The Human Microbiome: Composition and Change

Reflecting Health and Disease

Sarah Cooper, Rachel Mathews, Lauretta Bushar, Brie Paddock,

Jennifer Wood, and Randy Tammara

Corresponding Author: coopers@arcadia.edu

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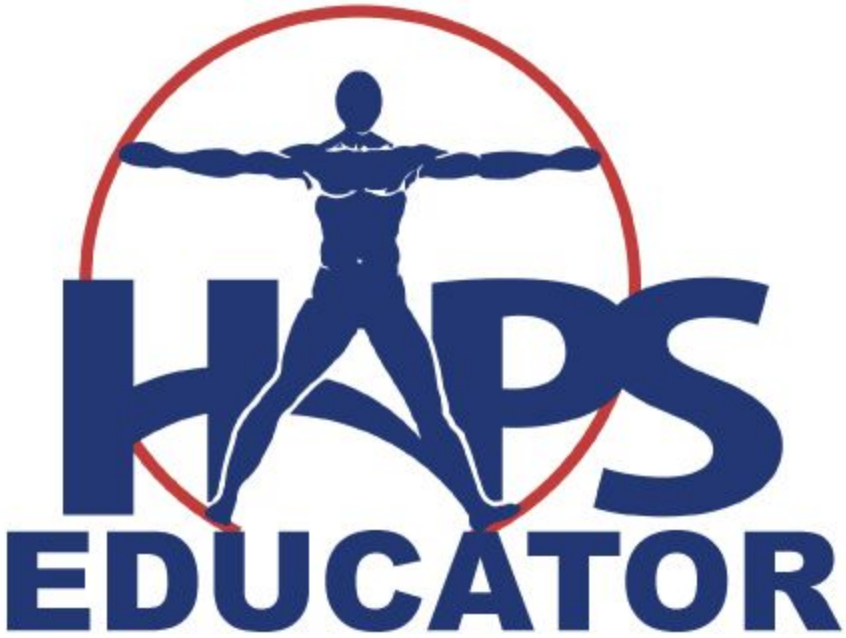

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The Human Microbiome: Composition and Change Reflecting Health and Disease

Sarah Cooper[1], Rachel Mathews[2], Lauretta Bushar[1], Brie Paddock[3], Jennifer Wood[4], Randy Tammara[5]
[1]Department of Biology, Arcadia University, 450 S. Easton Road, Glenside, PA 19038 coopers@arcadia.edu, busharl@arcadia.edu
[2]Arcadia University Physician Assistant Program mathewsr@arcadia.edu
[3]Southern Oregon University, 1250 Siskiyou Blvd., Ashland, OR 97520 paddockb@sou.edu
[4]University of Maryland University College and Certified Integrative Health Coach, Life Space Health, LLC. jen.j.wood@gmail.com
[5]Arcadia University, Masters in Public Health Program, 450 S Easton Road, Glenside, PA 19038 rantam111@gmail.com

Abstract
In humans, microbial organisms known as the microbiota, normally colonize airway passages, skin, the oral cavity, the gastrointestinal tract, and the vagina. There is a growing body of evidence linking the gut microbiota with the overall health of its host. Normally, the microbes that make up the microbiota coexist with a human or animal host without any noticeable difficulty. However, if the symbiotic balance is altered as a result of illness, stress, dietary changes, antibiotic treatment, or other disturbances, the result may be a disruption of normal interactions known as dysbiosis. As a result, the body may become more susceptible to disease. This article examines the most common means by which the microbiota are identified, the process by which the microbiome is acquired, fecal microbial transplantation, and the association of the gut microbiome with specific illnesses such as diabetes and autism. The utility of these applications in developing a teaching module that incorporates the microbiome into courses in physiology or pathophysiology is also reviewed. https://doi.org/10.21692/haps.2019.020

Key words: microbiota, microbiome, HPA axis, dysbiosis, FMT, 16s rRNA gene

Introduction
Healthy adults harbor microbiomes in five major body regions; microbial populations typically inhabit the airway passages, the skin, the oral cavity, the gastrointestinal tract, and the vagina (Proctor 2011). The largest microbiota population is found in the gut where every individual harbors 10 to 100 trillion non-human microbial cells (Figure 1). The study of these cells is relatively new and the terminology applied to them is still evolving. Collectively, the genes of these cells are known as the microbiome and the taxa of human-associated microbes, which include bacteria, bacteriophages, archaea, fungi, eukaryotic viruses and protozoa, are known as the microbiota (Jandhyala et al. 2015; Proctor 2011; Shreiner et al. 2015; Ursell et al. 2012). The vast majority of organisms making up the gut microbiota of adult humans, approximately 1,000 species of bacteria, represent just two bacteria taxa, *Bacteroidetes* (Gram-negative, non-motile forms) and *Firmicutes*, most of which are Gram-positive (Jandhyala et al. 2015; Shreiner et al. 2015). The term metagenomics, which was originally used to describe the total DNA present in all of the gut microbes, is increasingly used to refer to specific marker genes, such as the 16s rRNA gene, which is used to study the phylogeny and taxonomy of bacteria. With more than 8,000 species of bacteria named, an increase of over 450% in the number of described taxa since 1980, the field of bacterial genetics, particularly the genetics of the organisms related to the human microbiome, is exploding (Janda and Abbott 2007).

