Introduction
The microbiota-gut-brain axis has been explored broadly for its role in behavior and the mind, including pain perception, cognitive function, mood and emotion, temperament, stress management, and even social interaction. In the *Journal of Physiological Anthropology*, Selhub, Logan, and Bested hypothesize that increased prevalence of anxiety and depressive disorders can be attributed, in part, to a decrease in microbial diversity fueled by modern food choices such as decreased intake of dietary fibers acting as microbiota-accessible carbohydrates, citing population studies which link traditional healthy diets (such as Mediterranean or Japanese) with lower risk of anxiety or depression. Skillful application of therapeutic lifestyle interventions which strategically modify the microbiome, even if modifications are transient in nature, holds potential to address the significant burden of anxiety and depressive disorders.

The most recent data available from the Centers for Disease Control and Prevention indicates that 8 million ambulatory care visits (to physician offices, outpatient clinics, and emergency departments) carry major depressive disorder (MDD) as the primary diagnosis. Anxiety disorders, which include panic disorder and generalized anxiety disorder among others, are the most common class of mental disorders present in the general population. Anxiety is described as a frequent, negative emotional state characterized by feelings of worry and apprehension and accompanied by specific cognitive and behavioral manifestations. Anxiety is diagnosable as a disorder when “the anxiety, worry, or physical symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.” The experience of anxiety is accompanied by overactive activity in the amygdala and changes in prefrontal cortex, areas of the brain which regulate critical emotion. In addition to γ-aminobutyric acid (GABA), serotonin, opioid peptides, endocannabinoids, neuropeptide Y, oxytocin, and corticotrophin-releasing hormone are also implicated in stress disorders.

The Inflammation Connection
Through immune-brain signaling pathways, chronic low-grade inflammatory processes are thought to impact neurochemical changes involved in the pathogenesis of neuropsychiatric disorders including major depression and anxiety disorders. Dinan describes major depression as a “common, debilitating stress-related disorder whereby patients frequently experience hypothalamic-pituitary-adrenal (HPA) alterations such as elevated cortisol levels in plasma, elevated corticotrophin releasing factor (CRF) levels in the cerebrospinal fluid, and increased concentrations of pro-inflammatory cytokines.” Specifically, increased expression of IL-1β, IL-6, TNF-α, interferon gamma (IFN-γ), and C-reactive protein (CRP) have been repeatedly observed in depressed patients. Administration of probiotics can alter levels of circulating cytokines, as has been seen with *Bifidobacterium infantis* normalizing peripheral pro-inflammatory cytokine concentrations. In a study assessing the effects of probiotic-supplemented yogurt (*Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14) in 20 subjects with inflammatory bowel disease (IBD), the yogurt decreased concentration of TNF-α and IL-12–producing monocytes measured in peripheral blood. Additionally, decrease of those pro-inflammatory cytokines correlated with increased presence of regulatory T cells (CD4⁺CD25⁺), which are central to immune-based down-regulation of ongoing inflammation.
The potential to use targeted probiotics to modulate inflammatory mediators and impact mood via neuroendocrine systems or the vagus nerve is emerging.\textsuperscript{11}

**Probiotics and Psychobiotics**

Probiotics are defined broadly as microorganisms that, when consumed, maintain or restore beneficial bacteria to the digestive tract. In contrast, the term \textit{psychobiotics} describes “a live organism that, when ingested in adequate amounts, produces a health benefit in patients suffering from psychiatric illness.”\textsuperscript{11} Mechanistically, psychobiotics should have demonstrated behavioral effects, act as vehicles of delivery for neurochemicals, and have the ability to decrease pro-inflammatory cytokines and reduce HPA activity.\textsuperscript{11} The term \textit{microbial endocrinology} has also been used to describe “bidirectional neurochemical interactions between the host’s neurophysiological system and the microbiome.”\textsuperscript{12}

Essentially, the gut-brain axis consists of bidirectional communication between the gastrointestinal (GI) tract, or enteric nervous system, and the central nervous system, resulting in a linkage of peripheral intestinal functions with the emotional and cognitive centers of the brain.\textsuperscript{13} The enteric nervous system, often referred to as the “second brain,” consists of an estimated 200 million to 600 million neurons.\textsuperscript{14}

