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Chapter 1: INTRODUCTION

The human gastrointestinal tract is inhabited by over ten trillion microorganisms. These cells outnumber the cells in our body by tenfold and contain about 150 times as many genes as the human genome (Collado et al., 2012). While 75% of the phyla in the gut have been identified, largely in part by the Human Microbiome Project, 25% is still unknown. These cells facilitate a complex interaction between the intestinal microbiota, the gut, and the central nervous system that is being branded as the microbiome-gut-brain axis. This system plays an integral role in human health as homeostasis of microbiota in the gut regulates nutrient utilization, gut barrier function, and immune development; so much so that gut bacteria is responsible for 60-70% of immune system function (Mayer, 2011).

The microbiome-brain-gut axis functions as a bi-directional pathway between the nervous system and the digestive system. An alternate nervous system -the enteric nervous system-is being referred to as the "second brain" based on its size, complexity and similarity in neurotransmitters and signaling molecules with the brain (Mayer, 2011). Therefore, the bi-directional signaling that occurs between the gut and brain in regulating homeostasis can ultimately lead to disease processes such as inflammatory bowel disease, autism, and major depressive disorder. Alterations in the microbial composition of the gut, or dysbiosis, has also been implicated in increased risk of atopic diseases (such as allergic rhinitis, asthma), and celiac disease. Reduced bacterial diversity has been associated with obesity and reduced glucose tolerance (Koren et al., 2012).

Microbiota diversity is affected throughout the lifespan from conception to old age. According to Collado et al., (2012), the fetal programming hypothesis states that "maternal

exposures during pregnancy may induce long lasting or permanent changes in fetal physiology and thereby impact disease risk later in life." Microbiota in the infant depends on first inoculums, mother's microbiota, mode of delivery, type of feeding, and the environment, including weaning food practices and the use of antibiotics. Preterm, formula fed, c-sectioned, and those infants who required antibiotics and or intensive care treatment are implicated in cases of diarrhea, necrotizing enterocolitis, and even neonatal sepsis, due to decreased microbial diversity. As the human is exposed to infection, disease, diet, aging, and antimicrobials, the microbiome tends to revert to the diversity established in infancy without intervention (Dinan& Cryan, 2012). Consequently, scientists began investigating the use of probiotics as a means to reestablish homeostasis of gut flora and evade irreparable damage. This literature review will discuss the impact of the stress response on the gastrointestinal tract, with special focus given to the role of stress in the pathophysiology of common diseases affecting bowel to behavior; and the therapeutic role of probiotics as mainstay treatment.

Definitions

Comprehension of the complex *microbiome-brain-gut axis* (BGA) is necessary to realize its implications in the following literature review. The BGA includes the central nervous system (CNS), the neuroendocrine and neuroimmune systems, the sympathetic and parasympathetic arms of the autonomic nervous system, the enteric nervous (ENS) system, and the intestinal microbiota. This bidirectional network enables signals from the brain to influence the motor, sensory and secretory modalities of the gastrointestinal tract (GIT) (Konturek et al., 2011). Conversely, messages from the gut influence stress regulation in the brain via the hypothalamus (Dinan & Cryan, 2013). Stress causes changes in the composition of the microbiota, inducing changes in neurotransmitters (i.e. corticotrophin releasing factor, CRF) and proinflammatory

cytokine levels, modulating motility, permeability of the epithelial barrier, and visceral sensitivity. Glucocorticoids (i.e.cortisol/aldosterone) released from adrenal glands through activation of the *hypothalamic-pituitary-adrenal* (HPA) axis impact the immune system. *Proinflammatory cytokines* (i.e. interleukins and TNF alpha) stimulate the *vagus nerve*, which innervates areas of the gut, thereby, playing an integral part of the BGA communication between the gut microbiota and the HPA. Once CRF is released, the pituitary gland consequently releases adrenocorticotropic hormone (ACTH) to stimulate the release of cortisol by the adrenal glands. Serotonin (5-HT) and noradrenaline are other neurotransmitters essential to BGA signaling.

The following literature review will also discuss several other neurotransmitters, neuromodulators, and catecholamines that also affect the BGA. *Brain derived neurotrophic factor* (BDNF) is crucial for supporting neuronal survival and encouraging the growth and differentiation of new neurons and synapses and thus is involved in the regulation of multiple aspects of cognitive and emotional behaviors (Cryan & O'Mahony, 2011). *GABA* is the main inhibitory neurotransmitter of the CNS, which is mediated by $GABA_A$ and $GABA_{B,}$ which are pharmacological targets for anxiolytic agents (Bravo et al., 2011). Norepinephrine can increase the virulence of bacteria, E. coli.

This literature review will also discuss the therapeutic effects of probiotics, prebiotics, and fecal transplants with the preceding. *Probiotics* are live microbial organisms that beneficially affect the host animal by improving its intestinal microbial balance, as defined by the father of immunology, Elie Metchnikoff (Caepa et al., 2013). He theorized that lactic-acid producing bacteria could suppress the growth of autointoxicating bacteria, thereby, providing longevity of life. Rather, probiotics do not replace pathogens, but increase bacteriocins that inhibit bacterial translocation, enhance mucosal barrier function, generating signals within the gastrointestinal

tract's epithelium and immune system (Quigley, 2008). According to Tannock (as cited in Blaser, 2003), *prebiotics* can be defined as non digestible carbohydrates that benefit the host by selectively stimulating the growth and activity of bacteria in the colon. *Fecal transplantation* can be described as infusion of microbiota via various methods: gastric lavage, endoscopy, oral ingestion.

Theoretical Framework

The Health Belief Model (HBM) will serve as the theoretical framework for this literature review. The HBM was developed in the 1950s by the U.S. Public Health Services as a means of explaining low participation in prevention programs. The model theorizes that a person's perception of four areas- 1) severity of potential illness, 2) susceptibility to that illness, 3) benefits of preventative action, and 4) barriers to action- will drive health behaviors (Glanz et al., 2008). From a nursing standpoint, the utilization of the HBM is appropriate because it focuses on patient compliance and preventative health care practices. As it relates to gut health, there are many things that affect a person's microbial diversity, and have the potential to cause disease. However, many people are not aware of the existence of the BGA or what actions to take and avoid to ensure its prosperity. Ultimately, the patient's perception of the four aforementioned areas will determine their health behavior in regards to prevention and treatment of dysbiosis. This literature review will seek to describe the many possibilities affecting microbiota health and serve as information that nurse practitioners can utilize to educate and treat their patients, within the context of primary, secondary, and tertiary prevention.

Purpose Statement/PICO Question

In order to explore this phenomenon, a two-fold research question was formulated using the PICO-style format:

Throughout the lifespan, what are the principle concerns of dysbiosis and can probiotic treatment reverse its effects, as opposed to no treatment?

Chapter 2: LITERATURE REVIEW

Although numerous studies have been conducted regarding the BGA, it remains a veiled topic. Recently though, the BGA and its implications have garnered more attention as scientists grapple to explain the increase in prevalence of autoimmune disease and other disorders. This section will examine the most recent findings regarding dysbiosis and the BGA, and the effects of probiotic treatment. The goal was to identify modifiable risk factors in an effort to reduce the rate of occurrence of dysbiosis, thereby, decreasing the occurrence of disease & disorder, adverse outcomes, and associative costs.

For this literature review, useful key search terms included "microbiota," "brain-gut axis," "dysbiosis," "behavior," "disease," and "probiotics." Sources utilized in obtaining journal articles and other information dated from 2008 to the present. Cumulative Index to Nursing and Allied Health Literature (CINAHL) database, EBSCO Host, SciDirect, and Google Scholar were all utilized in retrieving journal entries. Several different sites were accessed for pertinent information- *Current Nursing, CDC, WHO, NIH,* and *Science Magazine*.

Methods and Results from Literature

Dysbiosis in rodents. Many studies have been conducted using rat/mice experimentation in regards to BGA, leaving few available research studies conducted on actual human patients to review. These preliminary studies still have great health implications, however.

