February 2008 Issue | Mary Ann Lila, PhD University of Illinois

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Welcome to *Functional Medicine Update* for February 2008. I'm going to state the obvious: we all know that plants make vitamins. What I mean by that is plants have the capability of biosynthesizing what we call vital amines, or vitamins (for example, thiamine, riboflavin, and pyridoxine). Plants have this capability of producing the complex array of substances that are necessary to support mammalian metabolism and serve as co-factors and/or antioxidants. They make tocopherols (vitamin E), and they make ascorbic acid (vitamin C).

This edition of *Functional Medicine Update* is going to be an update not only on the role of plant-derived vitamins on physiology, but also the thousands (literally) additional secondary metabolites plants make called phytochemicals and the role they play on physiology. Secondary metabolites make this lexicon of bioactive components from plant foods much, much broader than we previously recognized when we thought that plants only made vitamins. We now recognize the therapeutic potential of substances that come out of the plant kingdom is extraordinary. And I'm not just thinking of anti-cancer substances or anti-depressive substances, but I am talking about a whole array of hormetic substances (nutritional hormesis is a topic that has been identified and defined in previous editions of *Functional Medicine Update*).

The Mechanism of Secondary Metabolites
Secondary metabolites are small molecules found in foods that modulate signal transduction processes in a cell in a tissue-specific way to induce altered phenotype through altered effects on the epigenome. Through genetic expression, proteomics, and metabolomics, this complex process weaves its way and ultimately gives rise to the outcome of health of each individual (their function). It is the interface between functional medicine and nutrition (this transduction process) that is modulated, in part, through nutritionally hormetic substances. This is going to be the topic of this month's *Functional Medicine Update*.

Let's start, if we can, with a little back-to-the-future discussion. I want to take us back, in my experience, to the early 1980s when I had the privilege of working (on my sabbatical year) at the Linus Pauling Institute of Science and Medicine. As you know, Dr. Linus Pauling was a two-time Nobel Prize-winning laureate. As far as I know, even today he is still the only person to have won, solo, two Nobel Prizes in two different fields (one in chemistry and one in peace).

The Evolution of Pauling's Interest in Vitamin C
In the later years of his career, vitamin C (ascorbic acid) obviously became a major part of Dr. Pauling's focus, both for its nutritional and pharmacological effects. He was a proponent of vitamin C for the

common cold and flu, and his best-selling nutrition books changed the whole complexion of nutrition science and the nutritional supplement industry, and even medicine, to some great extent. Even those who railed against the concept that vitamin C taken in supplemental doses could have any positive health benefit still were challenged to the task of thinking about their discipline in a different way-looking at nutrition from a different perspective. We might consider that time as the dawn of the new era of nutrition, and now we are starting to witness it unfold in the 21st century. Pioneers like Roger Williams and Dr. Linus Pauling really helped guide us into this new era.

I was talking with Dr. Pauling in 1982 about vitamin C and asked him a question I'm sure he had been asked tens of thousands of times: How did he come to this vitamin C connection and his interest in it? He said there were two reasons that his interest peaked around ascorbic acid. One reason was his meeting of Dr. Irwin Stone. Some of you who have been in this field for many years will certainly remember him. Dr. Stone was a PhD who had looked at the evolutionary changes in biosynthetic capability for vitamin C in different animals and found a mutation in the human genome that resulted in a loss of the enzyme L-gluconolactone oxidase a few million years ago, which then resulted in the inability of humans to biosynthesize vitamin C from glucose. Vitamin C biosynthesis is able to be done in virtually all other animal species except the fruit bat, the guinea pig, and the human, who have lost the ability to manufacture ascorbic acid from the biosynthetic pathway coming out from glucose.

There are people who still maintain some resident ability in their own genome to biosynthesize vitamin C, which is a fascinating part of the story (it is kind of a legacy from the past). They still have a little bit of that L-gluconolactone oxidase activity, which is activated under stress. As Irwin Stone talked about in his early works, and then later Dr. Pauling talked about, animals like the goat synthesize 100 or so milligrams of vitamin C a day under low stress conditions. When they are put under high stress, biosynthesis of vitamin C will be activated some ten-fold and produce thousands of milligrams of vitamin C.

The goat has about the same body mass as a human, so why is it we don't feel that we need more vitamin C under stress? We can't make it, like a goat does. We can't turn on our liver to manufacture more ascorbic acid from glucose, so maybe it becomes a conditionally essential nutrient at higher doses for us under physiologic or environmental stress. That was a very interesting part of Dr. Stone's anthropological arguments and also Dr. Pauling's.

That was one feature that got Dr. Pauling very interested in the vitamin C/ascorbic acid story. The second was a personal experience (one of which many of you may not be aware of). The Linus Pauling Institute archives are now on the worldwide web. Much of his correspondence and early letters are now digitized, so those of you that are "Paulingophiles" can go back and find out a lot about the conversations and communications that Dr. Pauling was having with scientists, politicians, policy makers, and thought leaders around the world over this dynamic period in the 30s, 40s, 50s, 60s, and 70s.

I find it personally very interesting that so many of the letters, if you go back into his archives and you look at the letters that were written in the 50s, were actually typed on the same typewriter he was still using when I was at the Institute in the 80s. Each typewriter has its own unique kind of thumbprint, and you can actually see this in the letters that were written (in terms of the print style, the same compositional uniqueness was maintained into the 1980s).

The 1950s was an interesting period relative to Dr. Pauling's understanding of vitamin C. At that time,

you'll recall, he was at the California Institute of Technology in Pasadena. Around 1950 or 1951, he and his wife Ava Helen were attending a scientific congress on the east coast. Back in the 1950s, airplane travel between coasts was not common; therefore, to return from this meeting in New York to California they were on the train. He developed a very severe back problem during this trip, and later it became known to him that it really wasn't a back problem; it was a kidney problem.

By the time he arrived in California at the end of this trip, he had very severe kidney-related inflammation. He went to one of the most renowned nephrologists at Stanford University School of Medicine, who basically said, "You need to go on the Addis Program." The Addis Program (developed by Dr. Thomas Addis), was a protein-modified diet coupled with (very interestingly) supplemental vitamin C at high dose, which at that time was a very new substance that you could get in purified crystalline form.

The diagnosis at this time for Dr. Pauling was Bright's disease, which is what chronic glomerular nephritis was called back in this period (all kinds of kidney inflammatory disorders were lumped under one title called Bright's disease, named after the gentleman who discovered the origin of this condition). Dr. Pauling was placed on this specific intervention, which was extraordinarily successful for him. He became a real believer that this glomerial nephritis/Bright's disease treatment with the dietary intervention developed by Thomas Addis at Stanford University using supplemental vitamin C had some unique characteristic that deserved his attention. It was a combination, I think, of Irwin Stone and his own experience with Bright's disease and vitamin C that resulted in-for the rest of his life-interest intellectually, clinically, and scientifically in the role of vitamin C in human health and disease.