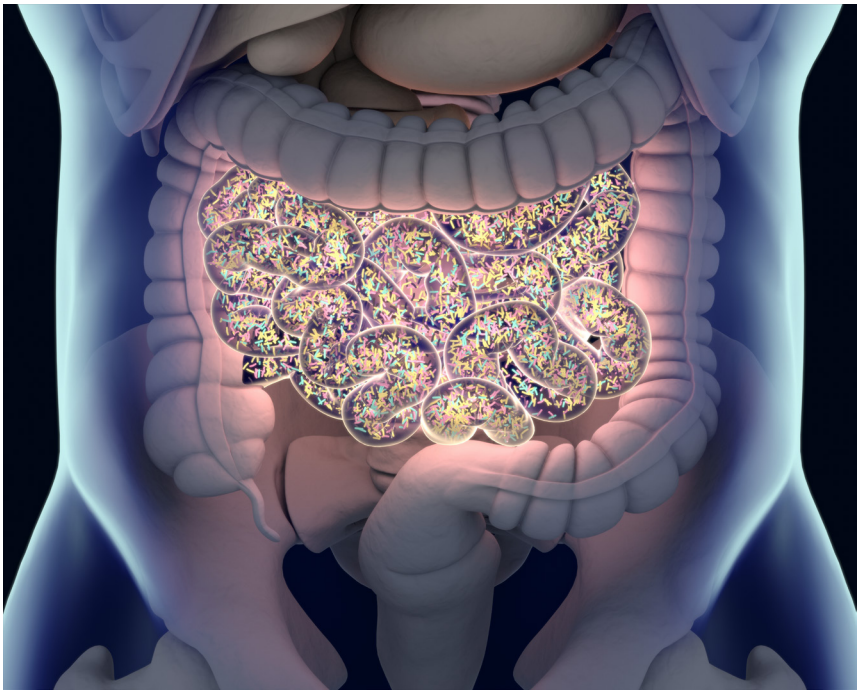

Figure 1. *The microbiome includes 10 to 100 trillion non-human microbial cells including bacteria, bacteriophages, archaea, fungi, eukaryotic viruses and protozoa. Depositphotos, 2019. Open access.*

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Antonie van Leeuwenhoek is credited with carrying out the first exploration of the human microbiome in the early 1680s when he compared his own fecal and oral bacteria. He observed and recorded the differences among the microbes in these two habitats and also noted the differences that he observed in samples taken from these sites in healthy and diseased individuals (Ursell et al. 2012). In keeping with the progression of scientific research since Leeuwenhoek's day, the primary focus of today's research is not merely to observe the obvious differences among site-specific microbial populations, but rather to use advanced molecular techniques to help explain why these population differences exist and to illuminate the physiological significance of the observed populations (Ursell et al. 2012).

The composition of the microbiota of the mammalian gut is unique to each individual and very different from free-living microbial communities. For example, bacteria associated with extreme external environments, such as thermoacidophils, are similar to communities in other environments, while mammalian gut bacteria appear to represent an extreme case of microbial existence confined to a specific internal environment that is warm, food rich, and relatively stable (Ursell et al. 2012).

As catalogued by the European Metagenomics of the Human Intestinal Tract (Meta-HIT) consortium in 2010, there may be as many as 3.3 million non-redundant genes in the microbiome of the human gut, compared with approximately 22,000 genes in the entire human genome (Shreiner et al. 2015; Ursell et al. 2012). The genetic diversity of the microbiome in any individual is immense. While individual humans are typically approximately 99.9% genetically identical to each other, each individual's gut microbiome can be as much as 80-90% different from all others. This suggests that, when technology and information become available, targeting the variations of the microbiome for personalized medical treatment may ultimately prove to be more efficacious than targeting the relatively consistent DNA of the human host genome (Ursell et al. 2012).

This article examines the use of the 16s rRNA gene in identifying the microbiota, the process of acquiring a microbiome, fecal microbial transplantation, and the association of the gut microbiome with diabetes, autism, stress, and the hypothalamic-pituitary-adrenal axis. The utility of these applications in developing a teaching module that incorporates the microbiome into courses in physiology or pathophysiology is also reviewed.

The use of 16s rRNA gene in identifying the microbiota.

The various species that comprise the human microbiota are most commonly identified by sequence analysis of the 16s rRNA gene (Gill et al. 2006; Nasidze et al. 2009; Lagier et al. 2012; Earl et al. 2018; Park et al. 2019). This highly conserved gene is ~1550 bp long and is transcribed to produce 16s rRNA, a critical component of the small ribosomal subunit found in all prokaryotes. Sequence analysis of the 16s rRNA gene has been useful in assessing phylogenetic relationships (Woese 1987; Clarridge et al. 2004). The gene includes highly conserved, variable, and hypervariable regions, which mutate at different rates and are quite diverse among different species of prokaryotes (Ludwig and Schleifer 1994; Van de Peer et al. 1996; Chakravorty et al. 2007). As a result, sequence analysis of the 16s rRNA gene can be used to identify the individual organisms comprising the microbiota using the readily available 16s ribosomal databases that include SILVA (Quast et al. 2013), RDP (Wang et al. 2007), and Greengenes (McDonald et al 2012). Most studies using this approach are able to classify organisms from phylum through genus, but only sometimes to the level of species (Gill et al. 2006; Chu et al. 2010; Yarza et al. 2014).

There is no generally agreed upon species concept for Bacteria and Archaea (Cohan 2001; Dykhuizen 2005; Staley 2006; Achtman and Wagner 2008). Eukaryotic species are typically defined as populations of morphologically similar organisms that are able to interbreed and that are reproductively isolated from other such populations (Sokal and Crovello 1970; Mayr 2000). However, this species concept is not easily applied to prokaryotes. Of the estimated millions of different bacteria (Curtis et al. 2002), most have not been cultured and are only known by DNA sequence (Venter et al. 2004; Giovannoni and Stingl 2005). As a result, "operational taxonomic units" (OTUs) are commonly used to approximate bacterial species. Typically, OTUs are characterized as those with a minimum of 97% 16s rRNA sequence similarity (Stackebrandt and Goebel 1994; Schloss and Handelsman 2006; Schloss 2010; Koeppel and Wu 2013).

There are a number of different approaches to sequencing 16s rRNA. The first methods of DNA sequencing involved chemical cleavage and electrophoresis (Maxam and Gilbert 1977) or primer directed synthesis and chain termination (Sanger et al. 1977). With the development of capillary gel electrophoresis (Swerdlow and Gesteland 1990; Luckey et al. 1990) and automated technologies (Wilson et al. 1988; D'Cunha et al. 1990), Sanger (chain termination) sequencing has become the standard method for sequence analysis and, due to its low error rate and read lengths of 700 bp or higher, remains the gold standard in applications that do not require a high throughput. The advent of next generation sequencing (NGS) or second-generation sequencing resulted in the ability to analyze a much larger number of sequences (millions of reads per sample) at a lower cost, although read lengths tend to be shorter than with Sanger sequencing (Caporaso et al. 2011; Slatko et al.

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2018). Newer third and fourth generation technologies such as nanopore sequencing (Branton et al. 2008; Liu et al. 2016) produce much longer read lengths with high throughputs but also with higher error rates (Schadt et al. 2010; Slatko et al. 2018). These newer technologies allow for full length sequencing of 16s rRNA, so this approach, coupled with NGS, is expected to be useful in the accurate identification of microbial diversity (Bashir et al. 2012; Shin et al. 2016).

