June 2008 Issue | Michael Fenech, PhD Theme Director, Food and Nutrition Food Science Australia

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Welcome to *Functional Medicine Update* for June of 2008. In the last few issues, we have talked about ways that one can validate the safety and efficacy of interventions. The gold standard has traditionally been the randomized, double-blind, placebo-controlled trial. We have talked about the strengths and limitations of that type of methodology when you are looking at network or systems biology interaction, because the placebo-controlled trial is very suited for single-agent-against-single-outcome types of studies. When you are doing studies that relate to polygenomic complexity (with multiple interventions looking at multiple outcome variables), the randomized, double-blind, placebo-controlled trial becomes less useful in terms of defining the effectiveness of a hypothesis, or the outcome of a hypothesis, or the safety and efficacy of an intervention.

A paper that demonstrates these limitations appeared in the *British Medical Journal* and was titled "Parachute Use to Prevent Death and Major Trauma Related to Gravitational Challenge: A Systematic Review of Randomized Controlled Trials."[1] This is by Gordon CS Smith and Jill Pell from the department of obstetrics and gynecology at Cambridge University and the Department of Public Health at Greater Glasgow (the institution of the National Health Service in Scotland) who I think did a marvelous job. In this article, the authors talk about how one would prove the efficacy and safety of the parachute under randomized controlled trial procedures. The conclusion of this study (which I think you will find fascinating) says the following: "As with many interventions intended to prevent ill health, the effectiveness of parachutes has not been subjected to rigorous evaluation by using randomized controlled trials. Advocates of evidence-based medicine have criticized the adoption of interventions evaluated by only using observational data. We think that everyone might benefit if the most radical protagonists of evidence-based medicine organized and participated in a double-blind, randomized, placebo controlled crossover trial of the parachute."

I think that sets the tone for this month's *Functional Medicine Update*. When we deal with systems biology-related issues, which are the focus of functional medicine, we are dealing with networks rather than pathways. We are dealing with interactions of complex, dendritic relationships (feedback pathways rather than single throughput steps that relate to A going to B), where it isn't very amenable to a randomized, placebo-controlled intervention trial system. We are also looking at human individuals over many years of living, which have complex lives and very diverse genotypes. All of these variables play a role in modulating and modifying the outcome within a population-based group of what you might call safety and efficacy of any therapeutic. And when we are dealing with a functional medicine systems biology approach, we are generally dealing with multiple things going on simultaneously, not just holding

all variables constant and modifying one thing, like the addition of one molecule (one new-to-nature-molecule).

With the complexity of this kind of thinking, what is the better methodology? We have been talking about the development of this new way of treating data that is related complexity, which is complexity science; it is related to network thinking; it is related to nearest neighbor analysis, cluster analysis, artificial intelligence, non-parametric solutions that relate to polynomial types of polyvariant rather than univariant type of statistics, and looking at things in a nonsymmetrical universe. Not everything is Gaussian (mid-line, symmetrical, about the average point). With all of the realities of the world in which we are living today and how those realities map against chronic age-related diseases, it is forcing us into different ways of thinking. Dr. James Wright, in his extraordinary interview in the April issue of *Functional Medicine Update*, used the example of statins as agents for primary prevention of cardiovascular disease. We learned that the outcome is not so easy to evaluate when we start looking at number to treat and actual cost-effectiveness of use of statin drugs in the primary prevention of cardiovascular disease.

Recently, a very interesting article titled "Future of Lucrative Cholesterol Drugs Murky," appeared.[2] This article says that physicians "are waiting for new preventive heart medicines beyond popular statin therapies, but a tough regulatory climate and fierce debate over the effectiveness of some of these newer drugs has clouded the future of cholesterol treatments. The uncertainty is roiling investors looking to cash in on what has traditionally been the most lucrative arena of the pharmaceutical sector. Sudden plunges in sales of newer cholesterol drugs, that had been expected to grow strongly for years, and unexpected US Food and Drug Administration rejections or delays of medicine have sparked deep declines in share prices. 'Today investors are probably feeling like a successful investment in a new lipid franchise is something that is going to take a long time to materialize,' said Leerink Swann, an analyst looking at the pharmaceutical industry. As you are probably aware, the Food and Drug Administration has received intense criticism over withdrawn drugs such as Merck's painkiller Vioxx and GlaxoSmithKline's diabetes PPAR-gamma agonist Avandia.

There is now debate over extensive use of cholesterol medicines such as Vytorin. That drug, sold in a joint venture between Merck and Schering-Plough, is facing plunging sales following publication of a failed study. Patients are now questioning whether these medicines are really safe and effective. Although heart disease remains the number one killer in the United States and many patients need something in addition to statins to get their cholesterol to target levels, we are asking about the real return on investment for a person taking statins in primary prevention. Are there subtypes of individuals who are either genotypically or phenotypically more sensitive and responsive to statins than others? Would some people be better candidates who we would target in a personalized medicine approach? Should we target those people who would best respond, rather than using a drug with a group and looking at the average-a blockbuster mentality, "one-size-fits-all"- type of medication?

All of these questions are really starting to come to the surface more and more frequently. Also related to this issue is the recent removal (or denial for acceptance) of the drug Torcetrapib by Pfizer, which was reputed to be a new HDL-elevating drug that would be added to statins to extend their therapeutic benefit. Studies found that although Torcetrapib did increase HDL, it also increased HDL of an inappropriate type. The drug didn't lead to reduced incidence of heart attack, but actually increased cardiovascular accidents.

Treating the physiology is what is important, not just treating a number. Of course, that is what we have been saying in functional medicine for nearly two decades: one needs to look at the functional outcomes that relate to any intervention and ask the question, do these functional outcomes relate to normalization of the physiological, physical, mental, emotional outcome of that patient and their performance level?

