Over the last century, a connection between gastrointestinal (GI) abnormalities and problems outside of the GI tract has become evident. For example, an association between GI problems and arthritis was described in 1910.\(^1\) Over time, a relationship between the GI tract and the brain (a gut-brain connection) also has emerged. As long ago as 1889, researchers reported “an exhaustional-confusional form of insanity proceeding from a dilated and over-filled colon.”\(^2\) Colonic irrigation was commonly used in the late 1800s and early 1900s, with some investigators reporting that colon cleansing improved certain mental diseases.\(^3\) Notwithstanding this history, it is only in the last decade or so that the gut-brain connection has become more widely acknowledged. Research in this area has greatly increased. While this article’s overall focus is on the interaction between the gut and the brain, it highlights mitochondrial function as one of the critical bridges between these two body systems. I first examine some potential mechanisms of a gut-brain connection. Next, I discuss mitochondrial function in detail and assess how problems with mitochondrial function (mitochondrial dysfunction) can contribute to both GI abnormalities and neurological sequelae. In the context of abnormal GI function, I also review the potential adverse effects on mitochondrial function of bacterial imbalances in the GI tract and discuss how this can adversely affect the gut-brain connection. I conclude with a discussion of the potential role of hyperbaric oxygen therapy (HBOT) in improving mitochondrial dysfunction as well as GI and brain function.

**EVIDENCE OF A GUT-BRAIN CONNECTION**
Over the last century, a connection between gastrointestinal (GI) abnormalities and problems outside of the GI tract has become evident. For example, an association between GI problems and arthritis was described in 1910.\(^1\) Over time, a relationship between the GI tract and the brain (a gut-brain connection) also has emerged. As long ago as 1889, researchers reported “an exhaustional-confusional form of insanity proceeding from a dilated and over-filled colon.”\(^2\) Colonic irrigation was commonly used in the late 1800s and early 1900s, with some investigators reporting that colon cleansing improved certain mental diseases.\(^3\)

Notwithstanding this history, it is only in the last decade or so that the gut-brain connection has become more widely acknowledged. Research in this area has greatly increased. While this article’s overall focus is on the interaction between the gut and the brain, it highlights mitochondrial function as one of the critical bridges between these two body systems. I first examine some potential mechanisms of a gut-brain connection. Next, I discuss mitochondrial function in detail and assess how problems with mitochondrial function (mitochondrial dysfunction) can contribute to both GI abnormalities and neurological sequelae. In the context of abnormal GI function, I also review the potential adverse effects on mitochondrial function of bacterial imbalances in the GI tract and discuss how this can adversely affect the gut-brain connection. I conclude with a discussion of the potential role of hyperbaric oxygen therapy (HBOT) in improving mitochondrial dysfunction as well as GI and brain function.

**POSSIBLE MECHANISMS OF A GUT-BRAIN CONNECTION**
Over time, a number of ideas have been developed to explain potential mechanisms of action for the gut-brain connection. One idea derives from evidence demonstrating that the central nervous system (CNS) and the GI tract share similar cells, including glial cells. In the GI tract, astrocyte-like glia are partly responsible for the proper functioning of the intestinal barrier and help to prevent larger food particles and other molecules from entering the circulatory system. Abnormalities in the GI glial cells may contribute to autoimmune diseases, enterocolitis, diabetes, irritable bowel syndrome (IBS), and inflammatory bowel disease (IBD).\(^4\)

The second body of evidence supporting a gut-brain connection comes from recent studies focusing on the bacteria in the GI tract. Whereas approximately 30,000 genes are found in the average human, more than 3 million genes from GI tract bacteria are present. The GI tract contains tenfold more bacteria (10\(^{10}\)) than the average number of cells (10\(^9\)) in a human body, and these bacteria serve important purposes. For example, the symbiotic relationship that exists between humans and their intestinal microbial flora is crucial for nutrient assimilation and important for the development of the innate immune system.
DANIEL A. ROSSIGNOL, MD, FAAFP, received his Doctorate of Medicine at the Medical College of Virginia and completed his residency in family medicine at the University of Virginia. Coming from an academic background, Dr. Rossignol searched the medical literature looking for a solution after both of his children were diagnosed with autism. He has made it his mission to research and publish in autism. In the last 6 years, he has had 23 publications and 3 book chapters concerning autism and related conditions. Dr. Rossignol has a special interest in autism spectrum disorders, PANDAS, cerebral palsy, and related neurological and developmental disorders as well as medically complex children and adults. Dr. Rossignol is a Fellow of the American Academy of Family Physicians (FAAFP) and is president of the Medical Academy of Pediatric Special Needs (MAPS), which provides education and long-term support for practitioners, ensuring the quality and consistency of medical care for children with autism and related medical conditions.
Exposure to "good" bacteria in the GI tract programs the immune system to more effectively fight infections. While it was already established that bacteria communicate with each other through a process known as quorum sensing, it is now apparent that the bacteria in the human GI tract also communicate with human cells through hormonal signals. This communication is important because bacteria and humans share metabolic pathways that are essential for health.