Signaling molecules from the microbiome include amino acid metabolites, short chain fatty acids, and neuroactive substances.\textsuperscript{15} As peptides and hormones produced by bacteria can directly or indirectly regulate the host behavior,\textsuperscript{16} interventions targeting the gut-brain axis could be novel treatments for both anxiety and gastrointestinal disorders such as irritable bowel syndrome (IBS).\textsuperscript{9}

Cryan and Dinan outline several mechanisms by which microbiota affect central nervous system function\textsuperscript{9}:

- Altering microbial composition (allowing for improvements in gut barrier function)
- Immune activation through cytokine production: psychoneuroimmunology presents a cytokine theory of melancholic depression and postpartum depression, whereby cytokines and inflammatory messengers are predictive and linearly correlated with depression\textsuperscript{18}
- Vagus nerve (cranial nerve X)
- Tryptophan metabolism
- Microbial neurometabolites (generation of neurotransmitters and neuromodulators)

A contemporary challenge to firmly establish the impact of specific probiotic strains on
depressive symptoms is the wide variation in duration of intervention, quantity and strains of probiotics, and different criteria used to define depression. At a phylum level, the microbiome represents at least 70 bacterial phyla with Bacteroidetes and Firmicutes as the two dominant bacterial phylotypes. The total weight of our gut microbes is estimated at 1% to 3% of total body mass, or 2 to 6 pounds of bacteria in a 200-pound adult. In an analysis of the fecal microbiota composition, patients with MDD (as compared to healthy controls) presented with significantly different concentrations of 72 different bacterial communities when analyzed at the genus level—most significantly, those with MDD exhibited increased levels of Enterobacteriaceae and Alistipes with reduced levels of Faecalibacterium. Faecalibacterium has demonstrated anti-inflammatory activity within the gut.

Synthesis and release of neurotransmitters has been demonstrated from the following various bacteria:

- *B. infantis* can influence central 5-hydroxytryptamine (5-HT) transmission levels through elevating plasma tryptophan levels. Serotonin, also called 5-HT, is a metabolite of the amino acid tryptophan which plays an important role in the regulation of several bodily functions including mood. Although whether 5-HT acts as a primary deficit in depression remains unresolved, 5-HT deficiency does seem to be associated with severe depression and/or suicidality.

- *Lactobacillus plantarum* 299V has resulted in a significant rise in fecal *Bifidobacteria* levels. *Bifidobacteria* increases plasma tryptophan levels, impacting turnover of serotonin and dopamine in areas of the brain associated with depression and anxiety.

- GABA, the major primary inhibitory neurotransmitter of the central nervous system which counterbalances the excitatory neurotransmitter glutamate, can be produced by *Lactobacillus, Lactococcus, Streptococcus*, and *Bifidobacterium* strains.

Neurotransmitters produced by gut bacteria are able to cross the intestinal mucosa and can potentially affect physiological responses in the brain, while preclinical data in mouse models has helped to clarify other mechanisms through which gut-brain communication could be influencing mind and mood. One model for exploring bidirectional communication of the gut-brain axis is to use germ-free mice as a control. It has been demonstrated that, in germ-free mice, brain-derived neurotrophic factor (BDNF) expression is decreased in the hippocampus as compared with controls. BDNF is a plasticity-related protein which promotes neuronal growth and of which levels are reduced in the brain and serum of depressed individuals.

Although to this point preclinical animal studies represent the majority of microbiome research, human studies have begun to emerge to guide application. Of human studies completed, measurement has been achieved with a variety of measurement tools including the General Health Questionnaire (GHQ), the Depression Anxiety and Stress Scale (DASS), the Leiden Index of Depression Sensitivity-Revised (LEIDS-r), the Positive and Negative Symptom Scale (PANSS), the State-Trait Anxiety Inventory (STAI), the Development Behavior Checklist (DBC), the Beck Depression Inventory (BDI), the Beck Anxiety Inventory (BAI), the Hopkins Symptom Checklist (HSCL-90), the Hospital Anxiety and Depression Scale (HADS), the Perceived Stress Scale (PSS), the Coping Checklist (CCL), and the Profile of Mood State (POMS) questionnaire.