Probiotics. Strains of Lactobacillus and Bifiobacterium were mainly used to display the effects of good microbiota on the gut using several testing methods to determine the stress response, thus confirming the presence of a brain-gut connection. In a study by Ait-Belgnaoui et al. (2012), female Wistar rats were either given a 2 week administration of *L.farciminis,* treated

with ML-7, or treated with an antibiotic, in an effort to demonstrate a deregulation in stress response, thereby, preventing "leaky gut." *L.farciminis*, in fact, enhanced the epithelial barrier decreasing enteric permeability, and attenuating the stress response by the HPA axis. Similarly, Bravo et al. (2013) treated 36 BALB/c mice with *L.rhamnosus* for 28 days, and then exposed them to stress testing. Treated rats displayed improved memory, and reduced levels of corticosterone. The bi-directionality of the BGA was further confirmed when vagomotized mice failed to exhibit neurochemical or behavioral effects, identifying the vagus as a major modulatory pathway between the gut and brain. Desbonnet et al. (2010) investigated the antidepressant effects of *B.infantis* compared with citalopram in rats exposed to a Forced Swim Test (FST). Interestingly, *B.infantis* induced an elevation in the neurotransmitter serotonin (5-HT), implicating microbiota in the regulation of CNS and ENS. Bercik et al. (2011), Gareau et el. (2011), and Neufeld et al. (2011) explored the concept of the germ-free (sterile) paradigm and how enteric microbiota affects behavior and brain biochemistry as compared to specific pathogen free (SPF) mice in their studies. In a study by Neufeld et al. (2011), SPF mice and GF mice were simply exposed to a stressor, the elevated plus maze (EPM). The presence or absence of microbiota was proven influential in the development of behavior and neurochemical changes when GF mice exhibited anxiety in comparison with SPF mice, while increases in BDNF and decreases in 5-HT were displayed. Bercik et al. (2011) exposed SPF mice to antimicrobials (ATMs) for 7 days; conversely, they colonized GF mice with microbiota. Increased BDNF and exploratory behavior (anxiety) were seen with the administration of ATMs. Once ATMs were withdrawn, changes in behavior reversed. However, in GF mice ATMs did not alter behavior because there was no microbiota to alter. Gareau et al. (2011) examined the link between enteric infection and impaired learning & memory, by inoculating SPF and GF mice with *C.rodentium*

followed by a probiotic. *C.rodentium* colonization induced memory dysfunction in SPF mice, but GF mice were unaffected, providing support for the requirement of commensal gut microbiota in memory (Lee & Chua, 2011), while probiotic dispensation prevented memory dysfunction.

Diet. Nutritional habits are so imperative that diet during pregnancy appears to influence the diversity of initial colonization of microbes (Collado et al., 2012). Studies were also conducted to determine whether dietary manipulation affected bacterial diversity, and consequently, memory and learning. Li et al. (2009) randomly assigned 5 week old mice to receive standard diet or chow infused with 50% lean ground beef (BD) for 3 months, and found that the BD diet group had significantly higher bacterial diversity and displayed improved memory with reduced anxiety in comparison to its counterpart. Ohland et al. (2013) took it a step further by investigating whether the modulatory effects of probiotics were altered by diet. In this study wild and IL-10 deficient mice were fed either a chow diet or a western style diet infused with or without *L.helvticus* and discovered that effects were diet and genotype dependent. Overall psychological effects were alleviated; however, a combination of probiotic and western diet exacerbated effects, demonstrating that diet does factor into probiotic induced effect.

Dysbiosis in humans. Several studies involving humans have been conducted demonstrating microbiota and its effects on behavior; pregnancy & infancy, and implication in various other disease states.

Behavior. Intestinal dysbiosis contributes to psychiatric disorders in patients with bowel disorders (Bercik et al., 2011). In a 12 year longitudinal, prospective, randomized, population based study, Koloski et al. (2012) surveyed 1775 Australian participants in 1997 on functional gastrointestinal disorders (FGIDs). The participants were contacted 12 years later and completed

a follow-up survey in hopes of determining the directionality of the BGA in FGIDs. The study found that "among people free of a FGID at baseline, higher levels of anxiety but not depression at baseline was a significant independent predictor of developing new onset FGIDs 12 years later. Among people who did not have elevated levels of anxiety and depression at baseline, those with a FGID at baseline had significantly higher levels of anxiety and depression at follow-up" (Koloski et al., 2012). In other words, the existence of FGID's begets anxiety, and anxiety begets FGIDs, thus confirming the bi-directionality of BGA.

In 2008, Maes et al. sought to determine whether major depressive disorder (MDD) is accompanied by increased serum levels of IgM and IgA directed again LPS of gram negative enterobacteria. Fifty-one MDD patients completed a FibroFatigue Scale and chronic fatigue syndrome (CFS) scale to determine their severity from which a correlation with IgM and IgA against LPS was measured against. Increased levels were accompanied by increased gut permeability as the immune response system (IRS) was initiated against the LPS of enterobacteria. Similarly, Rao et al. (2009) randomized 39 CFS patients between 18-65 years of age to complete a two month study in which anxiety and depression were assessed using the Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI). They took an eight week course of either probiotics (*L.casei* & *Shirota*) or placebos. Stool samples were collected and analyzed before and after, with a reassessment using the BDI and BAI. The treatment group displayed moderate increases in fecal microbiota with significant increases in *Bifidobacterium* and *Lactobacillus* strains. Consequently, BDI and BAI were also improved in the treatment group. A double-blind, placebo controlled, randomized paralleled group study of healthy human volunteers by Messaoudi et al. (2011) sought to determine the anxiolytic effect of probiotics on anxiety, depression, stress, and coping. They were given a combination of *L.helveticus* and

B.longum for 30 days and then analyzed using behavior scales, as well as 24 hour urine analysis. In combination with the behavior scales, a reduction in cortisol was observed displaying the anticipated anxiolytic effect. In single centered, randomized, controlled, parallel-arm design study by Tillisch et al. (2013), 36 healthy women ranging in ages 18-55 years old were either given a fermented milk product with probiotic (FMPP), a non-fermented control milk product, or no intervention twice daily for 4 weeks in order to determine whether a FMPP could alter intrinsic connectivity and responses to emotional attention tasks. After undergoing subsequent before and after MRIs, it was confirmed that FMPP ingestion evoked brain responses and resting state networks in women tested, "involving activity reductions in brain regions belonging to a sensory brain network, as well as frontal, prefrontal, and temporal cortices, parahippocampal gyrus, and the PAG" (Tillisch et al., 2013).

Other disease states and disorders. The effects of dysbiosis can have far reaching effects on human health as results of other studies have indicated. For instance, Tan et al. (2011) examined 52 severely affected traumatic brain injury patients in a randomized single blind study to determine whether enteric administration of probiotics would affect Th1 & Th2 (helper T cells) imbalance and improve clinical outcomes. After a 21 day course of probiotics via NG tube, the treatment group displayed lower incidence of nosocomial infections and shorter ICU stays.

Qin et al. (2008) assessed and characterized the microbiota in type 2 diabetics in large two-staged metagenome-wide association study involving 345 Chinese volunteers. After a deep shotgun sequencing of the gut microbial DNA, 60,000 type 2 diabetic associated markers were identified and validated establishing a metagenomic linkage that type 2 diabetics had a moderate degree of gut dysbiosis, and an increase in pathogenic bacteria. Colonization increases glucose uptake in the gut implicating enteric microbiota as modulators of fat deposition in the host;

diversity will determine regulation of energy storage and may predispose to obesity (O, Hara & Shanahan, 2007).

With an increased prevalence of autism spectrum disorders (ASD), studies examining causal relationship between microbiota and ASD have increased as well. Adams et al. (2011) surveyed 58 ASD children compared to 39 typical children to assess their GI and ASD severity and then examined fecal samples for associated flora and biomarkers. GI symptoms were strongly correlated with ASD children and increased severity of the disorder. Similarly, Kang et al. (2013) compared gut microbiomes of 20 GI symptom-free neurotypical children to 20 ASD children mostly presenting with GI symptoms. Abnormal breakdown of carbohydrates appeared to affect the amount and type of nutrients absorbed resulting in ill digestive bacteria contributing to poor digestion, intestinal inflammation and more severe ASD. The ratio of glutamate to GABA was higher resulting in the display of hyper-excitation. In a study by Theije et al. (2011), the increased presence of T cells in biopsies of three ASD children indicates increased inflammation, and thus altered immunity. The biopsies also revealed that the colonization of *Clostridium* species was at the expense of *Bifidobacterium*. He hypothesized that gut inflammation genetically predisposes one prone to express the autistic phenotype or increase the severity of disease state. Exposures to high levels of IL-6 in utero alter brain development and leads to permanent behavioral impairments (Collado et al., 2012), that if microbiota corrections cannot be made within the very narrow and critical window behavioral phenotype cannot be reversed and the opportunity to achieve normal brain development is lost (Diamond et al., 2011).

Pregnancy and infancy. Microbiota development is dependent on first inoculums, mother's microbiota, mode of delivery, type of feeding, environment, weaning and food practices and the use of antibiotics (Collado et al., 2012). A prospective, randomized mother-

infant study of 91 pregnant women and their infants by Koren et al. (2012) sought to determine how pregnancy affects the gut microbiome via stool samples and data collections concerning diet in the first and third trimesters. They concluded that pregnancy was associated with a profound alteration of microbiota, with the third trimester mimicking composition found in the metabolic syndrome-dysbiosis, inflammation and weight gain. These metabolic changes are necessary, however, to support a healthy pregnancy as hyperglycemia provides a continuous supply of nutrients and adiposity prepares the body for the energetic demands of lactation. Interestingly, a child's microbiota is most similar to their mother's in the first trimester. Microbiota tends to revert back to this status once infection subsides or antimicrobial is withdrawn (Dinan et al., 2012). In a study conducted by Azad et al. (2013), records were obtained regarding the cohort of 24 Canadian infants investigating the mode of delivery, infant diet, and medication. Fecal samples of infants were obtained and DNA sequenced in order to determine species colonization. Azad et al. (2013) found that compared with breastfed and vaginally delivered infants, formula fed and cesarean born infants had increased presence of *C.difficile* and decreased presence of beneficial microbiota. In fact, breastfeeding is the single most important postnatal element in immunological programming according to Collado et al. (2012), because breast milk is a source of oligosaccharides, which exert a prebiotic effect (Matamoros et al., 2013). Fortner et al. (2014) examined the association of fetal membrane chorion thinning, and bacterial presence in 14 Preterm Premature Rupture of Membranes (PPROM) subjects as compared to term subjects, using membrane samples. Results confirmed that dysbiosis can have adverse affects on term pregnancy, demonstrating that bacterial count is greatest and inversely correlated with chorion thinning among PPROM subjects.

