# Maureen Hanson, PhD | Metabolism and ME/CFS

https://www.youtube.com/watch?v=hPAzInLeSx4&index=11&list=PLl4AfLZNZEQPxjqF4ojAO3wdCFMeriNBK&t=0s

[Raeka Aiyar] Our next speaker is going to be Maureen Hanson of Cornell University who is going to continue the discussions of metabolism in ME/CFS.

[Ron Davis] I think I first met Maureen at one of the ME/CFS meetings. It might have been the one in London. Then I saw her get up and tell the researchers that what they were doing was wrong. And I said, wow, this is a wonderful researcher because she was absolutely right. She's totally dedicated to figuring this out. She's a plant biologist. But she knows a lot about mitochondria because plants have mitochondria. So she's another one that I have felt when Maureen says I'm gonna be doing this—and I say fantastic! I don't have to do that, that's another one of those things that she can figure it out. That's just certainly great. Now that doesn't happen with all researchers. Some of the researchers I know and they say we're going to do this—and I say crap, we're going to have to repeat it. And so it's really, really wonderful to have some people like Maureen Hansen in this field. Jesus, she's super. Thank you, Maureen.

[Maureen Hansen] Thank you. I'm here representing not plant biology but the center that I direct at Cornell called the Center for Enervating Neuro-Immune disease. That's actually my favorite name for the disease we know as ME/CFS. Until we come to the final name that this disease should be [called], I'm using this one. Now we have a center that is in Ithaca, New York, at Cornell's school in Ithaca. We also have our medical college that's in Manhattan, New York and there are labs at both places that are part of our Center. Today though I'm only going to be talking about research ongoing in my own lab. And I want to start out by thanking my lab members who are working on the disease. I have two postdoctoral associates and three graduate students who are actively studying the disease. I'm only going to be talking about one of the several projects that they're working on. My lab actually does a lot of molecular genetics and this talk on metabolism is sort of a venture into biochemistry. It's not one that we have done in the past.

So—our ongoing research concerning metabolism. There's two aspects to it. One is metabolite profiling which is a lot of biochemistry. Another one is more cell biology looking at cellular metabolism, a combination of cell biology and biochemistry. I'm not going to talk about that last project. I don't have time but I did briefly speak about it last time when I gave a talk here. So the metabolite profiling that we're doing is by having a collaboration with Susan Levine, a well-known ME/CFS physician and in New York. And we have to thank her patients who were willing to donate blood for this study. The data analysis has been done by Arnaud Germain in my lab, as well as David Ruppert who is a statistician who's been working with us. This work was funded by the Solve ME/CFS initiative and partly by NIH.

I'd just like to briefly introduce you to the question of metabolites in your body—there is an excellent Canadian human metabolome database that has gathered together all of the metabolites that have been either identified or predicted in humans. There's quite a number there and in your blood, your serum, there's—in this room we probably have 25,000 metabolites circulating. Obviously it's very difficult to exam 25,000 metabolites. In fact, the typical metabolite studies actually analyze and identify only a fraction of this human metabolomics. Of course many of the metabolites that are analyzed are

the ones that are more common and more abundant in your blood and therefore more important than some of the other ones. But the numbers of metabolites that are identified depends on the methods that are used—how they're separated and how they're analyzed. So different studies will give you different results depending on how you're analyzing the data. So the source and type and handling of samples varies between different studies. Now most of us are able to age and gender match our patients and our controls. But it's often very difficult, especially with this patient group, to regulate the time after they've eaten, their diet, all the various medicines that people take, and also of course where you live, and other aspects of the demographics. Different groups collect different types of samples in different collection tubes. And you might collect serum versus plasma. Then you have to transport the samples and then store them before you analyze them usually. And then the extraction methods vary between labs. So you could imagine that there would be a lot of difference in the different studies that have been done on metabolites. But in fact, the metabolomics studies of ME/CFS are actually largely consistent. There are alterations in energy metabolism, the Krebs cycle, citric acid cycle. It's known there's disturbances in fatty acid and lipid metabolism and purine metabolism. And the metabolites are able to distinguish patients from controls with really quite high specificity despite all of these differences.

So our first metabolomics study was a very small one which we analyzed only 361 metabolites using the technique of a colleague who is generous enough to allow us to run samples on his machine. And we identified 33 metabolites that were significantly different. Those pathways that were affected were fat metabolism, energy and sugar metabolism, and amino acid and purine metabolism which you've heard about already earlier today. And in that particular study 29 of the 33 metabolites were lower in the cases than controls and there was greater variation in the patients.