As we move from blockbuster medicines to personalized medicine, this is becoming a theme we are seeing more and more frequently in the literature. An article just appeared in a new journal called *Personalized Medicine* that shows, once again, how this field is moving forward and is evolving very rapidly. This particular article that appeared in volume 1 of the new journal was titled "From Blockbuster Medicine to Personalized Medicine" and went on to say that the biggest challenges of the pharmaceutical companies in the 21st century will be to develop and deliver drugs that fit the individual patient's biology and pathophysiology.[3] This is what Roger Williams called "Biochemical Individuality" back in the 1940s. This change from blockbuster medicine to personalized medicine, the article says, "will, to a large extent, influence the way that drugs are going to be developed, marketed, and prescribed in the future. These changes can mean an end to the blockbuster philosophy and Big Pharma, and thereby impose major changes in company structures. The implementation of personalized medicine will be a step-wise process where the division of patients into biological subgroups will be the first important step." I might say (parenthetically) that this has been the focus of the Institute for Functional Medicine and functional medicine for many years: trying to define the processes that relate to the underlying pathophysiologies and looking at the origins (rather than just the name) of the condition and its outcome as a pathophysiology.

Going back to the article, the authors say "today this is already the situation for several cancer diseases, for example, breast cancer. In years to come we may see more and more drugs being prescribed based on the results from pharmacodiagnostic testing." (That is looking at the genetics of the individual and how they respond to a specific therapeutic agent.) "Within cancer medicine, which has been at the forefront of this field, it is expected that in 10-15 years time very few drugs will be prescribed without such a test," (pharmacodynamics, pharmacogenetics, and pharmacodiagnostics to type the individual to their medication).

Now let me move away from this article to talk about the model that we have been describing and we are going to talk more about in this issue of *Functional Medicine Update*. We talked about the fact that in the laboratory of nature-- this large experiment that has been going on for hundreds of millennia called natural selection--that we are starting to recognize that there are certain molecules that have emerged in our food supply and in our life in such a way as to speak to our genes and modulate both epigenetic and genetic expression. As such, these may be molecules that are well suited for normalization of function in people who have distorted physiological networks in their physiological function (such that any distortion at a point on the net then distorts the whole of the net).

Pathways are really just abstracted snapshots in the web of life. With a systems biology approach to that network, we see it is composed of pathways that are all interacting in real time, and differentiate it from cell type to tissue type based upon the functional characteristics of that tissue or organ. These molecules in nature, the things that have been in our food supply system historically, have a relationship that has evolved with our signaling processes (the so-called intercellular signal transduction) to induce and to modulate certain cellular outcomes that lead to normalization of the web. As such, we call these hormetic effects, meaning small amounts of substances may have larger than expected effects on the system. And

that hormetic effect-because these substances in our food are not natural to our human body (they are produced by plants, not by humans-they are what we would call "foreigners")-we call xenohormesis. Xenohormesis means foreign molecules from plants (produced through the biosynthetic machinery of plants in response to their environment) that are then consumed by humans in complex diets and have a hormetic effect upon the regulatory nodes in our network of physiology. This hormetic effect leads to stability, or to organ reserve (to go back to a term we have talked about since 1980, when Dr. James Fries authored his classic paper in *The New England Journal of Medicine* on aging, natural death, and the compression of morbidity).

The concept that we have certain molecules in our foods that have come up through natural selection and are smooth in the way they respond through signaling agents to induce-at the epigenetic or genetic expression level-influences on our network physiology to normalize function as xenohormetic agents is very powerful. I know I threw a lot of terms together in that sentence, and I may have lost some of you if you are not longtime listeners. This is a very, very new concept within cellular physiology, nutrition, and medicine and it is reframing our view of how nutrients might play a role in both prevention and management of chronic disease. This is very different than looking at new-to-nature molecules that block certain pathways, like an ACE inhibitor, or an SSRI, or an HMG-CoA-reductase inhibitor, or an H2 blocker. Here we are talking about agents that have evolved through time to modulate intercellular signal transduction at such a level as to lead to functional plasticity in the network, and more reserve capacity, and to help normalize the texture, composition, and shape of our complex web of physiology.

This model-this xenohormesis model-that I am describing was first discussed by David Sinclair at Harvard, and now is gaining much more traction as we see other people picking up on the theme and doing research. It was recently further described in a new article by Sinclair and Howitz in the journal *Cell*, a very premier journal in cellular biology. This article appeared in a May 2008 issue under the title "Xenohormesis: Sensing the Chemical Cues of Other Species," and the subject is how plant molecules interact with and modulate key regulators of mammalian physiology in ways that are beneficial to health.[4]

As described in this article, the proposal is that there are heterotrophs (animals and fungi) that are able to sense chemical cues synthesized by plants and other autotrophs in response to stress. The complex array of phytochemicals that are manufactured through the biosynthetic machinery of plants are made in response to stress in their environment, for which they produce their antistress compounds (like carotenoids and the whole family of flavonoids (including flavanols and anthocyanins), isoflavones, glucosinolates, and other polyphenols-the list goes on and on). Literally thousands of different phytochemicals are manufactured by plants specific to their species in response to stress in their environment.

If not refined away, phytochemicals (in plant foods) are consumed by humans (heterotrophs), which can then affect function and may provide more reserve to adversity in changing environmental conditions. This is the organ reserve concept so once again we come around full circle to James Fries (through the understanding of gene expression, epigenetics, and the role that xenohormetic substances might have on them). Moving away from just looking for more new-to-nature molecules that can block or inhibit specific downstream pathways in physiology is a profound new concept. Here we are looking at upstream regulators (what you might call the regulatory units) in the net of physiology that has to do with this hormetic effect (small amounts of substances having larger effects on physiology).