Chapter 3: DISCUSSION

Summary of Evidence

Many aspects of the brain to gut signaling that are addressed in this literature review, in particular the role of this signaling in emotional and cognitive function, remain speculative at this point but may guide future research. Preclinical rodent studies demonstrate strong correlations in the mapping of neuroanatomy of the ENS, but they are insufficient in providing information for human studies. In fact there are more questions that remain to be addressed. For instance, "What role does gut to brain signaling have in brain development during early life and what part does it play in adults? Does mucosally driven vagal input to the brain play a part in brain development?" (Mayer, 2011). While larger randomized, double blind trials implementing gut microbiota before and after intervention are necessary to show clinical outcomes in regards to disease states (Vrieze et al., 2013), most of these hypotheses can be studied in human patients, using stool sample for metagenomic sequencing of the microflora and gut tissue. Analysis of signaling mechanisms can be obtained easily and noninvasively using endoscopic biopsies to study brain structure, function, and signaling with non-invasive neuroimaging.

After performing the literature review, it became apparent that developing an intervention to impose upon care providers seemed futile as such an intervention would require comprehensive thought in regards to the numerous factors that might predispose one to dysbiosis. Primary prevention by way of education, holds, not only the practitioner accountable, but as the HBM suggests, the patient as well, as they are the ultimate determinants of their health. This also places the investigation of probiotic treatment in all modes of prevention- primary, secondary, and tertiary.

Limitations

The most glaring limitation regarding this literature review is the abundance of preclinical studies using rodents rather than human experimentation. Although rodents are often used in preclinical trials, it is not comparable to human trials simply because anatomy and pathophysiology is not the same. Secondly, for the available studies using human subjects the sample sizes were small and lackluster at best, as it is difficult to substantiate clinical significance with a small sample size. Interest in the BGA and its correlation to the increased prevalence of autoimmune diseases and other disorders will expectantly increase the number of randomized, double blind clinical trials being performed.

The HIP is actively working on identifying the remaining undetermined 25% of phyla within the gut. Once the HIP completes the sequencing, it will better serve the experimentation process as the selection of suitable bacterial strains with specific properties tailored to patient needs is necessary to achieve therapeutic benefits of probiotics (Caepa et al., 2013), such as medication synthesis is to Cytochrome P_{450}. Therefore, there will be an incomplete retrieval of identified research because of the unknown until sequencing is complete.

As demonstrated, microbiota colonization is multifactorial beginning at conception and continuing to be altered throughout the lifespan in response to lifestyle changes. For instance, Ohland et al. (2013) determined that probiotic effectiveness was dependent upon the synergistic effect of diet and mouse phenotype. Therefore, it is difficult to demonstrate the specific underpinnings of probiotic effectiveness, as it is difficult to isolate the probiotic effect only in experimentation.

Chapter 4: CONCLUSIONS

Great progress has been made in the understanding of the bidirectional communication between the brain and digestive system, including the mapping of ENS and how it is modulated. There are 200-600 million neurons in the ENS; and as it interfaces with the largest body surface (Mayer, 2011), the intestinal surface, it creates a barrier to entry by directly killing pathogen, bad bacteria, viruses, fungi, parasites, and yeast via its intact bacterial lining.

Maternal exposure during pregnancy may induce long lasting or permanent changes in fetal physiology, and thereby impact disease risk later in life (Collado et al., 2012). Fetuses are born germ free but after birth, establishment of microbiota is determined by environmental factors such as mode of delivery, type of infant diet, hygiene levels, and medication (O'Hara & Shanahan., 2007).

Dysregulation of the BGA plays a central role in the pathogenesis of many disease states. As intestinal permeability increases, alteration of GI motility leads to profound mast cell activation resulting in release of proinflammatory mediators. Probiotics can attenuate the immune response. They are associated with reduction of risk in GI infections and lower frequency of colic (Theije et al., 2011). A study in China revealed that in children 3-5 years old treated with a probiotic combination 53% had lower rates of fever, 41% had reduced coughs, and 28% had decreased runny noses. It also demonstrated a decreased need for antibiotic treatment as usage declined by 84% (Barron, 2013). There are many possibilities for fecal transplantation as it was shown to improve MS symptoms (no encopresis, or neurological disability) anywhere from 2-15 years post treatment (Vrieze et al., 2013). Fecal transplantation also was found to increase

colonization and insulin sensitivity, and therefore decrease insulin resistance in male subjects with metabolic syndrome (Vrieze et al., 2012).

As the metagenomic sequencing of the microbiome reaches completion, discoveries are being made that will provide more crucial information. In a recent study for instance, dendritic cells were found to bind with the flagella fragments of bacteria in the gut, mobilizing other immune cells to fight rotavirus infection supporting the case for more stringent use of antimicrobials that can create dysbiosis (Leslie, 2014). Further examination of these pathways in human trials will solidify our understanding of microbiota gut-brain interactions, and the modulation of the gut flora can provide novel targets for people with various diseases associated with gut dysbiosis (Tillisch et al., 2013).

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Tan, M., Zhu, J., Du, J., Zhang, L., & Yin, H., (2011). Effects of probiotics on serum levels of Th1/Th2 cytokine and clinical outcomes in severe traumatic brain-injured patients: a prospective randomized pilot study. *Critical Care, 15*, 1-10.

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Vrieze, A., de Groot, P., Kootte, R., Knaapen, M., van Nood, E., Nieuwdorp, M. (2013). Fecal transplant: A safe and sustainable clinical therapy for restoring intestinal microbial balance in human disease? *Best Practice & Research Clinical Gastroenterology 27*, 127–137.

Vrieze, A., Van Hood, E., Holleman, F., Salojarvi, J., Kootte, R.S., Bartelsman, J., Dallinga-Thie, G., Ackermans, M., Serlie, M., Oozeer, R., Derrien, M., Druesne, A., Van Hylckama Vlieg, J., Bloks, V., Goren, A., Heilig, H., Zoetendal, E., Stroes, E., DeVos, W., Hoekstra, J., & Nieuwdorp, M. (2012). Transer of intestinal microbiota from leand donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology, 143*, 913-916.

Appendices

#1 Matrix for Research Articles

	Reference	Research Focus	Population	Variables & Measures	Findings
	Adams, J., Johansen, L., Powell, L., Quig, D., & Rubin, R. (2011). Gastrointestinal flora and gastrointestinal status in children with autism-comparisons to typical children and correlation with autism severity. *Gastroenterology, 11*(22), 1-13.	Gut flora (beneficial and pathogenic) and GI biomarkers investigated in ASD children compared to typical children	58 ASD children (2-18y/o) compared to 39 typical children who have not used antibiotics within last month.	Survey completed to assess GI status and severity as well as ASD severity. Single Stool sample assessed for bacterial and yeast cultures, lysosome, lactoferrin, secretory IgA, elastase, digestion markers, short chain fatty acids, pH, and blood presence.	GI symptoms strongly correlated with ASD children and increase with severity. Also had lower level of short chain fatty acids
	Ait-Belgnaoui, A., Durand, H. Cartier, C., Chaumaz, G., Eutamene, H., Ferrier, L., Houdeau, E., Fioramonti, J., Bueno, 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*, 1885-1895.	Does Lactobacillus Farciminis affect the HPA axis stress response, reverse changes in lipopolysaccharide translocation and central cytokine release, and prevent the "leaky" gut and LPS upload in the mucosa?	Female Wistar rats exposed to PRS model or sham-PRS model after receiving 1 of 3 experiments.	#1 Given L. farcimis for 2 weeks. Cytokines (IL-1B, IL-6, and TNF-a) levels measured. LPS concentration in portal vein measured. #2 treated with myosin light chain kinase(MLCK) inhibitor, ML-7. Then, LPS	L.farciminis and ML-7 enhances the epithelial barrier avoiding LPS upload decreasing intestinal permeability --> blocked the stress-induced hyperpermeability, endotoxemia and attenuated HPA axis stress response and central neuroinflammation.

