July 2011 Issue | Edwin Lephart, PhD Brigham Young University

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Welcome to *Functional Medicine Update* for July 2011. In this issue we're going to talk about some extraordinary new work in phytochemistry—plant-derived chemistry—and how this relates to cellular function. The complex nature of secondary metabolites in plants, this rich array of compounds that is unique to plants that has to do with things like chlorophylls, xanthophylls, carotenoids, flavonoids, isoflavones, glucosinolates, and polyphenols. There are literally thousands of compounds that are manufactured by the biosynthetic machinery of the genes of specific plants that then are being found to have unique effects on human physiology when consumed as part of the diet.

Secondary Metabolites in Plants

I find this to be a very remarkable chapter in the evolution of our understanding of nutrition and health. We've known from epidemiological work that plants, when consumed in the diet on a regular basis, are associated with lowered incidence of a variety of diseases. We've said that plant-based diets are healthy. And as we've more commercialized diets and processed plant foods to produce white starch and sugar and extracted fat (so-called compartmentalized foods), we have lost a lot of the value of what's found in "natural foods" that then are health-promoting. What is it in plants that has this influence on health in a beneficial way that upon their removal somehow lowers the health benefit of that food product? There has been wide-ranging discussion, speculation, and sometimes even controversy around what these secondary metabolites in plants do, and what role they have to play in human physiology. One of the simplest explanations that we've kind of last 20 years is that many of these compounds in plants are antioxidants, which means that they help to soak up adverse effects of activated forms of oxygen, such as singlet oxygen, superoxide anion radical, hydrogen peroxide, hydroxyl radical. In this role they serve as cellular protectants.

Phytochemicals Are More Than Antioxidants

Most of us came to say, "Well, that sounds reasonable. Good, I'll accept the facts that these phytochemicals are antioxidants. But as more work has been ongoing, particularly over the last decade since the human genome project was completed, we've started to recognize from more intimate cellular studies that these phytochemicals have a much more specific role than this general kind of catch-all phrase called antioxidation. They have very specific functional characteristics based on the structure of the secondary metabolite of the plant, meaning the individual phytochemical or phytonutrient, and how they regulate specific cellular function at the genomic level. So they have the ability to speak to genes indirectly, through regulatory mechanisms that ultimately control both genetic and epigenetic expression into what we call the phenotype of the cell, or its functional state. And it is this emerging story that I think is so fascinating because it puts a whole different level of interest and importance clinically as it pertains to the complex nature of phytochemicals and their effect on human health. It also ties nicely back to the

longstanding epidemiological associations between higher plant food diets that are minimally processed being associated with lower incidence of chronic disease. It gives us a mechanistic hypothesis as to how these substances—this rich array of secondary metabolites in plants—in a diverse diet that is plant-based, can have very dramatic health benefits, and how the absence of these compounds in the diet by over processing or eating foods that are devoid of these phytochemicals may have lost some of the beneficial attributes of the diet through the loss of the normalization of genetic expression or cell signaling.

We're going to have the privilege this month of talking with an expert in this field, a person who has been studying—at the cell biology and immunological level—the role that these phytochemicals play in various cellular functions. We'll specifically focus on one of them as a representation of the field in general, and deeper drill into this specific phytochemical. This phytochemical is really even more interesting because it is a secondary metabolite in the gut of a primary phytochemical produced by plants.

A Review of the Biochemical Processes Related to Phytochemicals

Let me go back and trace that again because this shows you this story is even more complex than we might have initially thought it. The plant, under conditions of its own physiology and the environment in which it is grown, induces its genes to produce these metabolites called specific phytochemicals. Those are then consumed by humans (in those edible portions of the plant). The human then chews them, and subjects them to stomach acid, and they go down the intestinal tract. Those secondary metabolites get converted, by the process of digestive exposure, into a new class of secondary metabolites that really I guess we would call tertiary metabolites that have names like indole-3-carbinol, and diindolylmethane, and phenyl isothiocyanate, and sulforaphane. These are byproducts of digestive juice influence on glucosinolates made by the plants in response to their environments. Now we have an even more complex story of the rate of conversion of those glucosinolates into substances that then have the ability to be absorbed into the body and influence human cellular function.

We find this story being reproduced with many other phytochemicals. For example, a family of phytochemicals called lignans is found in many plant foods. The soybean is known to have high lignan content. When ingested, it gets broken down from lignands into lignans, and those then get converted by gut bacteria by their own metabolism into a set of additional new phytochemically-derived metabolites with names like equol, or enterodiol, or enterolactone. These compounds have the ability to be absorbed and influence cellular function.

They might seem to be removed from the plant because they are produced by the digestion of the plant material by gut bacteria (certain families) to produce these new chemicals which then influence human health. The story sounds very ecological: we're connected to our soil and plants through this very interesting chemical cellular matrix that has to do in part with the production of these secondary metabolites in plants called phytochemicals, and they're further influenced by digestive process and gut microbial fermentation by specific types of symbiotic bacteria into compounds that then influence human health in beneficial ways.

I love this story because it ties us into this web of life in a very different way than just thinking about a pill for a problem, or a molecule for a diagnosis. We're really talking about a symphonic orchestration of interrelationships among the soil, the plant, the environment, the human, and ultimately to even its gut microbiome and how that influences the production of these secondary products that can have a favorable

effect on human health and physiology. You're going to learn much more about this from an expert, Dr. Edwin Lephart from Brigham Young University, who will tell us a lot about the evolving story around equol.