How does an individual acquire a microbiome?

Human babies *in utero* do not possess a microbiome. Newborns are colonized by microbiota during the birth process. Within twenty minutes after delivery, the microbiota of infants delivered vaginally is markedly similar to the microbiota of the mother's vagina. Infants who are delivered by Cesarean section have microbiota that are typically associated with the skin (Barko et al. 2018; Fuentes et al. 2016; Ursell et al. 2010). Babies continue to acquire microbiota during the first few years of life and by the time they are approximately a year old, the microbiome of the digestive tract begins to resemble that of the adult gut microbiome (Barko et al. 2018; Fuentes et al. 2016; Ursell et al. 2010).

As the baby grows, there is a steady increase in the phylogenetic diversity of the gut microbiome. Changes in the composition of the microbiota of babies have been observed to occur in tandem with dietary changes such as the introduction of breast milk, the addition of rice-based cereal, the introduction of formula, and eventually, the introduction of table food (Jandhyala et al. 2015; Ursell et al. 2012). For example, when babies transition to an adult diet, genes associated with vitamin synthesis and the digestion of polysaccharides typically make an appearance (Ursell et al. 2012).

Changes in the composition of the gut microbiome

The gut microbiome is not a static entity. It changes and adapts over the course of a person's lifetime. Changes in the microbiome are associated with antibiotic treatments and with diseases such as inflammatory bowel disease, obesity, asthma, metabolic syndrome, cardiovascular disease, immune related conditions and even autism spectrum disorder (Barko et al. 2018; Proctor 2011; Ursell et al. 2012).

Antibiotics have a significant impact on the composition of the gut microbiome. For example, treatment with ciprofloxacin, a broad-spectrum antibiotic that functions as an inhibitor of the bacterial enzyme topoisomerase, results in a decrease in the number of taxa and the diversity of the gut microbiota within three to four days (Shreiner et al. 2015). Typically, the gut microbiota begin to resemble the pre-treatment state approximately a week after antibiotic treatment is stopped but there are significant individual differences in how long the process of returning to normal takes and sometimes, certain taxa do not return to the internal community at all. In extreme cases, it can take up to four years following treatment with antibiotics for some microbiota species to become re-established (Jandhyala et al. 2015; Shreiner et al. 2015; Ursell et al. 2012). Due to ethical issues associated with administering antibiotics to healthy people the mechanisms by which the gut microbiome is replenished after a major disruption and the substantial differences in the process from person to person have not yet been fully described (Shreiner et al. 2015; Ursell et al. 2012).

Fecal microbial transplantation and the treatment of Clostridium difficile infection.

Clostridium difficile infection (CDI) is a human disease that develops as a direct result of changes in the gut microbiome (Shreiner et al. 2015; Ursell et al. 2012). *Clostridium difficile* is a Gram-positive, spore-forming, anaerobic bacterium of the taxa *Firmicutes* that may carry antibiotic resistance and is known to produce toxins that are associated with severe diarrhea and colitis (Fuentes and de Vos 2016). CDI is the most common cause of nosocomial antibiotic–associated diarrhea with approximately 3,000,000 cases reported each year (Cole and Stahl 2015). The infection can sometimes be cured with oral and intravenous antibiotics but an increased incidence of the disease and the appearance of more virulent strains have led to more cases of persistent, recurrent, or relapsing CDI, which can lead to years of escalating health care problems and death. CDI spores may persist for long periods on contaminated hospital surfaces and may be carried by health-care workers. In fact, these spores are so hardy that alcohol-based antiseptics are not enough to effectively kill them and hands must be washed with chlorhexidine soap to decrease the risk of spreading the infection (Cole and Stahl 2015). Risk factors include being older than 65, being treated with antibiotics, having a severe illness, and being hospitalized (Cole and Stahl 2015). CDI is characterized by a disturbed, low-density community of microbiota that is antibiotic induced (Fuentes and de Vos 2016). Fecal microbial transplantation (FMT) is currently the most successful treatment option for this disease.

Fecal microbiota transplantation is a straightforward procedure in which healthy microbiota from a donor individual are transferred directly into the gastrointestinal (GI) tract of a disturbed, dysbiotic microbial community of a recipient in order to re-establish the normal gut microbiota (Barnes and Park 2017; Fuentes and de Vos 2016; Shreiner at al. 2015; Ursell 2012). The microbiota can be transferred in pill form, via gastronomy or nasogastric tube into the stomach, post-pylorically (distal placement of a gastronomy or nasogastric tube into the duodenum or jejunum), colonoscopically, or via enema (Barnes and Park 2017). The data do not yet support a superior means of microbiota introduction.

For successful FMT, the patient's GI tract undergoes bowel lavage or treatment with laxatives prior to the procedure.

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Stool samples are screened for parasites and a variety of pathogenic bacteria and blood samples are screened for transmittable diseases such as hepatitis and HIV. Donor questionnaires, similar to those used for blood bank donors, are used to screen for non-GI diseases that might prove to be problematic (Barnes and Park 2017; Fuentes and de Vos 2016; Shreiner at al. 2015; Ursell 2012).

FMT therapy is known variously as fecal bacteriotherapy, fecal transfusion, duodenal infusion, probiotic infusion and even, since the work of Petrof et al. (2013), as "rePOOPulating" (Fuentes and de Vos 2016; Petrof et al. 2013). The majority of published data describing FMT come from studies of the treatment of CDI but the process is being investigated to treat ulcerative colitis, acute graft-versus-host disease, and diseases of the bowel such as inflammatory bowel disease and irritable bowel syndrome (Barnes and Park 2017). FMT is also being considered as treatment for non-bowel diseases such as multiple sclerosis, metabolic syndrome, and diabetes (Barnes and Park 2017; Proctor 2011).