What I find interesting is that it is often said that supplemental vitamin C causes kidney stones and so people are very worried about vitamin C and kidney disease when taken at supplemental doses above 6 grams a day. But if you actually go to the literature and do a fairly significant and complete survey, you'll find there are virtually only a couple of clinical observational case reports on kidney stones being produced by vitamin C at high doses in people that were marginally undernourished, so you already wonder about the confounding variables. There are a number of other reports indicating that supplemental vitamin C taken by people with urogenital infections or who have kidney inflammation actually had positive clinical outcome and effects on their situation. I think Dr. Pauling's own case experience was a very interesting kind of point on the curve related to, starting back in 1951, his journey down the vitamin C path.

This journey ultimately led into all sorts of things, one of which was collaborative work with Ewen Cameron, a Scottish surgeon, on vitamin C and cancer. When I was at the Institute in the early 80s, this debate was heated because there was extraordinary work being done under the oversight of Dr. Moertel at Mayo, who found that vitamin C didn't have a positive impact on cancer outcome. The impact of vitamin C on cancer was debated among Moertel and Pauling and Cameron at some length. Coming now into the 21st century, we start to see retrospective studies being published that demonstrate that intravenous vitamin C does appear to have a positive benefit on patients with various forms of cancer, particularly noted in their energy and quality of life, and possibly even their survival (although that probably is still open to some level of controversy).

There was a nice review published in *Integrative Cancer Therapies* in 2005 titled "Orthomolecular Oncology Review: Ascorbic Acid and Cancer 25 Years Later."[1] Some very nice work done by Dr. Mark Levine at the National Institutes of Health has also recently been published. Dr. Levine is an

endocrinologist who has been studying, the effect vitamin C has on human physiology using *in situ* kinetics under rigorous conditions. He has been able to identify (once again) that there is (at least from a fundamental mechanistic understanding) a rationale for the use of higher-dose, supplemental vitamin C therapy during certain kinds of oncological events.[2] Although Dr. Pauling was discounted early on by many of his colleagues when he came to the concept of supplemental vitamin C as being beneficial, as we learn more over time, we recognize the multiple effects that these interesting small molecules derived from plants have on physiology, I think some of his conceptualizations look a lot more reasonable today in light of these new discoveries than they looked to some back in the 50s, 60s, 70s, and even 80s. I think we should keep the jury out on this, but certainly the vitamin C story is a lot more than just the prevention of scurvy. I think we can say that with absolute assurance."

Antioxidant" is too Simple a Term

When we question the role phytochemicals have on function, they are often relegated to a simple term, which is antioxidation. I think that is essentially an oversimplification because what we are starting to learn is that "antioxidant" is a very general term that doesn't relate specifically to how that molecule or collection of molecules interface with different cells, different tissues, or different organs with specificity to induce different cellular or functional outcome. I think what we are starting to recognize is that these "antioxidant effects" really surround bioenergetic effects that these nutrients have by regulating cellular physiology in a very specific way, through signal transduction processes. We shouldn't generalize, I think, to just say "antioxidants." We ought to be talking about the specific role that families of molecules derived from plant foods have on specific cellular functions.

With that in mind, let's talk a little bit about what happens when you get dysfunctional bioenergetics. Other than the person is tired, worn out, can't think straight, has muscle mass loss, and their immune system has kind of gone awry (the kind of gross effects of low bioenergetics), at the cellular level what we see is oxidative stress often occurring with free radical pathology. It could be from oxygen radicals or nitrogen radicals, but we have lost the ability to regulate these intermediary molecules that are high energy, promiscuous molecules called free radicals that can damage macromolecules like proteins, nucleic acids, or lipids and cause cellular injury.

The concept of keeping the mitochondrial bioenergetics intact is a very fundamentally important part of any positive intervention program in nutritional therapy. The reason I threw the term "mitochondria" in there is that the mitochondrion is the site of bioenergetics in the mammalian cell. In the plant, the chloroplast is where a lot of the energy is transduced from the capturing of photons of light from the sun. The comparable site of reaction for that function in mammalian cells, or eukaryotic cells, is in the mitochondrion, the energy powerhouse where oxygen is utilized, water is produced, and substrates such as glucose, fatty acids, and amino acids are burned as fuel in the process of respiration to give rise to an ultimate building of reducing power in the cell through ATP and NADPH and other high energy co-factors that help to maintain structure and function to the cell, and that are powered by this furnace, or energy powerhouse, called the mitochondrion. As the cell becomes dysfunctional as a consequence of breakdown in the integrity of the cellular processes, it then translates into altered mitochondrial function increased release of these secondary oxidants that we call free radicals, that then injure the cell and cause, ultimately, cellular death or suicide that we associate with apoptotic changes, leading to programmed cell death or to induced cellular death and pathology.

So maintenance of mitochondrial function, as we have all learned, is extraordinarily important for the

prevention of oxidative stress and the role that "antioxidants" are presumed to have in some way influencing bioenergetics and mitochondrial function and preventing oxidative injury. What is the effect of a sublethal mitochondrial stress on physiological function? That was the discussion in a *Medical Hypotheses* article in 2006 in which the authors talked about a term called "mitohormesis."[3] Mitohormesis takes the term "hormesis" (small substances having larger physiological effects) and applies it to the mitochondrion. In this particular article, the authors are looking specifically at the role that dietary phytonutrients (or phytochemicals) have serving as mitohormetic agents-agents that stimulate proper mitochondrial function. So rather than talking about these phytochemicals as antioxidants, we are (in this case) giving them a more specific role: they influence the signal transduction process (what translates the outside world to the inside function of the mitochondria) and then regulate mitochondrial function to prevent it uncoupling and producing oxidants and engaging in oxidative stress.

The Impact of Uncoupling Reactions

Under conditions where the mitochondria has lost its functional integrity and you start to have phase transitions in mitochondrial oxidative phosphorylation and the production of more oxidants (the so-called uncoupling reactions), what we can then have (even in mild mitochondrial uncoupling) is impact on myocyte function (on muscle cell function), and that induces sarcomeric changes, which then causes metabolic sarcopenia (the loss of flesh). You start to see injury to muscle, muscle apoptosis, and lowering of muscle mass, and this may be a contributor to the process that we see of lowered strength and vitality with aging (as we start getting this increased oxidative stress effect). I'm now quoting, actually, from a nice paper that ties together this mild mitochondrial uncoupling and its effect upon muscle cell integrity *in vivo* and how that relates to cellular aging. This was in the *Proceedings of theNational Academy of Sciences,* volume 104 in 2007.[4]

I think this is some very nice work using magnetic resonance spectroscopy studies and looking at phosphocreatine and ATP levels in muscle, which is an indirect measurement in the whole organism of mitochondrial function and muscle cell activity. The results showed that even mild uncoupling of mitochondrial function led to decreased muscle cell bioenergics and increased oxidative injury in muscle cells. This is work actually out of the University of Washington, Department of Physiology and Biophysics, using NMR technologies to evaluate, in whole, exercising muscle in intact human beings (their bioenergetics).

