

August 2011 Issue | Garth L. Nicolson, PhD Institute of Molecular Medicine

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Welcome to *Functional Medicine Update* for August 2011. I believe we have a very, very interesting and insightful issue for you this month, one that takes me back to—believe it or not—the 60s. This goes back to the completion of my doctoral work and when I was starting my career as a professor in 1970.

The Fluid Mosaic Model of Membrane Structure

In the late 60s, I attended a very interesting conference, which at the time was kind of groundbreaking. The conference was on the development of a new conceptual framework for the understanding of one of the most dynamic and important organelles in cellular physiology: the cellular membrane. In eukaryotic biology (meaning the biology of animals), the membranes of cells were known to be composed of lipids. The new model that was developed and discussed in the late 1960s by a professor at the University of California at San Diego—a very esteemed professor, Dr. Singer—and his postdoctoral research fellow, Dr. Garth Nicolson, was called the fluid mosaic model of the membrane.

That discovery and its acceptance have revolutionized our understanding of cellular biology in eukaryotic cells. It certainly has translated into revolutionizing our understanding of many diseases that are membrane-related or transport-related disorders. And it has ultimately led to the development of what could arguably be considered the most well-supported nutritional supplement in the world right now: fish oil supplements that are rich in omega-3 fatty acids.

Connecting the Membrane Model to the Role Nutrients Play in Human Health

You might wonder how I am making this expansive connection between the membrane model and a nutritional supplement, because it may sound to you like a leap of abstraction. But I think you will learn over the course of this issue that this dynamic field of membrane physiology is really at the cornerstone of understanding how nutrients play roles in human health and disease. Membranes of cells are in fact the boundary between the outside and inside. Dr. Sidney Baker often refers to: “Having the right things in and having the wrong things out of cells.” Barrier function and defining how substances are pulled into cells (through nutrients and effector molecules that influence positively the expression of genes and cells that ultimately regulate their function), and expels toxins and metabolites from cells (and debris) are related to membrane function and bioenergetics that power membrane pumping actions. This is at the fundamental nexus of understanding how nutrients work, how they get to the right place, how they influence intermediary metabolism and ultimately things as complex as cell replication, cell repair, and bioenergetics.

The bilayer lipid membrane of the eukaryotic cell has a relationship to other membranes within the cell and within other organelles that reside within the cell, such as the mitochondrial membrane. The mitochondrial membrane is its own barrier of defense that allows the mitochondrion to swim around or be attached within the intercellular milieu in such a way that they are the metabolic furnace of the cell. It produces the bioenergetic energy necessary to power functions, including membrane transport and all the other cellular assembly functions that relate to cell renewal and cell repair. The mitochondrial membrane is slightly different in its construction from the cellular membrane (the outer envelope of the cell). Its own unique composition and construction gives rise to its own characteristics of transport of substances that are necessary for serving as fuel or feedstock for bioenergetics, like fatty acids that are transported by acylcarnitine types of transport mechanisms, or glucose, or amino acids that are utilized within the mitochondrion as a source of a fuel for powering up energetics. And the membrane of the mitochondria is very important, in terms of its composition, for establishing the function of this transport process. So this emerging concept that was really initiated in the late 1960s has grown up now to be an extraordinary fundamental component of understanding health and disease: the integrity of membranes and barrier functions and how the structure and function ultimately translate into regulating complex processes.

For the sake of history, let's quickly build a model that relates to the question I raised: How does omega-3 fatty acid supplementation relate to function through cellular membranes? In the 1970s, a very important paper was published. I think it is arguably considered to be one of the most important cited papers. It was published in an issue of the very well-respected *Science* magazine in 1972. This paper was authored by SJ Singer and GI Nicolson, both of whom I have already mentioned, who were then at the University of California, San Diego. The title of the paper was: "The Fluid-Mosaic Model of the Structure of Cell Membranes."[\[1\]](#) In this paper a model was advanced that is now considered--I think globally--to be THE actual representation of the cellular membrane. The fluid mosaic model is kind of a plum pudding model.

Explaining the History and Connection

What do I mean by that? It is a bilayer of lipids with the hydrophilic heads of various types of phospholipids sticking out towards the water environment or sticking inside the cell towards the water environment, with the inside of the membrane being the oily portion where the hydrophobic tails of the long chains of fatty acids are connected to these polar head groups to make up phospholipids and also make up triglycerides. These particular unsaturated tails, or—excuse me—long fatty acid tails could be composed of either saturated fatty acids, monounsaturated fatty acids, or polyunsaturated fatty acids. The polyunsaturated fatty acids can be from a variety of different families, including omega-3 and omega-6 fatty acids.

So you have differing personalities of phospholipids and triglycerides that comprise the membrane and give rise to different levels of oiliness within the membranes. Embedded within this lipid bilayer (this two-layered sandwich) are the plum pudding components, which are proteins. These specific proteins can be of three different types. They can be proteins that are principally attached to the outer surface of the membrane, which have to do with certain things like receptor function. Or they can be transverse proteins that span the whole domain of the bilayer and have an outside and an inside personality within the cell, like cyclic GMP-related proteins, the G protein family with five different loops that span the lipid bilayer; they can attach to substances outside the cell and they can communicate influences inside the cell due to their changes in conformation across the membrane. And finally you have proteins that are attached to the inner surface of the lipid bilayer inside the cell that regulate other functions.

This complex plum pudding model of proteins embedded within membranes having specific functions as it relates to translation of information from outside the cell to inside the cell, and their ability to produce that effect, is dependent upon the composition of the sandwich structure, meaning the lipid bilayer. And that is in part connected to what type of fatty acids are in the tails of these phospholipids and triglycerides, and the composition of the phospholipids and triglycerides within the membrane. It all gives rise to what we call the fluid-mosaic model, meaning that this is kind of an oily composition. It is not static like a brick wall, but rather it is actually able to aggregate and disaggregate. It is more like a hummingbird's wings in terms of its function: things are moving around in the membrane.