The first recorded FMT procedure is credited to the work of Benjamin Eisman and his colleagues in 1958, although the practice may date back 1700 years to the Djong-ji dynasty (Fuentes and de Vos 2016; Khoruts 2017). Eisman et al. (1958) reported a case study of four patients with pseudomembranous enterocolitis, a condition caused by *Clostridium difficile*, who were cured with "fecal enemas". He noted that in the early days of oral antibiotics, physicians frequently encountered cases of severe diarrhea due to the over-use of antibiotics and lack of standard dosing protocols (Khoruts 2017). Pseudomembranous colitis was a terrible disease in the 1950's with a mortality rate approaching 75%. The treatment of choice was surgical removal of the colon, which is still done in cases of antibiotic-refractory severe-complicated CDI infections and, even today, this surgery carries a mortality rate of approximately 50% (Khoruts 2017). Using FMT, the cure rate for CDI may be as high as 91% with the resulting creation of a stable bowel homeostasis that prevents further infections (Fuentes and de Vos 2016).

One of the goals of current research in this area is to identify specific communities of microbiota that can be used successfully and predictably for treating specific diseases. Another goal is to develop synthetic microbial communities that can be reproduced without human donors and stocked for future off-the-shelf use (Fuentes and de Vos 2016; Shreiner at al. 2015; Ursell 2012). Ready-to-use solutions of microbiota are currently being prepared and tested for commercial use in the treatment of recurrent CDI. If they become widely available, synthetic microbiota solutions for treatment of a range of specific diseases would overcome the problem of constantly screening for ideal donors (Barnes and Park 2017). If at some point in the future FMT becomes an easier, less invasive, more esthetically acceptable process, perhaps with the use of encapsulated cryopreserved microbiota, it may one day help to reduce the use of antibiotics such as vancomycin in the treatment of multidrug-resistant organisms that reside in the bowel (Crum-Clanflone et al. 2015).

The Food and Drug Administration (FDA) classified human feces for medical use as a drug in 2013, in an attempt to regulate and ensure the safety of fecal transplantation. The action was prompted in part by the appearance of do-it-yourself online treatment videos appearing on the Internet (Fuentes and de Vos 2016).

In spite of the enormous success of FMT treatment, the process may have side effects, including abdominal pain, bloating, nausea, and vomiting; most of which are mild. More severe side effects may include post-transplant sepsis and intestinal perforation (Fuentes and de Vos 2016).

Further studies of FMT are needed to evaluate the potential risk with respect to the transmission of autoimmune diseases, metabolic diseases, and cancer. The primary concern with respect to FMT is the possible transmission of resistant bacteria, unknown viruses, or other as yet unrecognized infectious agents, from donor to recipient (Fuentes and de Vos 2016). The FDA issued a warning on June 13, 2019 following the death of a FMT recipient from an invasive extended-spectrum beta-lactamase (ESBL) infection that was caused by resistant *E. coli* (Yancey-Bragg 2019). The patient who died was known to be immune compromised and the donor stool had not been tested for drug-resistant bacteria prior to the transplant. After the patient died, the stored donor stool sample was tested and found to contain the resistant *E. coli*. The FDA now requires that all donor stool samples be tested for drug resistant bacteria in addition to standard pre-transplant testing (Yancey-Bragg 2019).

Diabetes and the microbiota of the gut

Diabetes has evolved into a potentially deadly public health epidemic of pandemic proportions, with a disease prevalence approaching ten percent globally (Aw and Kukuda 2018). Type 1 diabetes (T1D) is an autoimmune disease that occurs when T cells of the immune system attack pancreatic islet β-cells rendering the pancreas incapable of producing adequate amounts of insulin. T1D accounts for 10% of all cases of diabetes (Tai et al. 2015). Type 2 diabetes (T2D) is a complex metabolic disorder that includes insulin resistance, in which the body cannot effectively utilize the insulin it produces. Insulin resistance is associated with obesity. Genetic factors are known to be critical in the pathogenesis of diabetes and there is growing evidence that the gut microbiota may also be important in influencing the development of T1D and T2D (Tai et al. 2015).

Several studies have suggested that short chain fatty acids (SCFAs) play a role in the pathogenesis of T2D (Aw and Fukuda 2018). These studies report that the number of bacteria that

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produce SCFAs is lower in people with T2D diabetes. This is problematic because SCFAs, adhering to G-protein coupled receptors, which play a role in regulation of lipid and glucose metabolism, are known to have wide ranging effects in the body. For example, SCFAs promote secretion of glucagon-like peptide-1, which impedes secretion of glucagon, slow down gluconeogenesis in the liver, increase insulin sensitivity, and increase central satiety, which may result in weight loss (Aw and Fukuda 2018). SCFAs can also disrupt the low-grade inflammatory response caused by bacteria moving from the intestines into the surrounding adipose tissue and the blood (Aw and Fukuda 2018).

Lifestyle changes may have played a role in the increased incidence of diabetes in the United States over the last 50 years (Aw and Fukuda 2018; Ursell et al. 2012). During this period antibiotic consumption has increased and the Western diet has changed. Antibiotic consumption may result in a decrease in the overall diversity of the gut mictobiota, which has been implicated in reduced immune function (Aw and Fukuda 2018; Ursell et al. 2012). A reduction in the diversity of the gut microbiome has also been observed in T2D and recent studies have shown that people with T2D are less likely to have the gut bacteria that digest plant polysaccharides into SCFAs. The presence of these bacteria in healthy patients facilitates the production of SCFAs (Aw and Fukuda 2018; Ursell et al. 2012). A change in eating habits, primarily an increased dependence on highly processed food, has resulted in an increase in the consumption of carbohydrates and fats for many people, accompanied by a decrease in the consumption of dietary fiber to less than half of the recommended fiber intake of 30 g. daily (Aw and Fukuda 2018). Normally, indigestible fiber is fermented in the digestive system by organisms present in the gut microbiota, producing SCFAs that exert an anti-inflammatory effect by producing immunoglobin A and immunosuppressive cytokines (Aw and Fukuda 2018). A diet reduced in fiber is associated with a decrease in the number of SCFAs and the microbes capable of synthesizing them. A diet high in fiber may help to maintain the bacteria needed to produce SCFAs. Loss of diversity of the microbiota and a decrease in the number of SCFAs, result in dysbiosis that is implicated in an increase in the presence of inflammatory diseases, including diabetes (Aw and Fukuda 2018; Ursell et al. 2012).