				concentration, IL-1B, IL-6, & TNF-a hypothalamic expression determined. #3 treated with antibiotics to reduce LPS concentration. Corticosterone, cytokines, endotoxemia and hypothalamic pro-inflammatory cytokines mRNA expression measured.	Antibiotic reduction of LPS concentration prevented HPA axis stress response and increased pro-inflammatory cytokines.
	Azad, M., Konya, T., Maughan, H., Guttman, D., Field, C., Chari, R., Sears, M., Becker, A., Scott, J., & Kozyrskyj, A. (2013). *CMAJ, 185(5),* 385-394.	Characterize the gut microbiota of healthy Canadian infants and describe the influence of cesarean delivery and formula feeding.	Subset of 24 term infants from the Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort.	Mode of delivery was obtained from medical records, and mothers were asked to report on infant diet and medication use. Fecal samples were collected at 4 months of age, and we characterized the microbiota composition using high-throughput DNA sequencing.	Compared with breastfed infants, formula-fed infants had increased richness of species, with overrepresentation of *C.difficile, E.Shigella* and *Bacteroides* species were underrepresented in infants born by cesarean delivery. Infants born by elective cesarean delivery had particularly low bacterial richness and diversity.
	Barron, J. August 10, 2013 The Baseline of Health Foundation.	Gut bacteria responsible for 60-	Immunomodulators produce lactoferrin	Study in China revealed that in	

	The Benefits of probiotics-more than ever. http://jonbarron.org/natural-health/benefits-of-probiotics-immune-system?utm_source=iContact&utm_medium=email&utm_campaign=Jon%2520Barron&utm_content=Biweekly+Newsletter+8%252F12%252F13#footnote11_jpth0qf	70% of immune system function. Bacteria line every inch of intestinal tract creating a barrier to entry by directly killing pathogens, bad bacteria, viruses, fungi, parasites, and yeast.	which directly immune function and B vitamin production. 70% to as much as 90% of immune system in colon in lymphoid tissue below surface of epithelial cells.	children 3-5 years old treated with 2 probiotics 53% had lower rates of fever, 41% had reduced coughs, 28% has decreased runny nose, and antibiotic usage decreased by 84%.	
	Bercik, P., Denou, E., Collins, J., Jackson, W., Lu, J., Jury, J., Deng, Y., Blennerhassett, J., Macri, J., McCoy, K., Verdu, E., & Collins, S. (2011). The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. *Gastroenterology 141*(2), 599-609.	Examine whether the intestinal microbiota affects behavior and brain biochemistry in mice	Specific pathogen free(SPF) Germ free BALB/c mice given antimicrobials(ATMs)	SPF mice given antimicrobials x7days. Germ-free BALB/c and NIH Swiss mice colonized with microbiota. Behavior evaluated after exposed to step down test and light/dark test. Gut samples analyzed for levels of serotonin, noradrenaline, dopamine, and BDNF	1. Alteration of microbiota increased hippocampal BDNF and exploratory behavior. 2. Changes were reversible upon normalization of microbiota after withdrawal of ATMs. 3. ATM admin did not alter behavior in germ free mice. Intestinal microbiota influences brain chemistry and behavior independently of the autonomic nervous system, GI specific neurotransmitters, or inflammation. Intestinal dysbiosis might contribute to psychiatric disorders in patients with

					bowel disorders.
	Bravo, J., Forsythe, P., Chew, M., Escaravage, E., Savignac, H., Dinan, T., Bienenstock, J., Cryan, J. (2011). Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *PNAS, 108*(38), 16050-16055.	Show that chronic treatment with L. rhamnosus induces region dependent alterations in GABA mRNA in the brain with increases in cortical regions and concomitant reductions in expression in the hippocampus, amygdale, and locus coeruleus in comparison with control fed mice.	36 Adult male BALB/c mice given Lactobacillus.	Exposed to open field test, stress induced hypothermia (SIH), elevated plus maze (EPM), fear conditioning, and forced swim test (FST). Plasma corticosterone concentration measured.	Neurochemical and behavioral effects not found in vagotomized mice. L.rhamnosus has direct effect on behavioral and physiological responses in a manner dependent of vagus nerve. Identifying the vagus as a major modulatory communication pathway between the bacteria in the gut and the brain. L.rhamnosus fed mice also showed improved memory, reduced corticosterone levels. Bacteria is bidirectional in communication of the gut-brain axis.
	Caepa, C., Worpereis, H., Rezaiki, L., Kleerebezem, M., Knol, J., & Oozeer, R. (2013). Influence of fermented milk products, prebiotics and probiotics on microbiota composition and health. *Best Practice & Research Clinical Gastroenterology 27*, 139–155.	Describe the molecular mechanisms involved in the crosstalk between gut bacteria and the human host and review the impact of different nutritional concepts such as	Elie Metchnikoff-founding father of probiotics concept. Studied Bulgarian pop and usage of fermented milk as attribute for longevity. Tissier discovered Bifidobacteria in human milk and administered it to	Most important characteristic of probiotic are survival during passage thru stomach and intestine. Selection of suitable bacterial strains with specific properties and use of schemes	

		pre-, pro-, and symbiotics on the GI ecosystem and their potential health benefits.	infants with diarrhea in the 1950s.	for different patient groups.	
	Collado, M., Cernada, M., Baüerl, C., Vento, M., & Pérez-Martínez, G. (2012). Microbial ecology and host-microbiota interactions during early life stages. *Gut Microbes, 3*(4): 352–365.	Each person houses 160 species from 1000 to 1150 bacterial species whose collective genome aka microbiome contains at least 100 times as many genes as the human genome. Microbiota development of the infant is rapid and depends on first inoculums, mother's microbiota, mode of delivery, type of feeding and the environment, including weaning food practices and the use of antibiotics. Colonization found in umbilical cord, placenta, amniotic	Delivery mode will affect early stage of neonatal microbial colonization. Csection infants acquire those found on skin i.e. Staph, Corynebacterium and Propionibacterium. Increased risk for atopic diseases such as allergic rhinitis, asthma, and celiac disease. Vaginally delivered resemble mothers vagina i.e. Lactobacillus, Prevotella, Sneathia. Breastfed infants supplied constant supply of staph, strep, lactic acid bacteria, & bifidobacteria. Single most important postnatal element metabolic and inmmunological programming of the	Gestational age affects gut microbiota. Premature infants whom are hospitalized and separated from their mothers are colonized by their environment. Obesity associated with markedly reduced bacterial diversity, depletion of Bacteroidetes, and higher propoertion of Actinobacteria compared to lean subjects. High numbers of Bifidobacterium have positive correlation with normalization of inflammatory status and improved glucose tolerance and glucose induced	Gut microbes that interact with fetus before birth modulate brain developmental pathways. Exposure to high levels of IL-6 in utero alters brain development and leads to permanent behavioral impairments. Preterm, formula fed, csectioned, and those who required antibiotics and or intensive care are prone to develop diseases including infections such as diarrhea, necrotizing enterocolitis, atopic eczema and other allergic diseases. Infant faecal microbial composition is related to maternal weight and weight gain over pregnancy. Thus higher infant weight correlates with lower numbers of bifidobacteria.

		fluid and meconium. Early dietary and gut microbiological environments more profound effect on metabolic programming of a child. Nutritional habits in pregnancy appear to influence the type of microbes found in meconium- organic or biodynamic diet have lower numbers of E.coli.	child's health. Antimicrobials are not selective for pathogens and indiscriminately affect all members of the commensal gut,esp decreasing the levels of anaerobic bacteria such as Bacteroides, E.coli and beneficial bifidobacteria and increasing levels of harmful Klebsiella and clostridia.	insulin secretion. Atopic disease related to lower microbe exposure in early life. Fetal programming hypothesis states maternal exposures during pregnancy may induce long lasting or permanent changes in fetal physiology and thereby impact disease risk later in life.	
	Cryan, J., & O'Mahoney, S. (2011). The microbiome-gut-brain axis: from bowel to behavior. *Neurogastroenterology & Motility, 23*, 187-192.	Does a decrease in desirable gastrointestinal bacteria lead to a deterioration in gastrointestinal, neuroendocrine, or immune relationships and disease. Germ free mice are utilized to study the BGA because they are devoid of	Brain derived neurotropic factor (BDNF) crucial for supporting neuronal survival and encouraging the growth and differentiation of new neurons and synapses- regulate aspects of cognitive and emotional behaviors. The modulation of systemic		Specific modulation of the enteric microbiota may be useful strategy for stress-related disorders and for modulating the co-morbid aspects of gastrointestinal disorders such as IBS and IBD.