When we start looking at how this translates into clinical medicine, we have to recognize that there are many, many different functional regulatory systems in the body that can express dysfunction over time and that we later call a disease. In *Functional Medicine Update*, we have been focusing on things like the stress response in humans and the hypothalamus/pituitary/adrenal/thyroid axis and how that translates into a population of people who are stressed from environmental changes, time compression, less-than-high-quality diets, and chemicalization. All of these are things manifest through what Hans Selye called stress factors. They induce alteration in the network plasticity and network function to be then seen as a whole complex array of disorders: cardiovascular disease, hypertension, increased risk to cancer, musculoskeletal problems, digestive disorders, non-ulcer dyspepsia-the list goes on and on as it relates outcomes from these changes in the network of life

Recently I put together a short presentation on this. It is available through Synthesis by Jeffrey Bland and is titled "Managing Adrenal and Thyroid Balance Associated with Stress." I think this is a very nice review of this whole topic. It is a combination audio and visual program that reviews this complex clinical topic quite nicely for those of you who are looking to learn how this model I am describing (the functional medicine model) fits into both the recognition and management of chronic stress. It also really ties together with another product that we have just released through www.jeffreybland.com, which is "The Emerging Therapeutic Target: Improving Therapeutic Outcomes by Treating the Intersection of Osteoporosis, Cardiovascular Disease, Type 2 Diabetes, Arthritis, and Cancer." This presentation also focuses on this intersection of mechanisms that relate to the outcome that we call disease at an earlier stage than waiting until histopathology is seen. Earlier intervention can lead to milder intervention and prevent the trajectory heading on towards the crisis disease (or the more acute disease). So these two products, "Managing Adrenal and Thyroid Balance Associated with Stress" and "The Emerging Therapeutic Target" are both a combination (audio plus visuals) products that provide an overview of these topics and give some news to use, clinically, in these two areas. If you are interested in finding out more about these, I refer you to our website, which is www.jeffreybland.com, or you can give us a call at 866-272-5789.

If we take this concept I am describing-this hormetic concept, and how substances in our diet or substances in our environment influence our function-then it leads to recognize that the translation of messages from the outside world into interior functional changes in the body has something to do with how our book of life is read. Our book of life is our genome-23 chapters, written by our biological parents-which then gets translated ultimately into expression patterns that leads to our phenotype: how we look, act, feel, and function. What questions would you ask clinically? What is it that communicates those messages and how do they get translated into function? How can we therapeutically harness this conceptual framework in such a way as to produce more effective outcome in patients with chronic illness instead of just blocking downstream pathways with inhibiting drugs to manage symptoms? The answers are fairly complex and require more than just a few minutes of explanation, but let's try to cut it down into individual piece parts that we can manage.

Let's first ask this question: what are we going to be measuring in order to understand where that patient is in the progression from optimal function to complete absence of function (which we call death)? This is where biomarkers become very important. Biomarkers are variables we can assess that give us some indication of the trajectory that person is traveling. The most classic example of a biomarker is blood pressure. We know that blood pressure, as it elevates, is associated with an increased incidence of cerebral vascular disease and coronary events and has detrimental effects on kidney function and vascular

function. We would say that elevated blood pressure is a biomarker for later stage, more serious illness.

Similarly, we might use a chemical biomarker, like total cholesterol or the cholesterol HDL ratio, as a way of assessing trajectory towards a relative risk to cardiovascular disease. We would not say the total-cholesterol-to-HDL ratio is a diagnostic criterion, but we would say that it is a prognostic evaluator, a biomarker of a trajectory towards the potential risk of vascular diseases. We might use something like an EKG (an electrocardiogram) to evaluate the functional integrity and organ reserve of the cardiovascular system under stress. Or we might use something like the power EEG (the electroencephalogram) to measure certain aspects of brain mapping and brain biochemistry, indirectly, by utilizing spectral analysis of the EEG. New technologies are being developed that allow for more precise understanding of regional aspects of metabolism in the brain and how that maps against regulatory features of things like brain inflammatory patterns, or ischemia in the brain, or altered neurochemistry of the brain. All of these measurements become functional biomarkers for evaluating trajectory towards more serious illness, and also become markers that can be used for tracking the success of intervention (by seeing normalization in these biomarkers, the assumption is that a patient is on a path towards improved outcome).

Let's look at something like metabolic syndrome (insulin resistance) as an example of a condition that is a syndrome before a disease. The concept of functional somatic syndromes and the preclinical stage of a disease is really where a lot of the action is. A metabolic syndrome/hyperinsulinemic patient may not yet have a diagnosed disease, but they have a risk to things like type 2 diabetes, cardiovascular disease, renal disease, stroke, certain forms of cancer (like breast, prostate, and colon cancer), or things like nonalcoholic steatohepatitis (NASH) with fatty liver infiltration if they don't do something about their insulin resistance. All of these are manifestations of metabolic syndrome progressing to clinical disease states.

How can we evaluate a patient's status before they have an onset of these more severe conditions that require more aggressive intervention? The answer comes down to things like the triglyceride-to-HDL ratio as a surrogate marker. We can also look at the apolipoprotein A and apolipoprotein B levels to see what relative risk is because we now know that an apo B-to-A1 ratio, when it gets above 0.6 to 0.8, is a ratio associated with increasing incidence of vascular disease associated with insulin resistance. The ratio of apo B to apo A1 (that number when you divide apo B by apo A1, that quotient) should be less than or equal to 0.8. As this number gets larger, it is associated with an increasing relative incidence of vascular disease associated with insulin resistance. We can look at the particle number and the particle size in the lipids in the blood, either by NMR or by densitometry; we can actually measure particle size and particle number. We know that dense LDLs, as they grow in number, are associated with a much higher atherogenicity even if there is a reasonably low total cholesterol, and even if LDL, in and of itself, is reasonably low. So we would look at atherogenicity of the particles by looking at their size and number.

All of these examples are relative biomarkers for evaluating the potential pathology downstream from insulin resistance. Once these markers are established, what is done to improve the function of the individual and improve their biomarker analysis? We talk about diet and lifestyle, of course (the so-called "first-line therapy" that the NIH talks about). Before you intervene with pharmacotherapy, the NIH guidelines say that we should first intervene for a period of three months with lifestyle and diet intervention to see if, in fact, we can normalize function before relegating the patient to pharmacotherapy.

What type of dietary interventions would we use? We would get away from refined carbohydrate. We

would get away from excessive lipid in the diet as saturated fat. We would lower total fat and increase the omega-3s and omega-9s within the diet. We would lower the amount of high fructose corn syrup sweeteners (in fact, I would say lower it to the point where it becomes an insignificant part of the total diet).