The third idea supporting a gut-brain connection comes from evidence that GI tract abnormalities may adversely affect brain function. Dysbiosis is the term used to refer to either an increase in the number of abnormal bacteria or a disruption in the type of bacteria in the GI tract. Although the idea that dysbiosis may contribute to abnormalities inside the GI tract (such as diarrhea and constipation) is fairly straightforward, it is increasingly apparent that dysbiosis also has effects outside the GI tract, including effects on brain function. Recent evidence has demonstrated that atypical levels and types of bacteria in the GI tract contribute to metabolic abnormalities reported in neurological and psychiatric conditions such as autism spectrum disorders (ASDs). The pathogenic metabolites produced by these bacteria in the GI tract may contribute to the brain dysfunction and metabolic problems that have been observed. As other examples, hydrogen sulfide produced in part by GI bacteria has been shown to play a role in blood pressure regulation and the unique makeup of the microbial community in the GI tract also may help determine a person's weight by influencing fat storage regulation. Even a decade ago, the idea that the bacteria in the GI tract might influence blood pressure regulation or weight would have seemed untenable.

Fourth, dietary factors also point to a gut-brain connection. Perhaps nowhere is this more apparent than in the case of celiac disease, a condition defined by intestinal damage resulting from gluten reactivity. Unfortunately, it has taken centuries for humans to realize that gluten exposure also can impair brain function in some people and lead to conditions such as ataxia and schizophrenia. Interestingly, the prevalence of celiac disease is 3.5 times higher in children with ASD than in the general population. However, recent studies reveal that the general prevalence of celiac disease remains under-recognized.

Exposure to foods other than gluten-containing foods may also impair brain function. For example, a one-month study reported disruptive behaviors in an 8-year-old boy with autism after exposure to a number of common foods. Staff collected frequency data on behaviors such as object throwing, scratching, biting, and screaming. The study included periods of a normal American diet, a fasting period, and a period during which individual foods were reintroduced one by one. During the latter phase, it was observed that mushrooms, dairy products, wheat, corn, tomatoes, and sugar all provoked behavioral problems. Some children will manifest similar types of sensitivities. These reactions point to a connection between food that is ingested and effects in the brain, which then result in certain behavioral changes.

Exposure to cow's milk may also impair brain function in some people, including individuals with cerebral folate deficiency (CFD). CFD is a newly described neurodevelopmental disorder typified by low cerebrospinal fluid (CSF) levels of 5-methyltetrahydrofolate (5MTHF) (the metabolically active form of folic acid) in spite of normal systemic folic acid (folate) levels. CFD is a newly described neurodevelopmental disorder typified by low cerebrospinal fluid (CSF) levels of 5-methyltetrahydrofolate (5MTHF) (the metabolically active form of folic acid) in spite of normal systemic folic acid (folate) levels. The most common cause of CFD is circulating autoantibodies that bind to the cerebral FRα and inhibit the transport of 5MTHF into the CSF. Lowered levels of 5MTHF in the CSF can lead to neurological abnormalities, including spastic paraplegia, cerebellar ataxia, dyskinesia, seizures, acquired microcephaly, and developmental regression that can occur as early as 4 months of age. Central visual disturbances (optic atrophy and blindness) and hearing loss also have been described at later ages. To date, seven studies have reported CFD in children with ASD, and several studies have described CFD in Rett syndrome. CFD has also been reported in individuals with mitochondrial disease, perhaps because mitochondria are integral for providing the energy needed for the transport of 5MTHF into the brain. Treatment with oral folinic acid can lead to partial or complete recovery in some children. In one such case, a 12-year-old girl with progressive spasticity, abnormal gait, and speech problems who had been diagnosed as paraplegic recovered from this condition after she was discovered to have CFD and was treated with oral folinic acid.