In a recent double-blind, placebo-controlled, randomized parallel group study, volunteers received either the probiotic combination *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 or placebo for 30 days. Daily administration of the probiotic significantly reduced
psychological distress, as measured by the HSCL-90, the HADS, and by the CCL. The HSCL-90 is a symptom inventory which measures symptoms of anxiety and depression, while the CCL is a questionnaire which captures the ways in which individuals deal with the internal and/or external demands of specific stressful encounters.31

A triple-blind, placebo-controlled, randomized, pre- and post-intervention assessment study randomized 20 healthy participants to receive a multispecies probiotic to assess impact on cognitive reactivity of sad mood. The questionnaire used in this study was the LEIDS-r, a self-report questionnaire which measures vulnerability to depression. Over 4 weeks, study participants were provided with 2 g of freeze-dried powder containing *Bifidobacterium bifidum* W23, *Bifidobacterium lactis* W52, *Lactobacillus acidophilus* W37, *Lactobacillus brevis* W63, *Lactobacillus casei* W56, *Lactobacillus salivarius* W24, and *Lactococcus lactis* (W19 and W58). At time of assessment, those who had received the 4-week probiotic intervention experienced significantly lower cognitive reactivity to depression, especially to aggressive and ruminative thoughts.32

**Serotonergic Pathway**

Recent recommendations published in *American Family Physician* for the diagnosis and management of generalized anxiety disorder and panic disorder in adults position selective serotonin reuptake inhibitors (SSRIs) as well as cognitive behavior therapy as first-line treatments for both conditions.33 The serotonergic system originates in the raphe nuclei in the brainstem with additional 5-HT receptors throughout the brain and within the myenteric plexuses.34

Another mechanism whereby specific bacterial strains could modify mood is through the production of GABA which is the main inhibitory transmitter in the human cortex and which has receptors in the enteric nervous system.35 When *L rhamnosus* was dosed to a mouse model, animals demonstrated reduced anxiety as measured by various behavioral measures as well as altered expression of GABA receptors. However, when animals underwent vagotomy (preventing gut-brain communication), there was no change in GABA receptor expression and lack of anxiolytic effect.7 In an 8-week randomized crossover trial in healthy males assessed using self-report stress measures, cognitive assessments, and electroencephalogram, *L rhamnosus* (JB-1) dosed at 1x10⁹ colony-forming units (CFU) did not demonstrate advantage to placebo for modifying stress-related measures or HPA response.36

**Probiotics in Clinical Use**

Given that Selhub, Logan, and Bested attribute a degree of our limited microbial diversity (and subsequently altered gut-brain signaling) to decreased consumption of fermented foods,2 whole food sources of gut-healthy substrate are worth considering for their mood-boosting benefits. It is notable that food-based sources of probiotics (cultured and fermented foods) offer benefits which would not be seen with isolated probiotic supplements. In addition to enhancing activity of bacterial strains, traditional fermentation can also enhance protein quality and the bioavailability of nutrients relevant to mood, including folate and zinc.2 Preclinical and clinical studies have shown that zinc interacts with the serotonergic system to enhance antidepressant effects37 while suboptimal levels of both serum folate and erythrocyte folate have been associated with increased depressive symptoms and decreased treatment response.38

Clinically, close attention should be given to the quality and microbial content of fermented food sources recommended to promote efficacy. In a study on the effects of probiotics
on mental health and the HPA axis in petrochemical workers, Mohammedi et al compared the effect of probiotic yogurt, conventional yogurt, and probiotic capsules. The probiotic yogurt contained *L acidophilus* LA5 and *B lactis* BB12 while the conventional yogurt contained *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. The probiotic capsule contained 7 probiotic bacteria species as well as fructo-oligosaccharide. After 6 weeks of consumption, changes in DASS scoring demonstrated significant improvements in the probiotic capsule group and probiotic yogurt group, but not in the conventional yogurt group.39