This is not just an esoteric topic. As I connect this to other work, I believe we can actually observe it, even in fairly mild cases of mitochondrial uncoupling. These are not constitutive mitochondropathies, where we are talking about people who have inborn errors of mitochondrial genetics. Here we are talking about induced mild mitochondrial uncoupling that is a result of environmental factors-things like insulin resistance, metabolic syndrome, hyperinsulinemia, high uric acid levels, high inflammatory cytokines as a consequence of inflammation. I think this study is important because all of these induce alterations in myocyte mitochondrial function and cause mitochondrial uncoupling and oxidative stress. It is a little bit like a dog chasing its tail: once you get this started, it tends to run around on itself as a self-perpetuating cycle, so you have to break the cycle. You have to break the cycle and put back the integrity of the energy powerhouse of the cell, which is the role of the mitochondria.

Mitochondrial Dysfunction Can Affect Cognition

The mitochondria is the center for signal transduction as well as bioenergetics. It plays a very important role in regulating intracellular signals that modify organelle function within the cell and signal to the

nucleus in the genome messages that help regulate gene expression and ultimately proteomic effects within the cell. There was a very nice paper published in *Free Radical Biology and Medicine* in 2004 talking about mitochondrial dysfunction and how it relates to oxidative stress.[5] It can ultimately lead to things that impact cardiovascular function and neurological function, and is particularly seen in things like epilepsy, central nervous system problems, or seizure disorders, where the brain and heart are very dependent upon mitochondrial integrity for their function. We recognize that even modest interruption-or let's call it mutation-of mitochondrial DNA as a consequence of oxidative stress can have an adverse effect upon cognition. This has been shown in animal studies where inducing mitochondrial oxidative injuries to mitochondrial DNA leads to poor performance in mazes by animals and an increase in what you would call biological age with reduced cognitive and memory effects in the animal. By the way, this was found in *Nature Genetics*, volume 35, page 65.[6]

Mitochondrial Dysfunction and Insulin Resistance

An interesting paper published in the *New England Journal of Medicine* a number of years ago-this was in 2004-showed that impaired mitochondrial activity, as a result of type 2 diabetes and insulin resistance, has adverse effects upon muscle cell function and on neurologic function, and may contribute to the impairment and the progression of degeneration seen in type 2 diabetes.[7] In fact, mitochondrial dysfunction is associated very early on with insulin resistance. They are very closely tied together, the metabolic syndrome and mitochondrial dysfunction. Again, I want to emphasize I am not talking about constitutive inborn errors of mitochondrial function. I am talking about induced effects that occur as a consequence of altered environment and altered physiologic status, in this case hyperinsulinemia/insulin resistance being associated with mitochondrial dysfunction. This was published in *Science* magazine.[8]

I think we can say there is a very close correlation between chronic disease-neurologic, cardiovascular, metabolic disease-and the relationship to altered mitochondrial bioenergetics, oxidative stress, and how that may relate to the need for specific phytochemicals that modulate intercellular signal transduction and can put these functions back on the rails, so to speak. Phytochemicals can help to regulate altered control points that are set points associated with bioenergetic dysfunctions.

Metformin has a Positive Effect on Mitochondrial Metabolism

Looking at type 2 diabetes as an example, if you intervene with a drug like Glucophage (or metformin), what you find is that metformin has a positive impact on mitochondrial metabolism.[9] It reduces mitochondrial oxidative stress, which is one of the benefits it imparts in the management of type 2 diabetes and subsequently has an effect on improved insulin signaling. These are pleotrophic effects that these drugs (or these agents) have, similar to what we see with some of the phytochemicals (they don't just affect one function, they have effect across the broad array of function). That is why I think the term "antioxidant" that we apply to these plant-derived materials like vitamin C (we certainly could talk about a whole array of other phytochemicals) is too simplistic a term.

We know that once insulin has been normalized, it helps improve biochemical function and bioenergetics and lowering of oxidative stress within the mitochondria. It is kind of a push-pull argument. You could say that improving biochemical bioenergetics at the mitochondrial level will improve insulin signaling, or you could say improving insulin signaling improves bioenergetics and reduces mitochondrial oxidative stress. In some senses, I guess you would say that those therapies that improve insulin sensitivity are antioxidant by nature because they improve mitochondrial bioenergetics, as contrasted to those interventions that enhance mitochondrial dysfunction and are associated with increased insulin resistance.

High Sucrose Diet and Mitochondrial Bioenergetics

Let's have an example. Let's give a very high-sucrose diet to animals and look at what effect it has on mitochondrial bioenergetics, skeletal muscle, and liver function. What we find in these studies is that a very high-sucrose/high-sugar diet induces mitochondrial dysfunction, oxidative stress, and is associated with altered skeletal muscle and liver function and increased apoptosis (cellular death) in those tissues.[10] I want to emphasize that these are animal studies in which the sugar percentage calories have been greatly exaggerated to try to make a point, but I think what we are seeing (at least from the implied work of these studies) is that there can be an affect mitigated by environmental choices on mitochondrial bioenergetics. If you eat a diet that is devoid of phytochemicals, or you eat a diet that is very high in fat and sugar, you are pushing mitochondrial bioenergetics and intracellular signal transduction towards the state of oxidative injury and towards an altered state of inflammation.

We know this dysfunction occurs in the mitochondria of type 2 diabetics. We now have some sense as to the molecular mechanisms that may show how this occurs in the cell (the mitochondrial dysfunction) and its relationship to insulin signaling. And we know that insulin regulation of mitochondrial proteins adversely affects, or has an effect upon, oxidative phosphorylation in human muscle. This story has certainly expanded in its depth and density over the last 5 to 10 years such that I think we can say that the environment does, in fact, influence mitochondrial bioenergetics. That we don't have to use the story of mitochondrial dysfunction as only an inborn error of metabolism story, and that we can now look at an induced effect caused by altered lifestyle, environmental factors, exposure to chemicals, exposure to radiation, or exposure to stress-all of these, then, can have impact upon bioenergetics. If we then take away the signal transduction normalizing substances, which are the phytochemicals in the diet that were part of our human history, that then further tips the balance towards increased dysfunctional signaling and altered bioenergetics

Clinically, what does this map against? I've talked about cardiac disorders. I've talked about neurological disorders. I've talked about dysinsulinism associated with cardiometabolic syndrome or with type 2 diabetes. What about things like the disorders of the 21st century? Chronic fatigue syndrome, fibromyalgia syndrome, multiple chemical sensitivity syndrome, Gulf War syndrome? As you probably know from previous editions of *Functional Medicine Update*, we have talked at length with experts in the field on this very topic. Dr. Paul Cheney was one of our extraordinary contributors in our clinician-of-the-month discussion about oxygen being a potential antioxidant when given in the right concentrations, and low oxygen tension (ischemia) being a pro-oxidant.

We have talked with Dr. Martin Pall twice as our *Functional Medicine Update*clinician/researcher of the month column about the work of nitric oxide, its relationship to peroxynitrite, how that relates to oxidative mitochondrial uncoupling through the activation of the immune system and inflammatory response, and how chronic fatigue syndrome and fibromyalgia may be considered conditions associated with mitochondrial dysfunction and with this whole concept of inflammatory disorders and altered cellular signaling at the cell pathology level.

