Metabolic Features of Chronic Fatigue Syndrome

Naviaux RK, et al. PNAS MS# 2016-07571RR, August 31, 2016

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Q1. *Some people still argue that CFS is not a real illness but all in the mind. Does your discovery of a chemical signature help shatter this myth?*

Yes. The chemical signature that we discovered is evidence that CFS is an objective metabolic disorder that affects mitochondrial energy metabolism, immune function, GI function, the microbiome, the autonomic nervous system, neuroendocrine, and other brain functions. These 7 systems are all connected in a network that is in constant communication. While it is true that you cannot change one of these 7 systems without producing compensatory changes in the others, it is the language of chemistry and metabolism that interconnects them all.

Q2. *How does chronic fatigue syndrome fit in with other kinds of hypometabolic states or syndromes?*

All animals have ways of responding to changes in environmental conditions that threaten survival. We discovered that there is a remarkable uniformity to this cellular response, regardless of the many triggers that can produce it. We have used the term, the cell danger response (CDR) to describe the chemical features that underlie this response. Historical changes in the seasonal availability of calories, microbial pathogens, water stress, and other environmental stresses have ensured that we all have inherited hundreds to thousands of genes that our ancestors used to survive all of these conditions.

The body responds differently to the *absence* of resources (eg, caloric restriction or famine) than to the *presence* of pathogens and toxins. We can classify two responses: a single-step response to the absence of resources, and a two-step process in response to the presence of a threat. Both responses are completed by a return to normal metabolism and function. When resources are severely curtailed or absent, the full CDR is bypassed, and the flow of nutrients through metabolism is decreased to conserve limited resources in an effort to "outlive" the famine. This is often called a caloric restriction response. On the other hand, when the cell is faced with an active viral, bacterial, or fungal attack, or certain kinds of parasitic infection, exposure to certain toxins, or severe physical trauma, this activates the two-step response. The first step is to acutely activate the CDR. Innate immunity and inflammation are regulated by the metabolic features of the CDR. Activation of the CDR sets in motion a powerful sequence of reactions that are tightly choreographed to fight the threat. These are tailored to defend the cell against either intracellular or extracellular pathogens, kill and remove the pathogen, circumscribe and repair the damage, remember the encounter by metabolic and immunologic memory, shut down the CDR, and to heal.

In most cases, this strategy is effective and normal metabolism is restored after a few days or weeks of illness, and recovery is complete after a few weeks or months. For example, only a small percent of people who are acutely infected with Epstein-Barr virus (EBV) or human herpes virus 6 (HHV6), or Lyme disease go on to develop chronic symptoms. If the CDR remains chronically active, many kinds of chronic complex disease can occur. In the case of CFS, when the CDR gets stuck, or is unable to overcome a danger, a second step kicks in that involves a kind of siege metabolism that further diverts resources away from mitochondria and sequesters or jettisons key metabolites and cofactors to make them unavailable to an invading pathogen, or acts to sequester toxins in specialized cells and tissues to limit systemic exposure. This has the effect of further consolidating the hypometabolic state. When the hypometabolic response to

threat persists for more than 6 months, it can cause CFS and lead to chronic pain and disability. Metabolomics now gives us a way to characterize this response objectively, and a way to follow the chemical response to new treatments in systematic clinical trials.

Q3. *You talk about the chemical signature being similar to a state of hibernation. What sort of animals exhibit a similar signature in hibernation?*

I wouldn't use the term hibernation to describe chronic fatigue syndrome. Humans do not hibernate. But I can see how it would be a way that people might get a general idea of the chemistry that we found. Hibernation is just one of a handful of hypometabolic states that has been studied in different animals. There are many others that go by names like dauer, diapause, torpor, estivation, caloric restriction, etc. Many environmental stresses will trigger hypometabolism in humans. In our experience, the metabolic signature of dauer is more similar to CFS than some of the other hypometabolic states that have been studied. One of the main points of our metabolomics study of CFS was to give other scientists a new tool to analyze all of these hypometabolic states, developmental stages, and syndromes so that the similarities and differences can be objectively studied, and rational new therapies developed.