A recent study on human males with T2D revealed that they had fewer bacteria of the taxa *Firmicutes* when compared to a non-diabetic population and more *Betaproteobacteria*, some of which may be pathogenic (Aw and Fukuda 2018). This study also identified positive correlations between plasma glucose levels and the *Bacteroidetes* to *Firmicutes* ratio (which normally changes over a person's life time) as well as the ratio of the *Bacteroides–Prevotella* group (associated with a plant-rich diet and a high protein diet respectively) to the *Clostridium coccoides–Eubacterium rectale* group, which produces butyrate (Aw and Fukuda 2018; Prados 2016). Butyrate is a SCFA found in the colon. It has several known functions; it serves as the preferred energy source for epithelial cells of the colon, enhances the barrier function of the gut, and enhances the immune and anti-inflammatory properties of the gut (Riviere et al. 2016). The reduction of butyrate-producing bacteria in the gut microbiota is correlated with impaired insulin sensitivity and obesity and it may contribute to the disease pathology in people with T2D diabetes (Aw and Fukuda 2018). The Aw and Fukuda (2018) study suggests that Gram-negative *Bacteroidetes* and *Proteobacteria* might be implicated in the pathogenesis of T2D through an inflammatory response induced by endotoxins (Aw and Fukuda 2018). If further research supports this conclusion, the gut microbiome might become a reliable biomarker for predicting the onset of T2D in glucose-intolerant people. It has also been noted that treating people with metabolic syndrome with vancomycin reduces the number of Gram-positive butyrate-producing bacteria.

In studies of children who are at high risk for developing T1D due to the presence of islet autoantibodies, the gut microbiota has been shown to be consistently less diverse and less dynamic than that of healthy controls (Tai et al. 2015). It is characterized by low numbers of lactate- and butyrate-producing bacterial species and very low numbers of the two most prominent *Bifidobacterium* species, *Bifidobacterium adolescentis* and *Bifidobacterium pseudocatenulatum*, which are common inhabitants of the gut microbiota that are often used as probiotics (Tai et al. 2015). The disturbed microbiota in children at risk for developing T1D also displayed an increased presence of bacteria of the taxa *Bacteroides*, which normally function in the breakdown of complex organic molecules (Tai et al. 2015). These studies suggest that changes in the gut microbiota are associated with T1D and that perhaps targeted FMT to re-establish a more normal microbiome might one day be used to delay and/or prevent the onset of diabetes (Tai et al. 2015).

Further research is needed to determine the mechanisms by which the altered microbiota of the gut develop in people with diabetes and the manner in which the immune system might interact with the microbiota with respect to this disease (Tai et al. 2015). It has not yet been determined whether the gut microbiota initiate the development of diabetes, enhance the disease once it is present, or are a result of the disease (Tai et al. 2015).

The microbiome in stress and mental health

Stress is known to affect the physiology of the GI tract in several ways including: changes in motility and secretions, increased permeability, decreased ability to maintain the mucosal lining, and changes in the gut microbiota (Konturek et al. 2011). There is a growing body of empirical evidence to support a possible connection between the gut microbiome, stress, and cognitive function, primarily in the expression of depression and anxiety (Liu 2016). Several studies have found that psychosocial stressors may change the composition of the gut microbiota, resulting in the increased presence of cytokines, which have

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been associated with activation of the vagus nerve and the risk for psychiatric disorders such as depression (Dowlati et al. 2010; Liu 2016; Mills et al. 2013).

Support for a possible connection between the gut microbiota and stress-related psychiatric illness comes partly from demonstrating the anxiety-inducing effects of certain bacteria and the anti-depressive or anti-anxiety effects of probiotics in rodents (Liu 2016). For example, increases in anxiety-related behavior has been observed in rodents exposed to *Citrobacter rodentium* (the rodent equivalent of *E. coli*), *Trichuris muris* (a nematode parasite of mice), and *Campylobacter jejuni*, the most commonly reported cause of bacterial food poisoning in the US (Altekruse et al. 1999; Lyte 1998; Liu 2016), while *Lactobacillus* spp. and *Bifidobacterium* spp. (probiotics) have been demonstrated to reduce anxiety and depression-like behavior in rodent studies (Arseneault-Bréard et al. 2012; Lui 2016).

Very few human studies have been done in this area; however, two recent observational studies have examined the gut microbiota with respect to human psychopathology. In one study, adult patients with clinical depression were found to have higher levels of *Enterobacteriaceae* and *Alistipes* and lower levels of *Faecalibacterium* relative to healthy controls. *Enterobacteriaceae* is a large taxa of bacteria that includes *E.coli*, *Alistipes* species are associated with the serotonin precursor tryptophan, and *Faecalibacterium* is known to have anti-inflammatory properties (Jiang et al. 2015). In the second study it was found that depressed adults had higher concentrations of *Bacteroidales*, the most abundant beneficial gram-negative organism in the human gut microbiota, and lower concentrations of *Lachnospiraceae*in, an anaerobic bacteria that is believed to be protective against colon cancer by producing butyric acid (Coyne and Comstock 2008; Meehan et al. 2014; Naseribafrouei et al. 2014; Lui 2016). The results of these studies are consistent with the possibility that there are changes in the microbiota that accompany psychological disorders. More research with psychiatric populations will need to be done in order to determine the relevance of these differences (Lui 2016).

Several studies have examined the use of probiotics or prebiotics in humans. In a representative study, participants given a combination of the probiotics *Lactobacillus* and *Bifidobacterium* species, demonstrated lower scores on measures of psychological distress, hostility, anxiety, and depression compared to a group given a placebo (Messaoudi et al. 2011). These studies are limited by the fact that the investigations were confined to psychiatrically healthy participants (Liu 2016). Further research is needed to directly evaluate the relation between the gut microbiota and clinically significant psychopathology.

While research on the microbiome continues, adopting a program to manage chronic stress may be one way to positively impact the gut microbiota. Mindfulness and other meditation practices are now considered standard methods to build resistance to the impact of stress on the body (Bergland 2016; Cooper et al. 2018). Additionally, exercise is a well-known approach to lowering blood pressure and cortisol levels and increasing overall feelings of well-being and there is evidence to support a relationship between the diversity of the gut microbiota and exercise (Fard 2014; Georgia State 2018). While more studies are needed to confirm the exact relationship between stress management and the microbiome, the impact of stress on the body and the potential benefits of mindfulness and exercise for overall health are well documented and undeniable (Cooper et al. 2018).

