January 2011 Issue | Mark Tarnopolsky, MD, PhD McMaster University

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I promised you that you were going to be in for a high energy experience. I bet your mitochondria were enlightened and activated through that journey that we just took with Dr. Tarnopolsky. It was really an amazing discussion and tour de force as it pertains to so many areas that we touched upon in our discussion with him.

I'd like to follow up with a few closing, news-to-use comments. You might need or desire a little bit more follow up on some of the specifics we talked about in the interview. I want to focus on agents that cause mitochondrial dysfunction, and those agents that Dr. Tarnopolsky alluded to that might improve mitochondrial function. I'm going to go back, in this discussion, to my meeting at Emory Medical School (the Center of Molecular Medicine), focusing and specializing on mitochondropathies in children, which occurred in the late 1980s. I'll discuss the evolving understanding (both my understanding and, more importantly, that of the field) since that period of time and how that interrelates with Dr. Tarnopolsky's comments.

First of all, what can we say about mitochondria? We can talk about the fact that really it establishes the redox potential of the cell (the reduction/oxidation potential). It raises the potential energy of the cell through these high energy cofactors, these reducing factors (ATP, NADPH, FADH2), the carriers of energy in the cell. As the mitochondria—the energy powerhouse of the cell—is under stress, that stress can come from various factors, including chemical stress and immunological stress, as well as sedentary stress.

As we learned very beautifully from Dr. Tarnopolsky's concepts, the best therapy of all is activity and movement and conditioning. All create cellular stress, which then alters the redox potential of the cell and its ability to do work, in terms of redox signaling. What happens is potential energy that could have put into good work gets put into promiscuous work. We call that oxidative stress, and it causes injury through oxygen, nitrogen, and sulfur radicals, on other biomolecules that cause cross-linking of proteins, or mutations of DNA, or oxidation of unsaturated linkages and fatty acids and so forth.

We recognize there are a variety of agents that can cause this kind of cellular stress to modify

mitochondrial function, and we hit upon a number of those, including things like the nucleoside reverse transcriptase drugs that alter mitochondrial activity, uncouple the cofactors involved in the electron transport chain, and set in motion what we see clinically as buffalo hump, or as lipodystrophy and metabolic storage disease. When the body can't use the energy of food, it stores it for a rainy day that might never come.

There is an argument that one could also apply to obesity—that maybe some factors related to obesity--like the AZT connection to lipodystrophy in the HIV patient--may also interrelate to the problem of toxicity in the average individual from other sources that alters their metabolic efficiency at the mitochondrial level, their ability to process calories efficiently, and store that, then, as energy storage and triglycerides in contractile tissues we call the adipocyte, for rainy days that never come, meaning obesity could come from toxicity. There is going to be a lot more we'll be discussing on this topic over the months to come because this is a very big area of research that is occurring presently.

Oxidative processes can injure biomolecules like omega-3 fatty acids, and in so doing produce secondary byproducts like hydroxyl nonenal, and these go on to further serve as agents that interrupt mitochondrial function and cause uncoupling. This is like the dog chasing its tail: it gets worse as it cycles into greater inefficiency in terms of energy processing by the electron transport chain. So agents that cause exposure to these toxic byproducts of fatty acids may interrupt mitochondrial function. These would be things like rancid food products, or oxidative stress on the high omega-3 intake.

The redox environment established by the mitochondria also regulates adipcyte differentiation, and therefore has an influence on insulin sensitivity, and on adipocyte signaling through adipokines like adiponectin. Therefore, if you alter the redox environment of the mitochondria, the cell becomes less efficient, its intercellular signal transduction changes, and it starts altering its friendly personality to that of a personality under stress by altering insulin sensitivity, changing the economy of energy production from that of energy of activity to energy of storage. Now you get what's called the "thrify" genotype—basically the phenotype of the cell is transitioned into storage rather than into energy processing, which I think is very interesting. When you think of patients who have high BMIs, you might think, "Wow, they've got all this energy they have stored; they must be very energetic." Each pound of fat is about 4000 calories of stored energy. You might say, "Wow, they've got more than enough energy to do all sorts of things." Yet these patients often present themselves as having low energy, fatigue, and hypotonia, because there is a switch metabolism: energy is going into storage rather than into utilization, and that can occur as a consequence of these defects in mitochondrial oxidative phosphorylation.

There are many drugs (not just the AZT-like drugs) that can be engaged in mitochondrial interruption and can create toxicity of various tissues, both in acute stages and in chronic stages. The chemical environment plays a role. It's interesting to note that the redox status of the mitochondria, as we mentioned, is regulated through things like transcription factors such as the nuclear receptor factor 2, which activates mitochondrial function and is engaged in an interrelationship between the antioxidant response element and the xenobiotic response element. The xenobiotic response element is the portion of our book of life in our genome that controls the production of the detoxifying enzymes, and thus is

connected, through similar gene promoter regions, to the antioxidant response element that regulates the production of the superoxide dismutase catalase superoxide or glutathione peroxidase/glutathione reductase, the antioxidant enzyme system. There is an interconnection between regulation of antioxidation and detoxification that are associated through mitochondrial function.

There are a variety of environmental chemicals that can engage in altered mitochondrial function. The one that really came to light dramatically back in the 1980s was MPTP, which is a byproduct from the metabolism of an herbicide, paraquat. When marijuana was found to be contaminated with paraquat and people smoked it, they were exposed to this chemical MPTP that caused a unique form of Parkinsonism.[17],[18]

Neurological Toxicity and Mitochondrial Poisoning

We recognize that neurological toxicity due to mitochondrial poisoning can be very dramatic in the acute case, but what about in the chronic state? Is there a connection between low grade chemical exposures that are not properly detoxified and altered mitochondrial function that is seen as—I guess you would call it—a degeneration of neurological function over time? There is a very interesting paper that was published in *Science* magazine back in 2004 titled “Biomedicine: Parkinson’s Divergent Causes Convergent Mechanisms” that talks about this whole connection.[19] This came out of the Emory School of Medicine, where a lot of this mitochondrial work was first pioneered. In *Neuroscientist* back in 2002 there was a wonderful paper titled “Environment, Mitochondria, and Parkinson’s Disease” that really started looking at the first level at this connection between excitotoxic death in neuronal cells as a consequence of complex I and complex II deficiencies in mitochondrial bioenergetics, and how the environment could contribute to altered toxicity and poisoning of mitochondria.[20]

So there is this mitochondrial paradigm for degenerative diseases and aging. An author by the name of Dr. Wallace wrote a very nice paper in the *Novartis Foundation Symposium* in 2001 looking at a mitochondrial paradigm for degenerative diseases and aging and how that relates to all sorts of different animal species, not just humans but going all the way into mice.[21] When you expose animals to agents that interrupt or poison mitochondria, you get accelerated neurological and immunological problems. That ties together things like dystonia that we saw with Dr. Tarnopolsky’s discussion. Endotoxins are capable of inducing mitochondria interruption as well. Bacterial endotoxins have been shown to alter the mitochondrial respiratory change and capacity in hepatocytes. A leaky gut—a problem related to dysbiosis—may also contribute to alteration in mitochondrial bioenergetics and how that ultimately controls and regulates function.

