How Enteric Microbiome Modulates Mitochondrial Function
Richard E. Frye, M.D., Ph.D.

[Bio From MitoAction website]

Dr. Richard Frye is the Director of Autism Research at Arkansas Children's Hospital Research Institute, Director of the Autism Multispecialty Clinic, and Co-Director of the Neurometabolic Clinic at Arkansas Children's Hospital and Associate Professor in Pediatrics at the University of Arkansas for Medical Sciences. He received his MD/PhD from Georgetown University in 1998. He completed a residency in Pediatrics at the University of Miami, Residency in Child Neurology, and Fellowship in Behavioral Neurology and Learning Disabilities at Harvard University/Children's Hospital Boston and Fellowship in Psychology at Boston University. He holds board certifications in Pediatrics, and in Neurology with Special Competence in Child Neurology. Dr. Frye is a national leader in autism research. He has authored over 100 peer-reviewed publications and book chapters, and serves on several editorial boards of prestigious scientific and medical journals.

Over the past several years he has completed several clinical studies on children with autism spectrum disorder (ASD), including studies focusing on defining the clinical, behavioral, cognitive, genetic, and metabolic characteristics of children with ASD and mitochondrial disease and several clinical trials demonstrating the efficacy of safe and novel treatments that address underlying physiological abnormalities in children with ASD, including open-labels on tetrahydrobiopterin, cobalamin and folinic acid and a recent double-blind placebo controlled trial on folinic acid. Future research efforts are focused on defining physiological endophenotypes of children with ASD and developing targeted treatments.

Dr. Frye: Thank you so much for the invitation I really appreciate it and I am glad there is a lot of interest in this emerging topic. I feel honored to be asked to talk about it and I appreciate everyone taking the time to listen. We will move forward in the slide deck as we talk.

Slide 2:

On the second slide we can see our talking points:

The enteric (gut) microbiome has an important influence on health and disease states in humans. We will talk a little bit about its affect on function but also a broader view of its effect on health.

The enteric microbiome influences the human host using chemical mediators, some of which can directly affect mitochondrial function.

Short chain fatty acids produced by gut bacteria not only modulate mitochondrial function and cellular regulatory pathways, but can also be used as mitochondrial fuels. So they are extremely interesting so it is a way that our bacteria actually can influence our body but also influence metabolism.

Slide 3:

This is just a general slide that talks about the microbiome. The microbiome has really had a lot of hype lately. People are starting to realize that we have all of these bacteria that live in our body and when we talk about the microbiome we are talking about all of the bacteria but also other organisms live in and on our body. But the majority, 99% of these organisms live really in our gut and really if we think about our gut, the gut is really not inside our body. The lumen, the space in the gut is really considering outside our body. So we have these bacteria in our gut which is technically outside of our body and we are starting to learn that there are probably somewhere about as many as cells to many ten times as many bacteria cells as there are cells in our body.

That is kind of striking and when we think about it, we think about the DNA and the genetic material, each of the cells in our body has the same genetic material whereas bacteria all have different variations on their genetic materials. So when we look at genes that actually control the way cells work, bacteria have maybe 10, 100, maybe 1,000 time more genes all together than we have genes in our body. It is kind of daunting when we start to think about it. I think it is really starting to become clear that this is important to our health.

Slide 4:

So on the fourth slide here, is a slide that shows bacteria and how they interact. So one of the things that makes microbiomes so special is that these are individual organisms that are working together to form almost a super organ. They influence each other and they reinforce each other to help each other grow and also inhibit each other so that bad bacteria that may make the body sick don't grow. So that is one of the really important parts of the microbiome is that we have to keep it healthy, because it actually inhibits bad bacteria from growing and keeps our gut healthy. This is just a slide to show some of these different pathways, how these bacteria influence each other. They actually produce something that is known as a biofilm. They make a film where they are almost connected to make a layer on our guts, so they have the first interface with anything that goes through our gut including foods.

Slide 5:

On the fifth slide we talk about here how the microbiome influences the host, that is us, the body. One of the major ways that we think about the bacteria influences us is through changing our immune system This is becoming more and more realized that gut health is very much important in modulating our immune system and in autoimmune diseases. There are many ways that the bacteria in our gut do this. There is actually a very large immune system called the galt (gut associated lymphoid tissue) that is in our gut and is very important for modulating immune function and through that bacteria can directly influence our immune system. It can actually alter things called cytokine production which is over on the left side of the slide which are immune messengers that modulate the immune system.