Erythrocyte Damage in CFS Patients

Recently it has been reported that if you look in the red blood cells of chronic fatigue syndrome patients, you will find very significant evidence of erythrocyte oxidative damage. This was published in the *Archives of Medical Research* in 2007.[11] This study implies there is a strong likelihood that this increase in erythrocyte oxidation seen in CFS is a manifestation of what occurs as a consequence of

systemic increased inflammatory and oxidative response to the condition. So the identity of the triggering agent is still open for discussion, but the pathophysiology of chronic fatigue syndrome and fibromyalgia seems to have a relationship to the same story of activation of oxidative injury.

If you look in the literature for correlations between oxidative injury and multiple chemical sensitivity, or oxidative injury and fibromyalgia, you'll see a number of published papers that provide strong support for this model. In the *Federation Proceedings Journal* (the *FASEB*journal), a paper authored by Dr. Pall showing that N-Methyl-D-Aspartate (NMDA) sensitization and stimulation by peroxynitrate/nitric oxide, as well as organic solvents, may be a mechanism to explain chemical sensitivity and it ties together with mitochondrial uncoupling and oxidative stress.[12] Another paper that was published in *Environmental Health Perspectives* in 2003 discusses elevated nitric oxide/peroxynitrite as it relates not only multiple chemical sensitivity, but also to neurological disorders and ties together multiple chemical sensitivity to, again, mitochondrial uncoupling.[13]

I think we are starting to witness a very interesting evolution of our understanding of the etiology of some of these complex disorders that associate themselves with muscle pain, cognitive dysfunction, immunological dysfunctions or lymphadenopathy, generalized bone weariness, and inability to think clearly ("foggy brain" syndrome). All of these may be early manifestations of these conditions of oxidative stress/mitochondrial uncoupling. And that begs the question, of course: what role does altered diet (if any) play in both progression of these conditions, and maybe their remediation, by changing the diet to enhance the level of intake of specific cellular signaling substances (i.e., phytochemicals) that can modulate these processes?

Dr. Bruce Ames, a world-renown investigator in this field (Professor Emeritus at the University of California, Berkley, Department of Biochemistry, and recently now working extensively at the Children's Hospital Oakland Research Institute), has authored a series of papers offering up some of the therapeutic potential that derives out of this model, one of which is an article titled "A Role for Nutritional Supplements in Optimizing Health," what he calls "The Metabolic Tune-up." This article appeared in *Archives of Biochemistry in Biophysics* in 2004.[14]

Dr. Bruce Ames on Genomic Instability

I was very fortunate to be present at a recent Bruce Ames Symposium that was held in his honor at the University of California at Davis on nutrigenomics and the effect of nutrients on the epigenome, and to have a conversation with Dr. Ames. Dr. Ames and I were talking about the role that micronutrients can play-vitamins and minerals and various phytochemicals-on cellular function, mitochondrial oxidative phosphorylation, energetics, and promotion of healthy aging. He said that if we take a gross view of the role these complex families of nutrients, we see that they help to stabilize genome. They prevent genomic instability. Genomic instability is a consequence of all sorts of adverse physiological functions or processes going on, one of which is related to oxidative injury that causes genome instability. Genome instability associates itself with cancer. It associates itself with increased cellular death. It associates itself with cardiopathies and with dysfunctional insulin signaling. His concept is that if we feed the right nutrients in the right doses, we can provide the cofactors and signaling substances necessary to promote proper oxidative phosphorylation, bioenergetics, and mitochondrial energy production. In his article, he talks about the things we all know, but often forget about because they are so simple: vitamin B12, folic acid, vitamin B6, vitamins C, E, minerals such as iron and zinc, which he says appears to mimic the ability to prevent radiation damage in cells and help to stabilize the genome against environmental

enzyme difficulties.

Preventing Upcoupling with Co-enzyme Q10 Supplementation

We also know that there are other cofactors that play roles in these processes, and one of these is co-enzyme Q10 (ubiquinone). There are a series of papers that have been published demonstrating the role of therapeutic intake vitamin Q, or Co-enzyme Q10, has on these processes, one of which was titled "Nutritional Cofactor Treatment in Mitochondrial Disorders." This appeared in the *Journal of the American Dietetic Association* in 2003, and is research showing the important role Co-enzyme Q10 has in the oxidative phosphorylation profile, helping to prevent uncoupling of factor 4 and establish appropriate bioenergetics in individuals who have a functional Co-Q10 deficiency.[15] These may be people, for instance, on statin drugs. Or it may be people under higher oxidative stress as a consequence of chemical exposure, psychological, or environmental stresses. Co-enzyme Q10, which is not considered a vitamin, as such, becomes a functionally conditional nutrient. For individuals with specific need, doses between 50 and 100 milligrams a day of Co-enzyme Q10 become valuable.

We know Co-enzyme Q10 supplements will improve mitochondrial function and tissue levels, not only of CoQ10, but also vitamin E, which helps to maintain proper tocopherol levels in tissues. This was demonstrated in a study published in the *Journal of Nutrition* in 2003.[16] Co-enzyme Q10 works along with another interesting mitochondrially active nutrient (conditionally essential nutrient): L-carnitine. There are a number of papers that have been published over the years on the efficacy of supplementation with L-carnitine for individuals who have compromised nutritional status, increased mitochondrial oxidative stress, and fatigue as a presenting symptom. An interesting paper was published in the journal *Nutrition*in 2006 on the use of L-carnitine therapy, giving 6000 milligrams a day to patients who had fatigue and were undergoing anti-cancer therapies; the findings showed improvement in mitochondrial function by lowering oxidative stress.[17]

I think what we are starting to witness is a very interesting relationship between modified nutritional environment and promotion of proper intercellular signaling and bioenergetics, which ultimately regulates the integrity of cellular function. There is a program that has been put out there for treating chronic fatigue syndrome that a number of clinicians are using called The Marshall Protocol. It is built around trying to use some of these cofactor materials like NADH, in supplemental levels, to try to activate bioenergetics and reducing factor.[18,19,20,21,22]

I think the takeaway that we have had over the years from the papers we have published in this area indicate there is not a silver bullet of any one specific intervention. It is lowering the environmental stress that induces mitochondrial uncoupling and oxidative injury. It is improving intercellular signaling by putting a person back on a diet that is rich and complex with regard to phytochemicals; getting them away from sugar, fat, alcohol, and caffeine in excessive doses; and then properly supplementing specific nutrients, like those that Dr. Ames talks about in his "metabolic tune-up" and the rich array of phytochemicals that regulate bioenergetics.

With that as a conceptual framework, let's move to our researcher of the month, and I think we'll take this discussion to the next level of your understanding and open up all sorts of new potential doors for both therapeutics and prevention. I would like to introduce Dr. Mary Ann Lila.

INTERVIEW TRANSCRIPT

Clinician/Researcher of the Month
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We are at that portion of our monthly Functional Medicine Update that I know you and I both look forward to with great anticipation and that is our researcher or clinician of the month section. This month, I feel very privileged to have an individual who is a very well-respected professional in the area of phytochemistry. She is a professor at the University of Illinois, and doing work as the head of the College of Agriculture, Consumer, and Environmental Sciences program, the Global Connect portion of what is called ACES (Agriculture, Consumer, and Environmental Sciences).