Q4. *Are men and women really that different in CFS?*

Yes. About 40-50% of all the metabolites that we measure in our method have a different normal concentration in males and females. This is not all related to testosterone and estrogen. Literally hundreds of metabolites are tuned to different concentrations in men and women. At the pathway level, we found that men and women shared 9 (45%) of the 20 biochemical pathways that were disturbed in CFS patients. Eleven pathways (55%) were more prominent in males or females. We find that to do metabolomics properly, you need to have an adequate number of age- and sex-matched controls. If healthy males and females are lumped together as controls, the power to see metabolic differences in CFS and many other diseases is much decreased. Likewise, the metabolism of a 25-year old male is different from a 35-year old male, and categorically different from a 25-year old female. In each decade of life there are many metabolic changes that occur as part of normal development and aging. When proper age- and sex-matched controls are used, metabolomics is one of the most powerful new tools available to physicians and scientists to study chronic complex disease.

Q5. *How do the metabolic changes you identified in CFS relate to the recent interest in epigenetics and methylation pathways?*

All the covalent chemical modifications of DNA and histones that regulate gene expression are the result of metabolic changes controlled by mitochondria. For example, all DNA and histone methylation depends on the availability of S-Adenosylmethionine (SAMe). Phosphorylation reactions depend on the availability of ATP. Acetylation depends on the availability of Acetyl-CoA. Demethylation depends on the availability of oxygen and alpha-ketoglutarate. Other demethylation reactions require the availability of FAD+ and generate peroxide. Deacetylation depends critically on the availability of NAD+. DNA ADP-ribosylation also depends on the availability of NAD+. The master fuel regulator AMP kinase (AMPK) activity depends on the build-up of AMP or the *de novo* purine biosynthesis intermediate AICAR (aminoimidazole carboxamide ribotide). mTOR is another key barometer of cellular fuel status. mTOR activity requires the availability of leucine. All of these metabolites that regulate epigenetics and gene expression are controlled primarily by mitochondrial metabolism. This makes sense because all cellular activities must be responsive to local resource availability and remain flexible to respond

to potential threats that alter cellular health, and mitochondria are the prime monitors and regulators of cellular metabolism.

With regard to cytoplasmic methylation reactions that involve folate and B12 metabolism, mitochondria also play a key role by regulating the release of formate, the balance of NADPH to NADP+, NADH to NAD+, $FADH_2$ to FAD+, propionyl-CoA to succinyl-CoA, and glycine to serine. Ultimately, all of these mitochondrial reactions influence the tide of substrates available for methionine, cysteine, glutathione, and taurine metabolism. The ebb and flow of these metabolites determines the balance between cell survival and death, controlling epigenetic modifications and gene expression. These reactions are illustrated in supplemental online Figure S6 of our paper.

Q6. *How might your results help with treatment of CFS?*

This first paper was not focused on treatment. However, metabolomics reveals a new window into the underlying biology of CFS that makes us very hopeful that effective treatments will be developed soon and tested in well-controlled clinical trials. Metabolomics will be an important component of any clinical trial of new treatments for CFS. It will also play an important role in analyzing the similarities and differences of classical laboratory models of hypometabolic states like dauer.

Q7. *How would you respond to Dr. Ronald Davis's recent statement: "What is important to note is that in the absence of evidence of an active infection, it is plausible that the long-term antimicrobial treatments often used for ME/CFS patients are doing more harm than good."*

I am in complete agreement. Many antibiotics like tetracyclines, erythromycin, and the fluoroquinolones (eg, Cipro), and antivirals like acyclovir, fialuridine, AZT, and ddC also inhibit mitochondrial functions when used chronically (usually for more than about 3 weeks). Because mitochondria are descendants of free-living bacteria, their machinery for protein synthesis, RNA synthesis, and DNA replication are susceptible to many antibiotics, and for reasons unique to mitochondrial DNA synthesis, they are also sensitive to antivirals. Chronic use of these drugs can do more harm than good if there is no longer good evidence for an active infection. When mitochondrial functions are critically impacted by long-term use of certain antibiotics, a ripple effect in metabolism and gene expression is produced that can further impair energy production by mitochondria, converting an active cell danger response that occurs during active infection to a hypometabolic survival response.