Tillisch et al demonstrated the ability for fermented milk to modify changes in midbrain connectivity when a small group of women were given a fermented milk product with probiotic strains *Bifidobacterium animalis*, *B lactis*, *S thermophilus*, *L bulgaricus*, and *L lactis*. Over a 4-week period during which women consumed 125 g of yogurt twice daily, changes in activity (as measured by functional magnetic resonance imaging [fMRI]) in brain regions which control central processing of emotion and sensation were observed.15 In a study of 124 individuals, consumption of a milk-based drink with *L casei* demonstrated a statistically significant change in mood (using Profile of Mood States variables) only among those who scored in the third lowest tertile at baseline.40

Therapeutic use of probiotics may have an especially pronounced benefit for those with mood disorders which are comorbid to GI disease. After assessing case-control studies for frequency of anxiety and depression in those with IBS, Fond et al recommended that IBS patients be regularly screened for anxiety and depression symptomatology, given significantly higher prevalence of anxiety in IBS-C and IBS-D and of depression in IBS-D.41 A group of 44 adults with comorbid IBS (diarrhea or mixed-stool pattern) and mild to moderate anxiety or depression were given 1x10^10 CFU of *B longum* NCC3001 per day. At week 6, fMRI scans demonstrated less activation of the amygdala when exposed to fear stimuli, which also correlated with reduction in depression scores.42

**Clinical Applications and Cautions**

When attempting to guide patients, clinicians should remain attentive to emerging research indicating strength of the evidence for various bacterial strains and strengths. For those integrating fermented foods regularly into their practice, Dolan et al provide a helpful product summary which reflects professional and commercial products as well as food sources of various bacterial strains based on work established thus far for both depression and anxiety.43

Where are we now? Since interpreting results of human trials cohesively is challenging due to variability in bacterial strains and methods used, there is a need for additional research in populations where participants meet criteria for clinical psychiatric disorders or a validated level of psychological disturbance to move the field of microbial endocrinology further into clinical practice.44 Given already established influences of the gut microbiota on brain and behavior, gut-brain psychology is expected to enhance the study and practice of psychology, neuroscience, and psychiatry,1 with a focus on targeting the microbiome through prebiotics, psychobiotics, and microbiota transplantation to achieve improved mental health.1,28

**References**


1. Microorganisms that, when consumed in adequate amounts, have demonstrated behavioral effects and the ability to reduce HPA activity are best described as:
   a. Probiotics
   b. Prebiotics
   c. Psychobiotics
   d. Synbiotics

2. Decreased expression of BDNF in the hippocampus of germ-free mice compared to controls helps to demonstrate which of the following:
   a. The bidirectional communication of the gut-brain axis
   b. That BDNF promotes neuronal growth
   c. That BDNF is reduced in the brain and serum of depressed individuals
   d. Several mechanisms by which microbiota affect central nervous system function

3. In addition to having active bacterial strains, traditionally fermented foods offer which of the following benefits:
   a. Higher levels of tryptophan
   b. Increased bioavailability of zinc
   c. Contribute to increased fecal Bifidobacteria levels
   d. Enhance expression of GABA receptors

4. According to Selhub et al, which aspects of traditional dietary patterns, such as Mediterranean and Japanese, may be protective against depression and anxiety?
   a. High levels of folate, zinc and magnesium
   b. Low added sugar and high protein levels
   c. High omega-3 content from fish, nuts and seeds
   d. Traditionally fermented foods and high dietary fiber

5. Signaling molecules produced by the microbiota include which of the following:
   a. Fructo-oligosaccharides and corticotrophin releasing factor
   b. Dopamine and glutamate
   c. Amino acid metabolites, short chain fatty acids, and neuroactive substances
   d. Firmicutes and Proteobacteria

6. Probiotic supplements may modulate inflammation by
   a. Reducing pro-inflammatory cytokines
   b. Increasing neurotransmitter synthesis
   c. Increasing cortisol levels
   d. Altering microbial composition
Performance indicators

8.1.5 Applies medical nutrition therapy in disease prevention and management
8.3.6 Keeps abreast of current nutrition and dietetics knowledge and trends
10.4.4 Makes recommendations for the appropriate use of vitamin & mineral supplementation in the management of health and disease.

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