What are the therapies? We talked a lot about the interesting nutrient pharmacology, because there are really no drugs available today that specifically target, effectively, these metabolic functions. In fact, the orthomolecular substances are probably the best tools we have available today for modulating the role of these protective systems within mitochondria and keeping electrons on the wire, so to speak, and the insulation of the wire intact, if you think of the electron transport chain as being kind of a wire between the generator, like the turbine in a hydroelectric dam, and ultimately transmitting that energy and

electrons to the site of need as reducing power for metabolism.

How does that work? From Dr. Tarnopolsky you heard a very nice discussion of how these various substances participate in regulation of metabolic function at multiple sites along the mitochondrial bioenergetic area. In fact, it was very interesting that in *Pediatrics* in 2010, a review paper was published titled "Therapies for Inborn Errors of Metabolism: What has the Orphan Drug Act Delivered?" looking at, from the FDA perspective, how various substances have been found to have roles in modulating mitochondrial disorders.[22] On that list are things like resveratrol at very high dose; things like lipoic acid; things like N-acetylcysteine; coenzyme Q10; creatine; the various orthomoleculars that Dr. Tarnolpolsky was sharing with us. The doses that are often required in these families of nutrients, however, are generally far greater than that employed in traditional wellness medicine or prevention. In cases of inborn errors of metabolism associated with mitochondropathies, the doses may be a hundred or more times the nutrition dose to push through these metabolic blocks.

There is a very nice review paper published in *Nature Biotechnology* in 2010 looking at screening for agents that are able to modulate mitochondrial respiration and mitochondrial function.[23] It was found that out of the more than 3500 small molecules that were screened, the most active compounds were often found to be "natural substances" that provided potential neuroprotection. I think when you start talking about doses, these are generally much higher doses than one normally employs for prevention-focused applications (or a general wellness focus). These treatments are expensive. These are not inexpensive substances (these nutrient pharmacological substances), and so these are not something for everybody. These are targeted nutritional cocktails (mitochondrial cocktails, to use Dr. Tarnopolsky's language) that are designed to specifically try to overcome these metabolic blocks or these conditional interruptions in mitochondrial function.

Some Thoughts on N-Acetylcarnitine

Omega-3 fatty acids, by the way, also play a role here. I want to emphasize that membrane integrity and fluidity of membranes works along—in mitochondrial function—with the other conditionally essential nutrients that are used pharmacologically. Again, the DHA/EPA formulations are high dose. The other conditionally essential nutrient that we didn't talk about in our discussion which I think has some good literature is N-acetylcarnitine. N-acetylcarnitine has been shown to be helpful in preserving mitochondrial function in the elderly. There is a very nice paper on this in *Advanced Drug Development Reviews* in 2009, in which, (again, using animal models first--the aged rat heart and then later going to other animals) it was found that supplementation at fairly high levels of N-acetylcarnitine was helpful in preserving mitochondrial bioenergetics, both neurologically and cardiovascularly.[24]

The places where you most frequently see mitochondrial deficiency (or energy deficit symptoms) are in the cells of tissues that are most rich in mitochondria. That includes cells of the heart, so you see cardiovascular effects; cells of the muscles, where you see muscular deficiency problems; and cells of the respiratory system, where you see pulmonary and respiratory symptoms. And lastly—really at the head of

the list—should be neurologically. Neurons are associated with mitochondrial dimension, so you have a very crowded environment within the interplasmic reticulum of mitochondria in the neuron. There is a tremendous amount of neuronal mitochondrial function that is going on, and there is a lot of oxygen being processed. Remember, we can live without food for weeks (generally), without water for days, and without oxygen for a matter of minutes because oxygen is what powers the neuronal function and keeps mitochondrial function active. It is a very important nutrient, and it's probably the primary nutrient for powering up neuronal function.

If we put all of this together and we say, "What are the symptoms people present with when they start having some degree of mitochondrial aging?" it's the things that Dr. Tarnopolsky was sharing with us. This includes symptoms like forgetfulness, cognitive decline, memory loss, depression, altered respiratory function, poor V02 max (maximal oxygen uptake and utilization under work), muscle-related dysfunction, strength changes, musculoskeletal pain, fibromyalgia (which we have talked about previously) that relates to central metabolic disorders as well as relationships to the neuroendocrine immune system function. We start seeing a whole array of things, not just in the exercising athlete or in the ALS patient, but across the range of aging and age-related chronic diseases that are related to these issues of mitochondrial dysfunction.

I think probably the most important (probably) clinical takeaway from Dr. Tarnopolsky was this urgency—this mandate, this stand-up-and-be-counted advocacy—as it related to activity and exercise, both strength conditioning exercise and aerobic conditioning exercise. He talked about mitochondrial hypertrophy, increasing mitochondrial activity, increased oxygen processing into bioenergetic molecules. He spoke to the fact that when you do that along with strength conditioning you build size of mitochondria, function of mitochondria, and you build healthy muscle mass. Muscle mass is a bioactive tissue. It is very important for processing glucose and for stimulating insulin-regulated pathways, so as one starts to engage in altered mitochondrial activity, you start seeing, as he said, a decline in mitochondrial function within a matter of few days to weeks after putting a person into a sedentary situation. When that person's insulin sensitivity goes down, their glucoregulation goes down, their bioenergetics go down, their oxidant stress goes up, and their damage to biomolecules (including DNA and proteins and lipids) all start to increase, and so they move more into an oxidative stress and inflammatory state of function. The exercise component, both strength conditioning and aerobic conditioning is almost like a prescription pad for improving mitochondrial function and defending against biological aging across those mitochondrial-rich tissues that includes the skeletal muscles, the respiratory system, the neurological system, and the cardiovascular system.

Beyond that, we have these augmented nutrients—nutrient pharmacology—for those individuals who have a deep hole that needs to be filled in. There is obviously still a lot of work to be done, because Dr. Tarnopolsky shared with us the unusual example of a cocktail mix of very high ORAC antioxidant potential substances that in the test tube were extraordinarily useful in quenching free radicals, but when put into human trials were found to actually serve as pro-oxidants (increasing the oxidative stress). I think this is a lesson to us all as we close this issue, because there are many substances that are being sold and marketed as having very high ORAC activities as if they are the best and the be-all for antioxidants, and what we are learning is that the in vitro activities of antioxidants is not nearly as important as what

happens in the body. We need human clinical trials on these substances. We need to know how they affect function at the human level, not just in the test tube, and I think there is a lot of over-selling right now of various antioxidants as being the best in class based upon the wrong kind of data. What is really needed is human intervention data to show biomarkers are improved and mitochondrial function is enhanced.

I hope this is helpful in following up on some of the extraordinary contributions that Dr. Tarnopolsky shared with us, and I think we've opened the door to the next step forward in our understanding of mitochondrial bioenergetics and its relationship to health and disease.