Cow's milk contains soluble folate receptor antigen, which is 91% homologous to the FRα. Autoantibodies to the FRα cross-react with the soluble folate receptor antigen in cow's milk, which causes an increase in the circulating autoantibody concentration. Exposure to cow's milk has been shown to increase the concentration of the folate receptor autoantibody and lead to worsening of CFD symptoms, while elimination of cow's milk has been reported to lower the autoantibody concentration and improve CFD symptoms. Moreover, re-exposure to cow's milk after a period of being cow's milk-free substantially worsens the condition and increases the autoantibody concentration. These findings may help explain why some parents of children with ASD report improvements in their child on a cow's milk-free diet. Exposure to cow's milk also has been associated with constipation in children with ASD.

Besides the potential effect of dietary exposures on brain function, toxic byproducts produced by certain bacteria can also negatively affect brain function, especially in the face of liver dysfunction. For example, in individuals with hepatic encephalopathy, the intake of large amounts of protein can contribute to abnormal brain function. This occurs because protein is eventually broken down into ammonia, which is directly toxic to brain cells and can easily diffuse into the CNS from the bloodstream. High ammonia levels are associated with neuropsychological abnormalities in patients with hepatic encephalopathy. Because the mitochondria in the liver are responsible for the detoxification of ammonia, it is apparent that liver dysfunction contributes to high ammonia. Ammonia is also produced by bacteria in the GI tract. High protein intake and dysbiosis in the face of liver dysfunction, therefore, can contribute to elevated ammonia levels and subsequent brain impairment. The important role of dysbiosis in this impairment is further illustrated by the finding that use of an antibiotic (rifaximin) reduces the risk of hepatic encephalopathy when compared with a placebo by eliminating ammonia-producing bacteria in the GI tract.

From this section, we can infer that mitochondrial function plays at least two important roles in maintaining a proper gut-brain connection. First, mitochondria help to detoxify certain toxins produced by GI bacteria that may otherwise adversely affect brain function. Second, mitochondria provide the energy needed to pump nutrients such as 5MTHF into the brain, a process that is impaired by autoantibodies that increase upon exposure to cow's milk. In the following sections, I review mitochondrial function and discuss how mitochondrial dysfunction can contribute to both GI abnormalities and neurological sequelae.
OVERVIEW OF MITOCHONDRIAL FUNCTION

Mitochondria are distinct cellular organelles that generate adenosine triphosphate (ATP) from adenosine diphosphate (ADP) by oxidizing glucose and fatty acids. ATP is the energy carrier in most mammalian cells. In the simplest terms, mitochondria are the powerhouses of the cell, generating energy from the breakdown of food. Figure 1 depicts a mitochondrion and shows the pathways involved when mitochondria break down food and use oxygen to create ATP (the energy source for the body, analogous to gasoline for a car). (For a more detailed review of mitochondrial function, see Haas and coauthors.34)

As seen in Figure 1, the structure of the mitochondrion consists of outer and inner membranes, with a space (the intermembrane space) in between. The matrix is the innermost part of the mitochondria where many biochemical reactions occur, including the tricarboxylic acid (TCA) cycle (also known as the Krebs cycle or citric acid cycle). The inner mitochondrial membrane contains 5 complexes (known as complexes I through V) that make up the electron transport chain (ETC). On the bottom of the figure, you can see glucose, which is eventually broken down into pyruvate through the process of glycolysis. Pyruvate is then transported into the mitochondria and eventually is broken down into acetyl-CoA, which feeds into the TCA cycle.