We sometimes call these fatty acids lipid rafts, meaning you've got this lipid matrix—this fatty matrix—of triglycerides and phospholipids, and sitting on it and floating around are these protein and cholesterol islands that then are involved with the changing structure and function of the membrane with time, under different conditions. It's a very, very dynamic process, and I want to emphasize that its dynamic nature is in part related to the degree of unsaturation that is found in these lipid tails within the membrane. As you have higher degrees of saturated fatty acids, the lipid tails have a higher melting point: they're more rigid/more stiff, and they are less fluid. You have a high polyunsaturated fatty acid component of the lipid membranes, and now it is a lower melting point, more fluid, and more dynamic.

As we've learned over the last 30 or 40 years, the composition of the diet changes the incorporation of various components within the membrane structure. You change, actually, the dynamic processes by which membranes interact with their outside and inside environments, and can affect translation of information from the outside to the inside. This was all really beautifully outlined in the extraordinary paper published in *Science* magazine back in 1972 by Singer and Nicolson.¹ This became known as the Singer-Nicolson model of membranes. In this seminal, paper, some of the thermodynamics of how membrane function occurs through these dynamic processes are described. They look at the formation of these membranous materials as it relates to their biosynthesis, their insertion within the membrane, the construction of membranes, and how that also relates to things like mitochondrial membrane function and regulation of bioenergetics, which is related to the transport of nutrients inside the furnace of the cell, the mitochondria, and the expulsion of waste products. All of this was born from these discoveries in the late 1960s/early 1970s and became more well understood in the 1970s. This paper in 1972 in *Science* magazine was the most cited paper in all of science literature for a couple of years after its publication, and stands as one of those seminal contributions to our understanding.

There are many different families of phospholipids: phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine. These are triglycerides that have a phosphate at the polar head group, and then attached to them are different types of fatty acids at the 2 and 3 positions. They have different degrees of other groups attached to phosphates: a choline to make a phosphatidylcholine, or a serine to make a phosphatidylserine, or an ethanolamine to make a phosphatidylethanolamine. Each of those families of phospholipids has different personalities and characteristics when incorporated within the membrane. These are tightly regulated in terms of synthesis, but they are dependent also on substrate availability. In other words, if you don't have a supply of these materials needed to build these membranous components, then you can't build them. That would result in a situation of deficiency or insufficiency.

When we start really looking at how all of this fits together, there is a nutritional dependency/relationship that relates to the availability of these different types triglyceride or fatty acid building blocks. If your diet is heavily preponderant in saturated fatty acids, then the membrane construction will incorporate more of

these saturated fatty acids as a consequence of their availability. If you have more omega-3 fatty acids, then you'll incorporate more regulated balance between omega-3, -6, and saturated fatty acids. So again, there is this dependency on dietary quality that relates to the regulation of membrane construction.

Fatty Acids and Membrane Structure: Studies Demonstrate the Connection

This relationship was documented in a human trial that was published in the *Lancet* medical magazine back in the early 1980s. In this study, researchers looked at men from Edinburgh, Scotland and men from Stockholm, Sweden, who were the same age, and they examined dietary intake and found that the Swedish men consumed more fish and when looking at their membranous components they found much higher levels of omega-3 fatty acids in their membrane phospholipid composition than the Edinburgh, Scotland men, who ate more meat (lamb, in this case).^[2] They then also looked at things like membrane action, red cell stickiness, and tendency of cells to clot, and they found that in the Stockholm men, unsaturated fatty acids, when incorporated within cell membranes, had much less of a tendency to clot, or stick, or form aggregates than those in the Edinburgh, Scotland men. Based on these findings, I think there is some connection between relative risk to certain chronic-related diseases, like stroke or heart attack, and the composition of membranes and diet.

Cardiolipin: An Interesting Member of the Phospholipid Family

One of the interesting families of these phospholipids has the name “cardiolipin.” Cardiolipin is a very interesting member of the phospholipid family. It is basically a polymer of phosphate groups that relate to phospholipids, so it is a longer chain component of a normal phospholipid. Cardiolipins are specifically synthesized with cardiolipin synthase, , and that is, in part, controlled by a variety of different regulating functions within the cell, one of which is, interestingly enough, thyroid hormone. It turns out that hypothyroidism has a negative impact on cardiolipin biosynthesis, and that then alters the construction of this very interesting and important family of phospholipid-like materials.

Cardiolipins are found particularly in the cell membranes of liver, and within liver cells they are found principally within the mitochondrial membrane, so they play a very important role in function of liver cell bioenergetics. They are found, also, to be incorporated at high level at various types of nervous system tissue, and—obviously by the name “cardiolipin”—in cardiac membrane cell tissue, principally in the mitochondria, so they have a very important role to play in terms of mitochondrial membrane structure and function.

Because of their highly anionic charge relationships, cardiolipins bind protons, and they also bind a large number of proteins themselves to form a different kind of aggregate structure in this fluid mosaic model of the membrane. They are very important as a constituent of cellular membranes, particularly mitochondrial membranes, in regulating the function and the transport of ions in and out of the mitochondria, which is all part of the flux of energy. That's how the electron transport chain works: through proton efflux and transport.

