	95% CI
Outcome				
Age				
Overall Health	-.06	-.67	.506	-.22, .11
Frequency	.03	.33	.742	-1.61, 2.26
Severity	.02	.19	.851	-1.25, 1.52
Abdominal	-.02	-.24	.813	-1.42, 1.12
IL-6	.16	1.54	.127	-.02, .12
CRP	.06	.59	.557	-.09, .17
WtHR	.04	.44	.663	-.03, .05
Systolic BP	.03	.32	.747	-2.00, 2.77
Diastolic BP	.01	.09	.926	-1.53, 1.68
Diet				
Overall Health	-.03	-.26	.794	-.20, .15
Frequency	-.21	-2.10	.038	-4.28, -.12
Severity	-.17	-1.65	.102	-2.73, .25
Abdominal	-.07	-.66	.514	-1.82, .92
IL-6	-.07	-.66	.513	-.09, .05
CRP	.01	.12	.906	-.12, .14
WtHR	.04	.42	.678	-.04, .06
Systolic BP	.07	.71	.478	-1.63, 3.45
Diastolic BP	-.01	-.07	.949	-1.77, 1.66
Activity Level				
Overall Health	.37	3.84	<.001	.17, .54
Frequency	.07	.73	.469	-1.35, 2.92
Severity	.11	1.12	.265	-.66, 2.39
Abdominal	-.07	-.67	.505	-1.88, .93
IL-6	-.11	-1.00	.321	-.11, .04
CRP	-.04	-.37	.712	-.16, .11
WtHR	.01	.12	.908	-.04, .05
Systolic BP	.04	.41	.68	-2.07, 3.17
Diastolic BP	-.04	-.40	.687	-2.13, 1.41
Gender				
Overall Health	.01	.12	.906	-.17, .19
Frequency	-.26	-2.53	.013	-4.75, -.58
Severity	-.25	-2.39	.019	-3.30, -.31
Abdominal	-.16	-1.51	.135	-2.42, .33
IL-6	-.22	-2.04	.044	-.14, .00
CRP	-.23	-2.05	.043	-.27, .00
WtHR	-.18	-1.70	.092	-.09, .01
Systolic BP	.35	3.49	.001	1.95, 7.09
Diastolic BP	-.14	-1.30	.196	-2.87, .59

Table A7. *Aim 3: MMR Results for Covariates and Mental Health*

Predictor	β	*t*	*p*	95% CI
Outcome				
Age				
CES-Depression	-.02	-.21	.838	-1.96, 1.59
Internalizing Problems	-.06	-.72	.476	-2.79, 1.31
DSM Depression	-.04	-.51	.615	-.96, .57
DSM Anxiety	.00	-.02	.982	-.61, .59
DSM Somatic	.05	.56	.576	-.32, .58
Diet				
CES-Depression	.01	.10	.922	-1.82, 2.01
Internalizing Problems	-.07	-.79	.432	-3.08, 1.33
DSM Depression	-.13	-1.42	.158	-1.41, .23
DSM Anxiety	-.03	-.30	.764	-.74, .55
DSM Somatic	-.16	-1.60	.113	-.88, .10
Activity Level				
CES-Depression	-.14	-1.33	.185	-3.34, .66
Internalizing Problems	-.15	-1.71	.09	-4.21, .31
DSM Depression	-.28	-3.02	.003	-2.12, -.44
DSM Anxiety	-.20	-2.06	.042	-1.35, -.03
DSM Somatic	.01	.07	.945	-.48, .51
Gender				
CES-Depression	.03	.27	.787	-1.67, 2.20
Internalizing Problems	-.01	-.06	.952	-2.28, 2.14
DSM Depression	.05	.52	.605	-.61, 1.04
DSM Anxiety	.01	.05	.957	-.63, .66
DSM Somatic	-.20	-1.95	.054	-.95, .01

Table A8. *Aim 3b: Bifidobacteria and Depressive Symptoms*

Outcome	*R*	*b*	*t*	*p*	95% CI
Predictor					
CES-Depression	.19				
Bifidobacteria		.15	1.53	.129	.00, .00
Gender		.06	.54	.588	-1.39, 2.44
Age		-.06	-.66	.513	-2.34, 1.18
Diet		-.07	-.69	.492	-2.49, 1.21
Activity Level		-.06	-.59	.558	-2.62, 1.42
DSM Depression	.35*				
Bifidobacteria		.03	.28	.779	.00, .00
Gender		.10	.99	.323	-.43, 1.30
Age		-.09	-1.00	.32	-1.21, .40
Diet		-.23	-2.42	.017	-1.87, -.19
Activity Level		-.22	-2.23	.028	-1.92, -.11
DSM Anxiety	.21				
Bifidobacteria		.04	.36	.717	.00, .00
Gender		.06	.63	.531	-.47, .90
Age		-.06	-.59	.555	-.82, .45
Diet		-.11	-1.13	.261	-1.04, .29
Activity Level		-.15	-1.47	.145	-1.24, .19

Note. * = $p < .05$.

Table A9. *Aim 3c: Bifidobacteria, Traditional Victimization, and Depressive Symptoms*

Outcome	*R*	β	*t*	*p*	95% CI
Predictor					
CES-Depression	.33				
Gender		.04	.36	.719	-1.54, 2.22
Age		-.05	-.55	.585	-2.26, 1.28
Diet		.00	.03	.974	-1.86, 1.92
Activity Level		-.11	-1.02	.310	-3.05, .98
Bifidobacteria		.14	1.41	.162	-.53, 3.12
Traditional Victimization		.28	2.81	.006	.75, 4.36
Bifido X Traditional		-.04	-.43	.666	-3.94, 2.53
DSM Depression	.47**				
Gender		.08	.83	.411	-.48, 1.17
Age		-.07	-.80	.427	-1.11, .47
Diet		-.14	-1.53	.130	-1.49, .19
Activity Level		-.26	-2.74	.007	-2.07, -.33
Bifidobacteria		.03	.30	.762	-.68, .93
Traditional Victimization		.33	3.55	.001	.63, 2.20
Bifido X Traditional		-.02	-.25	.803	-1.59, 1.24
DSM Anxiety	.25*				
Gender		.05	.45	.651	-.51, .81
Age		-.03	-.31	.755	-.72, .53
Diet		-.04	-.40	.688	-.80, .53
Activity Level		-.18	-1.82	.071	-1.32, .06
Bifidobacteria		.03	.33	.739	-.53, .74
Traditional Victimization		.30	3.08	.003	.35, 1.60
Bifido X Traditional		-.04	-.37	.710	-1.33, .91

Note. * = $p < .05$; ** = $p < .01$.

Table A10. *Aim 3c: Bifidobacteria, Cyber-Victimization, and Depressive Symptoms*

Outcome	*R*	β	*t*	*p*	95% CI
Predictor					
CES-Depression	.44*				
Gender		.02	.17	.869	-1.65, 1.95
Age		.01	.12	.908	-1.61, 1.81
Diet		.00	.05	.964	-1.72, 1.80
Activity Level		-.09	-.92	.359	-2.77, 1.01
Bifidobacteria		.15	1.68	.097	-.26, 3.04
Cyber-Victimization		.42	4.41	.000	2.12, 5.60
Bifido X Cyber		.09	.98	.330	-1.10, 3.25
DSM Depression	.56**				
Gender		.07	.73	.465	-.49, 1.06
Age		.00	-.04	.966	-.77, .73
Diet		-.15	-1.70	.091	-1.43, .11
Activity Level		-.24	-2.78	.006	-1.94, -.33
Bifidobacteria		.03	.33	.739	-.59, .83
Cyber-Victimization		.47	5.44	.000	1.28, 2.74
Bifido X Cyber		.14	1.66	.100	-.15, 1.73
DSM Anxiety	.46*				
Gender		.03	.35	.728	-.51, .73
Age		.03	.34	.732	-.50, .71
Diet		-.04	-.47	.640	-.76, .47
Activity Level		-.17	-1.81	.073	-1.24, .06
Bifidobacteria		.04	.43	.666	-.44, .69
Cyber-Victimization		.44	4.74	.000	.82, 1.99
Bifido X Cyber		.15	1.72	.089	-.10, 1.41

Note. * = $p < .05$; ** = $p < .01$.

Appendix B:

Surveys and Scales

Eligibility Checklist

	Yes	No
Do you have a history of active or uncontrolled gastrointestinal disorders or diseases including:		
Inflammatory bowel disease (IBD) including ulcerative colitis (mild-moderate-severe)		
Crohn's disease (mild-moderate-severe) or indeterminate colitis		
Irritable bowel syndrome (IBS) (moderate-severe)		
Persistent infectious gastroenteritis, colitis or gastritis, persistent or chronic diarrhea of unknown etiology		
Clostridium difficile infection (recurrent) or Helicobacter pylori infection (untreated)		
Chronic constipation		
Use of any of the following drugs within the last 6 months:		
Systemic antibiotics (intravenous, intramuscular, or oral)		
Antifungals, antivirals or antiparasitics (intravenous, intramuscular, or oral)		
Oral, intravenous, intramuscular, nasal or inhaled corticosteroids		
Cytokines		
Methotrexate or immunosuppressive cytotoxic agents		
Large doses of commercial probiotics consumed		
For females: combination hormone vaginal ring for contraception		
Please answer the following questions:		
Have you received nasally-delivered flu vaccine within the past 28 days?		
Do you have a chronic (unresolved, requiring on-going medical management or medication) pulmonary, cardiovascular, gastrointestinal, hepatic or renal functional abnormality?		
History of cancer?		
Have you made major changes in your diet during the past month?		
Recent history of chronic alcohol consumption (more than five servings of beer, wine, or liquor per day)?		
Have you been diagnosed with immunosuppression or immunodeficiency conditions including HIV infection?		
Have you had major surgery of the GI tract (not including appendectomy) in the past five years or ever had major bowel resection at any time?		

Direct and Indirect Aggression Scale – Victim Version
(DIAS-VS; Bjorkvist, Lagerspetz, & Osterman, 1992)

Directions: Answer each question by bubbling in the answer which seems to most closely tell you about how your peers (i.e., friends, classmates, work colleagues) behave toward you.

Scale

1 = never	3 = sometimes	5 = very often
2 = seldom	4 = quite often	

How often are you hit by other peers?
How often are you shut out of the group by other peers?
How often do other peers yell at you or argue with you?
How often do peers become friends with another peer as a kind of revenge?
How often are you kicked by other peers?
How often are you ignored by other peers?
How often are you insulted by other peers?
How often do peers who are angry with you gossip about you?
How often are you tripped by other peers?
How often do peers tell bad or false stories about you?
How often do peers say they are going to hurt you?
How often do peers plan to secretly bother you?
How often are you shoved by other peers?
How often do peers say bad things about you behind your back?
How often are you called names by other peers?
How often do peers tell others “Let’s not be friends with him/her!”?
How often do other peers take things from you?
How often do peers tell your secrets to a third person?
How often are you teased by other peers?
How often do peers write small notes where you are criticized?
How often are you pushed down to the ground by other peers?
How often do other peers criticize your hair or clothing?
How often do other peers pull at you?
How often do peers who are angry with you try to get others to dislike you?

"Things that Happen to Me at School"
Children's Self-Experiences Questionnaire, Self-Report
(CSEQ-SR; Crick & Grotpeter, 1995)

Directions: Here is a list of things that sometimes happen to peers (i.e., friends, classmates, work colleagues) your age. How often did they happen to you?

Scale

1 = never	3 = sometimes	5 = all the time
2 = almost never	4 = almost all the time	

How often does someone give you help when you need it?
How often do you get hit by a peer?
How often do other peers leave you out on purpose when it is time to hangout or do an activity?
How often does another peer yell at you and call you mean names?
How often does another peer try to cheer you up when you feel sad or upset?
How often does a peer who is mad at you try to get back at you by not letting you be in their group anymore?
How often do you get pushed or shoved by another peer?
How often does another peer do something that makes you feel happy?
How often does a peer tell lies about you to make other peers not like you anymore?
How often does another peer kick you or pull your hair?
How often does another peer say they won't like you unless you do what they want you to do?
How often does another peer say something nice to you?
How often does a peer try to keep others from liking you by saying mean things about you?
How often does another peer say they will beat you up if you don't do what they want you to do?
How often do other peers let you know that they care about you?