Phytoestrogens: Molecules in Soy that Influence Estrogen Signaling

We started talking about equol in *Functional Medicine Update* some years ago when we interviewed Dr. Ken Setchell from the Cincinnati Children's Hospital, and before that Dr. Herman Adlercreutz from Helsinki University Medical School in Finland, who was actually the person that was credited with coining the term "phytoestrogen." Dr. Adlercreutz was the first person to actually find these molecules in soy that influenced estrogen signaling (things like genistein and daidzein—the isoflavones). He developed the conceptual framework of how these molecules—these phytochemicals—found in soy foods influence human health, which has been a part of the emerging story of how these thousands of different substances produced by a variety of different plants influence cellular physiology beyond antioxidant effects.

If you were to examine a contemporary textbook of nutrition that is used in university courses, in medical school training courses, or anywhere in the United States (actually anywhere in the world), and ask how much of that textbook is devoted to understanding the role of phytochemicals in cellular physiology, you would find a very small number of pages that have any real discreet explanation about the role that phytochemicals play in human health and cellular physiology. That's because this topic that I'm describing is rather new. It has been only ten years or so in which we've started to really understand how these complex mechanisms of action of compounds in plant foods influence health. Textbooks are generally at least ten years behind in reporting the cutting edge of contemporary new knowledge. The textbooks being studied today and the classes that are turning out nutritionists use information that is truly is outdated in terms of understanding the role that phytochemicals play in human physiology.

So there are still people going out into the world with degrees in nutrition who consider themselves experts, but unless they are reading the primary literature and listening to the right colleagues, they don't understand the story. In this issue you're going to be exposed to news to use at a clinical level that many people have yet to really understand. You'll have to become an ambassador for bringing this information to your colleagues, and if they—when you talk about it—have a funny look in their eye, like "I've never heard about this," don't be surprised because this is a fairly new area of evolution in the field of nutrition and health.

Retinoids: A Family of Phytochemicals with Unique Effects on Cellular Physiology

With that in mind, let's talk a little bit about the family of substances called the retinoids. The retinoid family is a classic example of how specific families of phytochemicals have unusual and unique effects on cellular physiology beyond their "antioxidant effect," meaning the ability to just soak up oxygen radicals and prevent free radical pathology.

You probably know the retinoid family to be associated with vitamin A, but also recognize that vitamin A retinol can be converted in the body into things like retinaldehyde and retinoic acid, which are interesting derivatives of retinol vitamin A. You also probably know that vitamin A can be derived from beta carotene through conversion in the body by an enzyme that cleaves the beta carotene molecule into two retinol molecules by breaking the bond right in the middle (the double bond) and oxidizing that into what ultimately becomes vitamin A. So the conversion of beta carotene into all-trans retinol requires an

enzyme. That enzyme is activated by thyroid hormone and it is dependent upon the trace element copper.

Through cellular physiology, retinol inter-converts members of this retinoid family into a variety of additional forms, including cis and trans isomers, because there are many double bonds in these molecules. Remember, they started off as pigments—as things that we could see, like the color red in carotenoid-containing food, and then they get converted by the cleavage reaction into retinol. Retinol loses its color, but it still maintains this unsaturated conjugated double-bond matrix that we found in carotenoids. Vitamin A becomes a part of the color characteristics of the carotenoids, and so these all-trans double-bonds that are conjugated are part of the vitamin A family and they can be isomerized by different kinds of processes that occur within the body into mixtures of cis and trans so we have all-trans retinoids. Those are the ones that are derived directly from all-trans beta carotene. And then you have cis /trans mixtures of different double-bond configurations that are members of the family, all of which have different physiological effects and different effects on cell membrane signaling. I hope I haven't made that too confusing. I'm trying to give you a sense that beta carotene in the body gets converted into a variety of members of the retinoid family through these enzyme conversion processes.

Once converted into retinoids, what role do these phytochemicals that are derived from plants (the carotenoids) have on cellular function? You probably know you can get retinol and vitamin A directly from animal food. The animals have already done the conversion of the carotenes into retinoids for us, so they are present in the food as vitamin A itself. Or, you can get them from plant foods as precursors (as beta carotene that gets converted into retinoids by cellular physiology). So animal foods can directly deliver vitamin A, and particularly because it is a fat soluble vitamin it is found in the fatty components of various animal products, such as whole-fat dairy and so forth, but the precursors are found in your orange-red vegetables (the carotenoid-containing plant products).

Retinoids and Xerophthalmia

Once you get to retinoids, now the question is: What happens in cellular physiology? Are these antioxidants? What do they do? We've been told that beta carotene is an antioxidant. We've been told that vitamin A is a substance that helps to strengthen or support the immune system. We know that in children that are vitamin A deficient they get a deficiency disease called xerophthalmia. Xerophthalmia is a condition that leads to the most common form of juvenile blindness in the world, which is the result of a vitamin A deficiency that can be treated by giving children, usually, one big dose of vitamin A that lasts for some time in the child that may not be getting adequate levels of either carotene in their diet or getting adequate vitamin A directly through animal foods. There are projects being done by philanthropic groups to try to improve vitamin A nutriture in the developing world—a not-for-profit organization called Vitamin Angels is very actively involved in providing supplemental sources, free of charge, for the nutrients necessary to prevent blindness in children. It has had a very, very nice track record over the last five years of saving the sight of literally tens of thousands of children.

So we know that vitamin A deficiency has something to do with the function of the rods and cones in our eyes (with visual function). But way before you get into a situation of frank vitamin A deficiency leading to a condition such as xerophthalmia, there may be a functional insufficiency of retinoids, and that's where the story gets more complex.

