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. 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.” Colonic irrigation was commonly used in the late 1800s and early 1900s, with some investigators reporting that colon cleansing improved certain mental diseases.

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.

Potential 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).

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^{13}) than the average number of cells (10^{13}) 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 (F AA FP) 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.
Recent evidence has demonstrated that atypical levels and types of bacteria in the gastrointestinal (GI) tract contribute to metabolic problems that have been observed in human cells through hormonal signals. Hydrogen sulfide produced in part by GI bacteria has been shown to play a role in blood pressure regulation, but it is now apparent that the bacteria in the GI tract also communicate with each other through a process known as quorum sensing. 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 reactions that were of interest. 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 also may 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 normally transported into the CNS through endocytosis by the cerebral folate receptor-alpha (FRα) in a process that is dependent on cellular energy. 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 age. 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 folic 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 folic 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.
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.)

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 enters 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).

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) through the inner membrane and 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.

**OVERVIEW OF MITOCHONDRIAL FUNCTION**

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**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 dysfunction, neurological sequelae, and GI abnormalities

Since mitochondria are predominantly responsible for energy production, organs with the highest energy demand are most adversely affected by mitochondrial dysfunction. Low-energy cells (such as skin cells) have fewer mitochondria, while cells with higher energy needs contain more mitochondria. Cells that have high energy demands and, thus, many mitochondria include muscle, liver, brain, cerebrovascular endothelium, and GI cells. Neuronal synapses (areas of high energy consumption) are especially dependent on mitochondrial function. This helps explain how mitochondrial dysfunction can lead to impaired brain function. Mitochondria are concentrated in the dendritic and axonal termini, where they play an important role in ATP production, calcium homeostasis, and synaptic plasticity. Mitochondrial dysfunction can lead to reduced synaptic neurotransmitter release. Neurons that have high firing rates, such as GABAergic interneurons, may be the most adversely affected.

Mitochondria also play an important role in cellular lipid metabolism, signaling, and repair.

The ETC is the predominant source and the major target of reactive oxygen species (ROS). ROS (also called free radicals) are molecules with an unpaired electron that can cause damage to cells and tissues by removing an electron from a surrounding compound or molecule. This damage is termed oxidative stress. Oxidative stress has been reported in many neurological disorders.

The ETC is protected from the damage caused by ROS by a mitochondrial-specific superoxide dismutase and by antioxidants such as glutathione (GSH). Under normal circumstances, GSH, the body’s main antioxidant, can donate an electron to the free radical and quench the free radical before it can cause damage. Unfortunately, mitochondria lack the enzymes to synthesize GSH and, therefore, are dependent on cytosolic GSH production. When the GSH in mitochondria is depleted, cells are more vulnerable to oxidative stress and damage from ROS originating from the mitochondria. Additionally, some factors can increase ROS production, including environmental toxins, infections, and autoimmune disease, which can directly and indirectly lead to impairments in ETC activity.

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GSH depletion, activation of mitochondrial and non-mitochondrial-dependent biochemical cascades that result in programmed cell death (apoptosis), and oxidative stress have been implicated in many neurological and psychiatric diseases, including neurodegenerative diseases.

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

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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, and some studies have reported improvements with the use of carnitine in ASD. 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 2.4 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 fructose-inhibited sperm cells from rats. Fructose is the sugar used by sperm for energy production. In rats, HBOT prevented apoptosis and improved neurological recovery after cerebral ischemia by opening mitochondrial ATP-sensitive potassium channels. In another animal model, hypoxia and ischemia led to diminished ATP and phosphocreatine production; the addition of HBOT restored these levels to near normal and increased energy utilization when compared with room air oxygen and pressure levels.

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 15 atmospheres and 100% oxygen significantly increased brain oxygen levels, increased cerebral blood flow, and decreased CSF lactate levels. It also improved mitochondrial metabolism and modulated brain function when compared with both room air treatment and 100% oxygen given in normobaric pressure.104

**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 significantly lower inflammation in the GI tract and improve BD.100-104 Other studies have reported improvements using HBOT for Crohn's disease105-108 and ulcerative colitis.109-111 In addition, HBOT can kill clostridial species, because these bacteria are anaerobic.112 In my clinical experience, children with evidence of clostridia often have significant improvements in GI function (especially in diarrhea) with the use of HBOT.

**REFERENCES**


**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.
nutritional supplements. Both formulas, based on the published scientific research of flavonoid powders.

- Many antioxidant components and is instrumental in helping the body absorb and deliver oxidative stress and inflammation, while reducing gut and brain barrier disruption.
- Unique flavonoids: Luteolin, Quercetin and Rutin. Research has shown these ingredients inhibit:
  - Inhibits mast cell activation
  - Reduces inflammation
  - Inhibits the release of IL-6 & TNF
  - Prevents blood-brain barrier disruption
  - Blocks oxidative stress
  - Modulates immune system
  - Reduces inflammation
  - Inhibits mast cell activation

**NeuroProtek**

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**NeuroProtek LP**

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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, Quercetin and Rutin. Research has shown these ingredients inhibit oxidative stress and inflammation, while reducing gut and brain barrier disruption. Unlike 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, whey/gluten and yeast.

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