		bacteria, so the impact of complete absence of GI microbiota on behavior is examined. Animal studies are not translational to human disease but still incite data regarding enteric microbiota altering behavior.	inflammatory cytokines and oxidative stress could potentially lead to increased BDNF, involved in anxiety and depression. Lactobacillus reuteri (known to modulate the immune system) decreases anxiety. It alters the mRNA expression of GABAA and GABAB receptors in the central nervous system suggesting that parasympathetic innervations is necessary.		
	Desbonnet, L., Garrett, L., Clarke, G., Kiely, B., Cryan, J.F., & Dinan, T. G. (2010). Effects of the probiotic Bifidobacterium Infantis in the maternal separation model of depression. *Neuroscience, 170*, 1179-1188.	Explore the potential antidepressant properties of Bifidobacterium infantis in the rat MS model of depression by comparing the effects probiotic treatment with citalopram, on the adult behavioral	Rats given either Bifidobacteria or citalopram	exposed to Forced Swim Test (most widely used model for predicting antidepressant activity in rodents and increased immobility is considered to reflect behavioral despair). Cytokine concentrations, monoamine levels in	Probiotic administration resulted in normalization of immune response, reversal of behavioral deficit, and restoration of basal NA concentrations in the brainstem.

		phenotype and key systems involved in depression and gut-brain communication		the brain, and central and peripheral hypothalamic-pituitary adrenal(HPA) axis measures analyzed.	
	Diamond, B., Huerta, P., Tracey, K., & Volpe, B. (2011). It takes guts to grow a brain. *Bioessays, 33*, 588-591.	1. Bacteria in the gut changes rapidly after birth and food consumption that modulates brain pathways. 2. Also activate the systemic immunity. Only limited production of TH 17(responsible for controlling extracellular and fungal infections) results in the absence of microbiota. 3. With no commensal bacteria, production of IL-10 is diminished; therefore, proinflammatory cytokines are	4. Reversal is dependent upon the stage of brain development. There is a critical developmental window which intestinal microbiota can reverse behavioral phenotype and achieve normal brain development. 5. Although in infancy, rapidly expanding field focused on IDing and mapping the expression of immune based molecules in brain cells. Established that immune related signaling occurs not only in glia which are r/t monocytes and macrophages, but also neurons and	6. The nervous system exerts significant influence over the immune system through a reflex control set point. A cholinergic anti-inflammatory pathway transduced through the vagus nerve, and culminating in the down regulation of the monocytes in the spleen etc. Through this neural circuit the brain exerts a tonic inhibitory influence that prevents overexpression of cytokines.	Immune system + central nervous system from a modulatory function to the synaptogenesis necessary for normal cognitive function. It's a sensory organ that informs the brain about the status of infectious or sterile immune activation throughout the body. The activation of the afferent pathway results in synaptogenesis, thus determining the strength of the immune response.

		uninhibited.	endothelial cells present in the brain vasculature.		
	Dinan, T., & Cryan, J. (2012). Regulation of the stress presonse by the gut microbiota: Implications for psychoneuroendocrinology. *Psychoneuroendocrinology, 37*, 1369-1378.	BGA- bidirectional communication networ4k enables signals from the brain to influence the motor, sensory, and secretory modalities of the GI tract and conversely, visceral messages from the gut can influence brain function, esp areas of brain devoted to stress regulation, esp hypothalamus. The vagus is an important line of communication between the gut and HPA. The ENS is a complex neuronal network with multiple neurotransmitters and neuromodulators	Germ free paradigm based on the fact that uterine environment is sterile during prenatal development and with surgical delivery replacing the normal vaginal delivery , the opportunity for postnatal colonization of the gut is eliminated once animals are maintained in sterile environment. Antibiotic approach- disrupt the microbiome with deliberate infection and administration of abx since they will perturb biodiversity and delay colonization. In germ free mice, a mild stress induces exaggerated release of corticosterone and ACTH compared to	Early life stress can also have long term effects on the microbiome. Rats exposed to maternal separation for 3h per day from post natal says 2-12 revealed an altered fecal microbiome when compared to non-separated control animals. Demonstrates a decrease in beneficial bifidobacterium and increase in pathogenic Clostridium in the cecum. (Bravo) study found that chronic treatment with Lactobacillus rhamnosus over 28 days produced animals with lower levels of	(Martin) 30 human subjects who were classified high and low anxiety traits. Blood and urine samples collected. Those with higher anxiety traits showed a distinct metabolic profile: different energy homeostasis; hormonal metabolism, and gut microbial activity. A dietary intervention reduced the urinary excretion of both cortisol and catecholamines and partially normalized stress related differences in energy metabolism and gut microbial activities. (Messaoudi) L.helveticus and B.longum given to healthy volunteers. Reduction in cortisol noticed. Possibly d/t decrease in proinflammatory cytokines, which activate the HPA or alternatively

		such as 5HT, acetylchline, and CRF. The gut has 1014 microorganisms; which is 10x the number of human cells in our bodies and contains 150x as many genes as our genome. Consists of greater than 1000 species and more than 7000 strains. Once threat (infection, disease, diet and antibiotics) has subsided, microbiome tends to revert to stable diversity established in infancy. The number of bifidobacterium decreases with age and parallels changes in health status and decreased plasticity within the HPA. HPA	the specific pathogen free SPF controls. The stress response in the GF mice is partially reversed by colonization with fecal matter from SPF mice and fully reversed by Bifidobacterium Infantis. This study demonstrates microbial content of the gut is critical to the development of an appropriate stress response later in life and also that there is an narrow window in early life where colonization must occur to ensure normal development of the HPA axis.	corticosterone and reduced depressive behaviors in the forced swim test. Lactobacillus strains (Rousseaux) could induce the expression of u-opioid and cannabinoid receptors in intestinal epithelial cells and mimic the effects of morphine in promoting analgesia. (Desbonnet) B. infantis shown to induce an elevation in plasma tryptophan levels, a precursor to serotonin (5-HT) a key neurotransmitter in the brain. The implication is that the microbiota might play a role in the regulation of CNS as well as enteric nervous system 5-HT synthesis.	by an alteration of neurotransmitter inputs such as 5-HT. Brain can alter microbiome. Signals molecules released into gut lumen from cells in lamina propria that are under the control of the CNS changes GI motility and secretion and permeability. Thus altering microbiome. Usually nonpathogenic bacteria in the colon are restrained by the intestinal epithelium. Psychological stress can increase permeability of the gut allowing bacteria and antigens to cross the epithelial barrier activating mucosal immune response altering proinflammatory cytokines and perhaps activate the HPA. Prenatal stress reduced the overall number of bifidobacteria and lactobacilli.

		strongly implicated in susceptibility to development of obesity and metabolic syndrome.			
	Fortner, K., Grotegut, C., Ransom, C., Bentley, R., Feng, L., Lan, L., Heine, R., Seed, P., & Murtha, A. (2014). Bacteria Localization and Chorion Thinning among Preterm Premature Rupture of Membranes. *PLoS ONE 9*(1): e83338. doi:10.1371/journal.pone.0083338	Examine fetal membrane chorion thinning and to correlate to bacterial presence in PPROM, preterm, and term subjects.	Paired membrane samples (membrane rupture and membrane distant) were prospectively collected from: PPROM = 14, preterm labor (PTL = 8), preterm no labor (PTNL = 8), term labor (TL = 10), and term no labor (TNL = 8), subjects.	Sections were probed with cytokeratin to identify fetal trophoblast layer of the chorion using immunohistochemistry. Fluorescence in situ hybridization was performed using broad range 16 s ribosomal RNA probe. Images were evaluated, chorion and choriodecidua were measured, and bacterial fluorescence scored. Chorion thinning and bacterial presence were compared among and between groups using	In all groups, the fetal chorion cellular layer was thinner at rupture compared to distant site. Further, chorion thinning was greatest among PPROM subjects compared to all other groups combined. Bacteria counts were highest among PPROM subjects compared to all other groups regardless of site sampled or histologic infection. In PPROM fetal chorion, we demonstrated pronounced global thinning. Although cause or consequence is uncertain, bacterial presence is greatest and inversely correlated with chorion thinning among PPROM subjects.

				Student's t-test, linear mixed effect model, and Poisson regression model.	
	Gareau, M., Wine, E., Rodrigues, D., Cho, J., Whary, M., Philpott, D., MacQueen, G., & Sherman, P. (2011). Bacterial infection causes stress-induced memory dysfunction in mice. *Gut 60*, 307-317.	Examine whether acute enteric infection or absence of gut microbiota has an effect on behavior specifically anxiety and non-spatial memory formation.	Determine whether Clostridium Rodentium (causing colitis) infection resulted in anxiety like behavioral or memory changes & whether probiotics provide protection.	Behavior observed in C57 lBL/6 and germ free mice after inoculation of C rodentium in the presence and absence of water avoidance stress (WAS). Probiotics administered and evaluated.	C rodentium causes stress induced memory dysfunction in mice Probiotics given before and during prevent memory dysfunction Germ free mice lack memory even in absence of stress
	Kang, D., Gyoon, J., Ilhan, Z., Wallstrom, G., LaBaer, J., Adams, J., & Krajmalnik-Brown, R. (2013). Reduced Incidence of Prevotella and Other Fermenters in Intestinal Microflora of Autistic Children. *PLoS ONE 8*(7): e68322. doi:10.1371/journal.pone.0068322	Define systemic changes in gut microbiome associated with autism and autism-related GI problems.	20 neurotypical and 20 autistic children accompanied by a survey of both autistic severity and GI symptoms.	We compared gut microbiomes of GI symptom-free neurotypical children with those of autistic children mostly presenting GI symptoms.	Abnormal breakdown of carbohydrates affects the amount and type of nutrients for intestinal bacteria, thereby, resulting in ill digestive bacteria-contributing to poor digestion, intestinal inflammation, & more sever autism. Seven metabolites noted, 3 of significance. 1. Homovanillate, normally produced when dopamine is broken down, 2. N,N-dimethylglycine was found at lower levels