So we now have a new study, another small fairly small study, with 19 healthy controls and 32 patients. Again we used all female patients to have a little more consistency between the sort of person who was analyzed. Their age was the same and their body mass index is also about the same in this group. This time we used the company Metabolon to do the analysis. And they were capable of analyzing and identifying 832 metabolites in plasma. And they group these metabolites into eight super pathways. Now you can see their lipids, amino acids, xenobiotics, nucleotides, energy, all these different super pathways were analyzed. We identified eight metabolites corresponding to four of the super pathways at different levels between patients and controls. Three of these are cofactors. One of them is involved in energy metabolism. Three of them are nucleotides and one of them is a peptide. What's interesting though is even though we identify different actual chemicals than some of the other people have identified in their studies, what's important is what pathways these different metabolites belong to. So even if one group identifies one set of metabolites and another group identifies a different set, sometimes those are in the same pathway and therefore it is the same pathway that's actually affected.

Now you just saw this diagram earlier in our keynote address. The citric acid cycle is affected. And if you compare the ratio of the C4 and C5 molecules, it's disturbed in the citric acid cycle. And as I just mentioned, alpha-ketoglutarate, one of the compounds of the citric acid cycle, has a significant difference in level between the patients and the controls. The other interesting thing about this study is that we can actually predict whether you're a case or a control, to the 95 percent level, just by looking at the levels of 41 metabolites. And that is actually a very good sensitivity and specificity. The ratio of just two metabolites each can identify 86 percent prediction rate of cases versus controls. These are ones that are involved in these ratios, again as fatty acid metabolism, so there again seems to be something disturbed about fatty acid metabolism.

So how does our new study compared to the other metabolomics studies in ME/CFS? This diagram shows all of those studies indicating how many metabolites were found. That's what's in those circles. Whether it was only females who are analyzed, whether it was males who are analyzed, or both males and females. Now obviously if we can't really compare our data carefully to the female data to the male data. There are known differences and if it was grouped together as was done in some of these studies, we can't really compare it. But we were able to compare our data to the studies that also separated the females from the males.

So one issue in comparing the metabolites found in different studies is that the nomenclature for these metabolites is different. And it can it can be very difficult to know that this complex metabolite identified in one study is the same as in your study. But they're using this HMDB database and ID numbers. We could identify a number of metabolites that were the same between our study, our Metabalome study that we just did, and our old study in 2017, the study done by Naviaux's group, and by Armstrong's group, so we were able to compare a number of metabolites to see if there was any difference. And you can see that in these experiments, the comparison of our studies to the Armstrong one's there are some differences. But that is easily explained by the fact that the Armstrong group analyzed serum while we were analyzing plasma. So there's some difference there. But if you look at the comparison of our study with the Naviaux study and with our earlier study, there's very little difference. So that shows it's actually quite reproducible and that's very comforting since clearly our patient population is very different than the one that was analyzed here in California.

So we also ask the question, can we find patient subgroups with respect to metabolite levels? We've heard a lot that there are subgroups. And we know that there are subgroups in response to drugs, subgroups in different types of symptoms, etc. We used the statistical test to evaluate the hypothesis that the patients can be placed in to different sub groups according to their metabolite profiles. And when we did this, with our study, our old study, in the Naviaux study, we couldn't find any subgroups. And with regard to the Armstrong study, it was again only two patients that differed from the other patients. And one thing I have to ask Chris again is if he's figured out what's different about those two patients that might explain why they were their own little subgroup. So this lack of subgroups to me suggests that that the blood metabolomics data could be detecting a fundamental difference between ME/CFS patients and controls. And the fact that this is very consistent between different groups that have done metabolomics studies also suggested that there could be something fundamental that we are detecting by doing the metabolomics.

We also did an experiment. We could only again use the HMDB ID identified compounds but we compared the four data sets that we had to 344 disease associated human plasma datasets that this Genome Canada group made available. And a number of the conditions that were significant that correlated with the ME/CFS data set suggested that patient tissue may be experiencing hypoxia which is inadequate oxygenation. And we've again heard that theme today and I think we'll be hearing it more later. So what happens when you have hypoxia? It affects your transcription factors. It causes production of reactive oxygen species, causing oxidative stress, this can cause vasoconstriction affecting your circulatory system, which is one feature that maybe is a characteristic of ME/CFS. So there is a lot of evidence for deficiency in tissue oxygenation in ME/CFS and the metabolite information is just, you know, one now another aspect of this. We know there have been cerebral blood flow studies that show poor circulation to the brain. There's studies showing oxidative stress in the brain. We're going to be hearing more about that this afternoon. There's insufficient oxygen delivery during exercise, potentially resulting in post exertional malaise. There's reduced blood volume. David Bell did a very interesting and

unpublished study showing that two-thirds of female and 1/3 of male patients that he analyzed had reduced blood volume.