Beyond that, she has done extraordinary work in understanding the phytochemistry of bioactive components of plants. What got me interested in finding an opportunity to speak with her was a wonderful paper that she authored in the Journal of Science of Food and Agriculture in 2006. It was a perspective paper titled "The Nature-versus-Nurture Debate on Bioactive Phytochemicals: The Genome versus Terroir."23

Dr. Mary Ann Lila, we welcome you to Functional Medicine Update and want to thank you for giving us some of your valuable time. Let me start with this question first: What got you, personally, into the whole area of bioactives from food and the role that phytochemicals have in nutrition?

MAL: It's interesting because I have been working in nutritional sciences here for the last 15 years, but my actual training-my PhD research-was in plant sciences. So it is kind of a strange, but very useful blend of expertise because most of my colleagues that I interact with in nutritional sciences are very good at doing bioassays on compounds, looking for their mechanisms of action in the human body, but really have very little understanding of how a plant makes these compounds and why a plant is enticed to make certain compounds. Plants are not doing it for altruism; they are doing it for their own survival. It has been a real good blend of talents to be able to use what I know about plants in order to control or to enhance what a plant can do for human health.

JB: Let's talk, just briefly, about the point you just made because for many of our listeners they may not be familiar with this concept about secondary metabolites from plants or even primary metabolites that we call phytochemicals. Why do plants make them? It seems like it takes a lot of their metabolic energy to do that work, and some of these molecules are very sophisticated in structure. Tell us a little bit about why phytochemicals are in plants, from kind of a teleological perspective.

Why Do Plants Make Phytochemicals?
MAL: That is really, really a good question. It costs the plant quite a bit, in terms of energy, to produce a secondary compound. They are called secondary compounds because they are not essential for the plant to grow and develop. So a plant that is growing under ideal conditions of sunlight and fertility will put all of its energy into simply growing and photosynthesizing. But when it has some kind of stress or insult in the environment, it will cease or slow down the growth process and start putting some of its resources

instead into producing secondary compounds that will protect it, perhaps against an herbivore, an insect predator, maybe protect it against intense UV light, salt stress-all these kinds of things that can otherwise damage plant tissues, the secondary metabolites can inhibit those stressors in the environment. A plant, being a sisal organism, really has no choice; it has to chemically protect itself, and it uses a diversity of different mechanisms, not just one magic bullet to do each task. It uses a lot of redundancy-a lot of overlapping chemicals or similar chemicals that all together will protect it. So there are a lot of interactions going on when a plant puts its chemical cocktail together to protect itself. And interestingly, these same compounds that a plant uses to protect itself will interact with human therapeutic targets and help to prevent or provide therapy against human disease, or just enhance the human metabolism, so it is really a huge benefit to the consumer of these plants.

JB: Just to give our listeners kind of a quick thumbnail as to the broad range of experiences you've had from your research-I'm looking at some of your recent publications that have titles like "Identification of Isoflavone Glycosized from Pueraria lobata...," "A Comparative Evaluation of Anticancer Properties of European and American Elderberry Fruits," "Beyerane Diterpenoids from the Heartwood..." and effects that they have, "Serum Testosterone Reduction Following Short-term Phytofluene, Lycopene, or Tomato Powder Consumption in F344 Rats," "Comparative Phytochemical Characterization of Rhodiola Species," and so forth.24,25,26,27,28

Obviously, you've had a very broad range of experiences looking at these secondary metabolites in plants. Have you found differences in, say, individual cultivars in how they produce the phytochemicals and then, beyond the cultivars, are there differences in how the environment influences gene expression and secondary metabolite biosynthesis in an individual cultivar? That gets into this question of nature versus nurture and how it reflects in the plant (their output).

Genotype Differences Affect Secondary Metabolite Production
MAL: There are absolutely huge genotype differences in how a single species of plant will produce a secondary metabolite. The reason I have that diversity of looking at all kinds of different phytochemicals that you have just listed is because I'm part of a botanical center for age-related diseases, which is actually based in Purdue, looking specifically at metabolites from plants that will help bone health, or dementia, or other age-related diseases. And then I'm also part of an international cooperative biodiversity group, which is working in Central Asia, looking at plants that have bioactive properties that have been-because they are former Soviet Union-totally hidden from Western medicine for all these years (the whole Soviet regime). Now that these countries (Uzbekistan, Kyrgyzstan, Kazakhstan, and Tajikistan) are independent countries and rather impoverished, they are looking for opportunities to really validate some of their natural medicines that have been hidden from the west and really have some tremendous properties.

Now the reason that I have been working specifically in Central Asia is that these are some of the most inland countries in the world. This gets to the whole point of why a plant produces secondary metabolites and what makes different plants produce different metabolites. They are inland countries. That means they are not buffered by the ocean at all. That means they have very hot summers and very cold winters. That means the plants that manage to survive in these environments are extremely stressed, and, as a result, will be extremely jam-packed with secondary metabolites. They may not be beautiful, but they definitely are full of metabolites that have benefit for human health.

You mentioned different cultivars, and one of the problems we have is that with conventional breeding-

this is not on purpose, but just because we have been selecting plants for beauty, for keeping quality, shipping ability, sweetness-we have been breeding out secondary metabolites in many of our wild species and producing a commodity for the supermarket that has good shelf life and is attractive. One thing that I have looked at quite a bit is going back to the wild, going back to plants that are enduring stress in the wild, that aren't pampered in the field, and seeing what we can learn about their secondary metabolites what we can do to maybe get some of these back into conventional mainstream vegetables and fruits for the American public. I have also looked at some commodities that maybe haven't been available (or are underappreciated) in the American marketplace, but maybe are known locally in different countries for their value as foods and medicines.

JB: Wow, you have said so much in that...

MAL: And I completely forget the original question...

JB: No, you didn't. It just kind of leads me to an exploding series of thoughts. We recently interviewed, on Functional Medicine Update, Dr. Christoph Westphal, who is the CEO of Sirtris Pharma, who has made these discoveries about the SIRT1 and 2 genes and their relationship to certain effects on the epigenome. He spoke about resveratrol, in particular, but he also identified there are many other compounds that they are working on as well.

The concept was born out of a discovery and a term that was coined by Dr. David Sinclair at Harvard that I know you are familiar with called "xenohormesis" (that there are small levels of substances that can initiate, through signal transduction processes, larger effects on physiology than we might have expected based upon the small amount that might have actually gotten in the body). So you get this amplification effect of the right molecule at the right place, or the right combination of molecules. You've just been speaking to what (probably) Sinclair and Christoph would call a xenohormesis concept, and you have also spoken, I think, to possibly the difference between a product produced by organic agriculture versus that which is produced by a less stressed, nonorganic environment. Is there any validity to that concept (that this might help explain the difference in composition between organic and nonorganic)?

Organic versus Nonorganic Composition of Plants
MAL: It may well. I have to say that there have been very few scientific, replicated trials to really validate this point. Logically, it makes sense, based on what we know about wild versus cultivated. Organic, being a very low-input crop (low pesticides, natural fertility, a lot more stresses), it makes good sense that organic crop would have higher content of natural phytochemicals, and therefore more health benefit. There really haven't been a whole lot of replicated trials. I'm not quite sure why that is because it is a real good argument and it needs to be done.