And on the right of the slide we talk about short chain fatty acids. These are very small fatty acids, that is why they are short chains, and they can actually modulate the way that cells work. And they can modulate mitochondrial function and they can be used as fuels. Some of them are actually part of our normal metabolism. Because they are fatty acids they can easily getting into cells and move around cells and influence the way that that function.

Slide 6: 10:40

Slide six is a nice slide that shows some of the theoretical mechanisms as to how the microbiome may influence our brain and brain function. Some of the ways the microbiome

actually changes the way that we process certain nutrients and some of these are precursors to neurotransmitters that are used by our brain so that is very important. It influences cytokines, cytokines are these immune messengers and our immune system can influence our brain by its modulation. Also the bacteria can produce these fatty acids and other types of precursors that affects how the brain works. It can also affect something called the vagus nerve which directly sends signals to our brain and can influence the way that the brain works. So there are many different ways that the bacteria can influence our brain theoretically and then there is some practical data I'll show you about how we think things actually work.

Slide 7:

One of the things that has been brought up and it concerning is that the microbiome is something that we develop from the beginning of life so really we inherit it from our mother. Most of it starts out from when we are delivered as a child and we pick up bacteria from the vaginal canal and that starts to build what will be our microbiome for the rest of our lives. We also get it through breastfeeding. However, one of the things that we have seen in the past several decades is that there are things that we do in medicine for certain reasons to improve outcomes that may influence how the microbiome actually develops from very early on in life including c-sections. Of course in c-section delivery everything is sterile and you are not exposed to the vaginal bacteria, there are antibiotics that are used many times, formula feeding prevents getting bacteria or other good nutrients from breastfeeding. And then of course we are living in a more sterile environment which changes what we are actually exposed to.

Slide 8: 13:54

If we go to slide eight there is this idea of what we call biome depletion that is because of sanitation we have unintended consequences of decreasing exposure to certain bacteria and other organisms. By doing that the immune system which is supposedly designed to protect us against outside organisms from the environment, is not exposed to these and thus the immune system can become hyper sensitive because it does not have the necessary stimulation it is actually designed to have. So some people do believe this modern, industrial culture of increased sanitation and sterilization could have unintended consequences on the immune system.

Slide 9: 15:00

This is what we call the three-hit paradigm, the change in society to make the immune system possibly hyper sensitive, plus other genetic predisposition and environmental triggers could cause an increase in immune disease. It is thought that if you look at the developed world vs. the developing world that countries that are developed have increased sanitation have higher rates of autoimmune diseases. So there is thought to be support for this.

Slide 10: 15:55

In slide ten here we think this could involve the development of autism - that is the biome depletion can predispose there to be inflammation and other factors can put things over the edge to increase inflammation to a significant extent and this can cause metabolic dysfunction which may result in autism.

Slide 11: 16:26

Slide eleven is a list of many of the different diseases that we see that are associated with changes in the microbiome. These include common diseases from asthma and allergies which we have seen an increase in the last decade to other problems including inflammatory

bowel disease, obesity, metabolic syndrome, cancers, and other diseases. So it is believed that the microbiome is very much involved in many diseases.

Slide 12:

We have recently published a review of all of the different childhood conditions where the microbiome may be disrupted and may be causing some issues and contributing to disease. This involves prematurity, and also problems that occur during prematurity that is premature babies are very susceptible to necrotizing enterocolitis and sepsis and we think that it be a microbiome depletion and changes in the microbiome that may have predisposed them for some of these things. Colic early on, then malnutrition, but then actually the more common childhood diseases including eczema, allergies, asthma, diabetes, and autism spectrum disorder also has been associated with changes in the microbiome.

Slide 13: 18:20

In another paper that we published we listed many of the different factors that can contribute to depleting the microbiome and changing the microbiome, including very common things like acetaminophen, but other things, environmental toxins and such, c-sections, vitamin D depletion, and changes in diet. So there are many different factors that can results in changes in the microbiome.

Slide 14:

So there has been a lot of talk about autism spectrum disorder and how it is involved in the microbiome and we will talk a little bit about that.

Slide 15:

One of the first ways I got into this is something called the propionic acid model of autism that has been developed by Dr. MacFabe up in Canada.