Fructose Consumption as a Biomarker for Nonalcoholic Fatty Liver Disease
It is more than just fructose in and of itself; it is a combination of fructose plus other ingredients that are found in high fructose corn syrup sweeteners that we are now seeing an increased intake of in the diet. There was an interesting paper just published in the *Journal of Hepatology* titled "Fructose Consumption as High Fructose Corn Syrup Sweetener is a Risk Factor for Nonalcoholic Fatty Liver Disease."[5] In this particular paper the authors looked at patients who had symptoms and signs and biomarkers associated with metabolic syndrome versus a control group. Then they looked at people with nonalcoholic fatty liver disease versus controls, and they found that those people who had NAFLD had a much higher intake of fructose coming from fructose corn syrup sweeteners. The amount they were consuming in their diet was about 100 grams per day (100 grams of fructose coming from high fructose corn syrup sweeteners). One hundred grams per day is a very, very high dose relative to what you would get from a normal diet as it relates to fructose. For instance, an apple has about 5 – 7 grams of fructose, so to give an apple equivalent, obviously to get 100 grams of fructose you would have to eat something like 20 apples. You could get 20 apples as apple juice, but it would be very difficult to get 20 apples worth of fructose eating whole apples. And, in fact, the apple also contains other agents that might help mollify or modify the way that fructose is actually metabolized. It is probably not even a very good example-comparing apples to high fructose corn syrup sweetener-because there you get the raw signal of fructose and its multimers versus the signal that you would get of fructose in apple or apple juice that is modified by many other phytochemicals.

In this particular study, the authors go on to talk about the pathogenic mechanism underlying the development of nonalcoholic fatty liver disease, and they say that it may be associated with this excessive consumption of dietary fructose, particularly in people with specific genotypes that have unique polymorphisms of the enzyme fructokinase. In this study, they actually looked at hepatic messenger RNA expression of fructokinase and asked how it relates to those individuals who have NAFLD who consume this high dose of fructose as corn syrup sweeteners everyday in their diet. What the investigators found was that there was a dose-dependent increase in the expression of fructokinase, a protein inactivity associated with this high level of fructose intake as corn syrup sweeteners, and that that tracked back to specific genotypes that had higher genetic susceptibility to fructokinase activity. Again, a complex interaction among diet (among altered modified diet) and genotypes to give rise to a risk factor in a trajectory towards a disease that we call nonalcoholic fatty liver disease. I think this is a very nice example of the complex interaction of genes and environment from a functional medicine/systems biology perspective versus just looking at the outcome of a disease called NASH.

The other thing I want to mention here is to make sure that we identify the difference between giving a highly chemicalized, purified form of high fructose corn syrup sweetener versus that of taking in fructose in a complex dietary array as a small constituent of the diet. We are going to get some monosaccharides and disaccharides in diets that are of natural origin. The question is, at what magnitude do we consume them? Do they elevate the risk factors of metabolic syndrome, and what are the other factors that come along with them in a complex diet that actually assist in their metabolism or in their regulation? That is where the complex diet story really becomes interesting from a systems biology perspective because the

more we abstract and isolate constituents out of the diet and separate them into what Roger Williams talked about as "partition foods," the more we get a different effect of that food than that in the natural state, where it was in a complex mixture in its natural origin.

If you use the example of apple juice versus the same amount of fructose that comes from high fructose corn syrup sweetener, apple juice contains (if it is a full, whole-pressed apple) all sorts of lignans and polyphenols and other kinds of phytochemicals that modulate the metabolism of the nutrients like fructose that are found within the apple itself. I think these are the kinds of thinking patterns that are leading us into a state of understanding, even as it relates to all the published studies on nutrition that talk about "this nutrient produces this problem." You have to say, "Well hold it. What was that nutrient delivered in? What matrix? Was that a purified chemicalized form of that nutrient that was then delivered in a very synthetic fashion into that individual animal's diet to evaluate its outcome, or was it that same nutrient, in a complex whole form, at a modest level of intake, that then resulted in a different effect on its outcome?"

With all of that in mind, let's move this discussion to a specific clinical takeaway. I would like to move this into the molecular origins of cancer. Cancer comes about as a consequence of several different steps: initiation, progression or proliferation, metastasis, and then lastly angiogenesis, which is part of the metastatic process. What we recognize is that a cancer cell is a cell that has undergone dedifferentiation. It has become a juvenile embryonic-like cell that multiplies rapidly (an unregulated cell growth). It develops its own blood supply (angiogenesis) when the tumor cell mass gets to a certain size (about 3 millimeters). And then it starts to be able to spin off its brethren (which we call metastasis, where it can travel to distant sites and initiate growth elsewhere). This whole process is what we then term as the "cancer process."

How does the body manage to transform cells that might be developed over the course of living? It has an immune system (a recognition system) that tries to understand the presence of these foreign cells that have undergone transformation and excise them from the body prior to them getting a foothold and getting to a stage where they can actually undergo angiogenesis and metastasis. This has to do with the upregulation within these transformed cells with certain function expression patterns that relate to the caspase genes (the death genes) that cause apoptosis, or cellular suicide. A transformed cell is recognized within an immune system that is functioning optimally as a "funny" cell, as a cell that really should have no business there, and regulating its function through this natural excision process that we call the apoptotic process. When you get multiple hits, however, and you get accelerated initiation and you get processes that are involved with stimulating propagation, and you get regulatory factors in gene expression that relate to the ability for that mass to undergo angiogenesis, now you start getting double or triple hits on the system, and now the body's regulatory mechanisms may not be able to keep up or compensate for this initiation process and this whole way that tumors ultimately develop into a clinical cancer.

It all starts at a molecular stage, doesn't it? Over the past decade, insights into the origins and behavior of human cancers have reshaped our understanding of these diseases. We now know that cancer is not singular, it is plural (cancers), and each cancer may have a different fingerprint based upon its genetic mutation that it has undergone within the book of life of that specific cell. And that specific cell's regulatory change through a mutation either at the epigenetic or the genetic level can then result in certain dedifferentiated cell regulatory processes being obviated or exhibited, which then we ultimately see over

time developing into a diagnosed cancer. What controls these personality characteristics of cells? In part these are controlled epigenetically ("epi" meaning "above the gene"), so not only are we talking about initiation being caused by mutation of a gene (like an oncogene that has undergone a mutation to kind of ignite its proliferation), but we also talk about the alteration of the epigenetic messages that sit on the regulatory regions of genes that actually control their function (like the stop-function gene regulators that we call methylated promoter regions). Undermethylation can increase the relative risk to carcinogen induction or initiation of tumors. Let me say it again: undermethylation of the promoter regions of specific genes (that is the silencing messages of genes) can lead then to the promotion of these processes.