Welcome to *Functional Medicine Update* for January 2011. Yes, it's the first issue of the new year, and what a start for the year we have. You want to start off the year with good energy, and fortunately we are going to do so. This month our topic is mitochondrial bioenergetics and its relationship to things like oxidative phosphorylation, which then translates into neurological function; musculoskeletal function; immune function, and metabolic function in various ways. Our extraordinary clinician/researcher of the month, Dr. Mark Tarnopolsky will discuss the extraordinary work he has been doing over the years at McMaster University in the neurology, psychiatric, and pediatric areas. We're going to let him tell his story first, and then we'll come back after you've had this kaleidoscopic exposure and follow up on a few of the details.

INTERVIEW TRANSCRIPT

Clinician/Researcher of the Month

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Once again we are at that place that I look forward to and I know you do as well, and that's our discussion with a researcher or clinician of the month who is doing something quite remarkable. This month we are very fortunate to have someone who is both a clinician and a researcher. He is unbelievably productive across a very wide landscape of medical disciplines. Dr. Mark A. Tarnopolsky is an MD/PhD at the McMaster Children's Hospital in Canada. He is a professor of pediatrics, and he is also the Chair in Neuromuscular Disorders and the Director of the Neuromuscular and Neurometabolic Unit. He has a tremendous publication record behind him (more than 200 publications) and is a world leader in mitochondropathies and the relationship of mitochondrial function to neuromuscular and neuroendocrine function.

I want to give a short thanks to one of our long-standing *Functional Medicine Update* supporters, Dr. Sheila Dean, for recommending to me some time ago that we follow up on this work of Dr. Tarnopolsky. Sheila, thanks so much for opening up this extraordinary world and thank you, Dr. Tarnoposky, for being available today.

MT: You're welcome.

The Endosymbiotic Hypothesis of the Origins of Mitochondrial DNA

JB: Let me start with the first question. Years ago I had the privilege of meeting Dr. Lynn Margulis—this was, I think, back in the late 70s—who, at that time, was talking about the endosymbiotic origin of mitochondria and how the mitochondrial DNA is circular and looks more bacterial in origin, and therefore this may be an evolutionary example of an infection that ended up being beneficial to the host eukaryotic cell, and this is why we get maternal transmission of mitochondria. What do you think of this whole origin of mitochondrial function and how does that relate to where we are today in understanding mitochondria and disease?

MT: It's an interesting hypothesis. Obviously we can't go back and prove it. The hypothesis is that

about 1.5 billion years ago, when the oxygen content of the environment was going up, we took on this endosymbiotic relationship where what was thought to be probably a purple photosynthetic-type of bacteria invaded what at the time was called a protoeukaryotic cell, which then went on to form eukaryotes. We have eukaryotic cells throughout our body. What they think is that as the oxygen content went up, these mitochondria, which were bacteria, allowed us to detoxify the oxygen in our environment so that we weren't producing as many free radicals. But probably more importantly, it allowed us to extract more energy from our food.

Some of the first energy pathways that we had were the anaerobic pathways, such as the breakdown of sugar through glycolysis, which is rather energy inefficient. In the mitochondria we can use fats, proteins, and carbohydrates, in the presence of oxygen, to extract much more ATP, or units of energy per gram of protein, carbohydrate, and fat. What is interesting is that throughout evolution, the some 1500 proteins that make up a mitochondria are now encoded for by our nucleus. So through this evolutionary process, the blueprints, if you will, to make this little engine for our cells were transferred to the nucleus.

Essentially how this works is the food is broken down and what are called reducing equivalents come into complex I and II. They flux through a chain of linked proteins called complex, I, II, III, and IV, which really pump what is called a proton from the matrix of the mitochondria to the intermembrane space, and much like water would flow down a waterfall and be trapped by a turbine, they come back through complex V to make energy. The core link between complex I, III, and IV, and some components of complex V still retain their genetic code in this little vestigial piece of DNA called the mitochondrial DNA. That's the part that you referred to that is passed on from mums to all of their children.

Certainly as a clinician, when we see a history of a kid coming in with seizures and strokes, and it appears to be maternally inherited and there is a strong maternal inheritance pattern, that's very helpful for us to rule in disease. But given that most of the genes are now encoded for by the nucleus, we're now finding many more diseases that have classic Mendelian inheritance patterns, such as autosomal recessive, autosomal dominant, and even some X-linked recessive diseases.

It is an interesting hypothesis. We're left with this vestige, if you will, which is this circular piece of DNA. One point about that circular piece of DNA is that it doesn't have histones, which we have in the nuclear DNA, and it is very tightly packed, so essentially everything in the mitochondrial DNA (with a few exceptions) codes for a protein or a ribosomal RNA or a transfer RNA. So, it's more susceptible if it takes, for example, an ionizing radiation hit, or some other mutagenic hit. It's more likely that you're going to get a coding region, and therefore you are more likely to get pathology from mutations in the mitochondrial DNA, and that's probably why they are still overrepresented as causes of primary mitochondrial diseases.

JB: That was a fantastic, concise explanation. My compliments. I notice in one of your more recent publications—I think this was in October 2010 in B*iochem Biophys Research Communications*—that you describe some work that you've been involved in for some time: mitochondrial encephalopathy with lactic acidosis syndrome, or MELAS syndrome, cardiomyopathy and rhabdomyolysis and how that may interconnect with autism and with mitochondrial DNA deletions.[1] Are we moving in the direction to understand the interconnectedness and how that translates to problems that we call, for lack of a better

word, autism?

At Least 300 Point Mutations Are Found in Mitochondrial DNA

MT: It is an interesting point. We had kind of an explosion onto the scene of this area of mitochondrial medicine back in the late 1980s, where people found point mutations responsible for Leber's optic neuropathy, which is a painless blindness usually affecting males in their late teens; the MELAS syndrome which you talked about, which is an acronym for Mitochondrial Encephalopathy, Lactic Acidosis, and Stroke-like episodes; and another disease called Kearns-Sayers syndrome. From that time, we have now evolved, if you will, to the point where we have at least 300 point mutations that we found in the mitochondrial DNA and an expanding repertoire of mutations in the nuclear DNA responsible for clinical phenotypes.

The first ones that we described were more neurologic disease: strokes, and seizures, and epilepsy and children with neurodegenerative disease called Leigh's disease or Alpers syndrome. And these really dramatic—for lack of a better term—and strongly suggest that those mitochondrial diseases with high lactic acids and very abnormal muscle biopsies were pretty easy to pick up, and the relationship between the gene and the phenotype was very strong.

What you are talking about is the complexities of the new emerging area. What about more subtle mutations, and more common diseases such as developmental delay, autism, and various other neurological and non-neurologic disease? What role could mitochondrial DNA mutations have in the pathogenesis or in altering the expression of some of these diseases? And what nuclear genes encoding for mitochondrial proteins have we found and might we find in the future that are responsible for—or at least modify the expression of—more common diseases?