					(used before to decrease autism symptoms). 3. The ratio of glutamine to glutamate was higher. An imbalance between glutamate and GABA transmission has been associated with autistic-spectrum type behaviors such as hyper-excitation. Fecal transplant studies?
	Kennedy, P., Clarke, G., Eamon, Q., Groeger, J., Dinan, T., & Cryan, J. (2012). Gut memories: Towards a cognitive neurobiology of irritable bowel syndrome. *Neuroscience and Biobehavioral Reviews, 36,* 310-340.	Summarize data on cognitive function in IBS-stress, immune activation and chronic pain-an how they manifest in IBS, impact central neurobiological mechanisms.	Fig 1 pg. 312. Pathways involved in the bidirectional comm.. the CNS modulates GI functioning and in turn, signals from the viscera to influence brain. CNS influences GI motility and secretion via descending autonomic pathways (sympathetic & parasympathetic) by modulating activity of the enteric system. Ascending visceral signals arising from the ENS are received by higher brain regions via ascending	Glucocorticoids released from adrenal glands through activation of the HPA axis impacts the immune system while immune parameters can stimulate HPA axis. Proinflammaotry cytokines impact centrally via stimulation of the vagus nerve. Immune system influences the functioning NES which impacts the CNS. Enteric microbiota has key role in development	Germ free rodents found to with reduced hippocampal and cortical BDNF levels and exaggeraged HPA axis response to stress which normalized with probiotic Bifidobacterium infantis (Sudo). Reduction in visceral pain perception exhibited by rats fed a lactobacillus(Kamiya)or B.infantis.

			autonomic, spinal, and vagal afferents. Stress mood, cognitions and emotion can influence descending pathways to modulate GI function and the perception of signals arising from the viscera via limbic, paralimbic and cortical brain regions.	of systemic and mucosal immune systems and also in the normal development of both the GI tract and CNS. Other mediators essential to brain gut axis signaling are neurotransmitters and neuropeptides such as serotonin, noradrenaline, and corticotrophin releasing factor.	
	Koloski, N., Jones, M., Kalantar, J., Weltman, M., Zaguirre, J., Talley, N. (2012). The Brain-Gut Pathway in Functional Gastrointestinal Disorders Is Bidirectional, *Gut, 61*(9), 1284-1290.	Determine the directionality of the brain gut mechanism in functional GI disorders (FGIDs) i.e. IBS & functional dyspepsia (FD).	12 year longitudinal, prospective, population based study, randomized. N=1775 participants in Australia	Survey completed in 1997 on FGIDs. N=1002 completed survey in 12 year f/u. Anxiety and depression measured using the Delusion Symptoms States Inventory at baseline and f/u.	The central nervous system and gut interact bidirectionally in FGIDs. Among people free of a FGID at baseline, higher levels of anxiety but not depression at baseline was a significant independent predictor of developing new onset FGIDs 12 years later. Among people who did not have elevated levels of anxiety and depression at baseline, those with a FGID at baseline had significantly higher levels

					of anxiety and depression at f/u. In IBS higher levels of anxiety and depression at baseline were predictive of IBS at f/u, while only depression was predictive of FD at f/u.
	Konturek, P., Brzozowski, T., & Konturek, S. (2011). Stress and the gut: Pathophysiology, clinical consequences, diagnostic approach and treatment options. *Journal of Physiology, 62*(6), 591-599.	Interactions between the CNS and The enteric nervous system or ENS(aka little brain) regulates physiological gut functions including secretion, motility, and release of neuropeptides and hormones.	Brain communication through parallel pathways- ANS, HPA, and the brain gut axis (BGA). Cross talk between gut micorbiota, immune system, & BGA causing diseases of gut if exposed to stress.	BGA and microbiota communicate via direct interaction with mucosal cells (endocrine message), via immune cells(immune message), and via contact to neural endings(neuronal message). Stress causes changes in composition of microbiota inducing changes in neurotransmitter and proinflammatory cytokine levels affecting microbiota.	Exposure to chronic stress is major risk factor for pathogenesis for GER, PUD. IBD, IBS. The dysregulation of BGA plays a central role in pathogenesis of stress induced diseases. Stress increases intestinal permeability, visceral sensitivity, alteration of GI motility and leads to profound mast cell activation resulting in release of proinflammatory mediators.
	Koren, O., Goodrich, J., Cullender, T., Spor, A., Laitinen, K., Backhed, H., Gonzalez, A., Werner, J., Angenet, L., Knight, R., Backhed, F, Isolauri, E.,	How does pregnancy alter the gut microbiome?	Prospective, randomized mother-infant study 91 pregnant women Infant microbiota studied at 1 month	Obtained stool samples, diet information, and clinical data from pregnant women in the first and third	Pregnancy associated with profound alteration of gut microbiota. 1st trimester similar to non-pregnant female and male controls. 3rd trimester, the

	Salminen, S., & Ley, R. (2012). Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell, 150*, 470-480.		old, 6 months old, and 4 years old.	trimesters.	structure and composition resembles dysbiosis. Dysbiosis, inflammation, and weight gain = metabolic syndrome. Same changes seen in pregnant women. Hyperglycemic state provides continuous supply of nutrients to fetus. Adiposity prepares female body for energetic demands of lactation. These metabolic changes necessary for healthy pregnancy. Children's microbiota most similar to mother's microbiota at T1. T3 had no bearing.
	Lee, Y., & Chua, A., (2011). Influence of Gut Microbes on the Brain-Gut Axis. Journal of Neurogastroenterology and *Motility, 17*(4), 427-429.	Intestinal microbiota influences central control of BGA. Gareau et al exposed link between enteric infection and impaired learning & memory in the presence of psychological stress. Memory	Probiotics improved memory deficits, normalized HPA and colonic injury, restored hippocampus brain derived neurotropic (BDNF) & c-Fos (responsible for neuronal activation), reduced serum corticosterone, ameliorated colonic injuries and		Gareau et al demonstrated the beneficial effect of probiotics in preventing stress induced memory deficits, normalizing alteration in the HPA axis and colonic injury. Associated with the hippocampus brain-derived neurotropic factor (BDNF) & c-Fos expression in the context

		impairment persisted after bacterial clearance and resolution of pathogens.	normalized changes in intestinal microbiota.		of normalized microbiota. Germ-free mice also displayed an absence of memory, providing support for the requirement of commensal gut microbiota in memory.
	Leslie, M. November 13, 2014. Cells can fight viruses, even when stimulated to combat bacteria. Science Magazine. http://news.sciencemag.org/biology/2014/11/cells-can-fight-viruses-even-when-stimulated-combat-bacteria	Can flagellum initiate immune response again rotavirus?	Mice injected with flagellin under skin.	Flagellin appears to stimulate intestinal epithelial cells to become more virus-resistant, the researchers found. Gewirtz says his team is investigating whether treatment with flagellin (or IL-18 and IL-22, the molecules it is acting through) may be helpful in fighting other viral infections such as norovirus.	Treatment with bacterial flagella could prevent rotavirus infection in mice as well as clear chronic infections. For this effect to occur, they say that both the flagellin receptors Toll-like receptor 5 (TLR5) and NOD-like receptor C4 (NLRC4) were needed. The flagella seemed to set off TLR5 activation in dendritic cells that then induced cytokine interleukin-22 (IL-22), which, in turn, led to changes in gene expression in intestinal epithelial cells.
	Li, W., Dowd, S., Scurlock, B., Acosta-Martinez, V., & Lyte, M. (2009). Memory and learning behavior in mice is temporally associated with	Whether bacterial diversity due to dietary manipulation is correlated with	5 week old male CF1 mice randomly assigned to receive standard diet or chow with 50% lean ground	Trained and tested on a hole-board open field apparatus. Behavioral testing. Colonic stool	The BD diet group had significantly higher bacterial diversity and displayed improved working and reference