What we are going to learn from our clinician/researcher of the month is that one of the ways that you can actually examine this morphologically is by looking at the genome under the microscope for what is called genomic instability. Genomic instability is not just connected to the potential risk to cancer, but also the potential risk to virtually every age-related chronic disease. In fact, in *The New England Journal of Medicine* in January 2008, there was an interesting paper that talked about the molecular origins of cancer and looking at the genotype of the individual (as to their cancer susceptibility), looking at the genotype of cells that have undergone transformation (which are upregulated to cancer cells), and also looking at epigenetic dysregulation (where you have altered promoter region methylation or acetylation patterns that lead and result ultimately to higher expression of cancer).[6]

At one point, there was an interesting paper published in *Advanced Cancer Research* called "Epigenetic Theories of Cancer Initiation."[7] This article talked about hypomethylation and a methyl-deficient diet being a potential higher-initiation-to-cancer-type of regime because now that individual's genome is more susceptible to carcinogenic injury and initiating tumors. If we start looking at a whole organism, are there ways of assessing the relative risk to cancer? At a functional level, are there ways of assessing the risk to heart disease or to autoimmune disease? This concept of genomic instability-our book of life, the most sacred thing that we should protect--becomes a very interesting focus as to how we might understand (early, before the appearance of these diseases) whether we are on the trajectory (and it may precede the onset of a disease by decades by recognizing an individual may have this genomic instability).

Once you understand it, what do you do about it? Are there any ways of averting or changing your book of life from this unstable kind of state of injury into a protected, highly controlled, genomic regulatory region that then helps to prevent the environmental perturbation and these changes that ultimately lead to chronic disease? What you will learn from our clinician/researcher of the month is that, yes, the research indicates that here is where diets and specific nutrients may play a role in modulating genomic stability by helping to protect and to become able to express messages under very controlled conditions (one message at a time), rather than being susceptible to injury by mutagens or carcinogens or radiation, which then induces in them certain kinds of changes that ultimately lead to increasing risk to disease. This genomic instability is related not just to the exposure to outside caustic agents, like radiation and chemicals, but also internal agents that help to protect the genome, which are nutrient-derived, like methylation patterns that lead to methylation of the promoter regions, and silence specific genes that we don't want to be over spoken, like oncogenes. This is a very dramatic step forward in our understanding, at an early stage, the functional changes that may ultimately relate to the appearance of age-related chronic diseases. When I say age-related, these agents can be in youth; they don't have to be just in aged adults. It depends upon the relative susceptibility and exposure to the offending agents.

What we are going to hear in this interview is how nutrients may actually help to counteract

environmental carcinogen exposure, and how DNA hypomethylation increases the relative risk. I'm now quoting from a wonderful paper that Randy Jirtle and his colleagues from the Department of Radiation Oncology and the program in genetics and genomics at Duke University recently published. This article was in the *Proceedings of the National Academy of Sciences,* and these researchers showed that maternal nutrient supplementation in animals was capable of counteracting a carcinogen-induced DNA hypomethylation that led to very serious risk to cancer in these animals.[8] By giving folate and B12 and other methylating nutrients, they were able to actually lower the relative risk to this carcinogenic chemical producing adverse effects. So here is diet neutralizing an adverse effect from the environment by protecting the epigenome. It is a very, very powerful new concept and this relates to what you are going to hear from our researcher of the month.

INTERVIEW TRANSCRIPT

Clinician/Researcher of the Month
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Once again we are at that place in Functional Medicine Update that we all look forward to, which is our clinician/researcher of the month. Over the 27 years I have been doing this, I have had the honor to talk with many of the world's leaders who are really carving out this paradigm-this frontier-that we are calling nutrition in the 21st century, or nutritional medicine, or maybe even health care in the 21st century.

I am very privileged this month to interview Dr. Michael Fenech, who is a geneticist by background. He has a PhD in genetic toxicology from Flinders University of South Australia, with his thesis being about genetic damage in human lymphocytes assayed by a micronucleus technique. From there, Dr. Fenech became a senior principal research scientist at CSIRO Human Nutrition, Adelaide, and a visiting fellow at the Centre for Mental Health Research at the Australian National University in Canberra. He is a highly published and prolific scientist in the area of genomic stability/instability and its relationship to environment and nutrition. Many of you might be saying, "I'm not exactly sure what that means." By the end of this interview, I can assure you that you are going to know a lot more about this than you did at the beginning. You are going to hear from someone I consider to be one of the world's leading experts in this area.

Dr. Fenech, thank you so much for joining us from all the way in Australia for Functional Medicine Update. I think maybe we'll just start by asking how you got in the field of genomic stability/instability? Obviously your thesis (back in your PhD days) was down this path, but you must have been led into it with some interesting insight.

MF: Although I ended up working in the genomic instability area, I was always intending to be a marine biologist. What really happened was an opportunity came up to do a PhD in Australia with Professor Alexander Morley at Flinders University. I grew up in Malta, which is a tiny island in the Mediterranean,

and the opportunities for research were limited there, but I managed to get a scholarship to come and study in Australia. The opportunity that was presented to me was to work with Professor Alexander Morley. He is a hematologist, and he had an interest in the genetic basis or the origin of hematopoietic cancers. At that time, the mutation theory of cancer was a hot topic of interest. In Dr. Morley's laboratory a variety of methods were being examined to study mutation in human blood cells, specifically the lymphocyte. My interest was also stimulated from the fact that I had been working for some years as a hospital scientist in hematology laboratories and that (together with my curiosity about genetics) led me to come to this laboratory, which is really where I did my PhD.

JB: Well, I think the field is very fortunate that you were led in that direction. One of your more recent publications that I had the privilege of reading was a 2007 publication in Food and Chemical Toxicology titled "Genome, Health, Nutrigenomics, and Nutigenetics: Diagnosis and Nutritional Treatment of Genome Damage on an Individual Basis."9 I shared that article with a number of the scientists in our group and they have all commented that they think this is high scholarship and a wonderfully written article that provokes all sorts of questions. Can you tell us a little bit about genomic stability and instability? What does this mean?