It's really complicated and I'll use autism because you brought that up as an example. Of all the kids with autism, there are probably going to turn out to be a hundred different genes that are responsible, because autism is a clinical or a phenotypic description (to make the diagnosis). That phenotype can come from a large number of genotypes. So if we start with kids with autism, we're probably going to find, as time goes on, that at most ten percent of all of those kids with that label have mitochondrial disorders. But if we look at kids with primary mitochondrial disorders as a starting point, a fairly high proportion will have autistic features or pervasive developmental features. So it depends if you start from the chicken or the egg—either from the disease perspective or from the autism perspective as to the relationship. But I think it's important to know about these and to look for them, because it helps you with family planning, with counseling, and something we've been involved in, which is, how are you going to treat these primary mitochondrial diseases, or even secondary mitochondrial diseases, which could be involved in aging, diabetes, obesity, which we'll get to eventually.

Can Mitochondrial Dysfunction Be an Acquired Condition?

JB: Thank you. That is really a beautiful job of laying a groundwork for these—I guess you would call

them—inherited, constituative mitochondropathies. There is another question which has arisen in greater controversy recently, and that is probably spawned a little bit by experiences like Greg LeMond. You probably recall the elite Tour de France bike racer who retired from competitive racing because he had lost what he said is his competitive edge because of a mitochondrial deficiency, and said he was still functional, but not at the elite level of his performance in bike racing. And so people said, "Oh, here's an example of an acquired problem." Because there was no family history of mitochondrial disease in his family, this was thought to be an acquired problem, not a constitutive problem. Is there such thing as acquired mitochondrial dysfunction through injuries that somatic mitochondria might experience over living?

MT: That's a complicated question. Specific to your example, I was at the American College of Sports Medicine meeting a long, long time ago. There was a huge symposium on this specific story (the Greg LeMond story). They presented absolutely no evidence whatsoever that there was primary or secondary mitochondrial dysfunction, and I guess they didn't expect to have a metabolic genetics person at an American College of Sports Medicine meeting to challenge their hypothesis. But there was absolutely zero evidence for this.

Essentially we get older, we do get mitochondrial dysfunction, which is aging. We're not all 23 and at the peak of our V02 max, and there is always going to be some young buck that is going to beat you. But to try and claim that it was a mitochondrial disease or dysfunction that caused him to no longer be winning the Tour de France is illogical. He was shot in the gut—I think by one of his relatives—and retained pellets in his belly, so I'm sure that's going to inhibit his performance much more than any supposed mitochondrial myopathy. They also talked about him doing two-hour ski races. That's not what you see with real mitochondrial disease.

There's no question that aging is associated with some mitochondrial dysfunction.[2] When we look at brain and muscles from older adults over the age of 65, we do see some point mutations. There are some deletions which start to occur, and that probably is due to a number of factors. Just through the normal process of living and eating we generate free radicals, which are in close proximity to the mitochondrial DNA, so it's not surprising that with some drugs that we might take throughout our lifetime, and with ionizing radiation and other hits, that we would get some damage to our mitochondrial DNA, which may not be repaired in the normal repair process. So we sort of stochastically, if you will, accumulate these problems throughout our lifespan in a very segmental region of the muscle, but eventually it becomes quantitative, because if we have these tiny little changes occurring scattered throughout a muscle fiber, ultimately that muscle fiber will slowly shrink, or the heart will slowly be less functional, or the brain will be less functional over time. Certainly our work and the work of many others does support that there is a role for mitochondrial dysfunction, and likely an interactive role with oxidative stress, which contributes to the aging process of humans.

Aging, we know, is multifactorial. But I think these are two important components of aging which are going to lead to a decrease in V02 max, and that's why people are not top sport athletes when they are 65 in endurance sports, and V02 generally declines, which is your maximal aerobic capacity. From your mid-20s until you are 50 or 60, you're probably going lose 30 to 40 percent of your V02 max from aging-associated mitochondrial dysfunction.

JB: This is an interesting paradox. I recall I interviewed Bruce Ames, whose name I'm sure you are familiar with, a number of years ago. He was saying that oxygen we breathe gets converted into activated forms of oxygen, superoxide hydroxyl, singlet oxygen, and so forth, so our body has to work very hard to protect itself against this main of oxygen, knowing that we have this boon of the added advantage of oxidative phosphorylation—this energy that you talked about (energy efficiency). Can you describe for us how the mitochondria protects itself from this oxygen that goes into these high oxidant forms?

Controversy Over Free Radicals

MT: We know that the electron transport chain does produce free radicals. I think where the controversy lies is the question of are those deleterious or are they adaptive? There is no doubt that in certain neurologic diseases free radicals can be at such a high concentration that they can damage fat, protein, or DNA, and can contribute to the pathology. However, there is accumulating evidence that in normal physiology—for example, if you get up and go for a run—that when we flux through the electron transport chain, the slight burst, if you will, of free radicals that are produced function as signaling molecules for our own physiologic adaptation. For example, if you go for a run, you get a pulse of free radicals. That activates a series of signaling molecules, which then activate genes, which then function to attenuate the production of free radicals in the future. [2]

That's standard physiological adaptation, or sometimes people use the term "hormesis." What happens in such a process is we increase what are called our endogenous antioxidants, and there is a host of them in the cell—things like manganese superoxide dismutase, copper zinc superoxide dismutase, glutathione, glutathione peroxidase system, which can serve to quench these free radicals and make them less toxic, or into nontoxic products. What we find is in older adults, who often show elevated basal levels of oxidative stress, when they finish exercise training, even though you would predict that you're going to flux more free radicals through and you may have higher markers of oxidative stress, in fact after exercise training they are lower, due to the fact that our bodies are homeostatic and they adapt by increasing antioxidant enzymes.[3] A whole area of controversy has emerged where people are very concerned that during normal physiologic adaptation, if you are to quench those free radicals by taking excessive amounts of antioxidants, you could actually attenuate the body's own endogenous production of antioxidants, or this term called hormesis.

JB: Boy, in what you just said is buried a huge amount of extraordinary, interesting information. We interviewed Dr. Edward Calabrese, who has arguably been called the father of hormesis; maybe you are familiar with his work. He talked about these nonlinear dose-response relationships where you get close to the origins of certain substances and their effects seem paradoxical relative to what you think of in a normal pharmacological model of increasing concentration, increasing dose. In my discussion with him, he talked a little bit about things like antibiotics at low dose, or chemicals in our environment (xenobiotics) and how they can have a xenohormetic effect, or even metals, like palladium, platinum, nickel, cadmium, mercury. It seems like all of those things that I just mentioned have some relationship to altered mitochondrial function. Is there potential for this environment xenohormetic effect on mitochondrial function?

MT: It's certainly possible. That's definitely not my main area of research. I think what we do find and where there is good precedence is that pulses of stress, which allow enough time for the body to respond, generally are favorable in that they cause the hormetic response and they cause the body to withstand future stressors. That's gone back for many, many years, where people did a thing called preconditioning of the heart, where in animals if you transiently decrease the blood supply to the heart and then you cause, essentially, a myocardial infarction, there is much less damage if the heart was preconditioned.[4] That's a very similar process.