JB: Let's also talk a little bit about another theme that you described for us, and that is the difference between, say, single molecules versus mixtures of molecules. The plant produces, as you said, these families of molecules. In fact, in one of your review papers-I can't remember if it was the recent Annals of New York Academy of Sciences paper "From Beans to Berries and Beyond: Teamwork between Plant Chemicals for Protection of Optimal Human Health" or if it was in "The Nature versus Nurture Debate on Bioactive Phytochemicals," but you made the point that plants may produce a different repertoire, or different portfolio, of phytochemicals based upon different environmental circumstances.29 They have the gene potential to produce different families and they select (based on certain growing conditions)

which family they will produce. That leads us to believe, then, that there must be some benefit in the "anti-stress" compounds for specific types of stressors as mixture, not as single molecules.

MAL: Absolutely the case. I think that is why America has been so slow compared to a lot of other countries in really embracing the concept of foods as medicine because we are kind of a pharmacy-oriented, "big pharma" country. The pharma industry will look at compounds that are of value using high-throughput screens that will look at the effect on one enzyme (multiple samples looking at one enzyme) and that is the bioassay that they use. A plant is very, very different (a plant extract) because it is maybe hitting multiple targets at once, and maybe at lower levels than a single synthetic compound, so big pharma has not been ready to embrace plants as a source of medicine for that reason. Plants change their own metabolism and their own output of secondaries very much in response to the environment.

Research on Berries in Alaska

I'll give you one example. We are looking at berries that are growing in Alaska. This is an extremely stressful environment. This is an EPA-funded project because we are looking at this tribal resource (it is native Alaskans who use berries for health, specifically diabetes) and what is happening to these berries in the face of global warming. We are looking at three different locations in Alaska: one that is very, very far north (a whaling village way up on the northern coast of Alaska); one that is halfway out on the Aleutian Islands; and one that is in southern Alaska. We are looking at how the same species of berry will differ in phytochemical composition given its location, and how climate change is actually impacting on not just the survival of these plants, but the compositions of the bioactive compounds in them. In some cases it is not all bad in terms of bioactivity. It is just dependent on how the climate is changing-if it is drought conditions, if it is flood. Warming, in itself, is something that is very much shifting the high antioxidant potential of these berries; it is changing because of global warming.

JB: As it changes have you seen different, I guess, ORAC value, or however we would measure (in a gross sense) antioxidant capability of the global warming grown berries? Does it increase the ORAC or does it decrease, or does it change certain characteristics of oxygen radical absorbing capacity?

MAL: That's a long-term study-to actually look at the impact of global warming because it is a slow process-but what we are doing is looking at different environmental locations right now in the state of Alaska (which is a huge state) which have natural changes in their climatic conditions, and measuring differences in the same species based on what is naturally there now and what is shifting, so we can predict how global warming will be changing some of the these.

The ORAC values tend...they are extremely high now in Alaska just because sometimes there is 23 hours day length during the growing season, so there is intense UV light. The prediction is that the ORAC may be decreased-that the antioxidant value should be decreased-if they are less stressed (if they have less cold temperature), but this remains to be seen. We need to really be able to look at this over a period of time.

JB: One of the questions I have had asked of me, and I think it is a good question that I think you would be much more likely (as an expert) to help us understand is: When a plant is stressed and it produces these anti-stress families of secondary metabolites (be it either to cold stress, UV stress, insect stress, whatever it might be-a different portfolio, maybe for different types of stresses), does it also run the risk, in human consumption, of raising the production of potentially toxic metabolites for the human? I am thinking of an example I learned about with celery several years ago. Celery, when grown under stress conditions, can

produce a higher level of compounds that can induce allergy in some people. I guess it begs the question: Does this always produce a beneficial effect for a human who might consume the food?

MAL: No, you are quite right. There is not always a beneficial effect, but I would say that the incidence of actually finding a toxic effect is very rare in these cases (under the stressed plants). We always tend to look (in our research) at plants that have a long history of human use. Maybe not known to the American market, but a long history of human use in whatever country we are visiting, so, you know, we aren't going for something that is going to be a natural toxin. Plants, of course, are wonderful chemists and they have some of the most deadly poisons that are out there, but we are always looking at those things that have a long traditional use.

The Bioavailability Question

JB: Now another area that I noticed you have been involved with from your publications related to your research is the concept of bioavailability and I think this is another big area of, seemingly, controversy in the field because people have said, "Well, a lot of these bioactive compounds from plants which seem active in a test tube and in vitro experiment and cell culture, when given to animals they are highly bio-unavailable and therefore they really don't have any effect on the organism. But, of course, that assumes that there is some kind of a direct dose-response kind of relationship and they don't go through some xenohormetic mechanism. Tell us a little bit about your understanding of this bioavailability question.

MAL: It is quite true that when we consume foods and we know a certain compound in the food is bioactive (for example, resveratrol in red wine), sometimes we cannot see the dose response effect that we are used to looking at with synthetic pharmacological compounds. This is, in part, because it is just beyond the limits of the sensitivity of our instruments, and in part because the real way that plants are working is this mixture of compounds, not one single compound that should be increasing in its dose response. Those are complications that have hurt the research, or hurt the acceptance of plants as a source of medicines in the medical community. What we are finding is that the medical industry (for example, the medical practitioners) were unwilling to accept the whole concept of red wine being heart-protective because they didn't see the bioavailability. Very little of it actually seemed to get into the bloodstream. Now, with more sophisticated methods of measuring metabolites in the bloodstream, we are seeing that what we eat-what we actually consume-is immediately being metabolized into something that we weren't expecting. In the blood, which has a rather neutral pH, the cyanin, for example, that is in red wine is going to be metabolized into other molecules like chalcones, which are not recognizable as the original compound that was eaten. There may be things we are not measuring because they become something else.

One thing we are doing in our lab, which is a little bit groundbreaking but it has been a lot of fun, pertains to some of these plants that produce bioactive compounds, like tomatoes that produce lycopene and all of the lycopene precursors that are beneficial for prostate cancer. We have been looking at grapes and resveratrol and flavonoids in the grapes that are beneficial for cardiovascular and anti-cancer effects and other things as well. And we have been looking at other sources of isoflavones, like kudzu, for example, which is a noxious weed in the south of the United States but it is a wonderful ingredient for isoflavones in Chinese herbal medicine. We have been looking at all of these things and growing them in plant cell culture, or in organ culture (like rapidly growing roots of kudzu in test tubes). When we feed these plants in tissue culture a radioactive source of sugar (radiolabeled source of sugar), the metabolites become radiolabeled. Then we are able to isolate radiolabeled isoflavones, for example, feed them to rodent

models, and absolutely see where that label goes. Is it isolated in the liver? Does it pass the blood-brain barrier? How long is it in the bloodstream and how quickly is it excreted? We have been able to see where some of these metabolites, which by our conventional measurements we really didn't think were getting in there, or we didn't think that measureable levels were getting into the bloodstream, we are able to see that something is getting in there. Some kind of metabolite from the original labeled compound is definitely passing through the animal and localizing in certain organs.