All-trans Retinoic Acid: Like Vitamin D, an Important Regulator of Gene Expression

So what role do retinoids play in cellular physiology that manufacture or manifest control of cellular function? I'm going to specifically talk about all-trans retinoic acid. All-trans retinoic acid, which is a product of retinol vitamin A conversion through cellular physiology, , is a very, very important regulator of gene expression like vitamin D is, which we've heard so much about over the last several years.

Vitamin D is converted into a hormonal form called 1,25-dihydroxyvitamin D3, which then binds to what are called the nuclear orphan receptors (specific vitamin D receptors that sit on the nuclear envelope). That triggers a signal from the cytoplasm of the cell into the nucleus of the cell. Of course we know what's inside the nucleus of the cell; the book of life (our human genome). This signal activates various transcription factors through this nuclear orphan receptor agonism that ultimately turns on specific genes. There is some controversy still about the specific number of genes that are under control of the 1,25-dihydroxyvitamin D3, but let's say in the range of 50 or more genes are turned on.

So vitamin D is much more than just a vitamin that promotes calcium metabolism. We now recognize that it has effects on insulin sensitivity, cell reparation, immune function, cell cycling, and oncogenesis. We've learned that it is much more dramatic even in its influence. Even vascular endothelial function is, in part, related to vitamin D function, as well as immune vigilance. These are all consequences of the pleiotropic effect of vitamin D and these nuclear orphan receptor influences on specific gene activation in different cell and tissue types. I hope I made sense of all of that.

Let me kind of simplify it: When a person is suboptimal with regard to vitamin D nutriture, what happens—well before they get rickets—is incorrect signaling through transcription factors that regulate gene expression, so the cellular function gets disturbed or distorted. And it can get distorted into states of suboptimal performance that are related to chronic illness that occur—in a differential way, in different tissue types—depending upon what specific genes are not activated in those cell types as a consequence of the hormonal form of vitamin D being deficient or insufficient.

With that as a model, now let's talk about retinoids. With retinoids we have a similar story. The difference here is that retinol (vitamin A) is not converted into a hormonal form, but rather it is converted into secondary members of the retinoid family, like all-trans retinoic acid. All-trans retinoic acid, then, also binds to nuclear orphan receptors (different ones). In fact, it can even co-hybridize with things like the vitamin D hormonal form (1,25-hydroxy D3) to activate regulation of transcription factors that turn on different genes.

So, retinoic acid is a gene response modifier. It influences gene expression, or how our book of life is read, and it does so in different ways in different tissues. Therefore, it differs in its function from vitamin D, but it has an analogous influence on regulating pleiotropic effects across wide ranges of different tissue types in outcomes. Insufficiency of vitamin A can influence (adversely) cellular function in the absence of xerophthalmia, just as vitamin D can do in the absence of rickets). So there is a difference between deficiency and insufficiency as it relates to optimal cellular function.

Clinical Implications of Retinoic Acid Insufficiency

With that in mind, let's go into a little bit more detail about what the clinical implications of this could be. What has been discovered over the last decade is that retinoids influence these retinoid receptors in the control of energy balance, and have influences, then, on obesity and diabetes. That might be new news to you. Maybe you've never thought about vitamin A or carotenoids having influence on obesity and diabetes, but the influence of the all-trans retinoic acid at the receptor sites and activation of the various

cell signaling mechanisms is dependent upon nutritional status. If you don't have the precursor (i.e., retinol or beta carotene), you don't have the molecules available to be converted into these signals that are going to regulate gene expression. So insufficiency, based on the genotype of the individual (meaning biochemical individuality), may have a variety of subtle effects on cellular physiology that map against obesity and type 2 diabetes.

Could There Be a Connection Between Vitamin A and Obesity and Type 2 Diabetes?

Now let's just take this a step farther, because this might be kind of mind-blowing information for you. You probably never thought about vitamin A and obesity and diabetes unless you are following this literature. Obesity and type 2 diabetes are closely related metabolic disorders which have increasing incidence worldwide, and there is no clear-cut pharmacological treatment available for these metabolic disturbances because they're generally not just a consequence of one thing going wrong. It's really a shift in the web of metabolism, and it distorts many, many different processes related to a variety of families of genes that are all modified in their expression. So it is not like a one-disease/one-drug/one-outcome type of a problem. That is why it is so challenging to manage a diabetic patient, because there is no one single therapeutic target that one can focus on to correct these distortions or disturbances in metabolism. New directions, however, as we've been talking about in *Functional Medicine Update* for some time, for the management of these disorders, are now starting to emerge from a more complex understanding of these alterations in cellular signaling that occur as a consequence of the interrelationship between the environment of the person and their genetic uniqueness. This is why diet and lifestyle play such important roles in conditions like obesity and type 2 diabetes, because the environment is what sends the signal to the cells which contain the unique genomic book of life of that person, to give rise to their cellular expression called their outcome.

Diabetes doesn't necessarily derive from obesity, but rather obesity and diabetes arise together as manifestations of altered metabolism occurring as a consequence of this relationship between the unique genome of that person with their environment. If their environment sends an alarm signal to their genes through their regulation of gene expression, a disturbed metabolism is then seen as the trajectory towards type 2 diabetes and obesity.

What is it that increases adiposity, alters so-called energy expenditure), and changes insulin signaling when the environment is sending a message to the genes of alarm? That's been a central question for investigators around the world over the last few years. One thing that is emerging is that all-trans retinoic acid is known to inhibit the adipocyte (the fat cell) differentiation by the signal it has to its genes that are expressed. So retinoic acid (all-trans retinoic acid), to say it again, is known to inhibit adipocyte differentiation from a pre-adipocyte into a mature fat-storing adipocyte. As a consequence, it also influences cellular signaling of the adipocyte through things like adiponectin and leptin, molecules that regulate things like appetite, energy expenditure, immune function, and insulin sensitivity, meaning it has an influence on the web of control of energy economy and inflammatory response.