Explanation of Genomic Instability

MF: Okay. So going back to those early days when I was doing my PhD, the interest then was to look at environmental functions, both chemical and physical. The technique I was working on at the time was the micronucleus assay. This assay is really about a biomarker that tells you whether chromosomes are broken, or being lost, or not being properly segregated between daughter cells when they divide. In fact, hematologists have known about this particular biomarker for many years; it is known as a Howell-Jolly body in erythrocytes. These small nuclei, or micro nuclei, or Howell-Jolly bodies, as they are known to hematologists, increase when the organism (when the bone marrow) is exposed to either radiation of genotoxic chemicals, as well as due to deficiencies in nutrients required for DNA synthesis and repair such as folate and B12. I suppose it is from those early days that I was starting to get ideas in my head that maybe nutrition can be as important as environmental toxins, to measure in terms of the damage to the DNA or genome that may appear.

JB: If we consider our book of life to be the genome, which needs to be protected both for our own integrity of mitosis as well as for our future generations, then this genomic instability, as you are implying, obviously has some fairly significant influences not only on genetics and future progeny, but also on individual cellular function and physiology of the host, I would presume?

MF: Yes, exactly. I think that is a very critical point that you have mentioned. In reality, we know that only a small proportion of embryos actually succeed in developing into a fetus and into a healthy baby. Those of us who are here and living are actually quite lucky we were selected in utero to begin to be viable. That privilege really comes from the fact that the genome we were lucky enough to acquire from our parents happened to be a viable genome. The problem is, of course, that we start life as just one cell, and from that cell, millions and millions of others have to be copied and produced to create the organism, the child, the adolescent, and eventually the adult that defines us. I usually give the analogy of a photocopier: the more copies you make of the same original (making a copy of a copy of a copy), the less accurate is the image that we get with the more copies that are made. Furthermore, the quality of the copy depends on the toner and the photocopier. I consider the toner to be, perhaps, the nutrients required to make good copies of DNA in the cell. That is just my analogy.

JB: I think that is a wonderful way of looking at it. This photocopying goes on everyday in our cells that are turning over during the course of our life, and also those copies (through developmental biology after fertilization of the egg) start going through this epigenetic development process, so you've got two levels upon which genomic stability or instability can play a role in the phenotype, it seems.

Contributors to DNA Damage

MF: Yes, that's right. There are really many ways that the genome can be harmed, both at the base sequence level (that's the sequence of the nucleotides in the DNA), as well as with gross chromosomal changes in the genome. These are usually due to breaks in the DNA and misrepair of those breaks so that different fragments of chromosomes get located in with other chromosomes, or a change in chromosome number, which again alters the gene dosage, and therefore ultimately the phenotype of the cell.

When I give my presentations, I often show results, for example, of measurements that we can do with molecular probes, where we can identify the proportional cells, for example, which are triploid 4/chromosome 21, which is the Down's syndrome genotype. People are often startled by the fact that each and every one of us actually has these types of cells in the body. Furthermore, we know that the frequency of these cells appearing in the body increases with age and also with folate deficiency. And all of this is really important to drive home the point that nutrition and aging are key factors that affect genome stability and the normal genome compliment of the cells.

I think the more interesting aspect of this research is that we are finding that while ionizing radiation, of course, is a really important concern and a contributor to genome damage, also of concern is ultraviolet light and exposure to toxic chemicals such as mercury, which is of concern these days through its accumulation through consumption of fish and by other environmental routes. Surprisingly, what we find is that the impact of moderate deficiencies in micronutrients such as folate, which we have studied extensively, are as important as those induced by significant doses of these environmental carcinogens. These would be doses that would be considered unsafe. I think that is really the key point: that environmental exposures, while they are important, are not necessarily more important than the DNA damage that we can induce by dietary imbalance or deficiencies, as well as by other factors in lifestyle, such as alcohol consumption, which, again, is another important contributor to the DNA damage that we observed in people.

JB: I would like to just make sure that our listeners picked up a very important point that you stated, which I think amplifies some of the things that we all learned about the role of nutrients such as folate and B12 and the hematopoietic system. We often read in textbooks how you assess nutritional deficiencies, thinking maybe that there is just one cell type. So we think, "Gee, I'll look under the microscope at the blood and that is the only cell type that is going to be adversely influenced by this agent." We put a nutritional deficiency disease like pernicious anemia, or macrocytic anemia, with a specific nutrient deficiency, and then we capture that in our mind as if that is the only effect. What you really said to us, I think is very important: that's a marker cell type that is turning over from the bone marrow fairly rapidly, but it is not the only cell type influenced by these insufficiencies.

Measuring DNA Damage in Lymphocytes

MF: No, not at all. In fact, you have probably noticed from our literature that most of the work we have done relates to the lymphocyte, and more recently and increasingly, to the epithelial cell, which is easily accessible and therefore practical to use. The reason for looking at lymphocytes (apart from the fact that it

is a very practical system to use) is because the lymphocytes can be easily isolated and stimulated to divide in culture, and therefore to express the DNA damage. The other key reason is, of course, the lymphocyte is a very important component of the immune response system. We know, in fact, that increased damage to the DNA and lymphocytes also correlates with a reduced capacity of these cells to function as immune cells and to increase the numbers. It is for these reasons, as well, that we specifically examine lymphocytes.

Furthermore, we know that a depressed immune function is also an important component of the risk factors that relate to cancer. A lot of the work that we have been doing is related to understanding the nutritional requirements to maintain a healthy genome in lymphocytes using the micronucleus assay, as I mentioned before, and more recently, expanding this assay, which is actually known as the cytokinesis block micronucleus assay. We block cells in the binucleated stage (after they have completed nuclear division) because it is at this stage that you can observe the DNA damage accurately. With this procedure, apart from looking at the fragments or whole chromosomes that are left outside the main nuclei (and, hence, the term "micronuclei"), we also can measure bridges between the daughter nuclei, which is an indication of abnormal chromosomes with more than one centromere being pulled to the poles, as well as nuclear buds, which are these protrusions from the main nuclei, and are an indication of genome instability because this is the mechanism by which the nucleus attempts to eliminate amplified DNA, which is a hallmark of genomic instability.