Now if you look at this diagram you'll see that there's a diagram showing a normal hematocrit and a low blood volume hematocrit and it's absolutely identical, 42%. But the amount of blood is much lower and so that patient can go in with low blood volume, have a hematocrit done by their general physician and it will not detect that they have a low blood volume. It's only detected if you have anemia. So the other thing that some of you are probably experiencing here is when you stand up, is blood pooling in your legs. And that also of course restricts your oxygenation when all your blood is in your legs instead of where it belongs.

There are also some additional disease associated metabolites sets. I just thought I would show one of them since we're talking a lot about fatty acid metabolism. There are these three deficiency diseases. This diagram I'm showing was drawn not for ME/CFS but for illustrating these diseases. And you can see that what it says is that your long-chain fatty acids and metabolism disrupted. And what does then happen: little or no energy and health problems. So that sounds very familiar I think.

So we want to find out how metabolites change when the patient condition declines or improves. As I mentioned there is still a challenge about the fact that healthy people and ME/CFS patients are very different. They have their own genomics, environment etc. and there is a large number of drugs and supplements and dietary differences that we have to deal with. So we want to do the same kind of thing that we heard we heard about from Alain, use the patient as his or her own control. You have a healthy person who undergoes a challenge and it doesn't really affect them. But you have a ME/CFS patient who is already in bad shape and give them a challenge and they get worse. And so they are serving as their own control. What did that challenge do to them? Of course the other thing you do so you can look at the difference in various measures, not just metabolites but other measures, to see what's happened to them. Of course you can compare the baseline patients as well as the baseline controls to the patients as well as the post challenge patients to the post challenge controls. So as most of you know we are using the two-day cardiopulmonary exercise test as a challenge. The idea is to compare the response of healthy individuals and patients before and after an exercise challenge. As part of our NIH ME/CFS, NIH funded Center we'll be looking at metabolites, cytokines, doing neuroimaging, examining gene expression, and also extracellular vesicle cargo: what's contained in those vesicles and their number and the release. That is a project not just in my lab but in the labs across our Center at Ithaca and in New York City.

I also want to mention another project that is a rare chance, a unique opportunity to compare patients when they're extremely ill and after treatment improves their condition. And the reason this is rare course is that there's very few treatments that improve anyone's condition. But Ampligen that most of you know is a non FDA-approved drug that can only be obtained through a clinical trial is a way to improve patients, certain patients, condition—the subset of patients that do respond to Ampligen. Dr. Daniel Peterson at Simarron Research has a group of patients that he has given Ampligen to in the past and they are known responders. Because of manufacturing problems these people have been off of Ampligen for a year. They've relapsed, they're back to their baseline status. And now that they can get Ampligen again, we can see and look at these patients at the time that they start out and after they've gotten better. And we can compare these to 26 patients who aren't receiving Ampligen, who are not getting better. The patients are going to be followed over six months. This has already started. Some of

the patients have had their blood collected at time zero, they'll have blood collected after they've had three months of Ampligen, and then after six months of Ampligen. Due to the funding by a generous donor we will be able to look at metabolites, cytokines, and gene expression. But importantly we'll be able to bank this blood so that many different kinds of analyses can be done in the future as well. We've labeled hundreds of tubes for this exercise challenge study and Ampligen restart studies. So we had two labeling parties in my lab. We actually got some undergrads to come in. My seat is there too, all of us sit there for an hour and a half labeling hundreds of tubes and then send these off to the labs that are drawing and processing the blood. We'll be making a biobank that can then be used as mentioned for collaborative studies that we hope to do in the future.

I'm going to end here with my summary. I think since my time is up I will just leave the summary. I've got one minute? Okay so I can maybe read it then. We have a new study showing eight metabolites are significantly different. We have the four studies for which we have this data. We only have some of the metabolites in the same study identified due to different methods and also the problem of nomenclature that we have. Like other studies, our metabolite analysis we revealed the disturbance in the TCA cycle. We have levels of 41 metabolites to predict case versus control at 95%. We have very consistent results between all four studies. We don't have evidence for subgroups and that really gets us excited that we could be looking at a fundamental difference between patients and controls. One of the hypotheses is reduced tissue oxygenation in patients, as well as problems in utilizing fatty acids which other groups have also found in their studies. Thank you.

[Applause]

*Thank you to our wonderful volunteer transcribers for transcribing the Symposium presentations.*