Autism spectrum disorder and the microbiome

Autism Spectrum Disorder (ASD) is a range of disorders affecting one in 59 children at age eight. It is an intensely researched disorder but the etiology remains unclear and there is, as yet, no approved treatment for the core symptoms (Baio et al. 2014). While social and repetitive behavioral abnormalities are among the most recognizable symptoms of ASD, patients also demonstrate elevated levels of inflammation and gastrointestinal abnormalities. Mounting evidence indicates that the microbiome may play a role in the development and severity of symptoms in ASD, and possibly in the causality of ASD in young children (McElhanon et al. 2014).

Several studies have demonstrated differences in the gut microbiome of ASD as compared to neurotypical children, including reductions in fermenting bacteria such as *Prevotella* and increased levels of the propionic acid-producing bacteria *Clostridia* (Song et al. 2004, Finegold et al. 2010, Kang et al. 2013). Propionic acid has been shown to lower the fatty acid content of the liver and blood plasma, reduce food intake, and improve insulin sensitivity. Consequently, an increase in propionic acid production may be associated with the prevention of obesity and T2D (Al-Lahham et al. 2010). Propionic acid may also increase the overall threshold for inflammatory responses and it may have a major role to play in the link between nutrition, the gut microbiota, and human physiology (Al-Lahham et al. 2010).

The differential microbiomes associated with ASD may impact the development and function of the nervous system through enrichment or depletion of specific neuroactive microbial metabolites. Specifically, the SCFAs produced by some of these microbes impact the maturation and behavior of microglia (Erny et al. 2015). Microglia, which have several functions in the brain including phagocytosis of pathogens and the removal of damaged cells, are known to be overly activated in patients with ASD (Pardo et al. 2005). They may be associated with the neuroinflammation that is present in ASD as well as in inappropriate developmental pruning, the process of eliminating extra synapses that is widely thought to be

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associated with learning and efficient functioning of the brain (Paolicelli et al. 2011).

Several promising studies have suggested that current research in the role of the microbiome in ASD may result in therapies for ASD patients. Fecal transplants in ASD patients have resulted in decreases in GI, social, and behavioral symptoms (Kang, Adams et al. 2017) that have continued for as long as two years after the initial transfer of microbiota (Kang et al. 2019). More specific treatments may include the transfer of a single species of microbe, such as *Bacteroides fragilis*, a human commensal organism that has been shown to improve behavioral abnormalities in a rodent model of ASD (Hsiao et al. 2013).

Not all proposed treatments for ASD rely on microorganisms since they can be unstable due to host-organism interactions with the patient. However, treatment of animal models of ASD with specific microbiome metabolites such as 5-aminovaleric acid (5AV) and the amino acid taurine, both of which affect gamma-aminobutyric acid (GABA) receptors in the brain and are known to be reduced in ASD, has been shown to reduce repetitive ASD-like behaviors and increase social behaviors in mice (Sharon et al. 2019). More research is needed determine whether clinically significant metabolites such as 5AV and taurine exist in human patients with ASD and if targeted metabolites produced by microbiota might become effective treatment therapies for disorders that are seemingly centered in the brain.

The effect of gut microbiota on the hypothalamic-pituitary-adrenal (HPA) axis during stress

The human microbiome has been linked to the regulation of several physiological processes including those that are altered under stressful conditions in order to maintain homeostasis (Frodl and O'Keane 2013; Luczynski et al. 2016). The hypothalamic-pituitary-adrenal (HPA) axis is a neuroendocrine bidirectional communication pathway between the gut and the central nervous system that produces cortisol. It is an important component of the stress management homeostatic system (Cussotto et al. 2018; Frodl and O'Keane 2013). Cortisol is a glucocorticoid that alters several tissues in order to mobilize or store energy under stress conditions (Frodl and O'Keane 2013). Glucocorticoids bind to glucocorticoid receptors (GRs) in the hippocampus and mineralocorticoid receptors (MRs) dispersed throughout the brain; both receptors function as transcription factors (Frodl and O'Keane, 2013). Cortisol has a higher affinity for the MRs than GRs, which allows MRs to help maintain the low cortisol levels in the blood under normal conditions (Stevens and Wand 2012). Under stress conditions, higher concentrations of cortisol cause the cortisol to bind to GRs with lower affinity, which ultimately causes GRs to terminate the stress response as a result of a negative feedback reaction that is described below (Stevens and Wand 2012). The modulation of glucocorticoids plays an important role in maintaining normal function of the HPA axis (Frodl and O'Keane 2013).

Under stressful conditions, the HPA axis causes the release of corticotropin-releasing hormone (CRH) from the paraventricular nucleus of the hypothalamus (Frodl and O'Keane 2013). CRH is then carried to the anterior pituitary gland where it binds to its receptors and causes the secretion of adrenocorticotropic hormone (ACTH) into peripheral circulation (Frodl and O'Keane 2013). ACTH causes the release of cortisol (in humans) and corticosterone (in mice) from the adrenal gland, which leads to negative feedback of ACTH and CRH secretion and restoration of homeostasis (Figure 2) (Frodl and O'Keane 2013). The negative feedback of these hormones helps prevent prolonged activity of the HPA axis (Stevens and Wand 2012). Imbalances in the HPA axis have been associated with mood and anxiety disorders as well as digestion, immunity, emotions, sexuality, and energy expenditure (Cussotto et al. 2018).