Cyberbullying Experience Survey
(CES; Doane, Kelley, Chiang, & Padilla, 2013)

Scale

0 = never	2 = a few times a year	4 = about once or twice week
1 = less than a few times a year	3 = once or twice a month	5 = every day/ almost every day

1. Has someone distributed information electronically while pretending to be you?
2. Has someone changed a picture of you in a negative way and posted it electronically?
3. Has someone written mean messages about you publicly electronically?
4. Has someone logged into your electronic account and changed your information?
5. Has someone posted a nude picture of you electronically?
6. Has someone printed out an electronic conversation you had and then showed it to others?
7. Have you completed an electronic survey that was supposed to remain private but the answers were sent to someone else?
8. Has someone logged into your electronic account and pretended to be you?
9. Has someone posted an embarrassing picture of you electronically where other people could see it?
10. Has someone called you mean names electronically?
11. Has someone been mean to you electronically?
12. Has someone cursed at you electronically?
13. Has someone made fun of you electronically?
14. Has someone teased you electronically?
15. Have you received a nude or partially nude picture that you did not want from someone you were talking to electronically?
16. Have you received a pornographic picture that you did not want from someone electronically that was not spam?
17. Have you received an unwanted sexual message from someone electronically?
18. Have you received an offensive picture electronically that was not spam?
19. Has someone pretended to be someone else while talking to you electronically?
20. Has someone lied about themselves to you electronically?
21. Have you shared personal information with someone electronically and then later found the person was not who you thought it was?

College Daily Hassles Questionnaire
(CDHQ; Kohn, Lafreniere, & Gurevich, 1990; Insel & Roth, 2006)

The following is a list of experiences which many students may have experienced at some time or other. Please indicate for each experience how much it has been a part of your life *over the past month.* Put a "1" if the space provided next to an experience if it was *not at all part of your life* over the last month (e.g., "trouble with mother in law – 1"); "2" for an experience which was only slightly part of your life over that time; "3" for an experience which was *distinctly* part of your life; and "4" for an experience which was *very much* part of your life over the past month.

Rate the Intensity of your Experience over Past Month:
1 = *not at all part of my life*
2 = *only slightly part of my life*
3 = *distinctly part of my life*
4 = *very much part of my life*

* indicates a non-social daily hassles

1. _____ Conflict with boyfriend's/girlfriend's/spouse's family
2. _____ Being let down or disappointed by friends
3. _____ Conflict with professor(s)*
4. _____ Social rejection
5. _____ Too many things to do at once*
6. _____ Being taken for granted*
7. _____ Financial conflicts with family members*
8. _____ Having your trust betrayed by a friend
9. _____ Separation from people you care about
10. _____ Having your contributions overlooked*
11. _____ Struggling to meet your own academic standards*
12. _____ Being taken advantage of*
13. _____ Not enough leisure time*
14. _____ Struggling to meet the academic standards of others*
15. _____ A lot of responsibilities*
16. _____ Dissatisfaction with school*
17. _____ Decisions about intimate relationship(s)
18. _____ Not enough time to meet your obligations*
19. _____ Dissatisfaction with your mathematical ability*
20. _____ Important decisions about your future career*
21. _____ Financial burdens*
22. _____ Dissatisfaction with your reading ability*
23. _____ Important decisions about your education*
24. _____ Loneliness
25. _____ Lower grades than you hoped for*
26. _____ Conflict with teaching assistant(s)*
27. _____ Not enough time for sleep*
28. _____ Conflicts with your family
29. _____ Heavy demands from extracurricular activities*

30. _____ Finding courses too demanding*
31. _____ Conflicts with friends
32. _____ Hard effort to get ahead*
33. _____ Poor health of a friend
34. _____ Disliking your studies*
35. _____ Getting “ripped off” or cheated in the purchase of services*
36. _____ Difficulties with transportation*
37. _____ Disliking fellow students
38. _____ Conflicts with boyfriend/girlfriend/spouse
39. _____ Dissatisfaction with your ability at written expression*
40. _____ Interruptions of your school work*
41. _____ Social isolation
42. _____ Long waits to get services (e.g., at banks, stores etc.)*
43. _____ Being ignored
44. _____ Dissatisfaction with your physical appearance*
45. _____ Finding course(s) uninteresting*
46. _____ Gossip concerning someone you care about
47. _____ Failing to get expected job*
48. _____ Dissatisfaction with your athletic skills*
49. _____ Long commutes to get to school*
50. _____ Conflicts with roommate
51. _____ Lack of privacy*
52. _____ Parking problems*
53. _____ Experiencing a high level of noise*
54. _____ Adjustments to living with unrelated person(s) (e.g., roommate)
55. _____ Trying to secure loan(s)*
56. _____ Unsatisfactory housing conditions
57. _____ Gossip about yourself
58. _____ Car problems*

Perceived Stress Scale
(PSS; Cohen, Kamarck, & Mermelstein, 1983)

The questions in this scale ask you about your feelings and thoughts during the last month. In each case, you will be asked to indicate how often you felt or thought a certain way. Although some of the questions are similar, there are differences between them and you should treat each one as a separate question. The best approach is to answer each question fairly quickly. That is, don't try to count up the number of times you felt a particular way, but rather indicate the alternative that seems like a reasonable estimate.

For each question choose from the following alternatives:

0. never
1. almost never
2. sometimes
3. fairly often
4. very often

1. In the last month, how often have you been upset because of something that happened unexpectedly?
2. In the last month, how often have you felt that you were unable to control the important things in your life?
3. In the last month, how often have you felt nervous and "stressed"?
4. In the last month, how often have you dealt successfully with irritating life hassles?
5. In the last month, how often have you felt that you were effectively coping with important changes that were occurring in your life?
6. In the last month, how often have you felt confident about your ability to handle your personal problems?
7. In the last month, how often have you felt that things were going your way?
8. In the last month, how often have you found that you could not cope with all the things that you had to do?
9. In the last month, how often have you been able to control irritations in your life?
10. In the last month, how often have you felt that you were on top of things?
11. In the last month, how often have you been angered because of things that happened that were outside of your control?
12. In the last month, how often have you found yourself thinking about things that you have to accomplish?
13. In the last month, how often have you been able to control the way you spend your time?
14. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?

Assessing Health Outcomes
(Knack, Jensen-Campbell, & Baum, 2011)

Directions: Rate the frequency and severity of the following health symptoms.
Scale:

Frequency:	not at all	sometimes	often	all the time
Severity:	does not hurt at all	hurts a little	hurts a lot	unbearable pain

Extreme fatigue (feeling extremely tired)
Allergic reaction
Sleep problems
Stomach ache
Nausea/vomiting (sick to your stomach/throwing up)
Diarrhea
Muscle aches and pains
Headaches or migraine
Weight gain of 5 or more pounds
Weight loss of 5 or more pounds
Respiratory congestion (cold in your chest)
Runny nose
Coughing
Sore throat
Sneezing
Blocked nose
Fever or chills
Dizziness
Double or blurred vision
Trouble catching breath
Having a cold
Chest pains
Numbness or tingling
Low energy
Ear infections
Getting sick
Heart beating too fast
Visits to the doctor
Visits to the school nurse

Abdominal Pain Index
(Walker, Smith, Garber, & Van Slyke, 1997)

1. How frequently over the past two weeks have you experienced abdominal pain?
2. In a typical day over the past two weeks, how frequently did you experience abdominal pain during the day?
3. When you experienced abdominal pain over the last two weeks, how long did it typically last?
4. When you experienced abdominal pain over the last two weeks, how intense was the pain typically?
5. When you experienced abdominal pain over the last two weeks, what was the maximum intensity of the pain?

Health Behavior in School-aged Children
(Iannotti, 2005)

1. About how many hours a day do you usually watch television (including videos and DVDs) in your free time? (Please mark one circle for weekdays and one circle for weekend)

None at all	About half an hour a day
About 1 hour a day	About 2 hours a day
About 3 hours a day	About 4 hours a day
About 5 hours a day	About 6 hours a day
About 7 or more hours a day	

2. Over the past 7 days, on how many days were you physically active for a total of at least 60 minutes per day?

0 days 1 day 2 days 3 days 4 days 5 days 6 days 7 days

3. OUTSIDE SCHOOL HOURS: How OFTEN do you usually exercise in your free time so much that you get out of breath or sweat?

Every day	4 to 6 times a week	2 to 3 times a week	Once a week
Once a month	Less than once a month	Never	

4. OUTSIDE SCHOOL HOURS: How many HOURS a week do you usually exercise in your free time so much that you get out of breath or sweat?

None	About half an hour	About 1 hour	About 2 to 3 hours
	About 4 to 6 hours	About 7 or more	

5. How many times a week do you usually eat or drink...?

Scale:

Every day, more than once	Once a day, every day	Never
5-6 days a week	2-4 days a week	
Once a week	Less than once a week	

a. Fruits
b. Vegetables
c. Sweets (candy or chocolate)
d. Coke or other soft drinks that contain sugar
e. Diet coke or diets soft drinks
f. Low fat/semi-skimmed milk
g. Whole fat milk
h. Cheese
i. Other milk products (like yogurt, chocolate milk, pudding)
j. Cereals (like Cornflakes, Rice Crispies, Cocoa Crispies)
k. White bread
l. Brown bread (whole grain bread)

m. Chips (like potato chips or sticks, Fritos, Doritos
n. French fries

6. How often do you eat in a fast food restaurant (for example, McDonalds, KFC, Pizza Hut, Taco Bell)?

Never	Rarely (less than once a month)	Once a month
2-3 times a month	Once a week	2-4 days a week
5 or more days a week		

7. At present are you on a diet or doing something else to lose weight?

No, my weight is fine
No, but I should lose some weight
No, because I need to put on weight
Yes

8. Which of the following things did you do to control your weight during the last 12 months?

	No	Yes
a. Exercise		
b. Eat less sweets		
c. Eat less fat		
d. Drink less soft drinks		
e. Eat less (smaller amounts)		
f. Eat more fruit and/or vegetables		
g. Vomiting		
h. Use diet pills or laxatives		
i. Smoke more		
j. Diet under supervision of a professional		
k. Other, namely		

9. Please read each statement carefully and evaluate how it relates to you by checking the degree to which you agree or disagree with it.

	I strongly agree	I agree	I am undecided	I do not agree	I do not agree at all
a. I am frustrated with my physical appearance					
b. I am satisfied with my appearance					
c. I hate my body					
d. I feel comfortable with my body					
e. I feel anger toward my body					

Adult Self Report
(ASR; Achenbach, 2003)

Please print your answers.

ADULT SELF-REPORT FOR AGES 18-59

For office use only
ID#

YOUR FULL NAME First Middle Last

YOUR GENDER ☐ Male ☐ Female

YOUR AGE

ETHNIC GROUP OR RACE

TODAY'S DATE Mo. ____ Date ____ Yr. _____

YOUR BIRTHDATE Mo. ____ Date ____ Yr. ____

Please fill out this form to reflect *your* views, even if other people might not agree. You need not spend a lot of time on any item. Feel free to print additional comments. ***Be sure to answer all items.***

YOUR USUAL TYPE OF WORK, even if not working now. Please be specific—for example, auto mechanic; high school teacher; homemaker; laborer; lathe operator; shoe salesman; army sergeant; student (indicate what you are studying & what degree you expect).

Your work _____ Spouse or partner's work _____

PLEASE CHECK YOUR HIGHEST EDUCATION

- ☐ 1. No high school diploma and no GED
- ☐ 2. General Equivalency Diploma (GED)
- ☐ 3. High school graduate
- ☐ 4. Some college but no college degree
- ☐ 5. Associate's Degree
- ☐ 6. Bachelor's or RN Degree
- ☐ 7. Some graduate school but no graduate degree
- ☐ 8. Master's Degree
- ☐ 9. Doctoral or Law Degree
- ☐ Other education (specify): _____

I. FRIENDS:

A. About how many close friends do you have? (Do not include family members.)

☐ None ☐ 1 ☐ 2 or 3 ☐ 4 or more

B. About how many times a month do you have contact with any of your close friends? (Include in-person contacts, phone, letters, e-mail.)