Slide 16:

Here on slide sixteen we can see a diagram of propionic acid which is short chain fatty acid and we find that it actually has many physiological effects, including causing neuroinflammation and it is known to alter mitochondrial function, it can directly alter gene expression, and it can actually change the electronic coupling between neurons through gap junctions. So even though it is a very small molecule it can have profound effects.

Slide 17:

One of the first demonstration of the fact that this short chain fatty acid can have any type of effects that were similar to autism was developed by Dr. MacFabe, he first did his work on adult rat models, and what he did was he injected a propionic acid, which is a short chain fatty acid that is produced by gut bacteria, and he injected it into the brain of adult rats to see what would happen. What he found is that they would develop autistic like behaviors.

Here is a couple of the different measures that he used. So what you can see, especially in the lower right hand corner, is that if you put a rat in a box with another rat is that they will circle the rat and come close to them because they are interested, so you can see that on the bottom left hand corner with these kind of lines circling around, and you can see that the one that is labeled PBS which is the placebo. That means that they just had saline injected into their brains. And you can see that that rat actually walked around the other rat interested, so in a social type behavior, whereas the one that received propionic acid which is to the left and

labeled PPA, is that they weren't so interested in that other rat, they just walked around the sides of the chamber and were not very interested. And you can see this also in their graphs as far as the proximity to the other rat, which is this middle graph on the bottom, shows that when they had propionic acid they weren't as social, they stayed away from the other rat, you can see on the right hand side on the bottom graph that they had problems in the T Maze which is a learning paradigm, so they had problems learning. Then you can see on the top graphs, on the number of movements, the number of repetitive movements, and the movement time, is that they just generally had more repetitive movements and they were more hyperactive. These are all characteristics of autism spectrum disorder. So it is though that this simulated autism in some ways.

Slide 18: 22:50

So if you we go onto the next slide, and we can also see that Dr. MacFabe looked at different markers to see what was going on in the brain. In this marker he looked at the brain to see if there was any signs of inflammation. He showed that the rats with propionic acid injected had increases of this marker of inflammation known as GFAP and this marker of reactive astrocytes which are supportive cells in the brain becoming reactive because they are becoming inflamed.

Slide 19: 23:33

He also looked at a marker of something called microglia cells and microglia cells are the cells in our brain that our thought to the immune cells. Usually they are very quiescent and very silent and it is only when we have inflammation that they start to react. Usually we don't think of inflammation in the brain as good so here he showed that the rats that received propionic acid had an increased number of microglial cells and it was showing neuroinflammation in the brain. So the propionic acid seemed to increase inflammation.

Slide 20: 24:19

On slide twenty you can see that different parts of the brain and the white matter in the hippocampus, the right side, which are the panels of the rats that received propionic acid, that this marker of IL6, or interleukin six, an inflammatory cytokine that keeps coming up over and over and over again in autism and seems to be associated with autism as well as inflammation in the gut, is increased in the brain because of this molecule called propionic acid.

Slide 21: 24:01

In the next slide he measured something called nitrotyrosine which is a marker of oxidative stress. This marker was seen to be in the hippocampus of the brain which is a very important structure, you can see on the right side, the high dose of propionic acid rats had higher levels of this nitrotyrosine.

Slide 22: 25:36

What was important in the extension of this model was of course is well you say that is all fine and good if you inject this molecule, propionic acid into the brain and it causes all these things that might be bad. But we don't have the microbiome, the gut bacteria producing propionic acid in the brain, that the brain doesn't have the same microbiome that you see in the gut and doesn't have what we call a cementing bacteria to produce propionic acid, that is happening in the gut. So the brain seems a little far away from the gut, so maybe if you inject it into the brain, bad things happen but in practical terms, is this something that really can

happen? And two of course, he was working with adult rats, so also you know autism is really neurodevelopmental problems, what about young rats or young pups. So what he did is he extended the model to look at young pups and what he actually did was feed food that had increased amounts of propionic acid to the mothers of these pups and then to the pups after they were born. What he showed was that they did have some of the same abnormalities that we see behaviorally associated with autism and animal models of autism.

We can see on the bottom of the graph on the x-axis is the post natal days, so after the pups are born, their development. And this is a graph of the number with eyes opening and unlike humans, rat pups don't open their eyes right away, and how long it takes them to open their eyes is actually a measure of development and how their brain is developing. And what see here is that this bottom line with the orange triangles, is the pups that were exposed to propionic acid before birth and then early in life. And what we see is that there is a decreased number of them, opening their eyes, and it takes them more days to open their eyes suggesting that they developmentally delayed because they were exposed to propionic acid in utero.