Now this is a very, very interesting part of the story. You might say, does that mean, then, that vitamin A is an anti-inflammatory? No, what it means is that beta carotene/vitamin A/retinoic acid then participates in the regulation of cellular function in such a way as to diminish the response of the genes to the environment to produce an inflammatory response. It is not that vitamin A all-trans retinoic acid is an anti-inflammatory. All-trans retinoic acid, at the proper place at the proper time, helps to regulate gene expression in such a way as to buttress against an adverse response that we call inflammation.

This is a very important new development in our understanding of the role that retinoids play in cellular physiology. What happens if a person is marginally insufficient with vitamin A then? Couldn't they start to develop a distorted metabolism in response to a triggering event? Could retinoids have an effect on the progression of autoimmune diseases? That seems like a logical hypothesis that you would take away from this discussion. Of course the answer is yes. Recent papers, such as that published in *Molecular Immunology* in 2009, have talked about how retinoids differentially regulate the progression of autoimmune diabetes in clinical models in animals.[1] I want to emphasize these are animal studies trying to demonstrate a proof of concept. When an animal is put on a marginal insufficiency of retinoids a more rapid progression of this model to autoimmune diabetes is observed. When an animal is supplemented with all-trans retinoic acid, a return of proper cellular physiology and a resistance in autoimmunity is observed. These results are found even in animals that are treated with substances that kill the beta cells, like streptozotocin, which is known to be a toxin to beta cells in the islets and cause diabetes. Retinoic acid insufficiency greatly accelerates diabetes in these cases. Data like these indicate that maybe there is something about the role that retinoids have in regulating energy economy, regulating insulin signaling, and regulating inflammatory response to a stimulus as a consequence. This could be similar to vitamin D and the role it plays in orphan nuclear receptor activation of transcription factors that regulate specific gene expression and ultimately control proper response to environmental triggers.

Evaluating Vitamin A Status Clinically

What does that mean clinically? It means that we should be very mindful about adequate evaluation of vitamin A status: making sure carotenoids are being consumed in the diet, making sure the person is not hypothyroid (that would lead to under conversion of carotenoids into retinoids), making sure that person has adequate copper in their diet because copper is necessary for the conversion. How do you help to support proper thyroid hormone conversion of T4 to T3? It is T3 that activates the conversion of carotenoids into retinoids, so you make sure that the deiodinase enzyme, which requires selenium, is being properly promoted by proper selenium nutriture.

Regulating cellular signaling through the retinoids shares aspects in common with the regulation of cell signaling through the cholecalciferol (vitamin D) family. As we move into this discussion with our researcher of the month talking about equol, I hope you'll keep in mind this discussion that relates to the retinoic family, and phytochemicals in general and how they have this remarkable influence on regulating function well beyond prevention of deficiency diseases. That is the story that we will be talking about, reapplying it now to the discussion of equol.

INTERVIEW TRANSCRIPT

Researcher of the Month

Edwin Lephart, PhD

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We just seem to be so privileged with having individuals share their thoughts and their hard work in pioneering the new concepts in medicine. We're privileged once again: Dr. Edwin Lephart is going to be our Researcher of the Month this month.

Let me tell you a little about Dr. Lephart. I think you'll find his background to be both fascinating and perfectly consistent with the topic of our discussion. He received his PhD in physiology from the University of Texas Southwestern Medical Center, a world-renowned research institute in Dallas, TX. He comes from a very good background, with two of his committee members being National Academy of Science members. He did postdoctoral training at the Department of Psychiatry and then took a position at Brigham Young University, where he is currently Professor of Physiology and Neuroscience in the Department of Physiology and Developmental Biology. He has authored over 85 publications across a wide range of topics, many of which we're going to touch upon in this discussion. He has over 8 book chapters or scientific reviews coving wide topics, including reproductive biology neuroscience. The topic of our discussion will be the area of phytoestrogens.

I want to introduce this concept of phytoestrogens. If you are long term Functional Medicine Update subscriber, you'll remember that we've previously spoken to two notable luminaries in this field. The first is Dr. Herman Adlercreutz, who was from the University of Helsinki Pathology Department and Medical School there and has been credited as the father of the term "phytoestrogens." We had a very wonderful and robust discussion over 15 years ago with Dr. Adlercreutz, in which he said that of all the things he had done in his some 600 publications over the years, the one thing that he regretted was calling these soy-derived and plant-derived materials "plant-derived estrogens," because he said this term causes a misunderstanding in a lot of peoples' minds, causing them to think of these things exactly as they think of 17 beta-estradiol. Their mechanisms of action are actually different. They have weak agonist activities as estrogenic substances, but unfortunately the transliteration of the term "phytoestrogens" in the minds of many led them to think of them as estrogen itself. We're going to talk more with Dr. Lephart about that, but I want to just set the tone that that's kind of a legacy that goes back of 25 years in the literature.

The other individual that we have had the fortune of speaking to in this area within about the last 10 or 12 years was Dr. Kenneth Setchell at the Cincinnati Children's Hospital. Dr. Setchell, who was a post doc for Dr. Adlercreutz many, many years ago, was credited in really discovering this lignan family of bioactives in plants and how they interrelate with things like soy effects of physiology. He has obviously been very, very active in the field that Dr. Lephart himself has been involved in that we'll be speaking more about, which is the secondary metabolite of these lignan materials derived from bacterial fermentation in the gut, which is called equol.