By genomic instability, we really mean chromosomal and molecular events that are causing a continuous and major change in the genome of that particular cell. This hallmark of genomic instability is exactly what we see in cancer cells, and we can measure it directly in human lymphocytes.

JB: You just stated so many extraordinary things. Talk about densely packed information and content-rich words. That was very, very strong. Let me pick up on a few things and just to go back over them for emphasis. The first is the relationship between cellular function and genomic instability. I think for many people who have been trained for the pathomnemonic view (or let's call it the histopathological view of medicine), they see disease often being as a "switch," kind of either on or off. We either have something or we don't, and this concept of degrading function over time that leads ultimately into what we, in medical taxonomy, later call a disease. That concept is fairly abstract in medicine. Clearly, the way you have described this tendency toward genomic instability that might be associated with biological aging (if I can use that term euphemistically because it doesn't necessarily line up against the number of birthdays), and how that relates to cellular function, which then tracks against later pathology is a very big, different concept than the way that many people learned disease and health.

The Genome Clinic Health Concept

MF: Yes. What I have been trying to suggest and promote is this idea that we should consider an abnormally high level of genomic instability as a disease in itself. If we consider the genome to be the most fundamental component of the cell, and that determines the phenotype (the normal or abnormal phenotype of the cell), then we really ought to consider damage to the genome to probably be the most fundamental disease that we can actually diagnose and nutritionally prevent. That really is the basis of all the concepts that we have been developing in the past 5-10 years, which then leads automatically to the concept-the idea-that perhaps we should now be considering the dietary requirements of individuals and populations based on what is required to minimize damage to the DNA in human cells. And furthermore, this also leads to the Genome Health Clinic concept, which is more recently evolved from the other ideas. Perhaps the way forward in disease prevention from here onward should really be around the

establishment of genome health clinics in which an individual has their DNA damage or genome stability index diagnosed, and based on that diagnosis, one can determine whether it is either low enough or too high, and given that, to intervene nutritionally to minimize the extent of genomic instability in the individual's cells in the body. That is really where we are at, at the moment. We are currently taking this idea into practice through a small company here in Adelaide called Reach 100. It is a group of doctors who are interested in commercializing and taking this idea into practice at their anti-aging clinics.

JB: When I look at this 2005 paper that you authored for Mutagenesis titled "The Genome Health Clinic and Genome Health Nutrigenomic Concepts," it is mind expanding.10 It describes a vision for a paradigm shift in disease prevention that is based on the diagnosis and nutritional treatment of genome or epigenome damage on an individual basis. You have established a frontier that surrounds this assay-the cytokinesis block micronucleus assay-that allows one to have their own genomic stability/instability determined so that (presumably) one can titrate the individual need of nutrients to that person to manifest not only improved genomic stability, but therefore the myriad functional, metabolic, and proteomic changes that occur as a consequence of proper protection of your genome. It then ties together with this whole nutrition concept and you authored another paper (I think also in 2005) in Carcinogenesis titled "Low Intake of Calcium, Folate, Nicotinic Acid, Vitamin E, Retinol, Betacarotene, and High Intake of Pantothenic Acid, Biotin, Riboflavin are Associated with Increased Genomic Instability."11 Maybe you can tell us a little about how this nutrient-specific research ties together with the assessment tool of genomic instability.

MF: Our research, as well as that of a couple of other groups (for example, Professor Bruce Ames' work is an inspiration with regards to this and the direction of our research), has indicated that a number of nutrients can have impact on the genome, either when they are deficient or in excess. I think this is actually very important because in the minds of many people, there is this idea that if a micronutrient or vitamin is good for you, the more the better. What we actually find is that is not the case. We know that with micronutrients, a U-shaped curve with regards to DNA damage is typical. An example where DNA damage may increase and vitamin intake may be excessive would be betacarotene. Although not published yet, we know that excessive organic selenium can increase DNA damage in cells. It is essential to be able to exactly identify, for an individual, not only the dose of each micronutrient that is required to maintain the genome in the healthiest state possible, but also the combination of micronutrients and their doses. That is really where the field is going.

We are modeling that using virtual systems. In 2004, we published a paper in the Journal of Nutrition where we showed how this could be done.12 In this study, we also took into consideration the genotype of an individual. We took cells from people with a mutation in the methylenetetrahydrofolate reductase gene (MTHFR). This is a gene that determines how folate is used in the cells and whether the folate is used primarily to prevent the uracil getting into the DNA, or whether folate is used primarily to maintain methylation of cytosine in DNA, which can have different consequences to the genome stability in the first place. With too much uracil, there might be too many breaks in the DNA. In the second case, with not enough methylation of the DNA, especially in the centromeric sequences, this could lead to chromosome loss.

In the study, we examined the cells of people who were either homozygous normal for the mutation at the 6 and the 7 locus, or who were homozygous mutant, and therefore had a slow variant (a slow activity enzyme, in this case). We examined how their cells interacted with either a low or high folate combined

with a low or high riboflavin. It was very interesting to note that it was evident that the individuals with the MTHFR genotype showed a different genome instability profile than those with the normal variant. Furthermore, it was interesting that in a low folate background, when there was too much riboflavin, DNA damage actually increased. This indicated to us that it is really the interactions and the combinations that tell you what is going to happen in the cells.

In this way, using these tools, we could define the combination for individuals where the DNA damage is minimized. What we are trying to do now is take that concept to the clinic. At this point in time we do not have enough knowledge to be able to say, based on genotype, what is the optimal nutritional combination to reduce DNA damage in an individual. Really, we can only rely on information we have from placebo-controlled trials that tell us this combination of nutrients or this other one could reduce DNA damage and then test whether that actually works in an individual. That is really where we are. We are also building up a database to know what the genetic basis is for an individual's response to supplementation (for example, folate and B12, and why one individual differs from another). There could be a number of reasons. One reason could be, for example, a defect in the ability to absorb vitamin B12 or to metabolize it to the form that is actually active in the body. It is this information that we really need to build up to be able to provide targeted information to individuals.

Functional RDA

JB: As we look at the potential clinical application, it takes us to the potential development of what you alluded to in some of your papers as a "Functional RDA" that is based on genomic stability. You have some very nice articles where you postulated this concept and have also given evidence to why it may be valid, and how it ties together with preventing age-related diseases associated with genomic instability. Have any of your colleagues championed this idea along with you of a functionally based RDA?