☐ Less than 1 ☐ 1 or 2 ☐ 3 or 4 ☐ 5 or more

C. How well do you get along with your close friends?

☐ Not as well as I'd like ☐ Average ☐ Above average ☐ Far above average

D. About how many times a month do any friends or family visit you?

☐ Less than 1 ☐ 1 or 2 ☐ 3 or 4 ☐ 5 or more

II. SPOUSE OR PARTNER:

What is your marital status? ☐ Never been married ☐ Married but separated from spouse ☐ Married, living with spouse ☐ Divorced ☐ Widowed ☐ Other—please describe: _____

At any time in the past 6 months, did you live with your spouse or with a partner?

☐ No—please skip to page 2.

☐ Yes—Circle ***0, 1,*** or ***2*** beside items A-H to describe your relationship ***during the past 6 months:***

0 = Not True 1 = Somewhat or Sometimes True 2 = Very True or Often True

0	1	2	A. I get along well with my spouse or partner		
0	1	2	B. My spouse or partner and I have trouble sharing responsibilities		
0	1	2	C. I feel satisfied with my spouse or partner		
0	1	2	D. My spouse or partner and I enjoy similar activities		
0	1	2	E. My spouse or partner and I *disagree* about living arrangements, such as where we live		
0	1	2	F. I have trouble with my spouse or partner's family		
0	1	2	G. I like my spouse or partner's friends		
0	1	2	H. My spouse or partner's behavior annoys me		

Please be sure you have answered all items.
Then see other side.

ASEBA, University of Vermont, 1 South Prospect St., Burlington, VT 05401-3456
www.ASEBA.org

1-03 Edition - 111

Please print. Be sure to answer all items.

III. FAMILY:

Compared with others, how well do you:		Worse than Average	Variable or Average	Better than Average	No Contact
A. Get along with your brothers?	☐ I have no brothers	☐	☐	☐	☐
B. Get along with your sisters?	☐ I have no sisters	☐	☐	☐	☐
C. Get along with your mother?	☐ Mother is deceased	☐	☐	☐	☐
D. Get along with your father?	☐ Father is deceased	☐	☐	☐	☐
E. Get along with your biological or adopted children?	☐ I have no children				
1. Oldest child	☐ Not applicable	☐	☐	☐	☐
2. 2nd oldest child	☐ Not applicable	☐	☐	☐	☐
3. 3rd oldest child	☐ Not applicable	☐	☐	☐	☐
4. Other children	☐ Not applicable	☐	☐	☐	☐
F. Get along with your stepchildren?	☐ I have no stepchildren	☐	☐	☐	☐

IV. JOB: ***At any time in the past 6 months,*** did you have any paid jobs (including self-employment and military service)?

☐ **No—please skip to Section V.**

☐ **Yes—please describe your job(s):**_____

Circle ***0, 1,*** or ***2*** beside items A-I to describe your work experience ***during the past 6 months:***

0 = Not True **1 = Somewhat or Sometimes True** **2 = Very True or Often True**

0 1 2	A. I work well with others	0 1 2	F. I do things that may cause me to lose my job
0 1 2	B. I have trouble getting along with bosses	0 1 2	G. I stay away from my job even when I'm not sick or not on vacation
0 1 2	C. I do my work well		
0 1 2	D. I have trouble finishing my work	0 1 2	H. My job is too stressful for me
0 1 2	E. I am satisfied with my work situation	0 1 2	I. I worry too much about work

V. EDUCATION: ***At any time in the past 6 months,*** did you attend school, college, or any other educational or training program?

☐ **No—please skip to Section VI.**

☐ **Yes—what kind of school or program?**_____

What degree or diploma are you seeking?_____ Major?_____

When do you expect to receive your degree or diploma?_____

Circle ***0, 1,*** or ***2*** beside items A-E to describe your educational experience ***during the past 6 months:***

0 = Not True **1 = Somewhat or Sometimes True** **2 = Very True or Often True**

0 1 2	A. I get along well with other students	0 1 2	D. I am satisfied with my educational situation
0 1 2	B. I achieve what I am capable of	0 1 2	E. I do things that may cause me to fail
0 1 2	C. I have trouble finishing assignments		

VI. Do you have any illness, disability, or handicap? ☐ **No** ☐ **Yes—please describe:**

VII. Please describe your concerns or worries about family, work, education, or other things: ☐ **No concerns**

VIII. Please describe the best things about yourself:

Please be sure you have answered all items.

Please print your answers. Be sure to answer all items.

VIII. Below is a list of items that describe people. For each item, please circle *0, 1,* or *2* to describe yourself *over the past 6 months*. Please answer all items as well as you can, even if some do not seem to apply to you.

0 = Not True 1 = Somewhat or Sometimes True 2 = Very True or Often True

0 1 2 1. I am too forgetful
0 1 2 2. I make good use of my opportunities
0 1 2 3. I argue a lot
0 1 2 4. I work up to my ability
0 1 2 5. I blame others for my problems
0 1 2 6. I use drugs (other than alcohol and nicotine) for nonmedical purposes (describe): _____
_____
0 1 2 7. I brag
0 1 2 8. I have trouble concentrating or paying attention for long
0 1 2 9. I can't get my mind off certain thoughts (describe): _____
_____
0 1 2 10. I have trouble sitting still
0 1 2 11. I am too dependent on others
0 1 2 12. I feel lonely
0 1 2 13. I feel confused or in a fog
0 1 2 14. I cry a lot
0 1 2 15. I am pretty honest
0 1 2 16. I am mean to others
0 1 2 17. I daydream a lot
0 1 2 18. I deliberately try to hurt or kill myself
0 1 2 19. I try to get a lot of attention
0 1 2 20. I damage or destroy my things
0 1 2 21. I damage or destroy things belonging to others
0 1 2 22. I worry about my future
0 1 2 23. I break rules at work or elsewhere
0 1 2 24. I don't eat as well as I should
0 1 2 25. I don't get along with other people
0 1 2 26. I don't feel guilty after doing something I shouldn't
0 1 2 27. I am jealous of others
0 1 2 28. I get along badly with my family
0 1 2 29. I am afraid of certain animals, situations, or places (describe): _____
_____
0 1 2 30. My relations with the opposite sex are poor
0 1 2 31. I am afraid I might think or do something bad
0 1 2 32. I feel that I have to be perfect
0 1 2 33. I feel that no one loves me
0 1 2 34. I feel that others are out to get me
0 1 2 35. I feel worthless or inferior
0 1 2 36. I accidentally get hurt a lot
0 1 2 37. I get in many fights
0 1 2 38. My relations with neighbors are poor
0 1 2 39. I hang around people who get in trouble
0 1 2 40. I hear sounds or voices that other people think aren't there (describe): _____
_____
0 1 2 41. I am impulsive or act without thinking
0 1 2 42. I would rather be alone than with others
0 1 2 43. I lie or cheat
0 1 2 44. I feel overwhelmed by my responsibilities
0 1 2 45. I am nervous or tense
0 1 2 46. Parts of my body twitch or make nervous movements (describe): _____
_____
0 1 2 47. I lack self-confidence
0 1 2 48. I am not liked by others
0 1 2 49. I can do certain things better than other people
0 1 2 50. I am too fearful or anxious
0 1 2 51. I feel dizzy or lightheaded
0 1 2 52. I feel too guilty
0 1 2 53. I have trouble planning for the future
0 1 2 54. I feel tired without good reason
0 1 2 55. My moods swing between elation and depression
56. Physical problems ***without known medical cause:***
0 1 2 a. Aches or pains (***not*** stomach or headaches)
0 1 2 b. Headaches
0 1 2 c. Nausea, feel sick
0 1 2 d. Problems with eyes (not if corrected by glasses) (describe): _____
_____
0 1 2 e. Rashes or other skin problems
0 1 2 f. Stomachaches
0 1 2 g. Vomiting, throwing up
0 1 2 h. Heart pounding or racing
0 1 2 i. Numbness or tingling in body parts
0 1 2 57. I physically attack people
0 1 2 58. I pick my skin or other parts of my body (describe): _____
_____
0 1 2 59. I fail to finish things I should do
0 1 2 60. There is very little that I enjoy
0 1 2 61. My work performance is poor
0 1 2 62. I am poorly coordinated or clumsy

Please be sure you have answered all items.
Then see other side.

Please print your answers. Be sure to answer all items.

0 = Not True **1 = Somewhat or Sometimes True** **2 = Very True or Often True**

0 1 2 63. I would rather be with older people than with people of my own age
0 1 2 64. I have trouble setting priorities
0 1 2 65. I refuse to talk
0 1 2 66. I repeat certain acts over and over (describe): _____
_____
0 1 2 67. I have trouble making or keeping friends
0 1 2 68. I scream or yell a lot
0 1 2 69. I am secretive or keep things to myself
0 1 2 70. I see things that other people think aren't there (describe): _____
_____
0 1 2 71. I am self-conscious or easily embarrassed
0 1 2 72. I worry about my family
0 1 2 73. I meet my responsibilities to my family
0 1 2 74. I show off or clown
0 1 2 75. I am too shy or timid
0 1 2 76. My behavior is irresponsible
0 1 2 77. I sleep more than most other people during day and/or night (describe):
_____
0 1 2 78. I have trouble making decisions
0 1 2 79. I have a speech problem (describe):
_____
0 1 2 80. I stand up for my rights
0 1 2 81. My behavior is very changeable
0 1 2 82. I steal
0 1 2 83. I am easily bored
0 1 2 84. I do things that other people think are strange (describe): _____
_____
0 1 2 85. I have thoughts that other people would think are strange (describe): _____
_____
0 1 2 86. I am stubborn, sullen, or irritable
0 1 2 87. My moods or feelings change suddenly
0 1 2 88. I enjoy being with people
0 1 2 89. I rush into things without considering the risks
0 1 2 90. I drink too much alcohol or get drunk
0 1 2 91. I think about killing myself
0 1 2 92. I do things that may cause me trouble with the law (describe): _____
_____
0 1 2 93. I talk too much
0 1 2 94. I tease others a lot
0 1 2 95. I have a hot temper
0 1 2 96. I think about sex too much
0 1 2 97. I threaten to hurt people
0 1 2 98. I like to help others
0 1 2 99. I dislike staying in one place for very long
0 1 2 100. I have trouble sleeping (describe): _____
_____
0 1 2 101. I stay away from my job even when I'm not sick and not on vacation
0 1 2 102. I don't have much energy
0 1 2 103. I am unhappy, sad, or depressed
0 1 2 104. I am louder than others
0 1 2 105. People think I am disorganized
0 1 2 106. I try to be fair to others
0 1 2 107. I feel that I can't succeed
0 1 2 108. I tend to lose things
0 1 2 109. I like to try new things
0 1 2 110. I wish I were of the opposite sex
0 1 2 111. I keep from getting involved with others
0 1 2 112. I worry a lot
0 1 2 113. I worry about my relations with the opposite sex
0 1 2 114. I fail to pay my debts or meet other financial responsibilities
0 1 2 115. I feel restless or fidgety
0 1 2 116. I get upset too easily
0 1 2 117. I have trouble managing money or credit cards
0 1 2 118. I am too impatient
0 1 2 119. I am not good at details
0 1 2 120. I drive too fast
0 1 2 121. I tend to be late for appointments
0 1 2 122. I have trouble keeping a job
0 1 2 123. I am a happy person

124. ***In the past 6 months,*** about how many times per day did you use tobacco (including smokeless tobacco)? _____ times per day.

125. ***In the past 6 months,*** on how many days were you drunk? _____ days.

126. ***In the past 6 months,*** on how many days did you use drugs for nonmedical purposes (including marijuana, cocaine, and other drugs, except alcohol and nicotine)? _____ days.

Please be sure you have answered all items.

Center for Epidemiological Studies Depression Scale
(CES-D; Lewinsohn, Seeley, Roberts, & Allen, 1997)

Directions: Below is a list of the ways you might have felt or acted. Please check how *much* you felt this way during the *past week*.