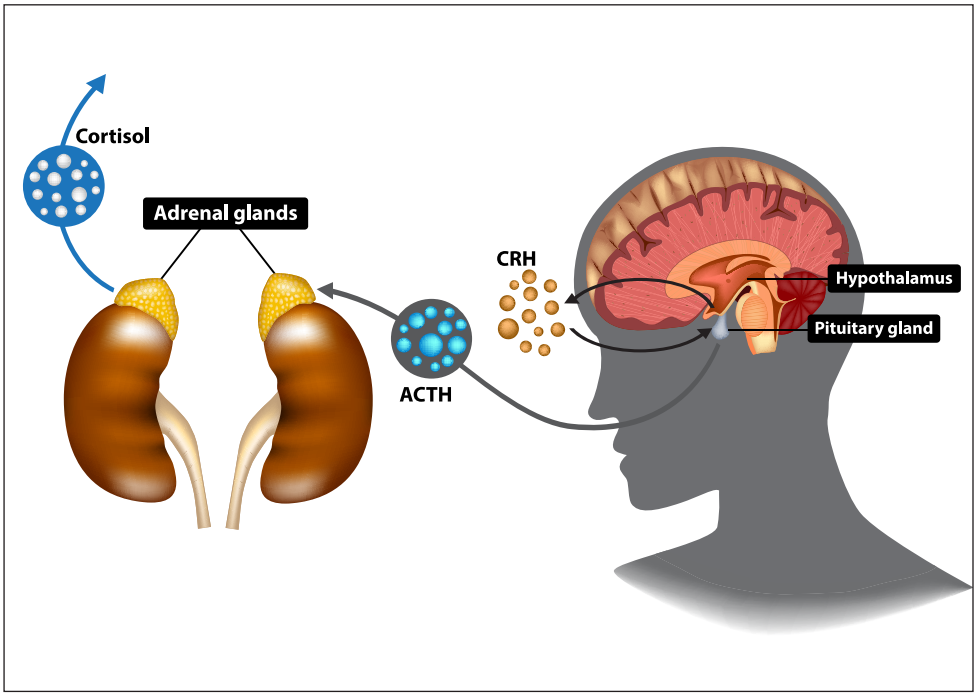

Figure 2. *The HPA axis is responsible for the neuroendocrine adaptation component of the stress response. Depositphotos.com*

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In investigating the link between gut microbiota and the HPA axis during stress conditions germ-free (GF) and specific-pathogen-free (SPF) mice are commonly used (Luczynski et al. 2016). GF mice are raised in an environment without exposure to microorganisms, while SPF mice have normal microbiota but are guaranteed to be free of certain pathogens (Luczynski et al. 2016). Sudo et al. (2004) undertook the first major study that linked gut microbiota to the stress response by comparing HPA axis activity of GF and SPF mice using an acute restraint stress protocol. The results of the study showed that GF mice under stress displayed higher levels of plasma ACTH and corticosterone compared to SPF mice (Sudo et al. 2004). The study concluded that the presence of gut microbiota influences normal regulation of the HPA axis (Sudo et al. 2004).

Clarke et al. (2013) observed the effect of gut microbiota on the serotonergic system, which is known to be associated with stress and anxiety. The study compared GF and SPF mice under environmental stress, which can include conditions such as isolation, noise, and low temperature (Clarke et al. 2013). The results of the study showed that GF mice had increased levels of 5-hydroxytryptamine (serotonin) and 5-hydroxyindoleacetic acid, the primary metabolite of serotonin. Serotonin is associated with feelings of well-being and happiness as well as reward, memory, learning, and other physiological processes and 5-hydroxyindoleacetic acid is the marker detected in urine that is used to determine the amount of serotonin present in the body. In addition, tryptophan (a precursor of serotonin) was also higher in GF mice (Clarke et al. 2013)

Diaz Heijtz et al. (2011) observed motor activity and anxiety-like behavior in GF and SPF mice using three types of stress tests: open-box, light-dark box, and elevated plus maze. The open box stress test is based on the preference rodents display for hidden, enclosed spaces. The light-dark box stress test is based on the aversion rodents display to brightly illuminated spaces and the elevated maze test is based on their aversion to open spaces. Rodents normally exhibit locomotion behavior in response to novel environments, which means they will move freely in a new environment seeking the place of least stress. During a typical light-dark box experiment, for example, the amount of time an animal spends transitioning from a light to a dark environment might be recorded. The results of the study showed that GF mice displayed decreased anxiety-like behavior and increased motor activity compared to SPF mice. The GF mice also displayed altered expression of genes related to anxiety and synaptic plasticity (Diaz Heijtz et al. 2011). Similarly, Neufield et al. (2011) compared the behavior of stressed GF and SPF mice using an elevated plus maze test. The study found that GF mice exhibited less anxiety-like behavior than SPF mice and increased levels of plasma corticosterone (Neufield et al. 2011). The results of these studies suggest that decreased anxiety-like behavior in mice may be due to hyperactivity of the HPA axis and modulation of anxiety-related genes (Diaz Heijtz et al. 2011; Neufield et al. 2011). Overall, these studies suggest that the presence of gut microbiota play a key role in normal behavioral response to stress (Diaz Heijtz et al. 2011; Neufield et al. 2011).

Two of the most recent studies, Huo et al. (2017) and Vodička et al. (2018), suggest that the absence of gut microbiota can alter the activity of the HPA axis, highlighting the importance of the gut-brain axis. When GF mice are compared to SPF mice, these studies revealed clear differences in how certain functions related to the HPA axis are either increased or decreased under stress conditions (Huo et al. 2017; Vodička et al. 2018). From the findings of the Huo and Vodička studies, it can be concluded that gut microbes modulate the HPA axis by impacting anxiety-like behavior, normal levels of HPA axis hormones, and the expression of hormone receptors and genes (Huo et al. 2017; Vodička et al. 2018).

Vodička et al. (2018) observed that GF mice spent less time in total defensive behavior compared to SPF mice and that SPF mice displayed more escape/flight behavior compared to GF mice when faced with a social defeat procedure during which mice are subjected to prolonged social stress by being exposed to large,
aggressive mice (Vodička et al. 2018). Escape/flight, which is defined as running or jumping away from resident mice, is an example of anxiety-like behavior. The results of this study suggest that GF mice are less likely to exhibit anxiety-like behavior under stress conditions (Vodička et al. 2018). Huo et al. (2017) found that stressed GF mice traveled a greater distance in an open field test, where they were permitted to move freely, and spent more time in the center of the open field test apparatus compared to stressed SPF mice (Huo et al. 2017). Greater traveling distance and expenditure of time in the central area are examples of anti-anxiety like behavior in mice (Huo et al. 2017). Although a few studies have shown an increase in anxiety-like behavior in GF mice, the majority of studies have found a decrease in this response and indicated it as an impaired behavioral response to stress (Rabot et al. 2016). The contrast in these results may be due to differences in methodology or the effect of genetic background of the mice (Rabot et al. 2016). It is important to note that the behavioral response results in the Vodička et al. (2018) study correlated with hormone changes of the HPA axis (Huo et al. 2017). Stressed GF mice showed less anxiety-like behavior along with over-activity of the HPA axis (Huo et al. 2017). This finding suggests that gut microbiota may regulate anxiety-like behavior through an endocrine response (Huo et al. 2017).