Slide 23: 28:48

We can see other measures of this acoustic startle response which is a measure of anxiety like behavior. What we can see is that the rats that were exposed to propionic acid which again is the orange bar and the ones that were exposed to LPS, which is another model of autism, had this exaggerated startle response as compared to the other control pups, suggesting that they developed anxiety like behavior because of being exposed to propionic acid. And what was very interesting too is that there was this interaction between male and female whereas the male pups seemed to be much more affected than the female pups. This is very interesting of course because we known that many developmental disorders like autism have a predisposition for males so in some ways this model is recapitulating this gender difference that you see in neurodevelopmental disorders.

Slide 24: 30:42

Of course that is kind of interesting but what about the mitochondria because that is one of the things that we here hear about. So how I actually got involved with this microbiome and getting involved with this animal model with propionic acid is that independently I was looking at metabolic disorders in children with autism. We will talk about how this propionic acid model parallels on children with autism.

Slide 25: 30:51

What I had found is that be measuring biomarkers of mitochondrial function in my clinic I had noticed that there were some children that had this unique pattern of elevations in something called acyl-carnitine which tells us about fatty acid oxidation metabolism. I had noticed that some children seemed to have this pattern of elevations in these biomarkers of mitochondrial dysfunction and that the pattern was actually not consistent with any known disorder of mitochondrial function. So for some time it was a head scratcher of saying 'well that is really interesting not sure what it means, it seems like the mitochondria might not be working well', but it was very nonspecific.

In this paper that we published in Translational Psych we reviewed the clinic that I had, this was from Houston, and we found that about 35% of the children where we measured fasting acyl-carnitines had three or more elevations and the acyl-carnitine are a whole panel of metabolites, and when we tried to confirm this because it is always good to confirm that what you measure is repeatable, is that overall 17% of the children that I saw in my practice seemed

to have this pattern of increased acylcarnitines.

Slide 26: 32:54

As I said I wasn't sure what it meant initially and then if we go to slide twenty-six, it just happened that I was at a conference and Dr. MacFabe was presenting this graph that is on the right here, of the elevations of acylcarnitines in the rats that he had injected propionic acid into. What he found, he wasn't sure what it meant, but he found that there were these elevations in what we call long-chain, that would be C-12 and above, and short-chain, which is about C-5/C-4 down, elevations in this particular acylcarnitines, and what we call the medium-chain acylcarnitines, from about C-10 to C-6 were not elevated. And this is not a pattern that is really known to be associated with genetic disorders of fatty acid oxidation because if you have elevations in short-chain fatty acids, you should also have elevations in these medium-chain fatty acids. So he showed this pattern that he found in his rat and was wondering what it meant, and at the same time I graphed out the elevations that we saw in the children with autism in these same biomarkers. And what we found was a very similar pattern, that is we found elevations in short-chain and long-chain acylcarnitines, but not medium-chain, which is very similar to the pattern that he saw. So we asked 'could this have to do with increased propionic acid in these children with autism' ?

Slide 27: 34:56

One of the other parallels we looked at which is on slide twenty seven, is abnormalities in glutathione. We actually found that the abnormalities that he saw in total glutathione in the rats were actually found in the children with autism and these elevations in acylcarnitines and that these were different than other children with autism. So that was an interesting parallel.

Slide 28: 35:28

What we also found is that children that were found to have more mitochondrial dysfunction and underwent muscle biopsy were found to have a decrease in a particular part of the mitochondria known as complex 1. We can see that here on slide twenty-eight.

Slide 29: 34:53

And we can see on slide twenty-nine, we consider the significance of this, and part of the significance is when you consider the biochemical pathways of the mitochondria and when we look at the Krebs cycle, the tricarboxylic acid cycle, we know that most of the substrates are metabolized to make NADH which then feeds into complex 1 whereas some make FADH2. What is interesting of course is that the first part of the citric acid cycle here tends to make two NADHs which fuel complex 1.

Slide 30: 36:46

What we see is that theoretically what would happen if we had an influx of propionic acid from clostridia or other sources what would happen is we would actually short-circuit this first part of the krebs cycle, and what that would do, is actually decrease the metabolites that the electron carriers, NADH, that would fuel complex 1 and result in a relative complex 1 deficiency. Just like we found in the children that actually had these patterns of elevations in these biomarkers that parallel rat models. We felt that this is evidence that suggests that there may actually be some parallels between this rat model of propionic acid and children with autism and potentially this could cause a mitochondrial dysfunction.