There's an intellectual lineage that traveled through Functional Medicine Update, through Dr. Adlercreutz into Dr. Setchell , and now we're very fortunate to have Dr. Lephart. With that kind of lengthy introduction, Dr. Lephart, thanks so much for joining us on Functional Medicine Update. We're going to be very pleased to hear your story, so thank you for being with us.

EL: Thanks for having me on. This is a real privilege.
JB: Let's start down the road here quickly. I think for a person with your background, as I have described it, it might not appear obvious to the listener why you would ultimately have gotten into the soy phytochemical research. It maybe sounds like kind of an interesting twist or turn as it relates to your background. What led you into this whole field?

Studying Phytoestrogens and Brain Chemistry

EL: My background really is a story of biochemistry. We had an associate of mine out to give a seminar,

and he was telling me about how he was studying phytoestrogens. I had no idea what phytoestrogens were and he was telling me how fascinating these molecules were. He really peaked my interest. After he left I looked up some names (that you just described) in the field and made contact with Ken Setchell and Herman Adlercreutz. We've published together in the past on these different topics, and they are fascinating molecules, having these polyphenolic structures that have similarities to natural stories, but as you described, are much different and have, in many ways, a broader range of biological activity. That's been over 15 years ago that I got this indirect introduction to phytoestrogens, and then a little bit on my own because my area is more involved in brain research. At that time, no one was looking at these particular molecules in depth in brain research, so it turned out to be a very fascinating area to study, and it has been very profitable as far as a research avenue over these past years.

The Origins of Equol

JB: The soybean is a very interesting plant that manufactures many secondary metabolites that are bioactive, and one of those that ultimately is seen in human physiology that has been the focus of your research is equol. Could you tell us a little about the origin of equol? Where does come from and how is it produced?

EL: Equol was first seen in fractions of biological samples from pregnant mare urine in 1932. It has a rich history but it really didn't gain any really high profile prominence in research until Ken Setchell, in the early 1980s, identified this molecule in human biological fluids, when humans consumed soy products like soybeans (as you just described). This particular molecule is a metabolite of daidzein, which along with genistein is found in the aglycone form soy or soybeans. Ken did a lot of work looking at production of equol in humans, which is much, much lower than animals, and then he came up with an equol hypothesis that soy-based diets could be enhanced if you could increase the efficiency of converting daidzein into equol. That could have health benefits. That was around 1996 (around there). And then if you look at the number of publications on equol, it has gone up dramatically since that time because equol is such a powerful antioxidant. We found that it can bind specifically 5-alpha-dihydrotestosterone (DHT), which is the most potent androgen in the body. And it is also expressed in two different isomers, R and S, and both of these isomers are biologically active, which is unique because it has a chiral carbon, and it is unique because genistein and daidzein aren't expressed in these particular biochemical properties.

JB: That's fascinating. Does both the right and left handed form of equol have the same biological effects or do they have slightly different effects?

EL: For binding specifically 5-alpha-DHT, R and S equally have a high affinity (for specifically 5-alpha-DHT). For example, we've studied more than 30 different steroid types of molecules, and it doesn't bind 5-beta-DHT, it only binds 5-alpha-DHT. And both R and S equally have high affinity for binding 5-alpha-DHT.

JB: That would be very, very interesting when you starting thinking that equol is really a secondary—almost a tertiary—metabolite, isn't it? Because first the plant makes it as a precursor, and then, as you said, in the gut, various microbes ferment that which is daidzein into this metabolite which is equol, and do so into…I guess two enantimers (the R and S form), which then are absorbed and have their effects on different receptors. That would suggest that maybe with its influence on DHT—obviously, in your work, you've published a number of papers that it might have favorable effects on prostate health.

In fact, there is a nice paper that you are a principal author on with Dr. Lund in Reproductive Biology and Endocrinology in 2011 on equol and its potential for improved prostate health.[2] Tell us a little bit about that connection between 5-alpha-DHT and equol.

Equol and Androgen Hormone Action

EL: Equol having these really unique biochemical properties (being a powerful antioxidant), but this unique property to bind 5-alpha-DHT is really important with aging, especially in men for prostate health. This is because it is thought that even though the principal circulating androgen, testosterone, decreases with age (say around 40 or 50), and it starts to decline, if you look at the enzymatic make up in the prostate, the 5-alpha-reductase enzymes actually increase their expression. So even though the substrate is going down, the enzyme expression is increasing, and so you're making more 5-alpha-DHT.This is the molecule that is causing proliferation, especially of the epithelial cells in the prostate, and causing the condition benign prostatic hyperplasia. So if we could bind that, we could decrease androgen hormone action at the androgen receptor. But what is really fascinating about equol is the S form has a relatively high affinity for estrogen receptor beta, and beta, in the prostate, along with breast tissue for women for breast cancer, if you bind beta then it indirectly will decrease the expression of androgen receptors. So in two different ways we're decreasing androgen hormone action. We are, in a modest way, binding 5-alpha-DHT, and then we are decreasing the expression of androgen receptor by activating estrogen receptor beta in the human prostate.

There is a great review looking at the difference between ER-alpha (the really traditional receptor) and then ER-beta that was discovered in about 1996 by Jan-Åke Gustafsson at the Karolinska Institute, who happens to be at the University of Texas in Houston right now.[3] By binding beta, both for the prostate and for breast tissue, that seems to be a positive influence for prostate health. That's the main mechanism of what's going on.