Cardiolipin plays a very important role in maintaining bioenergetics through mitochondrial integrity, and it is principally found in high energy tissues like nervous tissue, heart tissue, muscle tissue, and liver tissue. It also—as a consequence of its composition, in which it has fatty acid side chains and phosphatidyl head groups—is dependent, in its synthesis, on nutritional status; you can alter the composition of cardiolipin. There was a very nice review paper published in the *Journal of Lipid Research* in 2008 on cardiolipin biosynthesis and its assembly into mitochondrial membranes, again showing the important

role that it has in the structure and function of mitochondria.[\[3\]](#)

We recognize that cardiolipin has to be biosynthesized, obviously, and it also has to be transported and delivered to the place of need, like the construction of the mitochondrial membranes or the repair of mitochondrial membranes. There are transport proteins that appear to do that. One that has been talked about is a protein called ATP8B1, which is a cardiolipin importer. There are undoubtedly a number of different transport proteins within cells of specific cell type and specific tissues that are involved with transport and the regulation of cardiolipin's delivery into specific cellular membranes, particularly mitochondrial membranes.

However, as I mentioned, situations like hypothyroidism, can lead to poor cardiolipin biosynthesis and altered transport. Things that we start seeing with hypothyroidism include: impairment in bioenergetics, mitochondrial uncoupling, oxidative stress, cardiopathies, cognitive impairment, and poor muscle tone. Those are the very tissues I just talked about that are important for mitochondrial bioenergetics and cardiolipin integrity relative to mitochondrial membrane function that map against those conditions that are associated with hypothyroidism. I think this is not coincidental; there are some very distinct relationships. I don't want to put all the eggs in this one basket, but certainly some very interesting relationships between the conditions of energy dysfunction in various diseases and mitochondrial membrane compositional dysfunctions.

As another part of the story, these cardiolipin molecules--when they are oxidized, or injured, or damaged--can induce an immune response in which anti-cardiolipin antibodies are produced. In fact, there are anti-cardiolipin antibodies found in the sera of patients with chronic fatigue syndrome, suggesting that the integrity of mitochondrial membranes and the patency of cardiolipin is an important part of maintaining proper immune function and also lowering the relative risk to what we call autoimmunity that may be associated with energy-deficit types of problems, like chronic fatigue syndrome and fibromyalgia. There was a nice paper published in the *Journal of Clinical Laboratory Analysis* in 2009 about anti-cardiolipin antibodies found in patients diagnosed with chronic fatigue syndrome and fibromyalgia, indicating that there is a possible relationship between the expression of these anti-cardiolipin and the presence of chemically modified cardiolipin and how that interrelates with poor bioenergetics and mitochondrial interruption (or mitochondrialopathies).[\[4\]](#)

I think that there is a very interesting story emerging as it relates to the mitochondrial membrane integrity and the unique personalities of its composition as contrasted to the cellular membrane. How to maintain integrity of that important barrier defense and that transport characteristic of the mitochondrial membrane against agents that induce things like oxidative stress, or free radical injury, or immunological adverse response against certain constituents of the membrane is also very important.

How can you manage this? What can we do? That will be the topic of this month's clinician/researcher of the month interview. We are so fortunate to have with us, Dr. Garth Nicolson (of the Singer-Nicolson duo), who is going to tell us what has happened over the past 40 years in the evolution of the fluid mosaic model. There have been specific opportunities for new clinical therapeutics on fatty acid replacement therapy and specific targeted approaches towards cardiolipin integrity within mitochondrial membranes. This cuts across things like energy deficit disorders and the whole nature of oxidative stress for bioenergetics.

Mitochondrial Injury and Dietary Intervention

When studying injury to mitochondria, you must ask this question: How does this relate to overall lifestyle principles and dietary intervention? In days gone by we might have said, “Well, they just need more antioxidants. They need more vitamin E, or vitamin C, or carotenoids, or flavonoids, or polyphenols, or green tea (EGCG), or co-enzyme Q10.” All of those are important adjunctive supportive agents, but there is more to it than that alone.

We have to ask not just how do we trap the oxidant radicals, but why did they exist in the first place? What is the cause of increasing oxidative chemistry that causes membranous damage and ultimately loses the integrity of bioenergetics and causes this kind of free radical storm? What is emerging as a part of that answer is that alterations in things such as insulin signaling, and the things that keep coming back time and time again as it relates to the adverse effects of a high fat diet and the postprandial state seems to really drive some of these injuries.

The Case for a Fatty Acid Tolerance Test

What do I mean by the “postprandial state”? This means after eating. To measure blood glucose, we do what is called the oral glucose tolerance test, which means we fast a person, take a fasting blood sample, then we administer a Glucola drink (75 grams), and we then measure their blood sugar postprandially after the oral glucose load and we evaluate how much they can mobilize response based upon their organ reserve, and their endocrine pancreas, and their insulin regulatory process. How much they can control the postprandial load of glucose? With regard to fats, however, how do we measure fats? We generally bring people (fasted) in for a blood sample. We look at triglycerides, we look at cholesterol, we look at LDL, VLDL, HDL, and we make kind of an assessment as to whether they are hyperlipidemic.

Why don't we do the same thing with fats that we do with glucose, and that is, why don't we challenge that person with a fatty acid tolerance test? We know we can pick up many people in glucose dysregulation who show normal fasting glucose but become abnormal in a glucose tolerance test because they are having dysinsulinism that is a precursor to more dysfunctional states like diabetes. Why don't we use a fatty acid challenge test? There are people that are starting to do that. They are using certain graded doses of a standard lipid mixture to orally challenge a person. As in the case of an oral glucose tolerance test, a person has a fasting blood lipid level done, they are then given an oral dose of a liquid mixture of fats, they then have their blood drawn at times thereafter, and the relative regulation of their lipemia after a challenge is then a measurement of lipid tolerance..