Fatty acid metabolism is shown on the bottom right-hand corner of the figure. Short-chain fatty acids (SCFA) and medium-chain fatty acids (MCFA) can diffuse directly into the mitochondria, whereas long-chain fatty acids (LCFA) are transported into the mitochondria by attaching to carnitine, which shuttles these fatty acids across the inner and outer mitochondrial membranes. Once inside the mitochondria, the fatty acids, like pyruvate, are broken down and converted into acetyl-CoA, which feeds into the TCA cycle. However, some of the electrons released from burning fatty acids (fatty acid oxidation) can feed into complex II through FADH2, bypassing complex I in the process (which may partially explain why a ketogenic diet, which involves a high intake of fats, might be helpful for treating mitochondrial dysfunction).35

The three dotted lines with arrows coming off of the TCA cycle are electrons (negatively charged particles) that are transferred through NADH into complex I. Complex I then transfers these electrons to coenzyme Q10 (CoQ10) which, in turn, transfers the electrons to complex III. When the electrons pass through complex I, NADH is converted to NAD+. Hydrogen protons (positively charged hydrogen particles, H+) are pumped from the matrix (the innermost part of the mitochondria) into the intermembrane space, where they build up and form an electrochemical gradient. The electrons that passed to complex III are now transferred by cytochrome C (Cyt C) to complex IV. This process also pumps more hydrogen protons into the intermembrane space through complexes III and IV. During this process, oxygen is converted into water in complex IV. The hydrogen protons in the intermembrane space then diffuse back into the matrix through complex V (ATP synthase), and this generates ATP through a process known as oxidative phosphorylation.

MITOCHONDRIAL DYSFUNCTION

The concept of mitochondrial dysfunction is relatively new, and mitochondrial medicine is a rapidly evolving field of medicine. Mitochondrial disease, once thought uncommon, is now considered the most recognized cause of metabolic
Mitochondrial disease has a broad phenotypic presentation: children with mitochondrial disease can have normal intelligence, mental retardation, or developmental delay. One recent study reported that approximately 5% of children with ASD have mitochondrial disease. It is important to note that illness or stress will generally place mitochondria under more stress and increase dysfunction. Thus, stressors such as dehydration, fever, and infection can lead to a functional decline and neurodegenerative regression in individuals with mitochondrial disease.

To evaluate possible mitochondrial dysfunction, it is important to examine a patient’s clinical history. Occasionally, there will be a family history of mitochondrial disorder. Other clinical history that is often observed in mitochondrial dysfunction includes developmental regression (loss of previously acquired skills), seizures, fatigue or lethargy, ataxia (lack of coordination of muscle movements), motor delays, GI abnormalities (such as reflux, constipation, diarrhea, and inflammation), and cardiomyopathy (significant heart problems). Elevations in the various metabolites described previously are laboratory markers of mitochondrial dysfunction, making laboratory testing a helpful tool for identifying mitochondrial dysfunction. Generally, the higher the elevations and the more metabolites affected, the more likely it is that mitochondrial dysfunction exists.

The tests in question are typically covered by insurance and can be performed by any standard laboratory. The laboratory tests (ideally performed in the morning after fasting for 8-10 hours) may include those listed in Table 1. If the test results are abnormal, they may need to be repeated for confirmation. If the results are normal but mitochondrial dysfunction is still suspected, then repeating the tests when the child is sick or under stress may help unmask and identify mitochondrial dysfunction.

### Table 1. Laboratory markers of mitochondrial dysfunction

- Lactate (lactic acid)
- Pyruvate
- Carnitine (free and total)
- Acylcarnitine panel (fatty acids attached to carnitine)
- Quantitative plasma amino acids (for measuring alanine and lysine)
- Ubiquinone (also known as CoQ10)
- Ammonia
- Creatine kinase (CK)
- AST (aspartate aminotransferase) and ALT (alanine aminotransferase)
- CO2 and glucose

Since mitochondria are predominantly responsible for energy production, organs with the highest energy demand are most adversely affected by mitochondrial dysfunction.
The type of bacteria present in the GI tract may have a significant impact on the development of the brain and, eventually, on adult behavior.

such as Huntington's disease, Friedreich's ataxia, Parkinson's disease, and amyotrophic lateral sclerosis (ALS). Less obviously, mitochondrial dysfunction can also contribute to GI problems. For example, children with mitochondrial diseases are more likely to have GI abnormalities when compared with controls, and unexplained GI problems have been associated with mitochondrial disease. Constipation is a common symptom in children with mitochondrial disease as is the more severe condition of obstruction (an inability to produce stool and gas). Given the high energy demands of both the GI tract and the cerebrovascular endothelium, it is apparent that mitochondrial dysfunction may contribute to barrier dysfunction in both the brain and GI tract.