I think what would happen is if one were exposed to drugs which are known to increase oxidative stress (we use a whole bunch of these toxins, like rotenone and such, in our lab) chronically and you don't allow the system to have a period of time to adapt, they become deleterious. Whereas sometimes, for example, with rotenone, which poisons complex I of the mitochondria, if you give little pulses, after a period of time you almost can't kill the cells by giving extremely high doses of rotenone to try and poison the cells, which otherwise would be massively deleterious had you not preconditioned the mitochondria. And the same, I think, holds true of exercise. Even though, yes, there is damage that occurs and we do produce free radicals, when it comes in pulses and the body is allowed to adapt to that pulse and create its own endogenous detoxification system to up regulate, I think it generally ends up being a favorable thing. It gets back to things like, "No pain, no gain" when one does exercise. If you don't cause a little bit of damage, you don't get the adaptive response downstream. This even connects with Selye's theory of stress.If you have chronic pulses of stress which stays elevated in a sustained fashion, eventually the organism can't compensate, and you get failure of the system, and it's deleterious. The same analogies I think hold true for neurodegenerative diseases, and perhaps even for aging and some of the adaptive responses that occur.

HIV Drugs Can Be Related to Mitochondrial Injury

JB: Let's go away from the hormetic question for a second to the pharmacologic question. I've been told that there are some antibiotics that are mitochondrially toxic, which may suggest the bacterial origin of the mitochondria (sensitive to certain antibiotics). And I also think I have read that certain of the HIV drugs produce a lipodystrophy that's related to mitochondrial injury. Is this correct that there are these potential relationships in some classes of drugs?[5]

MT: I think the evidence is strongest for the HIV drugs, especially AZT, which is the quintessential HIV drug which really sort of changed management. Marinos Dalakas at the NIH was first to describe the ragged red fibers, which we usually use as a hallmark for primary mitochondrial disease, as starting to appear in muscle biopsies of HIV patients treated with the AZT.[6] That's not surprising because the polymerase which replicates the mitochondrial DNA is called polymerase gamma, and it has very similar structural properties to the DNA replication mechanism in the viruses. NRTIs (nucleotide reverse transcriptase inhibitors), which were developed for AIDS, also inhibit polymerase gamma. So as a consequence, if polymerase gamma can't go through and replicate the mitochondrial DNA, you therefore

would stop the ability for the mitochondria to repair themselves and to replicate, and eventually you would accumulate mitochondrial damage, which eventually causes these dysfunctional mitochondria building up and causing this histological hallmark of mitochondrial disease called the ragged red fiber.

JB: That's a very interesting point you made. Is it possible, through conditioning, to actually increase the number, or is it just the function of mitochondria? I'm talking about aerobic conditioning and strength conditioning. Do you increase number by replicating mitochondria or do you just increase function of mitochondria within trained cells (myocytes)?

Exercise Training and Endurance and Mitochodrial Biogenesis

MT: Turning to exercise training, we've done some studies with just two months of endurance training in young people. People talk about mitochondrial biogenesis as though these mitochondria are floating around as individual little parameciums or amoebas inside our muscle, and we replicate them and make more of them. We didn't find that that is the case. When we used electron microscopy, what we saw was that the size of the mitochondrial fragments were enlarging. In some cases they were doubling in size. Three dimensional tomography of muscle shows that in fact we don't have these small, little parameciumlike structures of mitochondria scattered throughout, but rather the mitochondria forms this reticulum, which intertwines its way through the various contractile proteins of muscle and forms almost a boardlike (for lack of a better term) interconnected reticulum. So mitochondrial biogenesis is to some extent a misnomer, I think. What we really see is that the mitochondria does enlarge, and we do get a biogenesis in that we get copies of mitochondrial DNA that replicate so those increase in number, but the mitochondria just enlarge; they hypertrophy, or get larger with endurance exercise training.[7]

JB: I want to go back and pick up on something else you said earlier related to signaling processes (intercellular signal transduction) and how signals from the outside reach the mitochondria and the genome. I remember that you published at least one paper looking at kinase signaling through various kinds of signaling molecules like Nuclear regulatory factor 2, and ECG-associated protein or Keap1, and how that interrelates with mitochondrial function.[8] Can you tell us a little bit about what we are learning about intercellular signal transduction and the signaling process of mitochondrial function?

MT: Sure. The Nrf2-Keap1 story more relates to—and that whole pathway is involved in—sensing oxidative stress and up regulating class II antioxidants. But specific to the mitochondrial biogenesis, there are a host of signaling pathways which can activate mitochondrial biogenesis. Most of these converge on a protein which was first described by Bruce Spiegelman called PGC1alpha. For example, there is calcium signaling pathways and we know with exercise calcium goes up, which can then signal through some of the CAM kinases (or the calcium-dependent kinases) to increase PGC1alpha's localization in the nucleus. PGC1alpha then is a cofactor (or co-regulator is probably a better term) of some of the

transcription factors which increase the nuclear encoded subunits, which then end up going to the mitochondria through mitochondrial targeting sequence. Now what is interesting is that PGC1alpha is a very ubiquitous co-regulator. When activated through a signaling process such as increased calcium transduced through CAM kinases, migrating into the nucleus it activates a host, in a coordinate fashion, of proteins which then are destined to go to the mitochondria and coordinately increase the electron transport chain components so that the total capacity of the mitochondria goes up as the mitochondria swells and starts to get larger and be more functional.

There is a host of other signals, however. For example, the cell can sense low energy charge, and one of the main pathways there is something called AMP kinase. ATP breaks down and forms AMP, the increase in AMP concentration activates AMP kinase, which in turn can phosphorylate the PGC1alpha, and that in turn can translocate to the nucleus and function as a co-regulator. And there is a host of other signaling pathways, including P38 MAP kinase, which can also activate PGC1alpha. So there is a lot of redundancy in the system, but generally all of those processes I'm talking about are things that occur in the context of physical exercise or some cellular stress, which—again—gets back to hormesis: if there is a stress, you need some disabling pathway that is going to counteract that stress in the future, and this represents a really nice integrated pathway, converging on PGC1alpha.

Correlation between Mitochondrial Function and Obesity and Type 2 Diabetes

JB: Now, of course, you've opened up the big door, at least for me. For the clinicians who are worried about this rising tide of insulin resistance, and pre-type 2 diabetes, and metabolic distortion, when we start talking about PCG1alpha and coactivators that takes us almost to crosstalk with the PPAR gamma system. It also takes us into mTOR and how that relates to insulin sensitivity and bioenergetics (Spiegelman's work crossing over into diabetes). It seems like there is a correlation here. Can you tell us a little bit about how these fit together?