Slide 31: 38:08

Other things that really point to this is another study that came out of Harvard and Columbia. They looked at the microbiome of children with autism and controls. What they found was that children with autism had an overrepresentation of clostridia bacteria and clostridia produced propionic acid as well as other short-chain fatty acids, also implicating the idea that propionic acid could be a culprit.

Slide 32: 38:45

But what is very interesting, is that in the same study, what they found is that the children with autism that had elevations in clostridia is this bacteria that produces propionic acid, were children that had GI symptoms that started before or at the same time as the onset of their autism symptoms, not those that had GI symptoms that started after their autism symptoms. This is important because many times many of the criticisms of some of the GI symptoms we see in children with autism is that maybe it is just behavioral and it is secondary to the behavior associated with autism and it very well may be in some cases. But here it actually suggests that this increase in clostridia that is specific to those that have GI symptoms that precede the onset of their autism symptoms suggesting that it could have a causative effect. So we think that is a very interesting piece of data. These are of course more correlative studies, but what about more direct studies that can show us that there really actually may be, not only a causative effect of gut bacteria, but, of course the thing that would really convince people that maybe gut bacteria had some type of effect on autism is if you could change that gut bacteria you could change the autism symptoms.

Slide 33: 40:44

So in the past couple years, we actually had some data that suggests that is actually the case. There was really a landmark study done at CalTech where they used something called the early immune activation model of autism that showed that they could actually change the microbiome and they could change the behavior of the mice.

Slide 34: 41:21

So this was really a landmark study published in Cell, one of the best journals.

Slide 35: 41:24

This is the theory of the immune maternal activation model and it comes from the idea that many neurodevelopmental disorders, particularly autism, seem to be associated with inflammation during gestation. This is a slide that some of these disorders, these inflammatory disorders that are associated with autism and inflammation. We believe that this can be caused through the production of cytokines or also antibodies may affect the fetus and may actually change brain development.

Slide 36: 42:26

This is an overview of the study and what the study found, that is that one, the female mice are injected with what they would call a mock virus that induces immunity and increases the immune system activity and inflammation when the pups come out, so two when the pups come out they displayed autistic like behaviors such as repetitive grooming and lack of interest in other mice. What is found is that these pups actually have a leaky gut, like has been indicated with individuals with autism, and altered microbiome. Then what these researchers did is they

actually replenished the bacteria, called bacteroides, and they found that it both sealed the leaky gut, and the pups stopped showing autistic like behaviors. Suggesting that they can both induce autism and they could actually treat autism by changing the microbiome environment.

Slide 37: 43:54

On slide thirty-seven we see the more technical graphs here. S is for the saline injected or those that received the placebo. P are the pups that were born to the mothers that had immune activation. What is seen here is that on the left the graph that say FTTC, those are measures of gut permeability. What we see is that the mothers that had their immune system activated, the pups had an increased leaky gut, and if we look on the right we actually see that these are different cytokines. We see that the pups that were exposed to their mothers that had an activated immune system were born and had higher levels of certain cytokines, particularly IL6 keeps coming up and that is something that tends to be important.

Slide 38: 45:22

We also see on slide thirty-eight that when we compare the microbiome communities and the bacteria that make up the microbiome, at the top the saline injected mothers and the poly(I:C) injected mothers which would be the ones that had inflammation we can see the communities in the gut bacteria are very different

Slide 39: 45:58

If we go to slide thirty-nine we see what happens when the probiotic bacteroides is actually replaced in these pups that had experienced inflammation in the mothers and we can see again that FTTC which is a measure of permeability in the gut, that decreases by replacing this bacteria. What you see in the middle is a measure of IL6, an important cytokine and controller of the immune system, we can see that decreases. So by replacing that bacteria both the permeability and the gut and the immune system is modulated.

Slide 40: 47:00

And we go to slide forty we can look at the different subsets of bacteria and see actually my giving this probiotic which is T+BF, that we actually changed the amount of certain key bacteria and decrease some of these bacteria by actually giving this probiotic.