MF: I presented this idea at the International Congress of Nutrition in Vienna about six years ago. The concept is essentially found to be acceptable by the wider community in the field of nutrition. While it is not being specifically promoted as the way to do it (because there are many different ways that one can measure health in the body), when you look through the literature you will find that the number of papers investigating the impact of nutrition and vitamins on DNA damage has increased. There is no doubt that DNA damage is increasingly considered to be a key biomarker to assess not only the optimal requirement of nutrients, but also to identify the toxic dose. I think this is really one of the very nice aspects of using DNA damage as biomarkers because you can not only determine the optimum to reduce DNA damage, but you can also identify the doses at which DNA damage starts to be induced or increased when the nutrient is in excess.

There are also wider aspects and applications that I haven't mentioned, which are increasingly important. For example, this concept relates to the important issue of whether consuming too much red meat is a risk factor for colorectal cancer. As you know from the World Cancer Research Fund Report, there is a recommendation to reduce the extent of meat consumption because there is an association with increased risk for colorectal cancer. Another way that we have been using these assays is to try and define the genotoxicity of the bowel contents as a way of determining which dietary patterns are most likely to create a safe (or a lower) genotoxicity environment in the colon. In fact, this has happened. We have shown that you can actually extract the water from the feces and test the toxicity of that water, depending on the diet. You can actually see, in animal models, quite clearly that if you take the fecal material or the fecal water from a rat that is on the high-risk side for colorectal cancer, the extent of DNA damage

induced by the fecal water is about seven times higher than that that you would observe on a diet that is considered at a low risk for colorectal cancer. So these are other ways that one can actually measure and assess genotoxicity in individuals.

JB: You have talked very beautifully about the relationship between genomic instability and cancer. Obviously there is a connection to inflammation-things like heart disease and diabetes. But it also begs, in my mind, as I am listening to you, the question concerning damage to DNA and the immune system related to autoimmunity. Do you have any sense as to whether genomic instability might be connected also to immunological responses to this transformed foreign DNA?

MF: There is evidence for this. We also obtained some direct evidence of immune responsiveness in the cytokinesis block micronucleus assay. The reason I say that is because we know that to do the assay in lymphocytes, we have to stimulate them with a mitogen. And the mitogenic response is a measure of immune responsiveness. We can actually measure this by counting the ratios of the mono- to-binucleated cells (and multinucleated cells) because of the nuclear division index. And we have been able to show that the mitogenic responsiveness is directly related to nutritional status, and in particular, to the levels of magnesium, zinc, selenium, and folate in the blood.

Furthermore, it is also known that as the nuclear division index of lymphocytes is reduced, the higher the DNA damage in the cells. From our point of view-from our experience-it is clear that nutrition does have an impact not only on DNA damage, but also on the responsiveness of the lymphocytes. Furthermore, we know that a number of immune deficiency disorders, such as the ICF Syndrome, which is a syndrome that exhibits a defect in DNA methyltransferase, also exhibits DNA damage, as measured by this index. It is also known that genetic defects in genome maintenance, such as ataxia-telangiectasia gene mutation, also results in a deficiency in immune response and elevated DNA damage. There have also been studies done on autoimmune diseases, such as systemic lupus erythematosus and DNA damage that was elevated under these conditions (whether that is actually a direct cause or just a consequence of the disease process is not known). With regards to inflammation, there is no doubt at all that the oxidative stress that is generated in the inflammatory process, as well as by activated neutrophils, causes a lot of DNA damage and is measurable by this index. We have actually modeled this in vitro, and the extent of DNA damage produced by activated neutrophils is really very, very high and can be easily measured.

JB: I know it is not fair to ask you to look into a crystal ball, but you are one among a small group of people in the world who probably have the best vision as to where this is heading. How long do you think it will take before this concept filters down into what might lead to effective clinical application? Do you think we are on the short edge, or do you think we are on the long frontier in terms of getting this concept into where it can really have meaningful effects on personalizing nutrition?

MF: I think it is really at the beginning. I have been really surprised, though. About two years ago, we had a story about this concept told (shown on television). This really wasn't our own initiative; we were actually approached to do this by the national science television program here known as Catalyst, which is a weekly program on new ideas in science and breaking new technologies. They approached us after they read one or two newspaper articles about our work, as well as some of the science articles. The story is known as "DNA Doctor" and I can make that available to anyone who is interested; it is also available on our website (on the CSIRO website). What happened was that we had a very strong response from a number of groups who wanted to take this idea to practice. Obviously CSIRO wasn't keen to have just

anyone take this idea into practice, so at the end of the day, our management decided to come to an arrangement with a group of doctors based here in Adelaide to put this idea into practice through their clinic, known as Reach 100. This is really an incubator activity that is going on for about 18 months not only to train the young doctors (of course, this idea is really being taken up by young doctors) with regards to these new concepts and ideas, but also to get a better understanding of what the expectations of the clients are and how we can communicate these results to the client.

JB: I want to tell you that this-to me-has been one of the most fascinating interviews that we have had the privilege hearing in our 27 years. It opens up a whole frontier. We are going to keep in close contact with this work and with the Reach 100 group. I think this has the opportunity to really make tremendous positive contributions. Dr. Fenech, I want to thank you for allowing yourself, at this early morning time, to be involved with this and wish you the very best. We are going to be checking back with you.

MF: Thanks very much for the opportunity, Jeffrey. I really enjoyed having the chance to discuss with you and with your colleagues the possibilities that emerge from this science. Thank you so much.

JB: Thank you very much.

My takeaway from this interview is this: it may be possible to evaluate our status before we actually end up with a diagnosis, at a point where we can actually intervene early to use complex lifestyle variables to modulate our genomic stability. We are going to take this model into next month's issue with another clinician/researcher of the month, Dr. Devra Davis, and we are going to look at mechanisms of oncogenic cooperation in cancer initiation and propagation and how it inter-relates to metastasis and angiogenesis. I hope you will be able to take this theme from the June FMU and take it with you into July, where we are going to explore a whole different approach towards cancer chemoprevention using xenohormetic signaling.

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