Scale: 0 = Rarely or none of the time (less than 1 day)
1 = Some or a little of the time (1-2 days)A little
2 = Occasionally or a moderate amount of time (3-4 days)
3 = Most or all of the time (5-7 days)

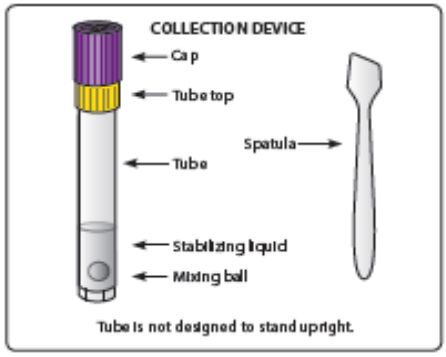
1. I was bothered by things that usually don't bother me.
2. I did not feel like eating; my appetite was poor.
3. I felt that I could not shake off the blues even with help from my family or friends.
4. I felt I was just as good as other people.
5. I had trouble keeping my mind on what I was doing.
6. I felt depressed.
7. I felt that everything I did was an effort.
8. I felt hopeful about the future.
9. I thought my life had been a failure.
10. I felt fearful.
11. My sleep was restless.
12. I was happy.
13. I talked less than usual.
14. I felt lonely.
15. People were unfriendly.
16. I enjoyed life.
17. I had crying spells.
18. I felt sad.
19. I felt that people dislike me.
20. I could not get "going."

Appendix C:

Instructions for at Home Collection

OMNIgene·GUT

For microbiome



Summary and explanation of the kit:

OMNIgene·GUT provides the materials and instructions for collecting and stabilizing microbial DNA from a fecal sample.

Warnings and precautions:

- FOR EXTERNAL USE ONLY.
- Do NOT remove the yellow tube top from the tube.
- Do NOT spill the stabilizing liquid in the tube.
- Wash with water if liquid comes in contact with eyes or skin. Do NOT ingest.
- If fecal sample is liquid or donor has diarrhea wait until the next bowel movement to collect the sample.
- Small items may pose a choking hazard.

Storage: 15°C to 25°C

Ship in accordance to applicable regulations covering transport of biological specimens.
See MSDS at www.dnagenotek.com

Label legend:

	Collect sample by (Use by)
REF	Catalog number
	Manufacturer
15°C 25°C	Storage instructions
	Caution, consult instructions for use
LOT	Lot number

USER INSTRUCTIONS

Read all instructions prior to collection

Procedure:

1 **IMPORTANT PREPARATIONS:**
- Empty your bladder before beginning the collection.
- Collect fecal sample free of urine or toilet water.
- Toilet paper or tissues may be required.

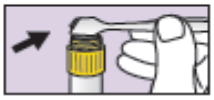
2 While holding the yellow tube top, unscrew ONLY the purple cap from the kit and set aside for later use.

IMPORTANT:
Do NOT remove the yellow tube top.
Do NOT spill the stabilizing liquid in the tube.

3 Use the spatula to collect a small amount of fecal sample.

4 Transfer the fecal sample into the yellow tube top. Repeat until the sample fills the yellow tube top.

IMPORTANT: Do NOT push sample into the tube.

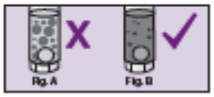


5 Scrape horizontally across the tube top to level the sample and remove any excess.

Wipe exterior of tube and top with toilet paper or tissue as needed.

6 Pick up the purple cap with the solid end facing down and screw onto the yellow tube top until tightly closed.

7 Shake the sealed tube as hard and fast as possible in a back and forth motion for a minimum of 30 seconds.



8 The fecal sample will be mixed with the stabilizing liquid in the tube; not all particles will dissolve.

IMPORTANT: Continue shaking if large particles remain as shown in Figure A.

9 Place spatula in original packaging or wrap in toilet paper and discard in garbage.

IMPORTANT: Send the sample for processing following the delivery instructions supplied separately by the kit provider.

DNA genotek

Superior samples
Proven performance

Made in Canada
DNA Genotek Inc.
3000 - 500 Palladium Drive
Ottawa, ON, Canada K2V1C2

Toll-free (North America): 1.800.613.6354
Tel: +1.613.723.5757 - Fax: +1.613.723.5067
info@dnagenotek.com
www.dnagenotek.com

Australian Sponsor: Emergo Australia, Level 20, Tower II, Darling Park, 201 Sussex Street, Sydney, NSW 2000 Australia

References

Achenbach, T. M. (2003). Adult self-report for ages 18–59. *Burlington, VT: University of Vermont, Department of Psychiatry.*

Ait-Belgnaoui, A., Durand, H., Cartier, C., Chaumaz, G., Eutamene, H., Ferrier, L., ... & Theodorou, V. (2012). Prevention of gut leakiness by a probiotic treatment leads to attenuated HPA response to an acute psychological stress in rats. *Psychoneuroendocrinology*, *37*(11), 1885-1895.

Al-Asmakh, M., Anuar, F., Zadjali, F., Rafter, J., & Pettersson, S. (2012). Gut microbial communities modulating brain development and function. *Gut microbes*, *3*(4), 366-373.

American College Health Association (2016). American College Health Association-National College Health Assessment II: Undergraduate Student Reference Group Data Report Spring 2016. Hanover, MD: American College Health Association.

Anderson, S. C., Cryan, J. F., & Dinan, T. (2017). *The Psychobiotic Revolution: Mood, Food, and the New Science of the Gut-brain Connection*. National Geographic Books.

Arana, A.A., Boyd, E.Q., Guarneri-White, M.E., Iyer-Eimerbrink, P.A., Liegey Dougall, A., & Jensen-Campbell, L.A. (in press). The Impact of Social and Physical Peer Victimization on Systemic Inflammation in Adolescents. MerrillPalmer Quarterly. Accepted June 2016.

Archie, E. A., & Tung, J. (2015). Social behavior and the microbiome. *Current opinion in behavioral sciences*, *6*, 28-34.

Arnett, J. J. (2000). Emerging adulthood: A theory of development from the late teens through the twenties. *American psychologist*, *55*(5), 469.

Arseneault-Bréard, J., Rondeau, I., Gilbert, K., Girard, S. A., Tompkins, T. A., Godbout, R., & Rousseau, G. (2012). Combination of Lactobacillus helveticus R0052 and Bifidobacterium longum R0175 reduces post-myocardial infarction depression symptoms

and restores intestinal permeability in a rat model. *British Journal of Nutrition*, *107*(12), 1793-1799.

Austin, E. Q., & Jensen-Campbell, L. A. (2018). The influence of peer victimization on health outcomes via perceived overall health and WtHR. Unpublished Manuscript.

Bailey, M. T., Dowd, S. E., Galley, J. D., Hufnagle, A. R., Allen, R. G., & Lyte, M. (2011). Exposure to a social stressor alters the structure of the intestinal microbiota: implications for stressor-induced immunomodulation. *Brain, behavior, and immunity*, *25*(3), 397-407.

Bailey, M. T., Dowd, S. E., Parry, N. M., Galley, J. D., Schauer, D. B., & Lyte, M. (2010). Stressor exposure disrupts commensal microbial populations in the intestines and leads to increased colonization by Citrobacter rodentium. *Infection and immunity*, *78*(4), 1509-1519.

Bäckhed, F., Roswall, J., Peng, Y., Feng, Q., Jia, H., Kovatcheva-Datchary, P., ... & Khan, M. T. (2015). Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell host & microbe*, *17*(5), 690-703.

Baron, R. M., & Kenny, D. A. (1986). The moderator–mediator variable distinction in social psychological research: Conceptual, strategic, and statistical considerations. *Journal of personality and social psychology*, *51*(6), 1173.

Barouei, J., Moussavi, M., & Hodgson, D. M. (2012). Effect of maternal probiotic intervention on HPA axis, immunity and gut microbiota in a rat model of irritable bowel syndrome. *PLoS One*, *7*(10), e46051.

Barrett, E., Kerr, C., Murphy, K., O'sullivan, O., Ryan, C. A., Dempsey, E. M., ... & Ross, R. P. (2013). The individual-specific and diverse nature of the preterm infant microbiota. *Archives of Disease in Childhood-Fetal and Neonatal Edition*, fetalneonatal-2012.

Bauer, H., Horowitz, R. E., Levenson, S. M., & Popper, H. (1963). The response of the lymphatic tissue to the microbial flora. Studies on germfree mice. *The American journal of pathology*, *42*(4), 471.

Bengmark, S. (2013). Gut microbiota, immune development and function. *Pharmacological Research*, *69*(1), 87-113.

Benton, D., Williams, C., & Brown, A. (2007). Impact of consuming a milk drink containing a probiotic on mood and cognition. *European journal of clinical nutrition*, *61*(3), 355-361.

Bercik, P., Park, A. J., Sinclair, D., Khoshdel, A., Lu, J., Huang, X., ... & Berger, B. (2011). The anxiolytic effect of Bifidobacterium longum NCC3001 involves vagal pathways for gut–brain communication. *Neurogastroenterology & Motility*, *23*(12), 1132-1139.

Bercik, P., Verdu, E. F., Foster, J. A., Macri, J., Potter, M., Huang, X., ... & Lu, J. (2010). Chronic gastrointestinal inflammation induces anxiety-like behavior and alters central nervous system biochemistry in mice. *Gastroenterology*, *139*(6), 2102-2112.

Bharwani, A., Mian, M. F., Foster, J. A., Surette, M. G., Bienenstock, J., & Forsythe, P. (2016). Structural & functional consequences of chronic psychosocial stress on the microbiome & host. *Psychoneuroendocrinology*, *63*, 217-227.

Bharwani, A., Mian, M. F., Surette, M. G., Bienenstock, J., & Forsythe, P. (2017). Oral treatment with Lactobacillus rhamnosus attenuates behavioural deficits and immune changes in chronic social stress. *BMC medicine*, *15*(1), 7.

Bhattacharjee, S., & Lukiw, W. J. (2013). Alzheimer's disease and the microbiome. *Frontiers in cellular neuroscience*, *7*.

Biagi, E., Nylund, L., Candela, M., Ostan, R., Bucci, L., Pini, E., Nikkila, J., Monti, D., Satokari, R., Franceshi, C., Brigidi, P., & De Vos, W. (2010). Through ageing, and beyond: gut microbiota and inflammatory status in seniors and centenarians. *PloS one*, *5*(5), e10667.

Biebl, S. J., DiLalla, L. F., Davis, E. K., Lynch, K. A., & Shinn, S. O. (2011). Longitudinal associations among peer victimization and physical and mental health problems. *Journal of Pediatric Psychology, 36*(8), 868-877.

Björkqvist, K., Lagerspetz, K. M. J., & Österman, K. (1992). The direct and indirect aggression scales. *Vasa, Finland: Abo Akademi University, Department of Social Sciences*.

Black, S., Kushner, I., & Samols, D. (2004). C-reactive protein. *Journal of Biological Chemistry*, *279*(47), 48487-48490.

Boström, A. M., Squires, J. E., Mitchell, A., Sales, A. E., & Estabrooks, C. A. (2012). Workplace aggression experienced by frontline staff in dementia care. *Journal of clinical nursing*, *21*(9-10), 1453-1465.

Braniste, V., Al-Asmakh, M., Kowal, C., Anuar, F., Abbaspour, A., Tóth, M., ... & Gulyás, B. (2014). The gut microbiota influences blood-brain barrier permeability in mice. *Science translational medicine*, *6*(263), 263ra158-263ra158.

Bravo, J. A., Forsythe, P., Chew, M. V., Escaravage, E., Savignac, H. M., Dinan, T. G., Bienenstock, J. & Cryan, J. F. (2011). Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proceedings of the National Academy of Sciences*, *108*(38), 16050-16055.

Brougham, R. R., Zail, C. M., Mendoza, C. M., & Miller, J. R. (2009). Stress, sex differences, and coping strategies among college students. *Current Psychology*, *28*(2), 85-97.

Bruce-Keller, A. J., Salbaum, J. M., Luo, M., Blanchard IV, E., Taylor, C. M., Welsh, D. A., & Berthoud, H. R. (2015). Obese-type gut microbiota induce neurobehavioral changes in the absence of obesity. *Biological psychiatry*, *77*(7), 607-615.