Slide 41: 47:26

Slide forty-one shows some of the behavioral parameters, one on the left we see the measures of anxiety and locomotion in the open field exploration test, you can see those that have been exposed to the mothers had their immune system activated which would be the P, do much more poorly on these tests whereas when they are treated with the probiotic which is T+BF that they show restoration back to control levels similar to the ones that were not exposed to mothers with inflammation, so the anxiety and locomotion abnormalities were actually ameliorated by using this probiotic.

Slide 42: 48:31

We can see on the left the stereotyped behavior is resolved, and if we look on the right we can see that they actually measured vocalizations also. They showed that actually by treating with this probiotic they normalized vocalizations also, which is a measure of communication in these mice.

Slide 43: 49:10

On slide forty-three is interesting most notably on the upper left is this metabolite 4EPS which seems to be a biomarker of this disruption in the microbiome. So this is an extremely interesting study because it shows that not only is the development of autistic behavior associated with disruption of the gut microbiome but that it can actually be treated by restoring some of the key bacteria in the gut.

Slide 44:

So a lot of people say, well that is all great, that is in mice, what about humans? Well we have been very lucky because Jim Adams has just completed a study where he used something called microbiota transfer therapy, previously known as fecal transplants. Now it is the key bacteria in the guts derived from the stool of donors can be refined and put in form that can be taken just like a drug, just like a pill, and restore the gut. At least that was the theory and this is a study that looked at that theory

Slide 45: 50:29

So in this great study, which was published in Microbiome, where they actually showed that this microbiotic transfer therapy will actually alter autistic symptoms and GI symptoms.

Slide 46: 50:46

So in slide forty-six we can see the design of the study. On the top left you can see a timeline, the first thing they used was something called vancomycin which clears out the gut and destroys a lot of the bad bacteria in the gut, it can also destroy some good bacteria too. Then they cleaned out the person with MoviPrep which is similar to what a lot of people use if they are going to have a colonoscopy they clean out their gut. They also gave the individuals prilosec, this is one of the limitations of these therapies and even if you take probiotics is that the acid in your gut can destroy some of the probiotics that you take. So you take prilosec to decrease the stomach acid. Then they treated them with first, a high dose of the microbial matter, and then they put them on maintenance for probably ten weeks. They then asked whether there was improvement in the actual symptoms and if there was changes in the microbiome.

Slide 47: 52:11

In slide forty-seven we see a really nice graph. The one on the top left is the GSRS which is a measure of GI symptoms and we can see that over that ten weeks that the GI symptoms decreased steadily from baseline, and even after the therapy was done at eighteen weeks the patient still had decreased GI symptoms. The PGI is a rating from the parents of improvement and we can see that steadily that during this treatment over the first ten weeks there was improvement and that this improvement lasted after the treatment was done at ten weeks to eighteen weeks. There are also some more formal measures of autism, the CARS, and below that is the SRS, the social responsive scale and at the bottom, the aberrant behavior checklist and you can see that in each cases, there was a significant drop in symptoms in all of these measures of autism symptoms.

Slide 48: 53:36

If we go to slide forty-eight we can see where they looked at the change in gut bacteria from the initial ASD when they started out. You can see on the bottom graphs that there are particular bacteria that they looked at and they can show that from the initial ASD to the final ASD that there was significant change in some of these key bacteria where they changed very

significantly. The top graphs really look at how diverse, the one on the left, the gut bacteria was and one of the things that they found was that there was this increase in diversity. On the right they actually showed that the gut bacteria actually became more like the donors. Although it wasn't exactly like the donors, which was very interesting that the children seemed to have a more diverse biome and it wasn't exactly like what the donors had. So it seemed like this actual treatment restored the diversity of the microbiome and autism symptoms. So that really is a nice study that shows that very potentially we are able to restore changes with the microbiome. We can change the way that they body works and symptoms of disease.

Slide 49:

With that, that is my last slide, forty-nine, I will open up to any questions.

MaryBeth Hollinger:

Wow Doctor Frye, thank you so much for that presentation I know many of us are aware of the emerging role of gut bacteria in some facets of health but I think that many of us have no idea the extent that the impact reaches and I am so glad that you have documented and outlined so much for us. I know for myself I am going to go back and look at these slide and cement a lot of this information because it is very impactful and I think that that field must be just blossoming with information. I will go through some of the questions that have come to me already and then I will open up the lines.

The first question is about antibiotic use. Sandy from Arizona is wondering - her child is required to go on antibiotics and she is now wondering, oh she is wiping out her microbiome, what is the balance there.