**DYSBIOSIS, MITOCHONDRIAL DYSFUNCTION, AND NEUROLOGICAL SEQUELAE**

The type of bacteria present in the GI tract may have a significant impact on the development of the brain and, eventually, on adult behavior. For example, the bacteria in the GI tract influence how the body uses vitamin B6, which then can affect the health of nerve cells. GI tract bacteria also have an influence on autoimmune diseases such as multiple sclerosis. A newly developed biochemical test that may help identify autism is based on the end products of GI bacterial metabolism.

Bacteria in the GI tract have been shown to influence anxiety. In one study, mice lacking normal gut microflora were compared with mice with normal gut bacteria. Investigators looked at behavior, brain development, and brain chemistry. Illustrating one way in which the composition of the gut flora affect brain function, the animals lacking normal gut microflora actually had less anxiety as measured by some behavioral-related tests and displayed increased motor activity when compared with mice with normal gut flora. Two genes that may play a role in increasing anxiety—brain-derived neurotrophic factor (BDNF) and nerve growth factor-inducible clone A (NGF-I-A)—were downregulated in the animals lacking the normal flora. In addition, the study demonstrated how the gut bacteria shape brain function by influencing brain cells to turn gene expression on or off, which in this instance affected the expression of almost 40 genes found in five different areas of the brain. These findings indicate that some brain-directed behaviors are influenced by the makeup of the GI flora.

Interestingly, some bacteria in the GI tract produce metabolites that may be potentially harmful. One example is propionic acid, an enteric short-chain fatty acid that is a fermentation end product of enteric bacteria. Propionic acid has been shown to inhibit mitochondrial fatty acid metabolism and function, contribute to seizure activity, and produce evidence of neuroinflammation, including reactive astrogliosis and activated microglia. Clostridia, which are anaerobic, spore-forming Gram-positive rod bacteria, are known to produce propionic acid, and a derivative of propionic acid recovered in the urine of individuals with ASD has been reported as a marker of clostridia. A recent rat model of ASD demonstrated that the administration of propionic acid induced mitochondrial dysfunction and led to brain, behavioral, and metabolic changes consistent with ASD, including clinical features such as repetitive behaviors, social interaction problems, hyperactivity, oxidative stress, lowered GSH levels, microglial activation, and altered carnitine levels.

Furthermore, significantly elevated concentrations of clostridia in the GI tract have been reported in some children with ASD when compared with controls, and in children with constipation when compared with controls. Treatment of clostridia improves brain function in children with ASD and has been shown to decrease hyperactivity and hypersensitivity as well as increase social interaction, eye contact, and vocalizations. Interestingly, clostridia levels increase with age and may lead to increased production of toxins that may affect liver function and play a role in cancer.

My clinical experience confirms that a number of children with ASD have evidence of clostridia. Symptoms commonly associated with increased clostridia include hyperactivity, irritability, aggressiveness, increased self-stimulatory behavior, and obsessive behavior. Treatment of clostridia is often associated with a reduction in these behaviors, usually within several days to a week. In the context of clostridia in children with ASD, treatment with carnitine may be particularly helpful. This is because carnitine deficiency has been implicated in ASD, some studies have reported improvements with the use of carnitine in ASD, and carnitine may lower the toxicity of propionic acid produced by clostridia. Carnitine has also been reported to improve mitochondrial function.

**HBOT AND MITOCHONDRIAL DYSFUNCTION**

Although treatments for mitochondrial dysfunction remain relatively limited, one treatment that has garnered interest in recent years is HBOT. HBOT involves inhaling up to 100% oxygen at a pressure greater than one atmosphere in a pressurized chamber. Since hypoxia is known to impair mitochondrial function, and because only approximately 0.3% of inhaled oxygen is ultimately delivered to mitochondria, increasing oxygen delivery to dysfunctional mitochondria through HBOT might aid in improving function.

Both animal and human studies have examined the effects of HBOT on mitochondrial function. In a mouse model with an intrinsic impairment of mitochondrial complex IV, HBOT at 2 atmospheres “significantly ameliorate[d] mitochondrial dysfunction” and delayed the onset of motor neuron disease when compared with control mice. In other animal studies, HBOT increased the amount of work done by mitochondria, improved mitochondrial function after brain injury, and prevented mitochondrial deterioration when compared with room air pressure and 100% oxygen levels. HBOT has also been reported to increase sperm motility by augmenting mitochondrial oxidative phosphorylation in fructolyis-inhibited sperm cells from rats. Fructose is the sugar used by sperm for energy production.