When you start looking at the postprandial relationships to fatty acid management, what you find is that there are peaks that occur after eating a high fat meal that really induce the significant potential for injury to cell function, and punch holes in membranes, and lead to mitochondrial alteration and oxidative stress. In fact, there are papers that have been published on this recently. Let me give you a couple of examples. In the *Journal of Clinical Science* in 2010,, a very interesting paper was published about a human intervention trial looking at postprandial oxidative stress by differing dietary fat consumption (in humans). In this study, people were put on different fat composition diets for 12 weeks: a high saturated fatty diet, a high monounsaturated (oleic acid or olive oil) diet, and two low fat/high complex carbohydrate diets, one supplemented with long chain omega-3 fatty acids, and the other with sunflower oil (high in oleic acid).^[5] Among 75 participants randomized across these diets, they found that the diet that was high in monounsaturated fatty acids had the least adverse effect on oxidative stress, meaning the

glutathione-to-glutathione disulfide ratio was normalized, the protein carbonyls were reduced, the inflammatory markers like hsCRP were reduced. It appeared as if monounsaturated fatty acids, as found in the Mediterranean diet, for instance, had a much more favorable effect on postprandial lipemia and oxidative injury and mitochondrial dysfunction than did the other three programs. They also point out that the Mediterranean diet probably has some desirable effects because of the diverse nutrients and biofactors in these foods that help to provide higher levels of NADPH, which is necessary for maintenance of proper bioenergetics.

This is very similar to another recent paper that was published in the *Journal of Hepatology* in 2010 page 727. This was a controlled study in animals, a specific type of rats that are bred to basically have a defect in their cholecystokinin signaling system, so they tend to overeat and become obese and diabetic.^[6] These rats basically have a problem with appetite regulation, not unlike—probably—some of us in the human species. What they found in these animals that tended to overeat was that as they got more obese and their fatty acid levels increased, they got hepatic steatosis, meaning infiltration of fat into the liver, and nonalcoholic fatty liver disease, which affects about 30% of all US adults presently, and 75 to 100% of obese and morbidly obese people. It is not an uncommon situation in the population of humans today. In fact, it is estimated that 10% of lean and 38% of obese children have fatty livers, and that this is a significant contributor to liver failure and ultimately the need for liver transplant, as well as having a very close correlation with the morbidity of insulin resistance and type 2 diabetes.

In this animal model, it was found that as the animal got more derangement in their postprandial fatty acid management, they got more and more oxidative stress. It is a two-hit model. The first hit is to interrupt the relative ability of the membranes to form proper integrity, and the second hit is in oxidative leakage and ultimately cellular derangement. They again call for the need for dietary intake that would regulate postprandial hyperlipemia (or lipemia postprandially), and reduce then the load of these untoward lipids on cellular chemistry.

I think these are some very important things to keep in mind as we move into this discussion with Dr. Nicolson. We must consider not only lipid replacement therapy, but dietary intervention using a modified Mediterranean intervention that is high in oleic acid and rich in the phytochemicals that modulate oxidative chemistry and mitochondrial function. This may play roles in conditions not just related to metabolic syndrome, but also things like chronic fatigue syndrome and fibromyalgia. About fifteen years ago, published work suggesting conditions such as these could be connected to mitochondrial dysfunction were heavily criticized. I recall presenting this at a chronic fatigue syndrome conference in San Francisco over 15 years ago and having some of the leaders in the field criticize this hypothesis as having no merit, even though I presented clinical and biochemical information suggesting that it was a relationship to the condition. It now seems much more respected that mitochondrial oxidative injury, oxidative stress, and toxicity (as it relates to energetics) are companions of the etiology of chronic fatigue and fibromyalgia. I think what you are going to hear from Dr. Nicolson will relate very nicely to the emerging literature. The beautiful take away from this discussion is that once you understand the membrane connections and the connection of diet to the manufacture and biosynthesis of these important structural and functional lipids

that are found in membranes, it now gives rise to the opportunity for selective intervention, nutritional therapy, and a targeted, personalized medicine approach—a functional medicine approach—to these conditions.

Let's now move into the extraordinary discussion with our clinician/researcher of the month, Dr. Garth Nicolson

INTERVIEW TRANSCRIPT

Researcher of the Month

Garth Nicolson, PhD

The Institute for Molecular Medicine

This is the section we all look forward to. This is the theme, this is the energy center, of Functional Medicine Update—our Clinician and/or Researcher of the Month section.

My history with Dr. Garth Nicolson actually goes back to 1970. In 1970, I was just finishing up my PhD in biochemistry and was at the University of Oregon. My thesis advisor said, “You know, we ought to go over to Corvallis [our kind of competitive institution on the other side of the state] and go to this seminar—this 3-day symposium, actually—on cellular membranes.” The concept as to the structure/function of membranes was just emerging. This concept of the fluid mosaic model, lipid bilayers, and embedded proteins (sometimes called the plum pudding model of the lipid bilayer) that we were exposed to at that 3-day meeting was the so-called Singer-Nicolson model of the lipid bilayer. It was a very, very revolutionary change in thinking about an organelle that had not just structure, but function—the cellular membrane. Of course, the Nicolson of the Singer-Nicolson duo was none other than our guest this month, , Dr. Garth Nicolson.

It turns out Dr. Nicolson and I overlapped at the University of California, Los Angeles. We were both chemistry majors. He graduated in 1965 and went on and got his PhD in biochemistry and cellular biology at the University of California at San Diego. He was involved with the Nobel Prize-nominated work that ultimately resulted in scientists understanding much more about the nature of this boundary layer, this compartmentalization that we call the membrane, and how it plays a functional role in cellular physiology.

We're extraordinarily fortunate to have Dr. Nicolson tell us more about how this field has evolved over 40+ years. He's presently the President, Chief Science Officer, and Research Professor of Molecular Pathology at the Institute of Molecular Medicine in Laguna Beach, California. As most of you know, I had the fortune of spending a couple of years on sabbatical with Linus Pauling, who used the term “molecular medicine” in his landmark paper in 1949 in Science magazine, when he wrote about (with Charles Itano) the concept of sickle cell anemia.”[7]

With great privilege, Garth, nice to have you on Functional Medicine Update and welcome.