In the field of mitochondrial medicine we are particularly sensitive to these issues of iatrogenic toxicity because some of the drugs that inhibit mitochondrial functions are very commonly used in patients without mitochondrial disease. For example, statins, valproate, and metformin can each produce problems in patients with pre-existing mitochondrial dysfunction. Most doctors do not think about how some antibiotics, antivirals, and other common drugs can inhibit mitochondrial function when they are used chronically. Our patients with mitochondrial disease are often the ones who educate their doctors about the mitochondrial dangers of many common drugs.

I know this is a sensitive area for many people struggling with CFS. It is important to emphasize that individual medical decisions must be governed by individual responses to treatment. Medical decisions should be informed by science, but cannot be based solely on abstract scientific concepts without also considering the many other clinical variables relevant to the care of each specific patient treated as an individual. Some patients do better on drugs that we would

consider to be inhibitors of mitochondrial function. This may not have anything to do with the conventional pharmacologic classification of the drugs as antibiotics, antivirals, anticonvulsants, antidepressants, neuroleptics, or anticholesterol agents. Most drugs have metabolic effects beyond their primary action. Because the field of metabolomics is so new, these "pharmacometabolomic" effects of drugs have not yet been studied well.

Q8. *Since mitochondria have two main jobs in the cell—energy metabolism and cellular defense—is it possible the one function can be overactive at the expense of the other?*

Yes. This is a key concept. Our lab classifies all complex chronic disease as being the result of either mitochondrial underfunction or mitochondrial overfunction. Each type has both genetic and environmental causes, but environmental causes outnumber genetic causes in the clinic 10:1. Only expert centers in mitochondrial medicine will typically see the many genetic forms of mitochondrial oxidative phosphorylation and metabolic disorders. Most academic centers will see more of the "ecogenetic" mitochondrial disorders caused principally by environmental factors. These disorders range from autism to asthma, depression and autoimmune diseases, to Parkinson and Alzhemier disease, and many more.

Mitochondria lie at the hub of the wheel of metabolism, coordinating over 500 different chemical reactions as they monitor and regulate the chemical milieu of the cell. It turns out that when mitochondria detect "danger" to the cell, they shift first into a stress mode, then fight mode that takes most of the energy-producing metabolic functions of mitochondria off line. Even normal exercise stresses mitochondria transiently and reminds the cell how to heal. Cells "go glycolytic" under conditions of stress, using oxygen less and sugar more for energy production. Mitochondria are highly dynamic in the cell. They will fuse with one another and divide, moving about the cell, changing their location according to cellular needs. Sometimes mitochondria will proliferate so a cell has more mitochondria than normal. Other times they will become hypersensitive to minute changes in one or more chemicals in the environment, overreacting to a stimulus that would normally be undetected by cells that have a normal mitochondrial setpoint.

What does all this mean? It means that mitochondria don't do just one thing. Sometimes, when one function is overactive the other is decreased. Energy production and cellular defense are two sides to the same coin—when you are looking at one side, the other side is temporarily hidden. Mitochondrial cannot perform both energy and defense functions at 100% capacity at the same time. Health requires a dynamic balance of both these functions. It is plausible that when a particular patient seems to benefit from long-term use of a drug known to be toxic to mitochondria, that the drug helps rebalance cell defense and cell energy functions by decreasing the over-activity of one function thereby permitting an increase in an underactive function. My experience is that this is rare in CFS, but exceptions occur and are important to understand if doctors are to get better at treating all patients. Both patients and doctors should carefully evaluate the pros and cons of long-term antimicrobial therapy if the signs of an objective infection have disappeared. Any drug has the potential to be therapeutic or toxic.