MT: Oh, there's no question. It sounds like I'm really pushing exercise, but the safest and easiest way to have a favorable effect on all of these pathways, and probably the most effective preventative strategy for obesity and type 2 diabetes, is physical exercise. And we know that the only way to safely and consistently increase PGC1alpha content and translocation to the nucleus is physical activity. People hand wave about different medications which might do this, but usually the medications have significant side effects, which is a real issue. Even things like the PPAR gamma agonist which people have worked on, or the PPAR alpha agonist which people can use clinically, are not without side effects. But unfortunately people don't want to hear that message. They want a pill to increase PGC1alpha's abundance, not the safe, effective way, which is through various types of physical activity. We know this epidemiologically, but we know at the biochemical level why it is so effective. And what is very interesting, too, is that there is also data that if you over express PGC1alpha (this is genetically, in an animal model—work by Carlos Moraes which was published in *Cell Metabolism* about a year ago) with a muscle-specific promoter, so it only expresses in muscle, it actually ameliorates much of the diabetic phenotype in the animals.[9] What that says is that there are signals that come from muscle which have a systemic effect, probably through a variety of hormone-like substances like myokines. It has a favorable effect on the rest of the body and

reduces insulin resistance.

JB: Let me, if I can, just trace back to one other thing we talked about earlier, and that was the work you've done on Nrf2 and Keap1. It is interesting to me that those both correlate, I believe, with co-localization of the ARE and the XRE in the genome, the antioxidant response element and the xenobiotic response element. It would seem, as you mentioned, the redox signaling controlled through those nuclear regulatory factors tie together antioxidant response and also xenobiotic response to toxins. Does that have a teleological explanation or rationalization?

MT: Hmmm. It's an interesting question. I hadn't really thought too much about that specifically from a teleological perspective, but I suspect that probably from an evolutionary perspective those two probably go hand in hand, and certainly when there is some deleterious stressor on the cell, upregulating both of those processes may be beneficial. But, again, I haven't really thought intelligently about that specific component of things.

JB: I know when we up regulate cytochrome P450s, because they are monooxygenases we increase oxidant stress, so maybe it makes sense in a teleological fashion that the antioxidant opportunities come up to prevent hepatic injury or something of that nature. They are kind of coupled.

MT: That certainly is as good an explanation as I've ever heard or thought of. That does make a lot of certainly phylogenetic and teleological sense.

Clinical Assessment of Mitochondrial Disorders

JB: Let's move now to what a clinician might be interested in. We've done a very good job, I think, of setting some background, but now let's talk about assessment of mitochondrial disorders. There are several levels that one might consider how we assess: histology, molecular genetics, functional effects, biochemical analysis. Maybe you could start on histology and molecular genetics. How do we understand whether a person has these mitochondrial underpinnings of some of their clinical presentations?

MT: Sure. I think it is first very important to separate primary from secondary mitochondrial disease. The primary mitochondrial disorders are, by convention, considered to be the disorders that primarily or secondarily affect the electron transport chain assembly or function. These are felt, at this point, to affect about 1 in 5000 individuals. I think as time goes on we are going to find many more diseases that we

didn't think were mitochondrial are going to fall under that umbrella, and that incidence of 1 in 5000 is probably going to increase. People think that primary mitochondrial diseases are rare, but 1 in 5000 is really not rare, because probably every listener will know about Lou Gehrig's disease or ALS, which has a prevalence of about 1 in 50,000. So these are ten-fold more common, and likely that number is going to keep going up.

The primary mitochondrial disorders usually present with some neurological dysfunction: stroke, seizures, developmental delay, developmental regression. It tends to be seen mostly in the neurology clinic. However, there are a host of other issues which can be seen, for example, hepatic failure in children, ataxia, visual loss (which, again, is the retina which is an extension of the central nervous system, but usually ends up going to ophthalmology). So the clinical picture, I think, can point one in the direction of these things like Leber's, and MELAS, and MERFF, and all of these other acronomic-type of mitochondrial diseases.

To approach someone, the first thing that we do is assess a history that is suspicious for mitochondrial disease, for example, under periods of stress like exercise or fasting the person's symptoms were to come out, one would be thinking in that direction. We would next do blood work. In the blood, probably the main chemical that we are looking at is lactic acid, which is reflective of an increased flux through anaerobic glycolysis, which is the cell's response to an aerobic energy crisis, and that is to try and flux through the anaerobic systems. In adults, the elevated lactate is seen in about 65 percent of our patients. So an elevated lactate can be used for ruling in--but it doesn't always rule out--disease. In children, most kids tend to have an elevated lactate in the presence of mitochondrial disease, so it has a bit more sensitivity.

We also find that due to alterations and increased flux through protein metabolism we get an increase in alanine, so measuring amino acids and finding elevated alanine is helpful. Many of the patients also have some damage to skeletal muscle and creatine kinase is variably elevated as a marker of muscle membrane damage. But, again, on its own, elevated CK can be reflective of any other muscle process. In the urine there are metabolites of the leucine pathway called 3-methylglutaconic acid, which can be elevated, and that's picked up on urine metabolic screens or urine organic acid screening.

Then, if we are suspicious of mitochondrial disease, the next thing we do is a muscle biopsy. Histologically what we find in adults is the accumulation of abnormal mitochondria in the subsarcolemmal region, called a ragged red fiber, which is, again, attempts by the cell to compensate for the mitochondrial dysfunction. They undergo massive mitochondrial biogenesis and we get this proliferation, or expansion, of the mitochondrial reticulum in the subsarcolemmal region, which comes up as the ragged red fiber with a stain called Gomori trichrome. One can also get reductions in various enzyme activities. The one that we use most commonly is called cytochrome C oxidase, or complex IV. So finding Cox negative fibers, as we call them, is reflective of mitochondrial dysfunction and often seen in mitochondrial disease.

An important note for the pediatricians out there: Often the classic hallmarks of ragged red fibers and Cox negative fibers aren't seen early on. It takes a bit of time; it takes some aging, if you will, with the muscle for those to occur. And it is on the electron microscope that we see the earliest manifestations of mitochondrial dysfunction, where the nice three-dimensional and two-dimensional architecture of the crystae become abnormal, the mitochondria become pleiomorphic, so we have large and small

mitochondria that have very bizarre shapes. And often they will start to accumulate abnormal densities within the mitochondrial membrane. One of the hallmarks is called the paracrystalline inclusion, which reflects oxidative damage to mitochondrial creatine kinase which then crystallizes in the mitochondria. That can be seen in various toxin exposures, which can damage the mitochondria, including the exposure to the anti-HIV drugs like the NRTIs, but is a hallmark of many of our patients with mitochondrial disease.

Once we have that, the next step is to start thinking from a genetic perspective. If there is a strong maternal history we would be thinking about something in the mitochondrial DNA. If there is a very specific clinical phenotype, like Leber's hereditary optic neuropathy, if you check just three mutations (the 11778, the 14484, and the 3460), you'll get 95 percent of your patients and you've got your diagnosis and you don't need to go any further. With a MELAS clinical phenotype, there are about 20 mutations, but you are going to get most people by just checking the 3243 and 3271 specific transition and transversion mutations. You can be really targeted about it, but if you have a nonspecific clinical feature, one may wish to sequence the entire mitochondrial genome, which is now very readily doable with garden-variety capillary sequencing. Next-Gen sequencing can do that very rapidly and to a high degree of coverage. And then, of course, there is a host of other diseases: the mitochondrial deletion syndromes, when you have multiple deletions; things like polymerase gamma nuclear gene mutations and Twinkle should be looked for; if there is a single deletion in an older person with failure of eye movements, that's pathognomonic for something called chronic progressive external ophthalmoplegia; and, again, the list goes on and on, but we don't have time to go through an entire diagnostic workup.