	diet-induced alterations in gut bacteria. *Physiology & Behavior, 96*, 557-567.	memory and learning	beef for 3 months	samples collected/analyzed via ARISA and bTEFAP for microbial diversity	memory, and reduced anxiety levels. Indicates correlation between dietary induced shifts in bacteria diversity and animal behavior that may indicate a role for gut bacterial diversity in memory and learning.
	Maes, M., Kubera, M., & Leunis, J. (2008). The gut-brain barrier in major depression: Intestinal mucosal dysfunction with n increased translocation of LPS from gram negative enterobacteria (leaky gut) plays a role in the inflammatory pathophysiology of depression. Neuroendocrinology, 29(1), 117-124.	To examine another immune pathway-whether MDD is accompanied by increased serum levels of IgM and IgA against LPS of 6 enterobacteria indicating an immune response directed to endotoxins secreted by gram-negative enterobacteria and which cannot be detected when the gut-intestinal lining is intact.	51subjects, 23 controls, 28 MDD patients. Excluding other psychiatric conditions, CFS pts, those taking psychotropic drugs, abnormal lab values, and acute allergic reactions.	Total sum of FibroFatigue scale and CFS rating scale to demonstrate severity. Correlation with IgM and IgA levels against LPS in CFS. Labs drawn to determine the IgM and IgA against LPS of 6 different enterobacteria.	MDD is accompanied by increased serum levels of IgM and IgA directed against LPS of gram negative enterobacteria. IgM-IgA values are r/t MDD/CFS symptoms. Increased levels accompanied by increased gut permeability and IRS against LPS of enterobacteria. The epithelial barrier 1.separates the luminal contents from the interstitium, and which protects against microorganisms including gram negative bacteria, larger toxic and antigenic molecules; 2. Transportation of fluids,

					electrolytes, and nutrients across the intestinal wall; 3. Secretion of IgA to bind to bacteria preventing attachment to epithelial cells. IRS activation causes loss of protective barrier with enlarged spaces between the cells of the gut wall aka leaky gut. Systemic increases in LPS cause central neuroinflammation with increased TNFalpha that remains elevated for 10 months. Activation of brain migroglia means elevated proinflammatory mediators (IL-6, IL-1B, TNFalpha) inducing MDD symptoms. Therefore, MDD pts screened for IgM and IgA levels against LPS of gram negative bacteria. If leaky gut present, treat with antioxidants and appropriate diet.
	Matamoros, S., Gras-Leguen, C., Le Vacon, F., Potel, G., & de La Cochetiere, M. (2013). Development of intestinal	Most of human microbiome located in digestive tract.-1014	Preterm infants Enterobacteriaceae & other pathogenic bacteria C.difficile or	Antibiotic therapy in infants-microbiota differences detected 1month after	Beneficial effects-protection against infections, diarrhea, necrotizing enterocolitis,

	microbiota in infants and its impact on health. *Trends in Microbiology, 21*(4), 16-173.	bacterial cells & >100x more genes than human genome. Human Microbiome Project investigating diversity of human bacterial population. Only 25% has been cultured so far. Initial low diversity and complexity, slowly develop and mature reaching adult state around 3 y/o. Classic pattern- born with E.coli and Enterobacteriaceae. When depleted of O2 gut becomes strictly anaerobic- Bifidobacterium, Clostridium, & Bacteriodes, Ruminococcus.	Kleb.pneumonia- indicating acquisition of hospital related microbiota. Full term- higher diversity Bifidobacterium, Lacobacillus, & Strep. Influenced by mother- microbiota functionally and phylogenetically close to mothers at 1 month of age. Mode of delivery- Csection vs vaginal. Bifidobacterium high in vaginal. Skin contact with Csection. Mode of feeding- Breast milk important for early gut colonization with bifidobacterium and lactobacillus w/low counts of bacteroides, clostridium coccoides, staph, and enterobacteriaceae. BM is source for oligosaccharides-exert a strong prebiotic effect for the neonates	treatment-reduced microbial fecal diversity & microbial counts. Antibiotic therapy in mothers (prenatal or breastfeeding) associated with less Bacteroides, Atopobium, and lower total sum. *reduction of phylogenetic diversity in infants linked with neonatal sepsis.	eczema, and atopic dermatitis. Csection increases chances of celiac dz, type 1 diabetes, and asthma- generally associated with excessive or aberrant T helper responses. Children exposed to antibiotics 0-6months have significantly higher body weight than unexposed children. (Tresande, L. et al. (2013). Infant antibiotic exposures and early life body mass. Int. J. Obes, 37, 16-23.

			microbiota. Environment- cultural and geographical factors influence development. Major source of bacteria that will colonize gut in 1st year.		
	Mayer, E. (2011). Gut feelings: the emerging biology of gut-brain communication. Nat Rev Neuroscience, 12(8), 1-30.	200-600 million neurons in ENS = to # of neurons in spinal cord. It interfaces with largest body surface-intestinal surface area is 100 times larger than the surface area of skin-with largest pop of commensal microorganisms of all surfaces-100 trillion microorganisms form 40k species with 100xthe number of genes in human genome.	With gut- associated immunity containing 2/3 of immune cells. And thousands of enteroendocrine cells containing more than 20 hormones. Three basic mechanisms by which sensory info is encoded: primary afferent, immune cells, and enteroendocrine cells.	Neuronal Signaling: Afferent Neurons Divided Into Extrinsic (Spinal & Vagal Afferents), Intrinsic, Primary Afferents (Ipans). Intrinsic And Extrinsic Reflex Regulation Of Intestinal Function Relies Primarily On Intrinsic Reflex Regulation, Mediated By Ipans And Enteric Motorneurons.	Endocrine And Paracrine Signaling: <1% of gut epithelial cells, comprise largest endocrine organ of body. Involved in both regulation of digestive and functions and CNS processes through the endocrine and paracrine signaling to vagal afferents. Immune related signaling: 70-80% of immune cells within gut lymphoid tissue. Immune cells remain immunologically hyposresponsive to commensal bacteria but respond to pathogens suggesting intestinal immune system recognize commensal bacteria and elicits basal or tonic signals in the

					absence of full activation of the innate and adaptive immune response.
	Messaoudi, M., Lalonde, R., Violle, N., Javelot, H., Desor, D., Nejdi, A., Bisson, J., Rougeot, C., Pichelin, M., Cazaubie,l M., & Cazaubiel, J. (2011). *The British Journal of Nutrition, 105*(5), 755-64.	The aim of the present study was to investigate the anxiolytic-like activity of PF in rats, and its possible effects on anxiety, depression, stress and coping strategies in healthy human volunteers.	Preclinical study – Rats Double-blind, placebo controlled, randomized parallel group study, healthy human volunteers	Rats administered probiotic formulation (PF) then tested using conditioned defensive burying test. Volunteers administered PF for 30 days then analyzed using Hopkins Symptom Checklist (HSC), Hospital Anxiety and Depression Scale (HADS), the Perceived Stress Scale, the Coping Checklist (CCL), & 24hr urinary free cortisol (UFC).	Daily administration of PF reduced anxiety like behavior in rats and alleviated psychological distress in volunteers. Taken in combination, L. helveticus and B.longum display anxiolytic like activity in rats and beneficial psychological effects in humans
	Neufeld, K., Kang, N., Bienenstock, J., & Foster, J. (2011). Reduced anxiety-like behavior and central neurochemmical change in germ-free mice. *Neurogastroenterology & Motility, 23,* 255-e119.	Does commensal intestinal microbiota normally communicate with the brain and does their presence influence CNS	Investigated the basal behavior of adult Germ-free (GF) mice, Swiss Webster female mice in the elevated plus maze (EPM) and compared this to conventionally reared	Tissue and blood samples collected. Corticosterone analysis performed. Locomotor activity assessed.	Germ free mice, compared to SPF mice, exhibited anxiety in the EPM. There was also a decrease in the N-methyl-D-aspartate receptor subunit NR2B mRNA expression in the central

		development and behavior?	specific pathogen free(SPF) mice.		amygdale, increased brain-derived neurotrophic factor expression and decreased serotonin receptor in the hippocampus. The presence or absence of conventional intestinal microbiota influences the development of behavior, and neurochemical changes in the brain.
	O'Hara, A., & Shanahan, F. (2007). Gut Microbiota: Mining for Therapeutic Potential. Clinical Gastroenterology and Hepatology, 5, 274-284.	Intestine diverse and dynamic bacterial community that is only separated by single layer of epithelial cells. Bacteria >genome 10 fold.	Mammalian fetuses are born germ-free but after birth, establishment of resident microbiota is driven by environmental factors such as mode of delivery, type of infant diet, hygiene levels, and medication.	Along epithelium, enteric bacteria form a natural defense barrier against exogenous microbes. Colonization increases glucose uptake in gut, and compared with colonized mice, germ-free mice require a greater caloric intake to sustain a normal body weight.	This implicates intestinal bacteria as modulators of fat deposition in the host. Individual's gut microbiota has specific metabolic efficiency, and differences in gut microbiota composition between individuals might regulate energy storage and predispose to obesity.
	Ohland, C., Kish, L., Bell, H., Thiesen, A., Hotte N., Pankiv, E., & Madsen, K. (2013). Effects of Lactobacillus helveticus on murine behavior are dependent on diet and	Determine whether the modulatory effects of probiotics differ depending on diet and mouse	Wild type mice and IL-10 deficient mice	Fed either a lab chow diet or western style diet with/without the administration of Lacotbacillus	L. helveticus effects depend on mouse genotype and diet. Overall alleviates psychological consequences of