For the first time, last summer, we were able to show, through collaboration with our colleagues at Purdue and the Center for Age-Related Diseases, that flavonoids from grape, when eaten (when ingested by rats), were able to pass through the blood-brain barrier and be localized in regions of the brain, which kind of works for why you have some anti-Alzheimer's and anti-Parkinson's disease effects from these metabolites, even though we weren't able to measure them getting into the brain because we didn't know what we were looking for once they are metabolized. So this radiolabeling has been a real key to show where these things are going and how they are actually working.

JB: That is really fascinating. In essence, it sounds a little like what you are doing is ADME work with natural phytochemicals (Absorption, Distribution, Metabolism, Excretion), which is like a Phase I-type of IND except here we are dealing with nature's laboratory of phytochemicals. Once you have determined the metabolites and their localization and excretion, then I guess the next question is what is their bioactivity? Could they be more bioactive than the original materials that were put in the mouth? Then you get into ex vivo analyses. You are probably looking at some studies along that line as well, I would imagine?

MAL: We are just now, yes.

Plant Extracts as Adaptogens

JB: That is very exciting. So that raises-for me-a very interesting, provocative question. There are so many interesting questions that are provoked by your work and your comments, but there is one that strikes me specifically. You have described the mixtures of molecules that are produced in response to environmental factors working on the genome of the plant and its unique, specific book of life, and that produces these secondary phytochemicals which then have an influence on the physiology of the plant. Then we consume that plant (possibly-let's say it is an edible plant for humans), and those anti-stress compounds in the plant then are absorbed at some level and have some impact, either directly or through metabolism as you have just described, and get localized in certain organs or tissues. And then there has been this long-standing question as to whether these mixtures of molecules serve as some adaptogens.

That is a term that has floated around in the botanical medicine literature for a long period of time. It is kind of one of those words that doesn't seem to have a specific definition, but it says that low levels of something help the body to adapt to stress. I am wondering about these compounds that are in these mixtures. Undoubtedly if we did a traditional pharmacological evaluation they would probably have very high IC50s (meaning they would be considered fairly low potency molecules in comparison to a new-to-nature molecule that had come out of drug discovery), but the mixture of these molecules, as you have described them, even though their individual potencies may be low, may influence physiology in such a way as to produce this normalization effect, which is called adaptogenic. Am I at all moving...?

MAL: You are exactly right. An adaptogen would be something that increases the nonspecific resistance

of an organism. That's a definition that we use, and actually that is one of the main foci of our work in Central Asia, in the stands, because the Soviet regime was very interested in plant extracts that would (as an adaptogen) help reduce fatigue, increase endurance, reduce hypoxia for soldiers climbing over the mountains, things like that that would enhance human metabolism. Many of the root crops that we have been working with there definitely have these adaptogenic effects. That is exactly one of our areas of research.

JB: So this would be like your Rhodiola rosea?

MAL: That is one of them and Ajuga is another one (Adjuga turkestanica), which is something that is actually used. In some countries, Ajuga leaves are used in salads, just like Arugula (they are very similar, actually). It is something we haven't embraced in the United States because we don't know about it, but in other countries it is used (different kinds of Ajuga). With these kinds of things, what they have is a compound called a phytoecdysteroid. It is a steroid-like compound; it has the same shape as a steroid without the bad effects of a metabolic steroid, so it is a natural, steroid-like compound that increases resistance. Some of these Ajugas have very high levels in their leaves. Spinach is another mainstream vegetable that has low levels of phytoecdysteroids. Maybe back when the Popeye cartoon was on-you know, before a lot of things were bred out of spinach-Popeye wasn't kidding when he ate his spinach and had the good muscle mass because those phytoecdysteroids really do help in endurance and muscle mass.

JB: I'm now going to ask the very provocative, kind of philosophical question. What you have just described obviously is a whole different way of looking at pharmaceutical science. It is a different way of looking at medicinal chemistry. It is a different way of looking at even therapeutic (let's call it preventive) and chronic disease management than the traditional method that we have taught health providers to practice their medicine from. How long will it take, do you think, to filter this message that you are describing into a place where it can actually make a difference in clinical practice?

MAL: You know, we are just at that point now because finally-just in the last few years-we have come up with screening methods that are high content, that we can look at the effect of multiple genes at once (putting a plant extract on a cancer cell line, for example, and look at the effect on multiple genes at once, or doing verse transcriptase PCR to look at multiple targets at one), so finally we have the tools to look at an extract not like a pharmaceutical compound, but has multiple targets and we can get to the bottom of what is working. We have always been very good breeders in this country (well, throughout the world). We can get traits into plants that we want. But we haven't known what to look for until now. We are just at the breakthrough point where we are figuring out what compounds and what mixtures of compounds are important, and with the correct tools, which we now have at our fingertips, we are able to either put new health-enhancing properties into mainstream fruits and vegetables, or introduce fruits and vegetables from other parts of the world that are underappreciated and under-recognized in the United States and get them into our marketplace so that we have wonderful, health-protective sources so people can responsibly select foods for their own diet and the diet of their families with health in mind, not just calories and nutrition, but health-protective properties.

JB: That's really very, very exciting. My last question is, given that you are an expert in this field, what plant foods do you consider, at this point, most interesting from a health perspective based on these phytochemical arrays and why?

The Phenomenal Wild Blueberry

MAL: The most exciting one for me has been the wild blueberry. This is a particular species of blueberry (Vaccinium angustafolium). It grows only-only, only-in maritime provinces of Canada and coastal Maine. The reason this is so exciting to me is this berry has such a variety of health-protective effects. We found anti-cancer effects, and not just anti-cancer in that one enzyme is knocked out. We have effects against the promotion, initiation, and proliferation stages of cancer from berry extracts, and different compounds in the berry are giving protection at all these different stages of carcinogenesis. There are cardioprotective effects with wild blueberry, anti-diabetic effects, anti-infection, dental health effects. One of my colleagues at Tufts University is doing cognitive and motor function with age-related diseases and how berries can actually help give relief to an aging patient who has memory loss; it can bring back memory. It is amazing to me that this little berry has such a variety of different benefits for human health. Now, all blueberries are great. All blueberries are in a class by themselves, but the wild blueberry, in particular, has been just phenomenal.

JB: That is a really great little insight for us. You make a wonderful comment-well, you make a number of wonderful comments, but one that stands out in the close here is the comment that it is very interesting that some of these phytochemical mixtures derive from plants have effect on physiological processes across a wide range of diseases. It is not like one plant for one disease. It is more of a mechanism, which I think is a very interesting concept.

MAL: It is absolutely true. The co-evolution of animals and plants probably contributed to that. It is on a timescale that is beyond my comprehension, but we find plants as sources of biologically active molecules and mixtures that we just simply don't find in any other source (any synthetic source).

JB: If there anything that you would want to leave if you were talking to several thousand health practitioners who are going out to give recommendations to their patients? Is there anything that you would want them to think about as they go into those exam rooms with their patients given all of this extraordinary new information?