JB: Thank you. That was brilliant. If we have a person who has, I would say, a functional mitochondrial problem--let's say the kind that we are talking about with aging--can you correlate the whole body (musculoskeletal function, strength, and respiratory function like FEV1 and cardiovascular fitness) at all with these decrements in mitochondrial function? Is there a clinical correlation?

Secondary Mitochondrial Dysfunction

MT: None of the things that you mention really correlate well. Weakness is seen in mitochondrial disease, but there are thousands of causes of weakness, so it's not really a good measure. And we do also have, for example, patients who have Leber's optic neuropathy or even severe MELAS syndrome who have totally normal muscle strength. The one thing that we do see in most of these disorders, especially if they affect skeletal muscle, however, is a decrease in maximal aerobic capacity. And that gets back to the whole thing with Greg LeMond. Greg LeMond and Lance Armstrong and all of these guys have high V02 max, which is the maximal amount of oxygen that they can consume in their body per minute. And really, if you think about it, at complex IV that's where all of the oxygen we breathe ultimately ends up, so any deficit in the electron transport chain right to complex IV or even complex V would cause it to back up. You're going to get a decrease in the ability of that oxygen to be reduced to water at complex IV, and hence your V02 is going to go down.

One of the reasonable screening tests, which has a sensitivity of about 70 percent, is a low V02 max, or a

low maximal oxygen consumption. People who are familiar with that type of testing know that often the respiratory exchange ratio goes up very rapidly during exercise, which is indicative of an early anaerobic metabolism shift, and those are probably the more sensitive tests. The difficulty, of course, is—as with everything—there are multiple causes of a low V02. If your heart isn't pumping, if your lungs aren't exchanging oxygen, and for other various different reasons your V02 is going to be down. But if you see a low V02 and you don't have a cardiovascular explanation for it, it is reasonable to put mitochondrial dysfunction on the list of things that one must consider.

I'll just say one more thing about that, and that is we've also published a paper recently where we took perfectly healthy 20 to 25 year old university students, we put their leg in an immobilization brace for two weeks, and in two weeks we lost 30 percent of their mitochondrial protein content and 30 percent of their mitochondrial enzyme activity.[10] What that points to is that if people are sedentary—in bed, inactive, not moving—we can get very rapid secondary mitochondrial dysfunction. I think that's probably the plague of most societies now—at least developed societies—is that people don't move, they get secondary mitochondrial dysfunction, and as a consequence they tend to get overweight, and they eventually become insulin resistant, and the whole thing forms a vicious cycle which spirals downward.

JB: Thank you. That was brilliantly said. That's really good news-to-use for the clinicians as they talk to their patients. Are there any correlations of what you have described as uncoupling of complex IV with biochemical markers (biomarkers) for oxidative stress like AOHDG or isoprostanes or lipid peroxides? Do they have a correlation, serologically?

MT: Yes, in a number of diseases, and in our patients with mitochondrial disease, we sometimes see—but not always—increased markers of oxidative stress, and there is a host of them that are available. Again, I don't believe anyone is doing this clinically, but certainly in the laboratory things like protein carbonyls, malondialdehyde, and the ones you mentioned—the 8-isoprostanes and 8-hydroxy-2-deoxyguanosines—are biomarkers that oxidative stress has occurred, and that will cover the lipid, the protein, and the DNA damage, as we talked about. And we can use those as markers of efficacy and intervention. And what we do see in our patients with some of the therapies--and one in particular is coenzyme Q10, alpha lipoic acid, creatine, and vitamin E—is those markers of oxidative stress went down when people were on those mitochondrial cocktails. We've seen that those markers of oxidative stress also go down in older adults. Where they are basally elevated, they come back down towards normal following exercise training. So either exogenous targeted antioxidant cocktails which we use for therapy or exercise training will reduce those biomarkers, but I don't think clinically, at this point, that that's going to really help you to differentiate patient from non-patient.

JB: You've really taken us to the next really important step and I bet everybody who is listening is just on the edges of their seats because everybody always wants to know, what do we do about these things? I recall in the late 80s I visited Emory Medical School. I went to their Center of Molecular Medicine where they deal with mitochondropathies, and I found that they were using at least some

empirical mixtures of things like sodium succinate, and creatine, and CoQ10, and lipoic acid. You recently published a very nice paper I saw on CoQ10.[11] Could you tell us what the status is of these things in nutrient pharmacology for these mitochondria-related dysfunctions?

Experimenting With Mitochondrial Cocktails

MT: Let's go back to the mitochondria. What happens when it doesn't work? First of all there is an increase in flux through alternative energy pathways. We know that you crank through glycolysis, but you also increase the breakdown of phosphocreatine. And the latter point is why we started to use creatine as a therapy. Way back in 1997 we published our first paper to try and give an alternative energy source, because we knew that brain and muscle in patients with mitochondrial disease were low, so that was the reason for the inclusion of creatine.[12]

Next, as we pointed out before, if there is damage to components of the electron transport chain, especially at complex I and III, and we get the excess generation of free radicals, it would make sense to quench them if they are present in excess. We chose CoQ10 because it is an integral part—essentially it receives electrons from complex I and complex III, and shuttles them to complex III—of the mitochondrial respiratory chain, and can function as an antioxidant.

Alpha-lipoic acid is another potent antioxidant which interestingly has been also used in type 2 diabetes, and it may be that the secondary mitochondrial dysfunction in type 2 diabetes is what was responding to the alpha-lipoic acid trials, which were used to treat diabetic neuropathy. Alpha-lipoic acid also localizes in the mitochondria, which was the reason why we used that.

The other reason why we throw in the antioxidants in combination is that every antioxidant can become a pro-oxidant. When they gain an electron and become reduced, they can give up that electron. There is good biochemical and biological precedence for combination antioxidants to function as what we call redox couples, and I'm sure when you talked to Bruce Ames he was talking about that as well. So that's why we usually include several antioxidants and not just one.