JB: You've raised some interesting kind of halo effect questions from that very insightful discussion. First of all, when you talk about epithelial tissue in the prostate having a favorable impact in males with the equol exposure then it also raises a question: What about epithelial tissues in the scalp? What about follicular loss? What about male pattern baldness, knowing that there is a 5-alpha-DHT connection there? Is there any interconnection between baldness and prostate problems, or between equol and all of this?

Equol: Hair and Skin Health Implications

EL: There certainly is because this particular receptor (estrogen receptor beta) is richly expressed in the base of the hair bulb and around the hair shaft. The expression of the 5-alpha reductase enzyme is not only in the prostate, it's in the hair follicle, it's in skin and fibroblasts. There is application not only to prostate health but to female and male pattern baldness. Also to skin health, as far as cosmetics and wound healing because androgens decrease wound healing, whereas estrogen-like molecules enhance it. So there are different target tissues or applications that have really great potential for utilization of equol.

JB: I note that you've been a principal author on another very interesting paper about the effects of equol in human skin as it relates to modulation of function of the extracellular matrix.[4] That sounds like it ties together very closely with this dermatological potential impact, and maybe even ties together with things like wrinkling, which we know is a consequence in part of oxidative damage to connective proteins that cause cross linking. Any connection there that is of value from this research?

EL: Yes, we've done a great deal of research examining equol and the enhancement of skin health, both in vitro data and also gene array data. We've looked at 40 different genes, and in general equol will

enhance collagen elastin, which is a really important dermal protein for skin health. And at the same time, it will decrease the matrix metalloproteases, and there are many different molecules. These matrix metalloproteases have the ability to break down collagen elastin. What we found in the gene array studies and also in the in vitro studies is that equol at very low concentrations (in vitro at 10 nanomolar) will enhance collagen expression and elastin expression, but also at the same time decrease this enzyme that breaks down collagen elastin (the MMPs). If we look at the antioxidant arm for equol, it's a great stimulator for many different anti-aging and antioxidant genes. This particular aspect of our research has been translated, and equol is actually used in the cosmetic product currently and it has really positive effects for skin health.

Males and Soy Consumption

JB: When we examine this pleiotropic effect of equol it takes us back to one of the questions I know has been on a lot of peoples' minds that don't understand the complexity of this field. They'll say: "Hold it. If these compounds are phytoestrogens, won't they—in males—block testosterone and cause feminization?" There are even some reports, I think, in the literature suggesting that males taking soy-based products have significant decreases in testosterone. What have been your observations as it relates to the male effects of equol?

EL: Yes. The question is not only for equol, but it has also been for soy, and I think a couple of really good reviews have come out, especially for male physiology, whether it is for soy or for equol. We don't see, either in animal studies or in the small clinical studies, any change in the steroid hormone pattern in males in the low effective doses that we have calculated for either prostate health, or for cosmetics, or for male pattern baldness, etc.[5] The properties, because of the modest way that it binds 5-alpha-DHT, and also the estrogen receptor, we have not seen any negative influences on steroid hormone patterns or other hormonal patterns in males.

JB: Let's go back, once again, to ask: where does equol come from, and if it is, in fact, the result of microbial modulation or a conversion of daidzein ultimately into equol, is there a variation from person to person based upon their gut microbiome or the speciation of gut bacteria?

High Variability in Equol Production

EL: That's an excellent question because a lot of research has gone into that area. The answer is that there is a lot of variation. It would appear that cultures that consume fermented soy products produce more equol, meaning that fermentation process has drawn the conversion process closer to equol (say the Asian cultures compared to western cultures). But even if you look within those particular environments, those study populations, there is quite a bit of variability in the ability or the levels of producing equol that might range from 20 nanograms to 50 nanograms per mL, up to a couple of hundred to maybe 500 nanograms per mL. So there is a lot of variation in humans compared to animals. Animals seem to have the ability to produce equol at incredibly high levels. If we just took the rodent or the rat as the experimental model, equol levels can represent 70 to 90% of the circulating isoflavonoid molecules, ranging from about 1500 to almost 2500 nanograms per mL. Humans produce low levels of equol, and the variation is quite high.

JB: Is there any data available that correlates the serum level of equol epidemiologically with relative reduction of risk to certain kinds of conditions? Do we have any sense of what the desired physiologic range would be?

The Equol Producer Concept

EL: Yes, the concept of equol producer was generated, I believe, by Ken Setchell and his associates.[6] Also, investigators out of Japan came up with this concept that if there could be a threshold level and it's an arbitrary level of around 20 nanograms per mL, they looked at prostate health studies and also breast cancer and osteoporosis.[7] If you stratify the data in those individuals that have the ability to produce equol at 20 nanograms per mL or higher have a lower incidence of prostate health issues (breast cancer, osteoporosis, etc.). This is still an evolving area, but right now it looks very promising, and that's really where the equol hypothesis came from. If you could enhance the efficiency of converting daidzein to equol, and so far that hasn't proved to be a very easy thing to do (meaning can you change a person's diet or would they consume probiotics, etc.?). It really points to the fact that if you could have sustained relatively high equol levels, there could be health benefits associated with that.

JB: That's very, very interesting. People might have jumped to the conclusion of, "Wow, I'll just give more good bugs by probiotic supplementation and that will improve the conversion of things like daidzein into equol and get them to the proper blood levels." But what you're saying is that—at least to date—oral supplementation with favorable symbiotic organisms hasn't resulted in a demonstrable increase in equol production. I think that's what I'm taking away from your comments.

EL: Yes, that's correct. There's a lot of research done in that area, and so far in the literature I don't think there has been a really good application to increase the efficiency of converting daidzein to equol by probiotics or even consuming combinations of foods, short of fermenting your food. And for Western cultures, that's a very difficult thing to accept.