Dr. Frye:

I think you hit it right on the head that it is really a balance. We know of course that antibiotics are very important, they are a life saving treatment that can profoundly help children with infections. But we also know that they can change the microbiome, sometimes for good sometimes for bad. We hope not for bad, but too much antibiotics especially in older individuals they can develop something called C. difficile infection which can cause profuse diarrhea. I recommend that antibiotics are important, so I am a big proponent of giving probiotics when you are giving antibiotics. Because the antibiotics are just going into your gut to be absorbed systemically so it is always important to treat with probiotics so you can try and keep the gut bacteria up to where they should be. There are some studies that are looking at that, to see if that is a viable alternative. It is thought by some to think it is probably something that should be done more often.

MaryBeth Hollinger:

Thank you, that is a helpful tip for sure. I know that slide thirteen had many more of those helpful tips so I want to refer everyone back, we will keep these slides up. I might print that one out.

Dr. Frye:

And those papers we have, both of those papers, about the current pharmaceutical design, I can send that to you if you want. The one before that, they are both open access papers so anyone can access them and read them for the details.

MaryBeth Hollinger:

We would love that. Thank you. I had another question from James, he wondered about biome depletion when you have mitochondrial disease, the more environmental depletion where we are limiting that exposure by creating that sterile environment. But yet he is told by all of his specialists to stay away from germs, get the flu shot, wash your hands, don't be around germy people because he has mitochondrial disease and it takes him so long to recover from any kind of viral insult.

Dr. Frye:

It is a really interesting balance because we know that inflammation is something that can trigger metabolic decompensation in individuals with mitochondrial disorders. It is a delicate balance to both stay away from germs, and to make sure that you stimulate your immune system enough so that is protects you. It depends on different individuals with mitochondrial issues. Some people with mitochondrial issues do have problems with their immune system which should be treated and should be investigated, and if he has frequent infections and such I would suggest that he reach out to a specialist to be evaluated but some people with mitochondrial disorders have just fine immune systems.

Of course it is this very delicate balance, it is recommended that individuals with mitochondrial disorders stay immunized because of that fact. It is thought that they are especially vulnerable to any of these kind of infections that most people would get over and those should be prevented. So it is a delicate balance, if there are any signs of immune dysfunction a specialist would be good for him to be evaluated by. Again there are important things like good gut health and such, probiotics and such that may actually help to keep his immune system healthy.

MaryBeth Hollinger:

I think that balance is a theme that most mitochondrial patients are well aware of, exercise, fatigue, there is so much balancing. I will ask one more that came to me. This caller wonderred what is the best way to test or assess your own microbiome, she wondered about commercial testing, and you mention that fasting acyl carnitine biomarker. Is it something along those lines?

Dr. Frye:

There are a number of different companies now that have developed tests, some of them are commercially available, that will test your biome. I would say the markers that I talked about are research markers, so I can't say that you can really use those. The commercial tests are analogous to 23 and me, which we call recreational genetics. You get this neat information, but ability of actionable items on it, that we know exactly what to do is limited. So there are a couple companies that will actually take your poop and they will analyze it for you. What you can actually do with that information is debatable at this time.

MaryBeth Hollinger:

Thank you, I will open up the lines.

Stephanie:

Hi MaryBeth, it's Stephanie. Do you have a particular probiotic that you recommend?

Dr. Frye:

I usually recommend VSL number three double strength.

Stephanie:

Is that something that you university sells?

Dr. Frye:

No it is commercial we don't sell any. But the one I usually recommend for my patients that a lot of people use, it is more of a medical grade probiotic VSL number three double strength, although there are many other ones. That is one that I particularly like, there are many other out there. One of the converovieris of probiotics, one of the things that we know is that a healthy microbiome is a diverse microbiome. So some people are argue that using the same probiotic for a long period of time might not be a good idea because you are getting the same bacteria over and over again. Some people think about rotating probiotics.You have to see what seems to work for you.

MaryBeth Hollinger:

Thank you Stephanie, great question. Susan is wondering if she can't tolerate meat, fish, or poultry, she is just eating eggs, she wondered if there was some sort of food substitute that help her gut if she can't tolerate fish, meat etc.

Dr. Frye:

So I come from autism perspective, but I think sometimes this can be helpful, in mitochondrial disorders we find these also is that in children with autism we find that their digestive enzymes are not being produced. So we have to replace the digestive enzymes to help them tolerate food. That is very common, that they decrease food intake because they won't digest the food and it makes them feel bad, just from having undigested food, and also it disrupts the microbiome.