Cani, P. D., Hoste, S., Guiot, Y., & Delzenne, N. M. (2007). Dietary non-digestible carbohydrates promote L-cell differentiation in the proximal colon of rats. *British Journal of Nutrition*, *98*(1), 32-37.

Cani, P. D., & Knauf, C. (2016). How gut microbes talk to organs: the role of endocrine and nervous routes. *Molecular metabolism*, *5*(9), 743-752.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., ... & Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature methods*, *7*(5), 335-336.

Chao, A. (1984). Nonparametric estimation of the number of classes in a population. *Scandinavian Journal of statistics*, 265-270.

Chapell, M., Casey, D., De la Cruz, C., & Ferrell, J. (2004). Bullying in college by students and teachers. *Adolescence*, *39*(153), 53.

Chaplin, T. M., Hong, K., Bergquist, K., & Sinha, R. (2008). Gender differences in response to emotional stress: an assessment across subjective, behavioral, and physiological domains and relations to alcohol craving. *Alcoholism: Clinical and Experimental Research*, *32*(7), 1242-1250.

Le Chatelier, E., Nielsen, T., Qin, J., Prifti, E., Hildebrand, F., Falony, G., Almedia, M. … & Leonard, P. (2013). Richness of human gut microbiome correlates with metabolic markers. *Nature*, *500*(7464), 541-546.

Cho, C. E., & Norman, M. (2013). Cesarean section and development of the immune system in the offspring. *American journal of obstetrics and gynecology*, *208*(4), 249-254.

Clarke, G., Cryan, J. F., Dinan, T. G., & Quigley, E. M. (2012). probiotics for the treatment of irritable bowel syndrome–focus on lactic acid bacteria. *Alimentary pharmacology & therapeutics*, *35*(4), 403-413.

Clarke, G., Grenham, S., Scully, P., Fitzgerald, P., Moloney, R. D., Shanahan, F., ... & Cryan, J. F. (2013). The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Molecular psychiatry*, *18*(6), 666-673.

Clarke, G., Stilling, R. M., Kennedy, P. J., Stanton, C., Cryan, J. F., & Dinan, T. G. (2014). Minireview: Gut microbiota: the neglected endocrine organ. *Molecular endocrinology*, *28*(8), 1221-1238.

Claesson, M. J., Jeffery, I. B., Conde, S., Power, S. E., O'connor, E. M., Cusack, S., ... & Fitzgerald, G. F. (2012). Gut microbiota composition correlates with diet and health in the elderly. *Nature*, *488*(7410), 178.

Cong, X., Xu, W., Janton, S., Henderson, W. A., Matson, A., McGrath, J. M., ... & Graf, J. (2016). Gut microbiome developmental patterns in early life of preterm infants: impacts of feeding and gender. *PloS one*, *11*(4), e0152751.

Conlon, M. A., & Bird, A. R. (2014). The impact of diet and lifestyle on gut microbiota and human health. *Nutrients*, *7*(1), 17-44.

Crick, N. R., & Grotpeter, J. K. (1995). Relational aggression, gender, and socialpsychological adjustment. *Child Development,* 66, 710-722.

Cryan, J. F., & Dinan, T. G. (2012). Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nature reviews neuroscience*, *13*(10), 701-712.

Cohen, S., Janicki-Deverts, D., Doyle, W. J., Miller, G. E., Frank, E., Rabin, B. S., & Turner, R. B. (2012). Chronic stress, glucocorticoid receptor resistance, inflammation, and disease risk. *Proceedings of the National Academy of Sciences*, *109*(16), 5995-5999.

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A global measure of perceived stress. Journal of health and social behavior, 385-396.

Cohen, S., Kessler, R. C., & Gordon, L. U. (Eds.). (1997). *Measuring stress: A guide for health and social scientists*. Oxford University Press on Demand.

Copeland, W. E., Wolke, D., Lereya, S. T., Shanahan, L., Worthman, C., & Costello, E. J. (2014). Childhood bullying involvement predicts low-grade systemic inflammation into adulthood. *Proceedings of the National Academy of Sciences*, *111*(21), 7570-7575.

Costedio, M. M., Hyman, N., & Mawe, G. M. (2007). Serotonin and its role in colonic function and in gastrointestinal disorders. *Diseases of the colon & rectum*, *50*(3), 376-388.

Crick, N. R., Casas, J. F., & Ku, H. C. (1999). Relational and physical forms of peer victimization in preschool. *Developmental Psychology, 35*(2), 376.

Crum, A. J., Salovey, P., & Achor, S. (2013). Rethinking stress: the role of mindsets in determining the stress response. *Journal of personality and social psychology*, *104*(4), 716.

Crumeyrolle-Arias, M., Jaglin, M., Bruneau, A., Vancassel, S., Cardona, A., Daugé, V., ... & Rabot, S. (2014). Absence of the gut microbiota enhances anxiety-like behavior and neuroendocrine response to acute stress in rats. *Psychoneuroendocrinology*, *42*, 207-217.

Cryan, J. F., & O'Mahony, S. M. (2011). The microbiome-gut-brain axis: from bowel to behavior. *Neurogastroenterology & Motility*, *23*(3), 187-192.

Cussotto, S., Sandhu, K. V., Dinan, T. G., & Cryan, J. F. (2018). The Neuroendocrinology of the Microbiota-Gut-Brain Axis: A Behavioural Perspective. *Frontiers in neuroendocrinology*.

DeSalvo, K. B., Fisher, W. P., Tran, K., Bloser, N., Merrill, W., & Peabody, J. (2006). Assessing measurement properties of two single-item general health measures. *Quality of Life Research*, *15*(2), 191-201.

Demaude, J., Salvador-Cartier, C., Fioramonti, J., Ferrier, L., & Bueno, L. (2006). Phenotypic changes in colonocytes following acute stress or activation of mast cells in mice: implications for delayed epithelial barrier dysfunction. *Gut*, *55*(5), 655-661.

De Palma, G., Collins, S. M., Bercik, P., & Verdu, E. F. (2014). The microbiota–gut–brain axis in gastrointestinal disorders: stressed bugs, stressed brain or both?. *The Journal of physiology*, *592*(14), 2989-2997.

Desbonnet, L., Garrett, L., Clarke, G., Bienenstock, J., & Dinan, T. G. (2008). The probiotic Bifidobacteria infantis: an assessment of potential antidepressant properties in the rat. *Journal of psychiatric research*, *43*(2), 164-174.

Dhabhar, F. S. (2014). Effects of stress on immune function: the good, the bad, and the beautiful. *Immunologic research*, *58*(2-3), 193-210.

Dinan, T. G., & Cryan, J. F. (2013). Melancholic microbes: a link between gut microbiota and depression? *Neurogastroenterology & Motility*, *25*(9), 713-719.

Dinan, T. G., Stanton, C., & Cryan, J. F. (2013). Psychobiotics: a novel class of psychotropic. *Biological psychiatry*, *74*(10), 720-726.

Dinan, T. G., Stilling, R. M., Stanton, C., & Cryan, J. F. (2015). Collective unconscious: how gut microbes shape human behavior. *Journal of psychiatric research*, *63*, 1-9.

Ding, T., & Schloss, P. D. (2014). Dynamics and associations of microbial community types across the human body. *Nature*, *509*(7500), 357.

Doane, A. N., Kelley, M. L., Chiang, E. S., & Padilla, M. A. (2013). Development of the cyberbullying experiences survey. *Emerging Adulthood*, *1*(3), 207-218.

Dominianni, C., Sinha, R., Goedert, J. J., Pei, Z., Yang, L., Hayes, R. B., & Ahn, J. (2015). Sex, body mass index, and dietary fiber intake influence the human gut microbiome. *PloS one*, *10*(4), e0124599.

Eckburg, P. B., Bik, E. M., Bernstein, C. N., Purdom, E., Dethlefsen, L., Sargent, M., ... & Relman, D. A. (2005). Diversity of the human intestinal microbial flora. *Science*, *308*(5728), 1635-1638.

Edwards, S. M., Cunningham, S. A., Dunlop, A. L., & Corwin, E. J. (2017). The maternal gut microbiome during pregnancy. *MCN: The American Journal of Maternal/Child Nursing*, *42*(6), 310-317.

Engel, G. L. (1980). The clinical application of the biopsychosocial model. American journal of Psychiatry, 137, 535-544.

Ewing, B., Hillier, L., Wendl, M. C., & Green, P. (1998). Base-calling of automated sequencer traces using Phred. I. Accuracy assessment. *Genome research*, *8*(3), 175-185.

Faith, J. J., Rey, F. E., O'donnell, D., Karlsson, M., McNulty, N. P., Kallstrom, G., ... & Gordon, J. I. (2010). Creating and characterizing communities of human gut microbes in gnotobiotic mice. *The ISME journal*, *4*(9), 1094.

Fekkes, M., Pijpers, F. I., Fredriks, A. M., Vogels, T., & Verloove-Vanhorick, S. P. (2006). Do bullied children get ill, or do ill children get bullied? A prospective cohort study on the relationship between bullying and health-related symptoms. *Pediatrics, 117*(5), 1568-1574.

Finegold, S. M., Dowd, S. E., Gontcharova, V., Liu, C., Henley, K. E., Wolcott, R. D., Youn, E., Summanen, P. H., Granpeesheh, D., Dixon, D., Liu, M., Molitoris, D. R., and Green III, J. A. (2010). Pyrosequencing study of fecal microflora of autistic and control children. *Anaerobe*, *16*(4), 444-453.

Ford, A. C., Talley, N. J., Schoenfeld, P. S., Quigley, E. M., & Moayyedi, P. (2009). Efficacy of antidepressants and psychological therapies in irritable bowel syndrome: systematic review and meta-analysis. *Gut*, *58*(3), 367-378.

Forsythe, P., Bienenstock, J., & Kunze, W. A. (2014). Vagal pathways for microbiome-brain-gut axis communication. In *Microbial Endocrinology: The Microbiota-Gut-Brain Axis in Health and Disease* (pp. 115-133). Springer New York.

Forsythe, P., Sudo, N., Dinan, T., Taylor, V. H., & Bienenstock, J. (2010). Mood and gut feelings. *Brain, behavior, and immunity*, *24*(1), 9-16.

Foster, J. A., & Neufeld, K. A. M. (2013). Gut–brain axis: how the microbiome influences anxiety and depression. *Trends in neurosciences*, *36*(5), 305-312.

Foster, J. A., Rinaman, L., & Cryan, J. F. (2017). Stress & the gut-brain axis: regulation by the microbiome. *Neurobiology of stress*.

Freestone, P. P., Williams, P. H., Haigh, R. D., Maggs, A. F., Neal, C. P., & Lyte, M. (2002). Growth stimulation of intestinal commensal Escherichia coli by catecholamines: a possible contributory factor in trauma-induced sepsis. *Shock*, *18*(5), 465-470.

Furness, J. B., Callaghan, B. P., Rivera, L. R., & Cho, H. J. (2014). The enteric nervous system and gastrointestinal innervation: integrated local and central control. In *Microbial Endocrinology: The Microbiota-Gut-Brain Axis in Health and Disease* (pp. 39-71). Springer New York.

Galley, J. D., Mackos, A. R., Varaljay, V. A., & Bailey, M. T. (2017). Stressor exposure has prolonged effects on colonic microbial community structure in Citrobacter rodentium-challenged mice. *Scientific reports*, *7*, 45012.

Galley, J. D., Nelson, M. C., Yu, Z., Dowd, S. E., Walter, J., Kumar, P. S., Lyte, M., & Bailey, M. T. (2014a). Exposure to a social stressor disrupts the community structure of the colonic mucosa-associated microbiota. *BMC microbiology*, *14*(1), 1.

Galley, J. D., Yu, Z., Kumar, P., Dowd, S. E., Lyte, M., & Bailey, M. T. (2014b). The structures of the colonic mucosa-associated and luminal microbial communities are distinct and differentially affected by a prolonged murine stressor. *Gut microbes*, *5*(6), 748-760.