MAL: Just stay tuned because I really think we are at the point of huge breakthroughs for fruits and vegetables, in particular. People now who want to be proactive about their health are so willing to go into health food stores and buy Echinacea extract and Gingko extract, and not that these things are bad, but they'll buy these little tablets with exotic things in them, but we have really some specifically health-protective phytochemicals in mainstream fruits and vegetables, and we can enhance those in mainstream fruits and vegetables and bring them to the diet, to the American table. So really, just stay tuned because these things are going to be coming out more and more now that the research is just flourishing.

JB: Well, Dr. Mary Ann Lila, I want to thank you so much, both for the extraordinary work you've done and for the eloquent way you present it. It gets us all pretty excited about what the future may be for both prevention and maybe even therapeutic medicine where these concepts get integrated more into the exam and treatment room. Thank you very, very much for the time that you spent with us.

MAL: Sure enough.

Natural Foods as the "New" Pharmacology

We want to thank Dr. Lila, once again, for that extraordinarily insightful and provocative series of comments and discussion about her own work and that of the field. Can you see the future of medicine and where it is going to integrate this "new pharmacology" which I find to be almost an ironic term ("new"). This is historic pharmacology. This is the way we ate in times before standing agriculture, when the foods kind of had evolved through time with us in the largest laboratory experiment that has ever been done: that of evolution and natural selection where the interaction of our food supply with human physiology and its genetic structure was that which had been evolved over millennia. We have obviously intervened and modified this relationship somewhat with standing agriculture and new cultivars, the way that we grow food products by taking the stress off the plants with herbicides and various biocides and fertilizers and sun protection and all of these things change the family of these secondary metabolites that then modulate human function in different ways, so it is a very fascinating chapter in nutritional medicine that we are starting to see opened up. This is providing support to a lot of what has been discussed within the natural foods field for some tens of years. In fact, going back into the 18th century, we saw the origins of these concepts of natural, minimally processed foods having a different effect on physiology than processed foods. It is a very, very interesting chapter in the evolution of our understanding.

There are all sorts of extraordinary papers coming from many highly competent research labs around the world that are adding depth and dimensions to this story. In the *Journal of Cancer Research*, an article titled "Grape Seed Extract is an Aromatase Inhibitor and a Suppressor of Aromatase Expression" was published showing, again, the wide, pleotrophic effects that many of these secondary metabolites have on physiological function in mammals.[30]

Aromatase is the enzyme that converts androgens to estrogens and it is expressed at higher levels in breast tissues, and (particularly more in cancerous breast tissue than normal breast tissue). There have been many synthetic compounds that have been studied, and some approved (Aromadex being one) for the blocking of the aromatase enzyme to result in lowered estrogen levels *in situ* in localized tissues. But the question has been raised for some time, were there natural substances in a complex, minimally processed diet that could influence this A-ring aromatization of the steroid nucleus from androgens to estrogens and therefore have estrogen modulating effects?

One that has been discovered and described in this particular paper is grape seed extract, containing high levels of procyanidin dimers that have been shown to be reasonably potent inhibitors of aromatase. This-again I want to emphasize-was a combination of *in vitro* studies and also animal study, looking *in vitro* at the MCF7 breast cancer cells (these are cells that are immortalized as a consequence of having undergone transition to cancer cells). It has been shown that grape seed extract has a very beneficial effect on preventing the production, locally, by these cells of estrogens from androgen precursors and also in animal interventions was shown to lower the production of estrogens in specific tissues (in this case, breast tissue).

It seems to be that these procyanidins have an aromatase inhibition effect, or let's call it modulating effect, and suppress, then, localized production of estrogen. So that's just another of the myriad pleotrophic examples that demonstrate the wide range of physiological functions these secondary metabolites can have.

Another of those that was described in this session with Dr. Lila was *Pueraria lobata* (or kudzu) isoflavones, and they have unique estrogen modulating effects as well, which are different than the soy isoflavones -- genistein and diadzein. A paper that was published in *Plant Medica* talks about isoflavones that are unique to the *Pueraria lobata/Pueraria mirifica*family and how they influence estrogen receptor activities alpha and beta (without having the estrogen drive for mitosis that you see with 17beta-estradiol) and can modulate estrogen functional status at the receptor site.[31]

Again, many different influences that these plant phytochemicals have (as we use this kind of complementary concept that agents in plants evolved as anti-stress compounds) then have physiological, normalizing, or anti-stress effects in the human. Of course, that raises the broad question about what kind of diet delivers the highest level of these phytochemicals and that is a diet that is minimally processed, rich in vegetable foods, fruits and vegetables, and whole grains.

So is there any difference we can ascribe to a vegetarian diet and health outcomes in the human? Although this has been a reasonably controversial topic in some bodies of literature, I think the data, over years, has come to pass to say that a well-balanced vegetarian diet providing adequate levels of protein, vitamins, and minerals, in fact, delivers, in population-based epidemiological studies good health outcome, looking at Seventh Day Adventists, looking at groups of people who elected to be on vegan diets-the health outcomes look very favorable. In fact, blood pressure regulation and vegetarian diets was the topic of a review paper in *Nutrition Reviews* authored by Susan Berkow and Neil Barnard.[32] This was in the January 2005 issue and focused on high blood pressure and its management with vegetable-based diets. It translates into the DASH-type diet (Dietary Approaches toward Stopping Hypertension). There are many papers-if you do a meta-analysis-of roles that vegetable-based diets have on blood pressure. With a search you are going to find over 20 papers that have all shown positive benefit as a consequence of the nutrient composition of vegetable diets, not only the mineral content of potassium and magnesium with low sodium, but also the high level of phytochemicals that serve as vascular-active substances that help to improve endothelial function.

We even know that diets that are very high in these phytochemicals have been ascribed as anti-cancer diets and have preventive effects. There is a very nice paper that appeared in the *Journal of Clinical Oncology* in 2007 titled "Greater Survival After Breast Cancer in Physically Active Women with High Vegetable/Fruit Intake Regardless of Obesity."[33] This paper again showed that there must be something of interest related to the portfolio or the profile of these phytochemicals and how they influence outcome in a secondary prevention trial in women who have had breast cancer.

I think that we are starting to see the convergence of various lines of observation and intervention research, ranging from epidemiological human work, to animal studies, to *in vitro*work, to cell culture work, to--even now--some intervention trials. There is a wonderful prospective study of the relationship between fruit and vegetable intake and the risk of prostate cancer that was recently published. This was in the *Journal of the National Cancer Institute* in 2007.[34] This study showed a very positive correlation between high intake of cruciferous vegetables (including broccoli and cauliflower) and a reduction in the relative risk of aggressive prostate cancer, particularly extra prostatic disease.

What we are starting to witness is a kind of a convergence of our observational-and almost intuitional-thoughts that high vegetable and fruit and whole grain diets play a positive role in health, and now the mechanistic underpinning of that and the fact that different cultivars grown in different conditions can

have differing levels of these beneficial phytochemicals. So it is an exciting story and we thank Dr. Lila very much for adding another chapter to our evolving understanding.

We'll look forward to seeing you next month in *Functional Medicine Update*.

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