Consequently, over the years, we put forth the concept of a mitochondrial cocktail to target some of the final common pathways of neurological and muscle dysfunction, including the oxidative stress, the alternative energy pathways, and also to try and bypass energy deficits. So if you have a deficit, for example, at complex I, succinate, which you mentioned, or CoQ10 in theory, could provide reducing equivalents distal to the site of the block. Our concept was if we're going to have any inroad on treating mitochondrial dysfunction—because there are so many pathological consequences—we need to target more than one pathway. Hence, we were in favor--back in 2001 in *Annals of Neurology* we published the hypothesis--that we should be having cocktails, hitting not just redundant pathways in one area, but targeting multiple pathways.[13]And we came up with the combination of creatine, CoQ10, lipoic acid, and vitamin E, which we used in a randomized, double-blind, crossover trial in 2007, published in M*uscle and Nerve*, and showed reductions in lactic acid, and we showed reductions in two markers of oxidative stress in patients with primary mitochondrial disease.[14]

Would these work in people who have secondary disease? We don't know at this point, but I think that

evidence was even stronger than the second paper you mentioned, where we just used CoQ10 and really didn't show the same consistent effects that we saw when we used the combination back in 2007. I think if we are going to have any in-roads, we have to take a cancer/chemotherapeutic approach, and that is to use combination drugs and not just single therapies to target the multiple pathogenic mechanisms in mitochondrial dysfunction.

JB: Thank you. That was very helpful. One of the conditionally essential nutrients that you didn't mention (or nutrient pharmacological substances) was N-acetylcysteine, which I know has a pro-glutathione biosynthetic effect. That's something that obviously doesn't hit on your target. Why?

MT: That's a very reasonable thing and I think there would be precedence and good biologic logic, as you pointed out, to use NAC (N-acetylcysteine), or Mucomyst is a brand name here in Canada, as a therapeutic strategy. Part of the reason why we didn't is that it is—I don't know if you have ever given it before, clinically—a little more challenging to give and some people don't like the taste, so we tended to stay away from it. But definitely I think that that would be another combination, and one could think, probably, of 40 or 50 other things. The real difficulty comes with how are we going to eventually test these different combinations when they are readily available, in most states and provinces in Canada, over the counter? Big Pharma (indeed most groups) really don't have that much vested interest in testing the number of potential combinations that one could come up with. Now, there may be some in vitro ways that we could do a form of high-throughput screening, and making a special cell called a cybrid out of our patient cells versus control cells, which we have done in two instances and published.[15] This one way that you can target, specifically for a mitochondrial disorder, what should go in your cocktail. I think certainly NAC would be something worthy of consideration in the future.

JB: My last question on that list of potential nutrient pharmacological agents has to do with this emerging understanding of phytochemicals. The list expands daily, it appears—resveratrol being in the news, curcumin, EGCG, ellagic acid, ferulic acid, and the list goes on. What is your thought about those as mitochondrial, conditionally essential substances?

MT: Again, I think that these are all reasonable things to consider and try. There are a plethora of studies out there using resveratrol as a mitochondrial enhancer, if you will, for lack of a better term. Resveratrol is limited, however, in terms of its absorption. You're probably familiar with the whole story of the company…the name escapes me right now…

JB: Sirtris Pharma

MT: Sirtris, yes. They were bought out by…

JB: GSK.

MT: GSK, yes. So anyhow, they were very cognizant of the fact that the resveratrol was not well absorbed and came up with small molecules that activate the same sirtuin pathway. So I think the absorption issues are something that one needs to consider, because what you see in vitro doesn't always translate. The second point I would make (and we're in the process of publishing it, so I can't give out all the details): we took the approach that you were talking about—we added curcumin and whole host of other antioxidants into a slurry, which in vitro was unbelievably potent as an antioxidant. We have a thing called the oxygen radical absorbance capacity (ORAC) assay where we essentially measure the ability of the media that we have to quench free radicals. We almost couldn't even generate free radicals when we added this slurry of different compounds into the media. However—because they were all available over-the-counter and they'd been mixed together and sold as supplements—we gave them to healthy individuals. And to our surprise—and I had my grad student go back because I didn't believe the data—it was actually a pro-oxidant in vivo. So I think we need to be very careful and we need to do animal studies and human studies to test the combinations, like we did with CoQ10, lipoic acid, creatine, and vitamin E, to prove that in the human it is functioning as an antioxidant, and show that biomarkers are moving in a favorable direction. Things may look good on paper, they may look good in cells, but they really need to transition to animals and eventually to humans before we should be convinced and we should be starting to consider giving these to our patients with disease.

Mitochondrial Function and Sarcopenia

JB: Thank you. You've been very, very kind in giving this amount of time. I want to close with probably the big 400 or 500 lbs. gorilla in the corner, which is the aging of our population in North America and the increasing risk to mobility-related issues, including sarcopenia, which is really this extraordinary loss of flesh, loss of muscle with age and with disease. Can you tell us a little bit about how the mitochondrial story connects to sarcopenia and maybe where you see medicine going to help take this new understanding and apply it clinically?

MT: I could talk for an hour on that alone. As I mentioned, there is no question that there is increased oxidative stress markers in older muscle and there is mitochondrial dysfunction. There are some pretty strong experimental lines of evidence suggesting that that can contribute to sarcopenia. Trying to enhance mitochondrial function is very likely beneficial. Taking that, then, to the epidemiological side of things, we know that there are numerous studies. Take, for example, the Stanford study published in 2008 on runners, where they compared a group of runners who were 51 years of age or older to either their

spouses or sedentary friends, and they followed them for 21 years.[16] The mortality rate in the non-runners was 34{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} over that 21 years, and 15{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} in the runners. So we do know that exercise is a countermeasure for mortality. I think that endurance exercise certainly has a plethora of benefits, not just in the muscle and with function, but also in terms of decreasing all-cause cardiovascular deaths, and in that study all-cause neurological deaths and all-cause cancer deaths as well. So right there I think, “Good evidence.” And many studies show that endurance activity is beneficial and possibly it is related to the increase in mitochondrial biogenesis in muscle, which has the systemic effects. We also know that exercise helps the hearts and the lungs.

Now, the problem is if you just do endurance exercise, it doesn’t target sarcopenia as well. In older adults there is a slight increase in muscle mass and a minimal increase in strength when you do endurance exercise, but the most effective countermeasure for the weakness of aging is weight training. Folks even in their 90s will respond to weight training in increased strength and muscle mass. So at the end of the day, my strong feeling is that for older adults we need to have a combination of both weight training and endurance exercise to truly combat the aging associated decline in function and in weakness that we see, and I think the two are related. Now, whether we can prolong life or not is open for question, but I think we certainly can compress aging and keep people functional and healthy for a much longer period of time, and keep them out of old age homes and not swamp the American and Canadian healthcare systems with people who need wheelchair assistance and have difficulty just getting up from the toilet.

JB: Dr. Tarnopolsky, this has been one of those—in my nearly 29 years of doing this—great moments of experience. We’ve gone over everything from the microscope to the telescope in this discussion. We’ve covered subjects that cross boundaries. We’ve had courageous discussions when probably most people who like to keep conversations in disciplinary-constrained boundaries would say, “How could you be so expansive in your thinking? You’ve cut across medical disciplines from basic science through clinical works and translational science.” But this is the way I think the big problems that we are confronted with today are going to be solved—by this kind of expansive thinking and sometimes taking a little bit of risk to move out of the disciplinary comfort zone. I think you’ve done it with grace and extraordinary wisdom. I think every listener has valued from your expansive discussion. Thank you very, very much.

MT: Thank you.

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