Gatchel, R. J. (2004). Comorbidity of chronic pain and mental health disorders: the biopsychosocial perspective. *American Psychologist*, *59*(8), 795.

Gatchel, R. J., Peng, Y. B., Peters, M. L., Fuchs, P. N., & Turk, D. C. (2007). The biopsychosocial approach to chronic pain: scientific advances and future directions. *Psychological bulletin*, *133*(4), 581.

Gareau, M. G., Silva, M. A., & Perdue, M. H. (2008). Pathophysiological mechanisms of stress-induced Intestinal damage. *Current molecular medicine*, *8*(4), 274-281.

Giovazolias, T., & Malikiosi-Loizos, M. (2015). Bullying at Greek universities. In H. Cowie & C. Myers (Eds.), *Bullying Among University Students: Cross-national Perspectives* (pp. 110-126). New York, NY: Routledge.

Glaser, R., & Kiecolt-Glaser, J. K. (2005). Stress-induced immune dysfunction: implications for health. *Nature Reviews Immunology*, *5*(3), 243-251.

Golubeva, A. V., Crampton, S., Desbonnet, L., Edge, D., O'Sullivan, O., Lomasney, K. W., ... & Cotter, P. D. (2015). Prenatal stress-induced alterations in major physiological systems correlate with gut microbiota composition in adulthood. *Psychoneuroendocrinology*, *60*, 58-74.

Gomez, A., Luckey, D., & Taneja, V. (2015). The gut microbiome in autoimmunity: sex matters. *Clinical Immunology*, *159*(2), 154-162.

Goodrich, J. K., Di Rienzi, S. C., Poole, A. C., Koren, O., Walters, W. A., Caporaso, J. G., ... & Ley, R. E. (2014). Conducting a microbiome study. *Cell*, *158*(2), 250-262.

Graff, L. A., Walker, J. R., & Bernstein, C. N. (2009). Depression and anxiety in inflammatory bowel disease: a review of comorbidity and management. *Inflammatory bowel diseases*, *15*(7), 1105-1118.

Graham, J. W. (2009). Missing data analysis: Making it work in the real world. *Annual review of psychology*, *60*, 549-576.

Grenham, S., Clarke, G., Cryan, J. F., & Dinan, T. G. (2011). Brain–gut–microbe communication in health and disease. *Frontiers in physiology*, *2*, 94.

Gruber, J. E., & Fineran, S. (2008). Comparing the impact of bullying and sexual harassment victimization on the mental and physical health of adolescents. *Sex roles*, *59*(1-2), 1.

Guarneri-White, M. E., Arana, A. A., Boyd, E. Q., & Jensen-Campbell, L. A. (2018). It's more than skin-deep: The relationship between social victimization and telomere length in adolescence. *Aggressive behavior*.

Gunnar, M., & Quevedo, K. (2007). The neurobiology of stress and development. *Annual Review of Psychology, 58*, 145-173.

Hamady, M., & Knight, R. (2009). Microbial community profiling for human microbiome projects: tools, techniques, and challenges. *Genome research*.

Hantsoo, L., Criniti, S., McGeehan, B., Tanes, C., Elovitz, M., Compher, C., ... & Epperson, C. N. (2018). F160. Cortisol Response to Acute Stress is Associated With Differential Abundance of Taxa in Human Gut Microbiome. *Biological Psychiatry*, *83*(9), S300-S301.

Haro, C., Rangel-Zúñiga, O. A., Alcalá-Díaz, J. F., Gómez-Delgado, F., Pérez-Martínez, P., Delgado-Lista, J., ... & Clemente, J. C. (2016). Intestinal microbiota is influenced by gender and body mass index. *PloS one*, *11*(5), e0154090.

Hawker, D. S., & Boulton, M. J. (2000). Twenty years' research on peer victimization and psychosocial maladjustment: a meta-analytic review of cross-sectional studies. *Journal of child psychology and psychiatry*, *41*(4), 441-455.

Hayes, A. F. (2013). Introduction to mediation, moderation, and conditional process analysis. *New York, NY: Guilford*.

Hefner, J., & Eisenberg, D. (2009). Social support and mental health among college students. *American Journal of Orthopsychiatry*, *79*(4), 491-499.

Herbert, T. B., & Cohen, S. (1993). Stress and immunity in humans: a meta-analytic review. *Psychosomatic medicine*, *55*(4), 364-379.

Hodges, E. V., & Perry, D. G. (1999). Personal and interpersonal antecedents and consequences of victimization by peers. *Journal of Personality and Social Psychology, 76*(4), 677.

Hoel, H., Sheehan, M. J., Cooper, C. L., & Einarsen, S. (2011). Organisational effects of workplace bullying. *Bullying and harassment in the workplace: Developments in theory, research, and practice*, 129-148.

Holzer, P., & Farzi, A. (2014). Neuropeptides and the microbiota-gut-brain axis. In *Microbial endocrinology: the microbiota-gut-brain axis in health and disease* (pp. 195-219). Springer New York.

Howard, D. E., Schiraldi, G., Pineda, A., & Campanella, R. (2006). Stress and mental health among college students: Overview and promising prevention interventions. In M. V. Landow (Ed.), *Stress and Mental Health of College Students* (pp. 91-123). Hauppauge, NY: Nova Science Publishers, Inc.

Huttenhower, C., Gevers, D., Knight, R., Abubucker, S., Badger, J. H., Chinwalla, A. T., ... & Giglio, M. G. (2012). Structure, function and diversity of the healthy human microbiome. *Nature*, *486*(7402), 207.

Hyland, N. P., & Cryan, J. F. (2010). A gut feeling about GABA: focus on GABA receptors. *Frontiers in pharmacology*, *1*.

Hyland, N. P., Quigley, E. M., & Brint, E. (2014). Microbiota-host interactions in irritable bowel syndrome: epithelial barrier, immune regulation and brain-gut interactions. *World journal of gastroenterology: WJG*, *20*(27), 8859.

Iannotti, R. J. (2005). Health behavior in school-aged children (HBSC), 2005-2006. Ann Arbor, MI: Inter-university Consortium for Political and Social Research.

Insel, P. M., & Roth, W. T. (1985). *Core concepts in health* (pp. 29). Palo Alto, CA: Mayfield.

Jackson, J. L., O'Malley, P. G., Tomkins, G., Balden, E., Santoro, J., & Kroenke, K. (2000). Treatment of functional gastrointestinal disorders with antidepressant medications: a meta-analysis. *The American journal of medicine*, *108*(1), 65-72.

Jiang, H., Ling, Z., Zhang, Y., Mao, H., Ma, Z., Yin, Y., ... & Li, L. (2015). Altered fecal microbiota composition in patients with major depressive disorder. *Brain, behavior, and immunity*, *48*, 186-194.

Jones, S. E., & Versalovic, J. (2009). Probiotic Lactobacillus reuteri biofilms produce antimicrobial and anti-inflammatory factors. *BMC microbiology*, *9*(1), 35.

Judd, C. M., & Kenny, D. A. (1981). Process analysis: Estimating mediation in treatment evaluations. *Evaluation review*, *5*(5), 602-619.

Kelly, J. R., Allen, A. P., Temko, A., Hutch, W., Kennedy, P. J., Farid, N., ... & Clarke, G. (2017). Lost in translation? The potential psychobiotic Lactobacillus rhamnosus (JB-1) fails to modulate stress or cognitive performance in healthy male subjects. *Brain, behavior, and immunity*, *61*, 50-59.

Kelly, J. R., Borre, Y., O'Brien, C., Patterson, E., El Aidy, S., Deane, J., ... & Hoban, A. E. (2016). Transferring the blues: depression-associated gut microbiota induces neurobehavioural changes in the rat. *Journal of psychiatric research*, *82*, 109-118.

Kelly, J. R., Kennedy, P. J., Cryan, J. F., Dinan, T. G., Clarke, G., & Hyland, N. P. (2015). Breaking down the barriers: the gut microbiome, intestinal permeability and stress-related psychiatric disorders. *Frontiers in cellular neuroscience*, *9*, 392.

Kim, D. Y., & Camilleri, M. (2000). Serotonin: a mediator of the brain-gut connection. *The American journal of gastroenterology*, *95*(10), 2698.

Kivimaki, M., Leino-Arjas, P., Virtanen, M., Elovainio, M., Keltikangas-Jarvinen, L., Puttonen, S., Vartia, M., Brunner, E. & Vahtera, J. (2004). Work stress and incidence of newly diagnosed fibromyalgia: prospective cohort study. *Journal of psychosomatic research*, *57*(5), 417-422.

Knack, J. M., Iyer, P. A., & Jensen-Campbell, L. A. (2012). Not Simply “In Their Heads”: Individual Differences Associated with Victimization and Health. Journal of Applied Social Psychology, 42(7), 1625-1650.

Knack, J. M., Jensen-Campbell, L. A., & Baum, A. (2011). Worse than sticks and stones? Bullying is associated with altered HPA axis functioning and poorer health. *Brain and Cognition*, *77*(2), 183-190

Knowles, S. R., Nelson, E. A., & Palombo, E. A. (2008). Investigating the role of perceived stress on bacterial flora activity and salivary cortisol secretion: a possible mechanism underlying susceptibility to illness. *Biological psychology*, *77*(2), 132-137.

Kohn, P. M., Lafreniere, K., & Gurevich, M. (1990). The Inventory of College Students' Recent Life Experiences: A decontaminated hassles scale for a special population. *Journal of behavioral medicine*, *13*(6), 619-630.

Kovacs, A., Ben-Jacob, N., Tayem, H., Halperin, E., Iraqi, F. A., & Gophna, U. (2011). Genotype is a stronger determinant than sex of the mouse gut microbiota. *Microbial ecology*, *61*(2), 423-428.

Kuczynski, J., Lauber, C. L., Walters, W. A., Parfrey, L. W., Clemente, J. C., Gevers, D., & Knight, R. (2012). Experimental and analytical tools for studying the human microbiome. *Nature Reviews Genetics*, *13*(1), 47.

Kraemer, H. C., Wilson, G. T., Fairburn, C. G., & Agras, W. S. (2002). Mediators and moderators of treatment effects in randomized clinical trials. *Archives of general psychiatry*, *59*(10), 877-883.

Kristensen, N. B., Bryrup, T., Allin, K. H., Nielsen, T., Hansen, T. H., & Pedersen, O. (2016). Alterations in fecal microbiota composition by probiotic supplementation in healthy adults: a systematic review of randomized controlled trials. *Genome medicine*, *8*(1), 52.

Kumar, R., Eipers, P., Little, R. B., Crowley, M., Crossman, D. K., Lefkowitz, E. J., & Morrow, C. D. (2014). Getting started with microbiome analysis: sample acquisition to bioinformatics. *Current protocols in human genetics*, *82*(1), 18-8.

Lachowicz, M. J., Preacher, K. J., & Kelley, K. (2018). A novel measure of effect size for mediation analysis. *Psychological Methods, 23*(2), 244-261.

La Rosa, P. S., Brooks, J. P., Deych, E., Boone, E. L., Edwards, D. J., Wang, Q., Sodergren, E., Weinstock, G., & Shannon, W. D. (2012). Hypothesis testing and power calculations for taxonomic-based human microbiome data. *PloS one*, *7*(12), e52078.

Latorre, R., Sternini, C., De Giorgio, R., & Meerveld, G. V. (2016). Enteroendocrine cells: a review of their role in brain–gut communication. *Neurogastroenterology & Motility*, *28*(5), 620-630.

Lee, S. H. (2015). Intestinal permeability regulation by tight junction: implication on inflammatory bowel diseases. *Intestinal research*, *13*(1), 11-18.

Lewinsohn, P. M., Seeley, J. R., Roberts, R. E., & Allen, N. B. (1997). Center for Epidemiologic Studies Depression Scale (CES-D) as a screening instrument for depression among community-residing older adults. Psychology and aging, 12(2), 277.

Ley, R. E., Bäckhed, F., Turnbaugh, P., Lozupone, C. A., Knight, R. D., & Gordon, J. I. (2005). Obesity alters gut microbial ecology. *Proceedings of the National Academy of Sciences*, *102*(31), 11070-11075.