	genotype and correlate with alterations in the gut microbiome. *Psychoneuroendocrinology, 38*, 1738-1747.	genotype.		helveticus. Intestinal immune function analyzed for cytokine expression. Memory and anxiety assessed using Barnes maze. Fecal microbiota analyzed.	intestinal disease. Combo of probiotic and western diet exacerbate. Diet is significant confounding factor in probiotic effect.
	Qin, J., Yingrui, L., Cai, Z., Li, S., Zhu, J., Zhang, F., Liang, S., Zhang, W., Guan, Y., Shen, D., Peng, Y., Zhang, D., Jie, Z., Wu, W., Qin, Y., Xue, W., Li, J., Han, L., Lu, D., Wu, P., Dai, Y., Sun, X., Li, Z., Tang, A., Zhong, S., Li, X., Chen, W., Xu, R., Wang, M., Feng, Q., Gong, M., Yu, J., Zhang, Y., Zhang, M., Hansen, T., Sanchez, G., Raes, J., Falony, G., Okuda, S., Almeida, M., LeChatelier, E., Renault, P., Pons, N., Batto, J., Zhang, Z., Chen, H., Yang, R.., Zheng, W., Li, S., Yang, H., Wang, J., Ehrlich, S., Nielsen, R., Pedersen, O., Kristiansen, K., & Wang, J. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature 490*, 55-60.	Assess and characterize the gut microbiota in type 2 diabetics	Two staged Metagenome-wide association study (MGWAS) performed on 345 Chinese volunteers	two-stage MGWAS based on deep shotgun sequencing of the gut microbial DNA	Identified and validated approximately 60,000 type-2-diabetes-associated markers and established the concept of a metagenomic linkage group, enabling taxonomic species-level analyses. MGWAS analysis showed that patients with type 2 diabetes were characterized by a moderate degree of gut microbial dysbiosis, a decrease in the abundance of some universal butyrate-producing bacteria and an increase in various opportunistic pathogens, as well as an enrichment of other microbial functions conferring sulphate reduction and

					oxidative stress resistance.
	Quigley, E. (2008). Probiotics in functional gastrointestinal disorders: What are the facts?: Current Opinion in Pharmacology, 8, 704-708.	probiotics do not replace pathogens; rather they increase bacteriocins, inhibit bacterial translocation, enhance mucosal barrier function, generating signals with the epithelium and immune system to modulate the IRS.	Probiotics offer considerable potential in treatment of functional GI disorders such as IBS. However, more large randomized double blind studies on human subjects are needed.		
	Rao, V., Bested, A., Beaulne, T., Katzman, M., Iorio, C., Berardi, J., & Logan, A. (2009). A randomized, double-blind, placebo-controlled pilot study of a probiotic in emotional symptoms of chronic fatigue syndrome. *Gut Pathogens*, 1-6	Determine how the administration of Lactobacillus casei Shirota to CSF (Chronic Fatigue Syndrome) patients influences mood related symptoms	39 CFS patients 18-65 y/o randomized suitable to complete two month study with depression and anxiety disorders only who are not bedridden	Initially completed Beck Depression Inventory (BDI) & Beck Anxiety Inventory (BAI). Stool samples taken. Consumed probiotic or placebo for 8 weeks. Then reevaluated using BDI and BAI and second stool analysis conducted.	Treatment group showed moderate increases in fecal total aerobes and anaerobes with significant increases in Bifidobacteria and Lactobacillus – contributing towards predominance of bacteria associated with healthy GI system. According to BDI and BAI scores, significant improvement in anxiety scores with treatment group.
	Tan, M., Zhu, J., Du, J., Zhang,	Examine whether	Prospective	Probiotic group	Probiotics group had

	L., & Yin, H., (2011). Effects of probiotics on serum levels of Th1/Th2 cytokine and clinical outcomes in severe traumatic brain-injured patients: a prospective randomized pilot study. *Critical Care, 15*, 1-10	the enteral administration of probiotics would adjust the Th1/Th2 imbalance and improve clinical outcomes in TBI patients.	randomized single blind study. 52 patients with severe TBI and Glascow Coma Scale (5-8).	given 21 day course of probiotics via NG tube	lower incidence of nosocomial infections with shorter ICU stays. Therefore probiotics could attenuate the deviated Th1/Th2 response induced by TBI.
	Theije, C.,, Wu, J., da Silva, S., Kamphius, P., Garssen, J., Korte, S., & Kraneveld, A. (2011). Pathways underlying the gut-to-brain connection in autism spectrum disorders as future targets for disease management. *European Jouranl of Pharmacology, 668*, S70-S80.	Enteric biopsies of 3 autistic children revealed: higher number of infiltrated helper and cytotoxic T cells an CD19+ B cells; more infiltration of helper T cells. ASD related microbial species mainly comprised of Clostridium strains, Ruminococcus, Bacteroidetes, Bacteroides, Firmicutes, and Desulfovibrio species.	Colonization of Clostridium species to the expense of Bifidobacterium associated with higher risks of allergy in children and pediatric IBD. GI disturbances, such as changes in the gut microbiota and T cell infiltration, indicate altered immune in ASD. Hypothesized that gut inflammation makes one with genetic predisposition for ASD more prone to express the autistic phenotype or increases severity.	Prebiotics not only have effect on enteric mucosa but also on systemic immunity by selectively stimulating the growth and or activity of Bifidobacteria and lactic acid bacteria in the colon. Human milk favors the growth of bifidus flora which activates the immune system and defends from pathogens. Non digestible oligosaccharides are examples of prebiotics and serve as substrates for bacterial metabolism.	Probiotics is associated with a reduction in the risk of nonspecific GI infections and lower frequency of colic or irritability. Lower levels of beneficial bifidobacterium were observed in ASD patients. Low levels associated with food allergy, inflammatory bowel diseases.

	Tillisch, K., Labus, J., Kilpatrick, L., Jiang, Z., Stains, J., Ebrate, B., Guyonnet, D., Legrain-Raspaud, S., Trotin, B., Naliboff, B., & Mayer, E., (2013). Consumption of fermented milk product with probiotic modulates brain activity. *Gastroenterology, 144*, 1394-1401.	Determine whether consumption of a fermented milk product with probiotic (FMPP) altered intrinsic connectivity or responses to emotional attention tasks.	Single center, randomized, controlled, parallel-arm design. Healthy women, 18-55 y/o, h no GI or psych symptoms randomly assigned to groups given FMPP (n=12), a nonfermented control milk product (n=11,control), or no intervention (n=13) twice daily for 4 weeks.	Underwent MRI before and after intervention measuring brain response to an emotional faces attention task and resting brain activity.	FMPP associated with reduced task related response of a distributed functional network containing affective, viscerosensory, and somatosensory cortices. Alterations in intrinsic activity of resting brain indicated that ingestion of FMPP associated with changes in midbrain connectivity that control central processing of emotion and sensation.
	Vrieze, A., de Groot, P., Kootte, R., Knaapen, M., van Nood, E., Nieuwdorp, M. (2013). Fecal transplant: A safe and sustainable clinical therapy for restoring intestinal microbial balance in human disease? *Best Practice & Research Clinical Gastroenterology 27*, 127–137.	Show the available evidence regarding the involvement of intestinal microbiota and human (autoimmune) disease. Also, show the beneficial or adverse effects of fecal transplantation on clinical outcomes of certain disease states.	(Borody) 3 pts with MS underwent daily fecal transplantation for 1-2 weeks were asymptomatic (no constipation or neurological disability) ranging from 2-15 years post treatment. Can alter insulin resistance and clostridium difficile diarrhea.	Fecal transplantation via enemas, infusion via duodenal or gastric tube, colonoscopy and self-administration via rectum.	Larger randomized double blind trials implementing gut microbiota before and after fecal transplantation intervention showing clinical outcomes in regards to disease states.
	Vrieze, A., Van Hood, E., Holleman, F., Salojarvi, J., Kootte, R.S., Bartelsman, J.,	Does rebalancing the obesogenic microbiota by	Treatment naive male subjects with metabolic syndrome	Underwent small intestine biopsies and bowel lavage	Significant modification in intestinal microbiota composition in fecal

	Dallinga-Thie, G., Ackermans, M., Serlie, M., Oozeer, R., Derrien, M., Druesne, A., Van Hylckama Vlieg, J., Bloks, V., Goren, A., Heilig, H., Zoetendal, E., Stroes, E., DeVos, W., Hoekstra, J., & Nieuwdorp, M. (2012). Transer of intestinal microbiota from leand donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology, 143*, 913-916.	small intestinal infusion of gut microbiota from a lean donor positively affect (host) metabolism and insulin sensitivity in subjects with metabolic syndrome?	randomized double blind trial	via duodenal tube then inoculation of autologous or allogenic gut microbiota infusion. insulin sensitivity tested 6 weeks post as well as measure gut microbiota	samples after allogenic gut microbiota infusion-2.5fold increased. increased gut flora increases insulin sensitivity and therefore decreases insulin resistance