Lee:

Hi this is Lee calling from Atlanta, and for years we have done rotating probiotics for my son, we have used for a long time VSL number three as well. The problem is he has a mild lactaid/high lactate in the body and every time we give probiotics there are lactate based probiotics like the VSL three, it seems he reacts very poorly and he has a lot of problems. So for children who have mitochondrial disease and they run all the time with the mild high lactate in the body, what kind of probiotics would you suggest since I have a problem with that.

Dr. Frye:

I think that you have to find the one that works for you, it may be, have you tried digestive enzymes?

Lee:

Yes we have, we have a prescription for it because he does have gastroparesis to, we have a prescription for digestive enzymes as well but it hasn't really done the trick. It seems the best probiotics like VSL three is the one that he can't handle. It makes him extremely tired because the lactate goes high in the body.

Dr. Frye:

Gastroparesis is a real problem, when you have severe dysmotility it is really a very big problem as far as disrupting the microbiome and the way that the microbiome works in the gut

because you really need that constant motion to turn over the bacteria in the food over again.

Lee:

Do you have a name of a lactate free probiotic? I know that some companies sell them.

Dr. Frye:

Do you have a good GI doctor a good GI specialist.

Lee:

I actually would take my kid to Children's Healthcare of Atlanta, however I don't know how well versed they are with the microbiome and so I think I see somebody else. But in the past I have taken my son to a doctor that looked at all of those problems and tried to address, but always with the probiotics with lactate.

Dr. Frye:

Well that is lactose, that is different from lactate.

Lee:

Lactate, yes, lactose is the milk protein.

Dr. Frye:

I would really suggest finding one of the really best GI specialist.

Lee:

Another question that I have, you have talked about the immune system and cytokines. So a lot of mitochondrial kids have problem with the immune system and myself and my son got severe attacks to the point of being on antibiotics ten times a year. But what has helped us a lot is the use of IVIG. I don't know if you know about it, but would IVIG replace or help the immune system with the IL6 cytokines and other immune system problems. Because it has been life saving, including the little problems with the high lactate and the behaviors it has helped tremendously, so much so that you can say okay the autism like symptoms are going away just by using the IVIG for four years now. What is you take on the use of IVIG for kids that have immune system problems.

Dr. Frye:

I definitely have a number children, especially ones that have frequent infections, what I find is that if they have mitochondrial disorders and frequent infections is a really bad combination because the infections run down the mitochondria, energy and the ability of the body to fight these infections and so they have this very long periods of really severe mitochondrial symptoms because of the infections because they can't get rid of it and once they get over it they get another infection. So yes, I have a number of children that have IVIG, that have had frequent infections and have mitochondrial disease and it has really helped them tremendously, because once they are well all the time, they can do things, like we talked about the delicate balance of exercise and keeping themselves healthy. I definitely use a lot IVIG when it is indicated for children with immune problems, it is appropriate.

Lee:

No more pneumonia and skin infections and all of those things, and in the end after four

years using IVIG, the healthy immune system, somehow helps the GI system as well. However it is not a cure but it is incredible help.

Dr. Frye:

I agree with you tremendously.

MaryBeth Hollinger:

Thank you Lee. I don't want to take up too much of your time, but one final question from Kristen. She knows that there are some evidence that ketogenic diets might be helpful with autism spectrum and mitochondrial disorders, do we know if a ketogenic diet actually changes the microbiome in a helpful way.

Dr. Frye:

I don't know that there is great evidence that is does occur, but I think that theoretically, it should decrease a lot of the bad players. A lot of the bad bacteria use carbohydrates to ferment, they create these short chain fatty acids. By decreasing the carbohydrates you intake it will decrease some of these short chain fatty acids. Also people worry about year, and have yeast overgrowth, that is another advantage of the diet because yeast uses carbohydrates to grow. So if you decrease your carbohydrates input, it should be helpful if people have those issues.

MaryBeth Hollinger:

Thank you so much! The bottom line is that we so appreciate your passion for the mito community, and your interest in this area. I really feel like this was a really great presentation, and I'm so glad you gave us your time today Dr. Frye.

Dr. Frye:

Well thank you so much. Thank you for you inviting me, I hope you found it useful, I hope everybody enjoyed in.