Ley, R. E., Turnbaugh, P. J., Klein, S., & Gordon, J. I. (2006). Microbial ecology: human gut microbes associated with obesity. *Nature*, *444*(7122), 1022.

Li, W., Dowd, S. E., Scurlock, B., Acosta-Martinez, V., & Lyte, M. (2009). Memory and learning behavior in mice is temporally associated with diet-induced alterations in gut bacteria. *Physiology & behavior*, *96*(4-5), 557-567.

Liu, R. T. (2017). The microbiome as a novel paradigm in studying stress and mental health. *American Psychologist*, *72*(7), 655.

Low, K. S., Radhakrishnan, P., Schneider, K. T., & Rounds, J. (2007). The experiences of bystanders of workplace ethnic harassment. *Journal of Applied Social Psychology*, *37*(10), 2261-2297.

Lozupone, C. A., Hamady, M., Kelley, S. T., & Knight, R. (2007). Quantitative and qualitative β diversity measures lead to different insights into factors that structure microbial communities. *Applied and environmental microbiology*, *73*(5), 1576-1585.

Lozupone, C., & Knight, R. (2005). UniFrac: a new phylogenetic method for comparing microbial communities. *Applied and environmental microbiology*, *71*(12), 8228-8235.

Lozupone, C. A., Stombaugh, J. I., Gordon, J. I., Jansson, J. K., & Knight, R. (2012). Diversity, stability and resilience of the human gut microbiota. *Nature*, *489*(7415), 220.

Luczynski, P., McVey Neufeld, K. A., Oriach, C. S., Clarke, G., Dinan, T. G., & Cryan, J. F. (2016). Growing up in a bubble: using germ-free animals to assess the influence of the gut microbiota on brain and behavior. *International Journal of Neuropsychopharmacology*, *19*(8).

Lund, E. M., & Ross, S. W. (2016). Bullying Perpetration, Victimization, and Demographic Differences in College Students A Review of the Literature. *Trauma, Violence, & Abuse*, 1524838015620818.

Lutgen-Sandvik, P., Tracy, S. J., & Alberts, J. K. (2007). Burned by bullying in the American workplace: Prevalence, perception, degree and impact. *Journal of Management Studies*, *44*(6), 837-862.

Lyte, M. (2013). Microbial endocrinology in the microbiome-gut-brain axis: how bacterial production and utilization of neurochemicals influence behavior. *PLoS pathogens*, *9*(11), e1003726.

Lyte, M., Li, W., Opitz, N., Gaykema, R. P., & Goehler, L. E. (2006). Induction of anxiety-like behavior in mice during the initial stages of infection with the agent of murine colonic hyperplasia Citrobacter rodentium. *Physiology & behavior*, *89*(3), 350-357.

MacDonald, C. D., & Roberts-Pittman, B. (2010). Cyberbullying among college students: Prevalence and demographic differences. *Procedia-Social and Behavioral Sciences*, *9*, 2003-2009.

Macfarlane, G. T., Blackett, K. L., Nakayama, T., Steed, H., & Macfarlane, S. (2009). The gut microbiota in inflammatory bowel disease. *Current pharmaceutical design*, *15*(13), 1528-1536.

Macpherson, A. J., & Harris, N. L. (2004). Interactions between commensal intestinal bacteria and the immune system. *Nature Reviews Immunology*, *4*(6), 478-485.

Macpherson, A. J., Hunziker, L., McCoy, K., & Lamarre, A. (2001). IgA responses in the intestinal mucosa against pathogenic and non-pathogenic microorganisms. *Microbes and infection*, *3*(12), 1021-1035.

Macpherson, A. J., Martinic, M. M., & Harris, N. (2002). The functions of mucosal T cells in containing the indigenous commensal flora of the intestine. *Cellular and molecular life sciences*, *59*(12), 2088-2096.

Marketon, J. I. W., & Glaser, R. (2008). Stress hormones and immune function. *Cellular immunology*, *252*(1), 16-26.

Markle, J. G., Frank, D. N., Mortin-Toth, S., Robertson, C. E., Feazel, L. M., Rolle-Kampczyk, U., ... & Danska, J. S. (2013). Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. *Science*, *339*(6123), 1084-1088.

Malan-Muller, S., Valles-Colomer, M., Raes, J., Lowry, C. A., Seedat, S., & Hemmings, S. M. (2018). The gut microbiome and mental health: implications for anxiety-and trauma-related disorders. *Omics: a journal of integrative biology*, *22*(2), 90-107.

Matud, M. P. (2004). Gender differences in stress and coping styles. *Personality and individual differences*, *37*(7), 1401-1415.

Mayer, E. (2016). *The mind-gut connection: How the hidden conversation within our bodies impacts our mood, our choices, and our overall health.* New York, NY: HarperCollins.

Mayer, E. A., Knight, R., Mazmanian, S. K., Cryan, J. F., & Tillisch, K. (2014). Gut microbes and the brain: paradigm shift in neuroscience. *Journal of Neuroscience*, *34*(46), 15490-15496.

McEwen, B. S. (1998a). Protective and damaging effects of stress mediators. *New England journal of medicine*, *338*(3), 171-179.

McEwen, B. S. (1998b). Stress, adaptation, and disease: Allostasis and allostatic load. *Annals of the New York Academy of Sciences*, *840*(1), 33-44.

McGinley, M., Rospenda, K. M., Liu, L., & Richman, J. A. (2015). Chronic generalized harassment during college: influences on alcohol and drug use. *Journal of youth and adolescence*, *44*(10), 1898-1913.

McIntyre, K. P., Korn, J. H., & Matsuo, H. (2008). Sweating the small stuff: How different types of hassles result in the experience of stress. *Stress and Health*, *24*(5), 383-392.

Messaoudi, M., Lalonde, R., Violle, N., Javelot, H., Desor, D., Nejdi, A., ... & Cazaubiel, J. M. (2011a). Assessment of psychotropic-like properties of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in rats and human subjects. *British Journal of Nutrition*, *105*(5), 755-764.

Messaoudi, M., Violle, N., Bisson, J. F., Desor, D., Javelot, H., & Rougeot, C. (2011b). Beneficial psychological effects of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in healthy human volunteers. *Gut microbes*, *2*(4), 256-261.

Miller, G. E., Chen, E., & Zhou, E. S. (2007). If It Goes Up, Must It Come Down? Chronic Stress and the Hypothalamic-Pituitary-Adrenocortical Axis in Humans. *Psychological Bulletin*, *133*(1), 25-45.

Miller, G. E., Cohen, S., & Ritchey, A. K. (2002). Chronic psychological stress and the regulation of pro-inflammatory cytokines: a glucocorticoid-resistance model. *Health psychology*, *21*(6), 531.

Moloney, R. D., Desbonnet, L., Clarke, G., Dinan, T. G., & Cryan, J. F. (2014). The microbiome: stress, health and disease. *Mammalian genome*, *25*(1-2), 49-74.

Morgan, X. C., & Huttenhower, C. (2012). Human microbiome analysis. *PLoS computational biology*, *8*(12), e1002808.

Morgan, X. C., Tickle, T. L., Sokol, H., Gevers, D., Devaney, K. L., Ward, D. V., ... & Bousvaros, A. (2012). Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. *Genome biology*, *13*(9), R79.

Murali, R., Hanson, M. D., & Chen, E. (2007). Psychological stress and its relationship to cytokines and inflammatory diseases. *Cytokines, Stress and Immunity*, 29-49.

Nakamoto, J., & Schwartz, D. (2010). Is peer victimization associated with academic achievement? A Meta-analytic review. *Social Development*, *19*(2), 221-242.

Nansel, T. R., Overpeck, M., Pilla, R. S., Ruan, W. J., Simons-Morton, B., & Scheidt, P. (2001). Bullying behaviors among US youth: Prevalence and association with psychosocial adjustment. *Jama*, *285*(16), 2094-2100.

Naseribafrouei, A., Hestad, K., Avershina, E., Sekelja, M., Linløkken, A., Wilson, R., & Rudi, K. (2014). Correlation between the human fecal microbiota and depression. *Neurogastroenterology & Motility*, *26*(8), 1155-1162.

Neufeld, K. M., Kang, N., Bienenstock, J., & Foster, J. A. (2011). Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterology & Motility*, *23*(3), 255.

Nguyen, T. L. A., Vieira-Silva, S., Liston, A., & Raes, J. (2015). How informative is the mouse for human gut microbiota research?. *Disease models & mechanisms*, *8*(1), 1-16.

Nielsen, M. B., & Einarsen, S. (2013). Can observations of workplace bullying really make you depressed? A response to Emdad et al. *International archives of occupational and environmental health*, *86*(6), 717-721.

Nielsen, M. B., Matthiesen, S. B., & Einarsen, S. (2010). The impact of methodological moderators on prevalence rates of workplace bullying. A meta-analysis. *Journal of Occupational and Organizational Psychology*, *83*(4), 955-979.

Nonis, S., Hudson, G., Logan, L., & Ford, C. (1998). Influence of Perceived Control over Time on College Students' Stress and Stress-Related Outcomes. *Research in Higher Education, 39*(5), 587-605.

O'Mahony, S. M., Clarke, G., Borre, Y. E., Dinan, T. G., & Cryan, J. F. (2015). Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. *Behavioural brain research*, *277*, 32-48.

O'Mahony, S. M., Marchesi, J. R., Scully, P., Codling, C., Ceolho, A. M., Quigley, E. M., ... & Dinan, T. G. (2009). Early life stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric illnesses. *Biological psychiatry*, *65*(3), 263-267.

Olweus, D. (1993). Bullying at school: What we know and what we can do. Oxford: Blackwell.

Padgett, D. A., & Glaser, R. (2003). How stress influences the immune response. *Trends in immunology*, *24*(8), 444-448.

Parnell, J. A., & Reimer, R. A. (2009). Weight loss during oligofructose supplementation is associated with decreased ghrelin and increased peptide YY in overweight and obese adults. *The American journal of clinical nutrition*, *89*(6), 1751-1759.

Perren, S., & Alsaker, F. D. (2006). Social behavior and peer relationships of victims, bully-victims, and bullies in kindergarten. *Journal of child psychology and psychiatry*, *47*(1), 45-57.

Pirbaglou, M., Katz, J., de Souza, R. J., Stearns, J. C., Motamed, M., & Ritvo, P. (2016). Probiotic supplementation can positively affect anxiety and depressive symptoms: a systematic review of randomized controlled trials. *Nutrition research*, *36*(9), 889-898.

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., ... & Mende, D. R. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *nature*, *464*(7285), 59.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2012). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic acids research*, *41*(1), 590-596.

Quigley, E. M. (2009). Do patients with functional gastrointestinal disorders have an altered gut flora?. *Therapeutic advances in gastroenterology*, *2*(4), S23-S30.

Rao, A. V., Bested, A. C., Beaulne, T. M., Katzman, M. A., Iorio, C., Berardi, J. M., & Logan, A. C. (2009). A randomized, double-blind, placebo-controlled pilot study of a probiotic in emotional symptoms of chronic fatigue syndrome. *Gut Pathogens*, *1*(1), 6.

Rex-Lear, M., Knack, J. M., & Jensen-Campbell, L. A. (2012). Beyond the playground: Bullying in the workplace and its relation to mental and physical health outcomes. In *Handbook of Occupational Health and Wellness* (pp. 219-240). Springer US.

Rhee, S. H., Pothoulakis, C., & Mayer, E. A. (2009). Principles and clinical implications of the brain–gut–enteric microbiota axis. *Nature Reviews Gastroenterology and Hepatology*, *6*(5), 306-314.

Rigby, K. (2003). Consequences of bullying in schools. *The Canadian Journal of Psychiatry*, *48*(9), 583-590.

Rivers, I., & Noret, N. (2010). Participant roles in bullying behavior and their association with thoughts of ending one's life. *Crisis*, *31*(3), 143-148.

Ross, S. E., Niebling, B. C., & Heckert, T. M. (1999). Sources of stress among college students. *Social psychology*, *61*(5), 841-846.

Rubin, D. B. (1976). Inference and missing data. *Biometrika*, *63*(3), 581-592.