JB: That might suggest that if people were consuming fermented soy, which I know in Asian cultures is one of their ways of actually consuming the product, that you would get some equol that is a byproduct of the fermentation process that you would not get if you ate the soy without fermentation. Is that the appropriate take away?

EL: I think that's correct. The fermentation process breaks down the molecule and converts it so there are fewer steps that the intestinal bacteria have to make in order to get to the equol molecule.

JB: That then raises the question: "Well, it sounds like we ought to be giving equol as a supplement for those individuals that are low equol producers, who are below the 20 nanograms per mL threshold." What's the regulatory status of equol? Is it a dietary ingredient that is considered, under DSHEA, unacceptable, or is it an NDI, or what is its regulatory status?

The Regulatory Status of Equol: Recent Discovery Complicates Things

EL: That's a great question. The status is…I can give you a really simple answer and that is I don't know because in 2009 equol was actually discovered in white cabbage and that's the first report where equol was discovered in a food product, and until that point it was thought that all equol had to be converted by intestinal bacteria. But in 2009 researchers were studying antioxidants, and they happened to select equol as one of the biomarkers in white cabbage. It's a very good antioxidant and it's very stable over 9 months of storage. So I can't say one way or another what the regulatory status is because now equol has been discovered in a food product and that has usually been a hallmark for the regulatory perspective, so I'm not sure how it would be evaluated to date.

JB: That's very interesting. Let's go back to the clinical effects, which I know for many of our listeners is really very, very important information. We've talked about prostate health. We've talked, indirectly, about breast health. You alluded a little bit to the osteoporosis effect (anti-osteoporotic effect) as a consequence undoubtedly of the favorable effect on ER-beta. And you've also talked about the effects in the skin in relationship to wound healing and wrinkling and so forth. In your background we know that you have a deep kind of both training and research experience in the neurology/neuroscience area. Tell us a little bit about what you have learned with regard to equol and neuroscience.

Equol and Neuroscience

EL: Yes, we just had a recent paper come out on soy diets and supplementing with equol in relationship to depression in animal models. We examined the rat model that uses the Porsolt forced swim test as an indicator (an index) of depression.[8] We found that when we supplemented animals that have naturally gone through ovarian failure (in rats, that takes place about 300 days of age), and then if we supplement equol on animals that are consuming a soy-free diet, we could actually enhance serotonin levels and improve the performance in this Porsolt forced swim test that is an indicator of depression in this animal model. And so, again, the concept isequol is binding estrogen receptor beta and it's very important in different brain sites for depression, but due to its polyphenolic biochemistry and ability to bind estrogen receptor beta, it also improves (possibly) the transport and the synthesis of serotonin, which is a very important neurotransmitter associated with depression. And so, at least in this animal model for this particular hormonal status of natural ovarian failure, it seems to be a very promising application for brain health.

JB: You know, it's very interesting because epidemiologically there is association in the literature between soy in postmenopausal or peri-menopausal women and the reduction of dysphoria and the depression associated with estrogen loss with ovarian loss of function.[9] It seems like what you've observed in the animals at least tracks with what has been observed epidemiologically with females.

EL: Yes, and not only in that area, but when we were talking about prostate health, an Asian study supplemented men with high PSA levels with soy, and when they looked at their 5-alpha-DHT levels (and this was with high supplementation), their 5-alpha-DHT level actually decreased by about…I'd say 10 to 20% with a corresponding positive decrease in PSA levels and improvement in BPH symptoms.[10] I believe these different tissue sites and applications throughout the body due to the antioxidant properties--binding specifically 5-alpha-DHT and then having two isomers that are biologically active—is really unique.

JB: In the paper in Neuroscience in 2011 looking at neuromodulation by equol, you also have another interesting observation that relates to its antiobesity effects. Could you tell us a little bit about how that connection is made through equol? I think it is fascinating.

EL: Yes, that connection, again, is made through the impact of estrogen homorne action. If you were to take a rat model and take away all the estrogen, the animal gets obese. If you give back estrogen, then you can control that obesity. When women experience menopause, one of the common challenges is that they gain weight, along with increased challenges in skin health and wrinkle formation, etc., because of the loss of estrogen. But if you look at the receptor subtypes (alpha and beta), alpha seems to be the important estrogen receptor in fat as far as the major modulator or control factor. However, when they knocked out estrogen receptor alpha in animal models, and then they gave estrogen to induced obese animals, they also lost weight. So the concept was, even though estrogen receptor alpha is the major receptor for

controlling fat deposition, the ERbeta component is also very important, and it is not only for the fat tissue site, but it is also for the hypothalamic influence of having molecules that would regulate metabolic hormones like thyroid. In other studies we've seen that thyroid slightly increases, associated with a decrease in body weight and also, with the hypothalmic connection, there seems to be an increase in activity, meaning the animals move more (there is more local motor activity). As we've talked about before, there are multiple actions to equol in binding ER-beta, having influences at the hypothalamic level, and at metabolic hormone levels, too.

JB: The more we talk, Dr. Lephart, the more we see this pleitropic effect of equol being quite amazing. I think you've touched on something, there, that I know our listeners want me to ask about, and that is if we're talking hypothalamic effects (central effects in the nervous system), then would that influence the hypothalmic-pituitary-adrenal thyroid axis. In other words, would it have an effect on stress response in animals as kind of a modulator of stress? Is there any evidence that it has a regulatory effect on stress modulation

EL: We haven't examined equol specifically in the pituitary-adrenal-stress axis, so I can't say for sure. What I could maybe speculate on is because of the influence on serotonin in the pilot studies that have been conducted people have reported that they seem to handle stress better, and not necessarily through the hypothalamic-pituitary-adrenal stress axis, but I think because of the overriding influence of increasing serotonin and activating estrogen receptor beta in various parts of the brain actually would be a stress modulator.