In an animal model, HBOT was recently shown to activate mitochondrial DNA transcription and replication and increase the biogenesis of mitochondria in the brain. It is possible that HBOT could be used to increase the production of mitochondria in humans. Although biogenesis has not yet been proven to occur in humans, I have had two patients with severe mitochondrial disease and abnormal mitochondrial function (as measured by muscle biopsy) who have improved clinically with HBOT at 1.3 to 1.5 atmospheres and who now have normal mitochondrial function (again as measured by muscle biopsy).

Some bacteria in the GI tract produce metabolites that may be potentially harmful.
In a recent human study of 69 patients with severe traumatic brain injury, HBOT at 1.5 atmospheres and 100% oxygen significantly increased brain oxygen levels, increased cerebral blood flow, and decreased CSF lactate levels. It also improved brain metabolism and mitochondrial function when compared with both room air treatment and 100% oxygen given at normobaric pressure. 97

HBOT AND GI FUNCTION
Because HBOT possesses potent anti-inflammatory properties, 98,99 it may be useful in ameliorating inflammatory conditions of the GI tract. Several published animal models of IBD have demonstrated that HBOT can enhance memory, 114 published animal models of IBD have demonstrated that HBOT can it also improved brain metabolism and mitochondrial function when compared with oxygen levels, increased cerebral blood flow, and decreased CSF lactate levels.

HBOT AND BRAIN FUNCTION
HBOT may help improve brain function in certain conditions. In animal models, HBOT improves learning and memory. 113 In healthy young adults, the addition of 100% oxygen when compared with room air significantly enhances memory, 114 cognitive performance (including word recall and reaction time), 115 attention, and picture recognition. 116 Several studies have shown improvements in traumatic or chronic brain injury with HBOT. 117-120

In a recent study of 16 individuals who had traumatic brain injury, individuals exhibited significant improvements with the use of HBOT at 1.5 atmospheres and 100% oxygen in their neurological exam, IQ, memory, post-traumatic stress symptoms, depression, and anxiety; they also displayed objective improvements in brain perfusion. 121 Studies also have reported behavioral improvements in children with ASD using HBOT at 1.3 to 1.5 atmospheres. 122,126 HBOT may bring about improvements even in conditions where permanent brain problems are thought to be present, including cerebral palsy 127,128 and fetal alcohol syndrome. 129

CONCLUSIONS
The evidence for a gut-brain connection has become stronger over time. Abnormalities in the GI tract, including dysbiosis, and abnormalities caused by intake of gluten and cow’s milk proteins may contribute to abnormal brain function. Mitochondria play an important role in the gut-brain connection, and abnormalities in mitochondrial function are found in many neurological and psychiatric disorders. Mitochondrial dysfunction can lead to GI abnormalities and brain dysfunction. Ultimately, mitochondrial dysfunction can have adverse effects on the gut-brain connection. Treatment of mitochondrial dysfunction with modalities such as carnitine and HBOT may be beneficial in maintaining and/or improving the gut-brain connection. Additional studies examining the gut-brain connection in neurological and psychiatric disorders are warranted.

REFERENCES
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NeuroProtek® and our new formulation, NeuroProtek®-LP are all natural, flavonoid based, nutritional supplements. Both formulas, based on the published scientific research of Prof. Theoharis, PhD, MD., contain an exclusive combination of three pure, plant based flavonoids: Luteolin and Quercetin and Rutin. Research has shown these ingredients inhibit oxidative stress and inflammation, while reducing gut and brain barrier disruption. Unique to Algonot’s formulas is micro-filtered, low acidity, Olive Kernel Oil (OKO). OKO contains many antioxidant components and is instrumental in helping the body absorb and deliver flavonoid powders. NeuroProtek® & NeuroProtek®-LP are free of the following allergens: artificial colors or dyes, flavors or sweeteners, corn, eggs, fish, heavy metals, milk/ casein, peanuts, preservatives, salt, shellfish, soy, starch, sugar, tree nuts, wheat/gluten and yeast.