GN: It's a pleasure to be with you.

JB: I know it sounds like it may be ancient history, but I'd like to take us back and start with the emergence of the work that you are engaged in and the development of your thoughts about the composition and the activity of the membrane. Could you take us back and work us up from there?

GN: When I first started my work on membranes I was actually interested in mitochondrial membranes. I did some work on mitochondrial membranes before I switched (with Singer) to the cell membrane. This ended up the fluid mosaic membrane model of cellular membranes, but it also held for all of the intracellular membranes in the cell as well. My interest in the mitochondrial membrane centered more around the function of the membrane in the production of energy inside the cells, and of course the mitochondria are the little batteries inside our cells that produce the energy. I've recently come back to that because of my interest in fatiguing illnesses and the role that mitochondria play in fatiguing illnesses. This really, again, is integrated into membranes because the whole apparatus of energy production takes place in a membrane matrix inside the mitochondria, and so we were very interested in some of the problems that occur in cells, particularly when they become diseased.

Mitochondrial Function, Chronic Illness, and Aging

During chronic illnesses we know that the mitochondrial function goes down, and we were very interested in how to restore mitochondrial function. The role that the actual membrane and the membrane lipids play in this whole process turned out to be incredibly interesting. The upshot of it all is that we were able to replace the damaged lipid membrane. I should say that lipids get damaged like other structures of our cells (the proteins and the DNA) by excess oxidative events (oxidative stress is what we generally call it), which is really the production of excess oxidative molecules that tend to damage molecules in our cells. Lipids turn out to be very sensitive to this whole process, and it turns out the inner mitochondrial membrane, which is the functional part of the membrane in terms of energy production, is exquisitely sensitive to these oxidative events that occur inside cells. When the lipids get damaged, the inner mitochondrial membrane becomes leaky, and because it becomes leaky the potential across the membrane can't be maintained and this is absolutely integral to the production of energy (that membrane dynamic and also the chemical potential across the membrane must be maintained for energy production). It turns out that when the lipids are damaged this can't be maintained and energy production goes down.

We've tried to think about how to fix this when it occurs, and it occurs naturally during aging. For example, it occurs during all types of disease processes: infection, chronic illnesses, cancer. All kinds of different situations cause destruction of the inner mitochondrial membrane lipid. It turns out we have a natural process for repairing lipids in our cells anyway, and by making use of that natural process and providing undamaged lipids for this natural process, we're able to actually see the complete circumvention of destruction of energy production and a sudden reemergence of mitochondria that, with their repaired membranes, are capable now of producing the energy at the levels that they normally should. We see this on an organismic level or holistic level as the resurgence of energy and vitality and the reduction of fatigue.

In a nutshell, that is what we've been working on the last few years.

JB: For the sake of many clinicians who may still be brushing up on some of their cellular physiology and biochemistry, let's go back and just make sure we have some of the terms all in line. You talk about the

fluid mosaic model of the membrane. Could you take us through a descriptive review so we're all looking at the same picture with those words? What does that mean?

GN: What it means is there is a lipid bilayer of phospholipids, and in that lipid bilayer are intercalated proteins and glycoproteins of the cell. This occurs not only in the cellular membrane but other membranes of the cell, including what we're going to talk about eventually (the mitochondrial membrane). These intercalated proteins and lipids are not static. They are in movement because it's a very dynamic structure. The lipids, in fact, form the matrix of that membrane, and that was the whole idea behind the fluid mosaic membrane model. That membrane actually is a two-sided membrane. The inner part of a membrane (in any particular membrane) is different from the outer part in terms of its composition, and in terms of the lipids, for example, and in terms of the proteins as well. To maintain that polarity, we have to have some kind of a structural arrangement, and to maintain the dynamics we have to have a structural arrangement. The best way that explained this was that it was a dynamic process where the lipids were in constant movement and so were the proteins, and they did maintain a polarity across this membrane. The dynamics of the membrane turned out to be very important for many processes of cellular function. Also--again--the fluidity was important, and also the polarity across the membrane and the fluidity were tied up with this chemical potential across the membrane (the electrical potential, essentially, across the membrane). So it all kind of fits together in an integrated sort of structure of membranes.

JB: Let's review. You talked about phospholipids. I think most of us remember that phospholipids are somehow like a glycerol backbone, where the three position has some kind of a phosphate-related group on it, and the first position and second position are occupied by fatty acids. Could you tell us a little bit about the classes of phospholipids and their composition?

GN: In terms of membrane phospholipids, we generally categorize both by their fatty acid chain and also by the constituent groups that are attached to the heads of the phospholipids (the hydrophilic parts). These are amphipathic molecules; they have a hydrophobic part or a more lipid part, which are the fatty acid acyl chains, and they have a hydrophilic part, which exists essentially in the water milieu of the cell. These constituents can vary. For example, they can contain attached serine, glycerol, and so on and so forth molecules. That turns out to change not only the dynamics of the membrane, but it is also important for the structure of the membrane and its function as well.

So these molecules are really very asymmetric molecules. They're very different in terms of each side of their structures, and also their functions are really quite different in terms of what they provide to the membrane and what they provide to the cell.

JB: And those fatty acids that are connected to the phospholipids, I presume they can be members of a number of different classes, such as the fully saturated, or they could be omega-3, -6, or -9 unsaturated. How does the composition of these fatty acid chains get established?