JB: Very, very interesting. The other interesting thing you kind of touched on is that often you hear people talk about the fact that soy is anti-thyroidal;(it's a thyroid antagonist). But you've just suggested at least one of the byproducts of soy metabolism, equol, might actually be a thyroid modulator in a positive way. It seems very interesting and almost paradoxical to some of the reputation that soy has.

Soy Allergies

EL: That's correct. You have to think about the soy allergies that are probably connected with the carbohydrate portion of soy, the protein portion, and then the lipid portion. When we talk about equol we're just talking about one isoflavonoid molecule compared to the total global soy concept of having hundreds, if not thousands, of molecules and all their interactions. And so far, when we've examined equol, the enhancement is either equal to or better than what we've seen with soy supplementation.

JB: Oh, very interesting. Thank you so much for your gracious sharing of this very, very important information. As you undoubtedly know, soy has been through an interesting consumer perception cycle, not uncommon in the nutrition area where things wax and wane and the pendulum swings back and forth. We went into a period of probably about 30 years when soy was almost considered anti-American because it was in things that Asian cultures ate (it was kind of xenophobic a little bit when I first started in the field of nutrition in the late 60s/early 70s). Then it went into favor and we saw soy become considered a very important part of the diet to regulate all sorts of functions and in fact even an NLEA (National Food Labeling and Education Act) claim was allowed for soy. And now the pendulum has started to swing back and people are now villifying soy, saying that it has all these dangerous and untoward effects of stimulating cancer, and causing pancreatic enlargement, and the list goes on and on. From your perspective as a research leader, do you have any sense as to how we put this in balance (when patients come in and want to know the soy story)?

EL: I think the media does a great job, but in science they fall short. I know they try very, very hard to do a good job, but part of the issue is—and it is also in the scientific literature—when researchers mix and match animal data with human data.I In some cases they go together, and in some cases they don't go together. For example, the levels that are used in some of the animal studies are incredibly high. It's difficult for someone in the general public to sort all this out. I believe that if someone is knowlegable, and I'll use individuals that are pure vegetarians (they are really sharp about health in general, and nutrition, and biochemical properties of different foods, and antioxidants, etc.), it takes a level of investment on the part of the media that is telling the story or the individial that is in the public to try to sort this out, and unfortunately I think it gets mixed up. If you look at the animal studies and the potential negative aspects you could look at the administration and especially the levels that they are using. My kind of evolution in this particular field is, "We're going to use the animal model as a starting point—as a screening tool—and then as we transition to the human study, whether it is negative or positive data, does it translate to humans?" If it does, the story is much easier to tell. But in many cases, the negative data has been reported in animals studies, whether it is for thyroid or for other topics, and if you translate that into the human model, that transition doesn't hold. It makes it much more difficult on the general public to sometimes make sense out of this.

JB: I'm sure you've probably had this kind of conversation offline as well. People will say, "Okay, well given all of this—this is really high-falutin' science—what do you do? Do you consume soy yourself?" Is it something that you or your family eat, if someone asks you? Because that's a where-the-tire-meets-the-road kind of question. I think we each have our own opinions as to how we translate the science into our own daily activities. What kind of recommendations do you have about soy as a part of the standard diet?

EL: From all the literature, and especially from the recent reviews, in general, unless someone has a soy allergy, or they have just an unusual medical history for cancer or for some other really novel aspect of their health, in general I believe that soy is safe and that all of the studies we've done using a very low effective dose of equol in our animal studies and also in our pilot human clinical studies, I believe that in general for a very broad population covering human health that it is safe.

JB: I think you've touched upon so many interesting areas. We use the word "pleiotropic" which means "multiple effects." You've covered, in this short discussion with me, prostate, and breast, and skin, and obesity, and its effect on brain and neuroscience as it relates to depression, and osteoporosis. These are a fascinating array of web-like effects that are connected with the cellular signaling associated with equol, and how it influences gene expression, and things that you have been researching and publishing papers on for some time. It is certainly a fascinating story. If you think that we eat a food that has a precursor in it that then is further consumed by bugs in our gut into a secondary metabolite from those bugs, and it is then absorbed and has this really remarkable effect of normalizing functions across this wide range of tissue types. There is something about an ecological model of nutrition, about a web-like physiology, about our kind of deep interconnection through cell signaling with our plant world that is quite fascinating. It is really an amazing chapter in our evolving understanding of where health and disease comes from and how it relates to our diet.

EL: I agree with you. And because it's so abundant it is really almost impossible for those individuals that may have concerns because exposed to these molecules all the time. It might be at low levels, but they are in corn and wheat, and in many, many plant products and so it is very difficult to try to exclude these molecules (if not impossible) in your dietary consumption. With that perspective, we are always being exposed to these molecules, and in the right dose or right levels, and with correct knowledge, they could have health benefits, but I think it is going to take a little time to tell the story where people can

grasp on to the difficult, multi-faceted action for molecules like equol.

JB: Personally, I'm pretty persuaded I'd like to be on the threshold of my serum levels of 20 nanograms per mL. Your work looks pretty convincing to me. I want to thank you so much. This has been a very, very fascinating discussion and one that I think opens up all sorts of further chapters of review as we move down the road. Keep up the great work and thank you for sharing this with us.

EL: Thank you for having me on.

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