The normal regulation of HPA axis hormone levels is also impacted by gut microbiota (Huo et al. 2017). The regulation of these hormones is important since corticotropin-releasing hormone (CRH) causes secretion of ACTH, which leads to the release of cortisol (Frodl and O'Keane 2013). Huo *et al*. (2017) found that the absence of gut microbiota caused the upregulation of both CRH and ACTH in stressed GF mice compared to stressed SPF mice (Huo et al. 2017). This finding

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was similar to the Sudo et al. (2004) study of GF and SPF mice subject to stress. In addition, the study found an increase in cortisol (CORT) and aldosterone (ALD) levels (Huo et al. 2017). There is debate about whether corticosterone levels are changed in GF mice since some studies find an increase in concentration while others observe a normal range (Luczynski et al. 2016). These contrasting results may be due to the type of microbiota examined, the sex of the mice, or differences in the experimental procedure (Luczynski et al. 2016). Overall, the increase in these hormones in the Huo et al. (2017) study may explain the decreased anxiety-like behavior observed in stressed GF mice, whereas stressed SPF mice exhibited normal behavior following a stress protocol (Huo et al. 2017).

Gut microbes regulate the expression of hormone receptors and genes under stress conditions (Huo et al. 2017; Vodička et al. 2018). In the Huo et al. (2017) study, it was found that GF stressed mice had increased levels of CRH receptor Type 1 (Crhr1) mRNA and decreased levels of Nr3c2 mRNA, which encodes the mineralocorticoid receptor that regulates the action of aldosterone (Huo et al. 2017). Huo et al. (2017) also found that GF stressed mice had decreased MR/GR (Huo et al. 2017). Previous studies have suggested that changes in MR/GR expression may indicate HPA axis dysfunction in mood-related disorders (Webster 2002). Overall, the results from the Huo et al. study suggest hormone dysfunction in the hippocampus of GF mice under stress (Huo et al. 2017). In addition, Vodička et al. (2018) found that stress increased the expression of proopiomelanocortin (POMC), the pituitary precursor of melanocyte stimulating hormone and ACTH, but did not significantly affect corticotropin releasing hormone receptor 1 (Crhr1) (Vodička et al. 2018). Since POMC is a precursor of ACTH, its increased levels matched the increase in ACTH in the Huo et al. (2017) study (Huo et al. 2017; Vodička et al. 2018).

Vodička et al. (2018) also found that the expression of the Fkbp5 gene in the pituitary gland was decreased in SPF mice and upregulated in GR mice. The function of the Fkbp5 gene is to encode a protein that controls negative feedback by decreasing the affinity of GR for corticosterone (Vodička et al. 2018). It is suggested that higher expression of Fkbp5 in GF mice may result in decreased efficiency of negative feedback via GR (Vodička et al. 2018). Therefore, the upregulation of Fkbp5 in GF mice may be one reason that an exaggerated HPA response was observed in these mice (Vodička et al. 2018). This finding correlates with previous studies that have shown an increased expression of Fkbp5 and the GR levels in the cytoplasm during chronic mild stress (Guidotti et al. 2013). The results of the Vodička et al. (2018) study also suggest that the absence of microbiota increased the expression of genes encoding proteins involved in steroidogenesis (Star and Cyp11a1) and biosynthesis of catecholamines (TH and PNMT) in the adrenal gland (Vodička et al. 2018). In contrast, stress only affected genes encoding epinephrine synthesis (Vodička et al. 2018). These findings suggest that the presence of microbiota help regulate normal catecholamine biosynthesis and steroidogenesis, and the absence of these microbes can lead to dysregulation of these processes (Vodička et al. 2018).

Further research is needed to provide much needed knowledge about how the gut microbiota influence and interact with the function of the HPA axis and stress-related disorders (Huo et al. 2017; Vodička et al. 2018).

Conclusion

An unprecedented amount of growth in our understanding of the microbiome has come about relatively quickly as a result of the availability of powerful research tools, but there are still many questions that remain unanswered. Emerging technologies such as 16s rRNA sequencing and FMT raise the prospect of a future in which specific treatments for diseases such as diabetes, metabolic syndrome, and mental disorders can be targeted to individual humans. Determining the relationships between the gut microbiota, systemic diseases and diseases of the bowel, stress-related mental illnesses, and physiological processes such as those that function within the HPA axis, is clinically extremely important. Preliminary evidence suggests that the use of probiotics and FMT may be free of some of the side effects and addictive properties that are associated with pharmacological medications; this bodes well for their potential safety and tolerance as a form of treatment. As always, inherent in the world of today is an ever-present need for the development of new treatment options for the future.

The information contained in this article provides a physiological framework for studying the gut microbiome and a means of illustrating the normal and pathophysiological structure and function of the digestive and endocrine systems. Using this model, students can be encouraged to learn the microbial identification of samples of their own oral microbiome. Additionally, information from this article could be used in a module that explains sterile technique, allows students to design experimental approaches to the study of microorganisms, communicates experimental results, and critically evaluates scientific findings related to the composition and function of the microbiome. Students might also be prompted to learn how to estimate the frequency of antibiotic-resistant cells in natural populations of bacteria and create and test their own hypothesis about patterns of antibiotic resistance. They can be encouraged to learn about the trillions of microbes living on and in their bodies and to explore the role of diet, environment, and antibiotic use in forming and maintaining individual human microbiomes, while learning to appreciate the foundations of digestive health.

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About the Authors

Sarah Cooper is an Associate Professor of Biology at Arcadia University and Managing Editor of the *HAPS Educator*.

Rachel Mathews, Arcadia '19, is currently attending the Physician Assistant Graduate Program at Arcadia University.

Lauretta M. Bushar, PhD is a Professor of Biology at Arcadia University. She conducts research in the ecology and genetic structure of threatened and endangered snake populations.

Brie E. Paddock, PhD, is an Assistant Professor of Animal Physiology at Southern Oregon University.

Jennifer Wood, PhD, teaches non-profit management at University of Maryland University College where she is an Associate Professor. She is a certified integrative health coach with a family practice, Life Space Health, LLC, specializing in stress management and anxiety reduction.

Randy Tammara is a pharmacist who has been working in the Philadelphia area for over twenty-five years. He is a certified diabetes educator who gives presentations to caregivers through Northampton Community College in Bethlehem, PA, where he is an adjunct faculty member in the Community Education Division.

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