GN: Of course there are enzymatic mechanisms to produce these. What's important is the overall mix of these different molecules in a structure like the membrane of the cell. Whether they have double bonds or single bonds, and then whether they are saturated or unsaturated turns out to be very important for the structure and validity of the membrane. And when this changes—for example, by oxidation—this can result in a slight change in the structure, which eventually causes these lipid moieties not to fit exactly the way they should, and not to be quite as fluid as perhaps they should be. And this causes discontinuity of the

membranes and eventually this might cause a discontinuity that results in leakage across the membrane. When that sort of leakage occurs, that can destroy the chemical electrical potential across the membrane. So that is something that we have to be well aware of because the membranes are constantly being turned over, repaired, and replenished all the time. So when this damage occurs that could affect the overall structure and the potential across the membrane, we have to exchange out those damaged lipids for lipids that are undamaged so that we can return back to the physiologic state that is imperative for various processes that occur in the membrane.

JB: I want our listeners to understand that you're an individual who has not only been in this field since its beginning, but has been one of the emergent contributors to our understanding of membrane structure and function. Dr. Nicolson is approaching 600 publications across this vast array of research experiences in the field. I hope you're all listening carefully because you're getting news-to-use from the right person here.

As it relates, then, Garth, to this composition, presuming then that you could have more fluidity by more highly unsaturated fatty acids, and less fluidity (more rigidity) with more saturated fatty acids, in a normal individual how much of these fatty acids would be, say, the more highly unsaturated, like the omega-3s?

GN: Well, of course, there is the balance between them. The balance is really what is important. When this balance goes out of sync, more or less, what happens usually is that the lipids get oxidized. And when the lipids get oxidized they get converted to a different chemical form. The different chemical form, then, really perturbs the membrane, more or less, when enough of the lipids get oxidized. This is, for example, what happens during aging. This is what happens during a variety of chronic disease processes. This is something that we have to be aware of because it is very important for our physiology.

JB: Let's take that and ask the simple question. I know it is implied in what you've said, but let's make it clear for everyone listening. Does the status of nutrition have any role to play in the composition of those membranes or are they controlled by other factors that are nutritionally independent?

Nutrition and Membrane Composition

GN: Oh, absolutely nutrition is really important in this. We have to provide our body with the correct constituents in order to replace the damage that occurs on a daily basis, and it is damage that is accelerated during disease processes. If we don't have the correct precursors, for example, for our lipids and for the other structures of our cells, then we can't keep up with the damage that occurs, and eventually it throws our cells out of sync. And so with the membranes, we have to repair this damage because if we don't the membranes can become less fluid, they can become more leaky, other things could happen, there is less functionality of the membrane, and so we can see that with this type of damage, our cells can no longer function as they should.

JB: With that in mind, one might ask the question: What's best? Should we give the precursors to these membrane constituents like the fatty acids, or should we give structured lipids that contain all the requisite requirements to make these phospholipids, which means more of a complete phospholipid molecule in order to stimulate proper membrane construction. What's the research tell us?

Stimulating Membrane Construction: What is the Best Method?

GN: The research tells us that it depends on the speed in which you want this to occur, because they are

natural processes in our cells which can interconvert all these things anyway. But it turns out when it is diseased it doesn't always have the capacity to do this in a timely manner. In other words, the damage may outstrip the ability to repair this whole process from occurring. As we get out of sync in our bodies, and our cells get out of sync, we then can accumulate damaged molecules in our cells which are not being replaced rapidly enough. So it is a whole dynamic process. If you're very healthy you can get by with less. But if you are ill—if you are sick and if you have damage inside your system and damage inside your cells—then you may not be able to keep up with that. In that case, it actually helps to have a more complete set of molecules that are very capable of repairing—without a lot of chemical changes occurring inside the cells—the damaged structures within the cells. If you're really very, very healthy you can get by with less than if you are in a process where you're not so healthy and a lot of damage has accumulated inside your cells.

JB: That then leads clinically to something everyone that is listening is familiar with, I think, and that is we have seen a very, very interesting rise in fatty acid therapy becoming kind of a standard of care or an accepted therapy (almost like a pharmaceutical therapy). GlaxoSmithKline acquired the company that manufactures what they now call Lovaza, which is a medical delivery form for omega-3 fatty acids. Its condition of use is for hypertriglyceridemia. This seems to have a much broader implication in terms of health and disease.

GN: I think it does because we're actually providing the direct precursors for membranes rather than one of the constituents of the precursors, so it is one step further in the whole process (the whole metabolic process) of generating membrane structures. We feel that this is an important dance because people that are ill don't always have the capacity that they should to perform all the enzymatic features necessary to make a fully functional membrane.

JB: So now let's go to—I'm running forward really quickly from 1970 to 2003, but I want to take us up to one of the many of your very extraordinary publications—the Journal of Chronic Fatigue Syndrome in 2003. It is an article talking about a clinical intervention in severe fatigue patients that are older aged and who have altered mitochondrial function and lower bioenergetic potential, who then responded very favorably to a structured lipophospholipid dietary supplement.[8] Could you tell us about this study? It is very interesting.

Studying a Natural Approach for Solving Problems of Loss of Energy Production

GN: We've been working on this for a few years now. Again, it is a very natural approach for solving the problem of loss of energy production, which occurs inside the cells of people that are having any chronic illness, and also with aging and with other processes that can go on that can damage membranes. This is a very common occurrence these days in people, essentially because of all the environmental insults they are exposed to, as well as aging and other natural processes that occur. What we've tried to provide is a very balanced dietary supplement called NTFactor™, which provides the glycopospholipids in a format that essentially compositionally matches the membranes of the cell fairly closely. This provides a very rapid way to repair the damage that occurs, and to keep above and beyond the damage that will continue to occur to these cells during normal day-to-day living, and help repair the process inside the mitochondria, the little energy production centers of our cells, and return them to a more physiologic state where they are capable of producing enough high-energy molecules to perform all the functions necessary in the cell. And what this dietary supplement does is it really utilizes the natural transport features that are present inside our bodies to distribute these precursor molecules to all the cells of the body, and then

replace the damaged molecules that are there in a more efficient way and therefore return the cells to a more normal physiologic state more rapidly, and to maintain them in that situation.