Sankaran-Walters, S., Macal, M., Grishina, I., Nagy, L., Goulart, L., Coolidge, K., ... & Flamm, J. (2013). Sex differences matter in the gut: effect on mucosal immune activation and inflammation. *Biology of sex differences*, *4*(1), 10.

Savignac, H. M., Kiely, B., Dinan, T. G., & Cryan, J. F. (2014). Bifidobacteria exert strain-specific effects on stress-related behavior and physiology in BALB/c mice. *Neurogastroenterology & Motility*, *26*(11), 1615-1627.

Savva, S. C., Tornaritis, M., Savva, M. E., Kourides, Y., Panagi, A., Silikiotou, N., ... & Kafatos, A. (2000). Waist circumference and waist-to-height ratio are better predictors of cardiovascular disease risk factors in children than body mass index. International journal of obesity, 24(11), 1453-1458.

Schenk, A. M., & Fremouw, W. J. (2012). Prevalence, psychological impact, and coping of cyberbully victims among college students. *Journal of School Violence*, *11*(1), 21-37.

Scheperjans, F., Aho, V., Pereira, P. A., Koskinen, K., Paulin, L., Pekkonen, E., ... & Kinnunen, E. (2015). Gut microbiota are related to Parkinson's disease and clinical phenotype. *Movement Disorders*, *30*(3), 350-358.

Schmidt, K., Cowen, P. J., Harmer, C. J., Tzortzis, G., Errington, S., & Burnet, P. W. (2015). Prebiotic intake reduces the waking cortisol response and alters emotional bias in healthy volunteers. *Psychopharmacology*, *232*(10), 1793-1801.

Schwartz, S., Friedberg, I., Ivanov, I. V., Davidson, L. A., Goldsby, J. S., Dahl, D. B., ... & Chapkin, R. S. (2012). A metagenomic study of diet-dependent interaction between gut microbiota and host in infants reveals differences in immune response. *Genome biology*, *13*(4), r32.

Sender, R., Fuchs, S., & Milo, R. (2016). Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. *Cell*, *164*(3), 337-340.

Shalev, I., Moffitt, T. E., Sugden, K., Williams, B., Houts, R. M., Danese, A., Arseneault, L., & Caspi, A. (2013). Exposure to violence during childhood is associated with telomere erosion from 5 to 10 years of age: a longitudinal study. *Molecular psychiatry*, *18*(5), 576-581.

Shannon, C. E. (2001). A mathematical theory of communication. *ACM SIGMOBILE Mobile Computing and Communications Review*, *5*(1), 3-55.

Söderholm, J. D. (2010). The intestinal barrier and its regulation by neuroimmune factors. *Neurogastroenterology & Motility*, *22*(7), 718-733.

Söderholm, J. D., Yates, D. A., Gareau, M. G., Yang, P. C., MacQueen, G., & Perdue, M. H. (2002). Neonatal maternal separation predisposes adult rats to colonic barrier dysfunction in response to mild stress. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *283*(6), G1257-G1263.

Sokol, H., Pigneur, B., Watterlot, L., Lakhdari, O., Bermúdez-Humarán, L. G., Gratadoux, J. J., ... & Grangette, C. (2008). Faecalibacterium prausnitzii is an anti-inflammatory

commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proceedings of the National Academy of Sciences*, *105*(43), 16731-16736.

Sonnenburg, E. D., Smits, S. A., Tikhonov, M., Higginbottom, S. K., Wingreen, N. S., & Sonnenburg, J. L. (2016). Diet-induced extinctions in the gut microbiota compound over generations. *Nature*, *529*(7585), 212.

Steenbergen, L., Sellaro, R., van Hemert, S., Bosch, J. A., & Colzato, L. S. (2015). A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood. *Brain, behavior, and immunity*, *48*, 258-264.

Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X. N., ... & Koga, Y. (2004). Postnatal microbial colonization programs the hypothalamic–pituitary–adrenal system for stress response in mice. *The Journal of physiology*, *558*(1), 263-275.

Suez, J., Zmora, N., Zilberman-Schapira, G., Mor, U., Dori-Bachash, M., Bashiardes, S., ... & Horn, M. (2018). Post-antibiotic gut mucosal microbiome reconstitution is impaired by probiotics and improved by autologous FMT. *Cell*, *174*(6), 1406-1423.

Smith, P. K., Mahdavi, J., Carvalho, M., Fisher, S., Russell, S., & Tippett, N. (2008). Cyberbullying: Its nature and impact in secondary school pupils. *Journal of Child Psychology and Psychiatry, 49*(4), 376-385.

Spor, A., Koren, O., & Ley, R. (2011). Unravelling the effects of the environment and host genotype on the gut microbiome. *Nature Reviews Microbiology*, *9*(4), 279.

Strandwitz, P. (2018). Neurotransmitter modulation by the gut microbiota. *Brain research*, *1693*, 128-133.

Takizawa, R., Danese, A., Maughan, B., & Arseneault, L. (2015). Bullying victimization in childhood predicts inflammation and obesity at mid-life: a five-decade birth cohort study. *Psychological medicine*, *45*(13), 2705-2715.

Takizawa, R., Maughan, B., & Arseneault, L. (2014). Adult Health Outcomes of Chilhood Bullying Victimization: Evidence From a Five-Decade Longitudinal British Birth Cohort. *The American Journal of Psychiatry, 171*(7), 777-784.

Talham, G. L., Jiang, H. Q., Bos, N. A., & Cebra, J. J. (1999). Segmented filamentous bacteria are potent stimuli of a physiologically normal state of the murine gut mucosal immune system. *Infection and immunity*, *67*(4), 1992-2000.

Tap, J., Mondot, S., Levenez, F., Pelletier, E., Caron, C., Furet, J. P., ... & Dore, J. (2009). Towards the human intestinal microbiota phylogenetic core. *Environmental microbiology*, *11*(10), 2574-2584.

Teitelbaum, A. A., Gareau, M. G., Jury, J., Yang, P. C., & Perdue, M. H. (2008). Chronic peripheral administration of corticotropin-releasing factor causes colonic barrier dysfunction similar to psychological stress. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *295*(3), G452-G459.

Tetel, M. J., De Vries, G. J., Melcangi, R. C., Panzica, G., & O'Mahony, S. M. (2018). Steroids, stress and the gut microbiome-brain axis. *Journal of neuroendocrinology*, *30*(2), e12548.

Tillisch, K., Labus, J., Kilpatrick, L., Jiang, Z., Stains, J., Ebrat, B., ... & Mayer, E. A. (2013). Consumption of fermented milk product with probiotic modulates brain activity. *Gastroenterology*, *144*(7), 1394-1401.

Tsigos, C., & Chrousos, G. P. (2002). Hypothalamic–pituitary–adrenal axis, neuroendocrine factors and stress. *Journal of psychosomatic research*, *53*(4), 865-871.

Turnbaugh, P. J., Bäckhed, F., Fulton, L., & Gordon, J. I. (2008). Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell host & microbe*, *3*(4), 213-223.

Turnbaugh, P. J., Ridaura, V. K., Faith, J. J., Rey, F. E., Knight, R., & Gordon, J. I. (2009). The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. *Science translational medicine*, *1*(6), 6ra14-6ra14.

Underwood, M.K., Beron, K.J., & Rosen, L.H. (2009). Continuity and change in social and physical aggression from middle childhood through early adolescence. *Aggressive Behavior, 35*, 357-375.

Vaez, M., & Laflamme, L. (2008). Experienced stress, psychological symptoms, self-rated health and academic achievement: A longitudinal study of Swedish university students. *Social Behavior and Personality: an international journal*, *36*(2), 183-196.

Vaillancourt, T., Hymel, S., & McDougall, P. (2013). The biological underpinnings of peer victimization: Understanding why and how the effects of bullying can last a lifetime. *Theory into Practice*, *52*(4), 241-248.

Van der Wal, M. F., De Wit, C. A., & Hirasing, R. A. (2003). Psychosocial health among young victims and offenders of direct and indirect bullying. *Pediatrics*, *111*(6), 1312-1317.

Van de Wiele, T., Van Praet, J. T., Marzorati, M., Drennan, M. B., & Elewaut, D. (2016). How the microbiota shapes rheumatic diseases. *Nature Reviews Rheumatology*, *12*(7), 398-411.

Vartia, M. A. (2001). Consequences of workplace bullying with respect to the well-being of its targets and the observers of bullying. *Scandinavian journal of work, environment & health*, 63-69.

Watts, L. K., Wagner, J., Velasquez, B., & Behrens, P. I. (2017). Cyberbullying in higher education: A literature review. *Computers in Human Behavior*, *69*, 268-274.

Walker, L. S., Smith, C. A., Garber, J., & Van Slyke, D. A. (1997). Development and validation of the pain response inventory for children. *Psychological Assessment*, *9*(4), 392.

Whitehead, W. E., Palsson, O., & Jones, K. R. (2002). Systematic review of the comorbidity of irritable bowel syndrome with other disorders: what are the causes and implications?. *Gastroenterology*, *122*(4), 1140-1156.

Wilder, R. L. (1995). Neuroendocrine-immune system interactions and autoimmunity. *Annual review of immunology*, *13*(1), 307-338.

Williams, B. L., Hornig, M., Buie, T., Bauman, M. L., Paik, M. C., Wick, I., ... & Lipkin, W. I. (2011). Impaired carbohydrate digestion and transport and mucosal dysbiosis in the intestines of children with autism and gastrointestinal disturbances. *PloS one*, *6*(9), e24585.

Willing, B. P., Vacharaksa, A., Croxen, M., Thanachayanont, T., & Finlay, B. B. (2011). Altering host resistance to infections through microbial transplantation. *PloS one*, *6*(10), e26988.

Winter, G., Hart, R. A., Charlesworth, R. P., & Sharpley, C. F. (2018). Gut microbiome and depression: what we know and what we need to know. *Reviews in the Neurosciences*.

Wooley, J. C., Godzik, A., & Friedberg, I. (2010). A primer on metagenomics. *PLoS computational biology*, *6*(2), e1000667.

Xia, Y., & Sun, J. (2017). Hypothesis testing and statistical analysis of microbiome. *Genes & Diseases*, *4*(3), 138-148.

Yano, J. M., Yu, K., Donaldson, G. P., Shastri, G. G., Ann, P., Ma, L., ... & Hsiao, E. Y. (2015). Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell*, *161*(2), 264-276.

Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., ... & Heath, A. C. (2012). Human gut microbiome viewed across age and geography. *nature*, *486*(7402), 222.

Young, S. N., & Leyton, M. (2002). The role of serotonin in human mood and social interaction: insight from altered tryptophan levels. *Pharmacology Biochemistry and Behavior*, *71*(4), 857-865.

Zhou, J., Martin, R. J., Tulley, R. T., Raggio, A. M., McCutcheon, K. L., Shen, L., ... & Keenan, M. J. (2008). Dietary resistant starch upregulates total GLP-1 and PYY in a sustained day-long manner through fermentation in rodents. *American Journal of Physiology-Endocrinology and Metabolism*, *295*(5), E1160-E1166.

Zijlmans, M. A., Korpela, K., Riksen-Walraven, J. M., de Vos, W. M., & de Weerth, C. (2015). Maternal prenatal stress is associated with the infant intestinal microbiota. *Psychoneuroendocrinology*, *53*, 233-245.

Zmora, N., Zilberman-Schapira, G., Suez, J., Mor, U., Dori-Bachash, M., Bashiardes, S., ... & Federici, S. (2018). Personalized gut mucosal colonization resistance to empiric probiotics is associated with unique host and microbiome features. *Cell*, *174*(6), 1388-1405.

Biographical Information

Erin Quinn Austin is a native of Fort Worth, Texas who graduated from Abilene Christian University in Abilene, Texas with a Bachelor of Science in Biology and Psychology in 2012. She began her graduate career at the University of Texas at Arlington under the mentorship of Dr. Lauri Jensen-Campbell and received her Masters of Science in Experimental Psychology from UTA in 2014, and her Ph.D. in Experimental Pscyhology in 2018 from UTA.

She is currently a resident of El Paso, Texas and a Visiting Assistant Professor of Psychology at New Mexico State University.