With the mitochondria it is very important that we maintain the chemical potential across the membrane and that's what these help to do very quickly (quickly, meaning now that we've had our new formulation this could occur within a week). So we can see very dramatic increases in energy production and decreases in fatigue now within a week of administering our newer lipid supplement.

How Do You Measure Mitochondrial Function?

JB: A lot of individuals probably would be interested to know about the design of this study. How do you measure mitochondrial function in human subjects? What is the readout? I think that would be an interesting thing to share with our listeners in terms of this clinical study. I found the way that you actually assessed the before and after effects on mitochondrial function very fascinating.

GN: One of the ways to do this is to measure the ability of mitochondria to produce high-energy-reducing molecules in the cell. We can take cells, for example, from the blood of patients and show that their blood cells have the capacity to do this to various degrees. People that are aged, or people that are sick, or people that have chronic illnesses of various types lose the ability to produce these high-energy reductive molecules. By putting in a fluorescent dye of a potential redox potential so that it will access electrons (but only if they are high-energy energy electrons produced by the inner mitochondrial membrane), we can actually show that the mitochondria will fluoresce if we feed this dye to the cells, if they can produce the high-energy molecules. If they can't, they'll fluoresce to a lesser degree, or not at all if they have completely lost function. So we can actually get a quantitative measurement of mitochondria this way. There are other ways to do this as well, such as measuring high energy molecules like ATP and so on. But all these measurements are really focused on the ability of mitochondria to produce the high energy molecules or to reduce the high energy molecules that are needed for various enzymatic features inside ourselves. So that's the one way that we've done it. By using a cell sorter we can separate out the white blood cells and measure on a cell-by-cell basis the ability of the mitochondria to produce these high energy molecules or reduce them by the dye we put in, and we can see that when the dye fluoresces. So we can get a quantitative measurement of return of mitochondrial function this way.

JB: So that takes us now to 2006 and another really interesting paper. You have been authoring a series of papers in the Journal of Chronic Fatigue Syndrome. This paper is about an intervention trial using the NTFactor supplement for restoring mitochondrial function in fatiguing illnesses. This is with Dr. Ellithorpe as your co-author/clinician. I find this paper is really beautifully written. I think it has a very sensible introduction to this complex topic of mitochondrial bioenergetics, and redox potential, and membrane boundaries, and leakiness of electron, and all those kinds of things that relate to oxidative stress and free radical pathology. But for the clinician, the outcome in this study I think once again showed very, very significant effects on clinical outcome in the patients correlated with the improved mitochondrial function.[9] How did you measure the clinical outcome in these patients with fatiguing illnesses?

GN: What we used was a validated instrument for studying fatigue. It was actually developed for cancer patients who suffer fatigue. By the way, these products are also very useful in cancer—particularly during cancer therapy—to reduce fatigue, which is the number one complaint of the patients during cancer therapy. Basically it is a questionnaire-type of instrument and it has been validated in thousands and

thousands of patients for being a valid instrument for looking at fatigue. Fatigue is a multi-dimensional phenomenon and it has very different sorts of aspects to it, which I won't go into in detail about. This instrument covers the various aspects of fatigue so we can get a pretty good and accurate measure of fatigue. This is not really our development. Barbara Piper and her colleagues, over many, many years, had developed this to study fatigue in cancer patients. We simply utilized her very useful instrument to look at chronic fatigue in chronic fatigue syndrome patients and other patients that suffer from tremendous fatigue. We would give these patients the lipid supplements and not only look at their mitochondrial function, but also look at fatigue at the same time. Where we found the mitochondrial function was restored, the fatigue level went down, and was reduced anywhere from 35 to 45% in different studies. So this turned out to be a very useful instrument.

JB: We move from that to 2010. This is a paper that appeared in the Journal of the American Nutraceutical Association looking at this upgraded NTFactor intervention with a glycopospholipid antioxidant vitamin formulation that then seemed to compress or reduce the time to efficacy quite significantly.[10] What was the nature of improvement that you had learned about to actually hasten or to make more quick the outcome?

GN: The biggest improvement, in my own mind, was the fact that we put in more immediate precursors of some of the most important molecules in the membrane. So that's the first thing that we did. For example, in mitochondria there are molecules like cardiolipins, which are exclusively sensitive to oxidation. When they are oxidized, they really modify the function of the mitochondria very dramatically. Cardiolipin has important constituents, such as phosphatidylglycerol. By supplying the precursors to cardiolipins we are able to speed up the entire process, which before depended in part on interconversion of some of the cardiolipins into the cardiolipin precursors. As I mentioned earlier in the conversation, if you apply the more immediate precursors to membrane functional molecules then you seem to get a faster process because it doesn't require the enzymatic interconversion in between, so these things can functionally work sooner than if we apply less intermediate or less immediate precursors. By doing that and by also adjusting some other aspects of the mixture, we tended to speed up the entire process. That's the good news, and we're tinkering with this all the time. My colleague, Bob Settineri, who has really been doing a lot of tinkering with the lipid composition has really come up with some really outstanding formulations, which I think will push this even further.

JB: As you talk about oxidation of membrane lipids it reminds me of the reactions that occur due to oxidation that produce aldehydes like malondialdehyde, and how those can have effects on intracellular proteins by tanning the proteins, forming shift spaces and cross linking. Do you ever have occasion to measure some of these of these oxidation products, like the aldehydes that are produced? I think you can measure these, even things like the thiobarbituric acid reactive intermediates. Do you see a correlation between the reduction of these reactive aldehydes and the preservation of membrane integrity?

GN: It is interesting that you bring that up because it is one of the next things that we are actually going to get involved in. The lipids that we're providing also have an antioxidant effect as well, and, of course we do put in antioxidant with them. This is an important aspect, I think, of the whole process: preventing and eventually reversing the oxidative events that occur inside a cell. These are not only to lipids, but they are also to other structures in the cell as well (to proteins, and to DNA eventually). I think in this entire process, we're going to look at it in much more detail. We haven't yet, but we plan to do that.

Mitochondrial Function, Functional Foods, and Cancer Therapy

JB: That leads me, then, to 2011, and what I call (in my vocabulary) a “seminal” paper that you’ve just put into the literature that is available by open access in the *Functional Foods in Health and Disease* journal. This is a wonderful review titled, “Lipid Replacement Therapy: A Functional Food Approach with New Formulations for Reducing Cellular Oxidative Damage, Cancer-Associated Fatigue, and Adverse Affects of Cancer Therapy.”[11] Again coming back to what you talked about earlier: that many of the cancer therapies themselves cause mitochondrial perturbation and induce oxidative injury and the cancer process even in the absence of therapeutics increases oxidative reactions due to the anaerobic metabolism that is often occurring within cells that shifts mitochondrial function into an oxidant production situation. Could you highlight this extraordinary review?

GN: Thanks for plugging the paper because it is a fairly new journal. We have been kind of interested in the functional food aspects of this because that’s really what we are talking about in terms of a lot of the dietary supplements. If we can provide them as a functional food then more and more people would have access to this technology. I think that’s the way of the future. So we have been looking at the wide variety of different ways that these lipid supplements, and antioxidants, and so on can be provided in the future, to not only patients but to people to generally improve their lives and generally help to protect against the environmental insults that they are seeing on an increasing basis these days.

This is just kind of a review of what lipid replacement therapy has done, and it concentrates on cancer because it is an interest of mine and it has been for some time—how to reduce some of the morbidity of cancer. Of course, a lot of this, as I mentioned, is due to the direct effects of not only the cancer, but also the drugs that are taken to treat the cancer. We’ve tried to figure out ways to reduce not only the effects of the cancer on morbidity, but also the effects of cancer therapy on morbidity. This could be radiotherapy, chemotherapy, or whatever. We know that these processes result in a lot of oxidative stress in our systems. The difference—and the reason why we are not interfering with the actual therapy against the cancer itself—is that that therapy actually takes place in a fairly short window. There is a very short window of opportunity to kill a cancer cell. The time we are talking about is a much longer time period, and that is the effect on the normal cells and tissues that occurs due to the cancer therapy—the residual problems that are associated from the cancer therapy. And this is what we want to improve, to improve the quality of life of cancer patients, and to also help them overcome the morbidity associated with therapy.

JB: I find this very, very interesting. Probably like you, we’ve been around in this field long enough now to see cycles within cycles, and wheels developing, and lineage of thought from an initial kind of a-ha discovery ultimately into a broader kind of formalism. It was more than 15 years ago in *Functional Medicine Update* that we had the opportunity to interview Dr. Martin Pall, who presented to us what, at the time, was a very provocative concept: that fibromyalgia syndrome and chronic fatigue syndrome were associated with oxidative stress, and peroxynitrite formation, and activation of the immune system that induced nitric oxide-mediated free radical pathology and a cascade of events, including oxidative chemistry as well as nitrogen chemistry. He is a biochemist at Washington State University and had been studying himself. He, himself, had had chronic fatigue. It just came out of the blue for him after a flu that he had at a science meeting in Spain. So he made this the topic of his work for the last 20+ years. At the time a lot of people said, “Well that sounds like a very audacious concept that doesn’t seem to really ring true. That’s not the dominant theme of the origin or the pathophysiology of chronic fatigue or

fibromyalgia. It sounds like just a wild speculation.” But he continued on with this work. He published a number of papers in this area. That seems to converge very nicely with your emerging concept as well. Are you familiar at all with Dr. Pall’s work?

GN: Oh, yes. In fact we cite his seminal references in our papers. In his case he is concentrating on nitrogen oxidation/nitric oxide oxidation, but it is all part of a general scheme that occurs inside our cells, and that general scheme occurs when the cells are damaged. Our part of it is, how do we repair this damage? How do we return the cells back to a more normal state so they can be fully functional?

JB: So then that leads to another therapeutic question. There are those people that are detractors of this whole lipid replacement concept that say, “Just a minute. I don’t understand how this can work. You’re administering therapeutically a few thousand milligrams of lipid to the person per day, yet their body is composed of 20{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} or more of fat (of their whole body weight), so if they are a 70 kilogram person they have from 14 to 25 kilograms of lipid and that’s like 14,000 grams and you’re only giving them just a couple of thousand milligrams, so that’s like a breath of wind in a storm. How could that have any therapeutic effect?” What would be your answer to that?

GN: For one thing, we’re not talking about the total lipid stores of a body, which are considerable. We’re really talking about some very important functional membranes of the body, which is a minute percentage of that total store. Then there is even a more minute percentage of that, which are actually the functional lipids, which are the target of oxidative events inside the cells. So we are really talking about something that is really very, very small compared to the total amount of lipid in a body. But those are the functional lipids that really determine—in terms of energy production—whether it will occur or not. We’re really going after the functional molecules.

JB: Again, to kind of trace back to our history of Functional Medicine Update, this sounds very similar to an interview that we had with Dr. Edward Calabrese, who is kind of the father (at least, arguably) of hormesis, this concept that small things can have bigger effects than expected if they hit regulatory regions within cell physiology. He has talked a lot about the nonlinear effects of a dose response curve when you get down near the origin at high dilution, that sometimes substances have different signaling effects. It seems like we’re almost talking about a hormetic effect:

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