December 2000 Issue | Dr. Donald L. Hayes, President

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Welcome to *Functional Medicine Update* for December 2000. The year ends, but the excitement continues in the changing field of medicine. This issue of *FMU* will be packed with friendly news regarding anti-senescence medicine and improving health span. This area of medicine is sometimes called anti-aging medicine. Can it deliver the goods, or is it more hype than hope? We will deal with these questions in this issue.

The top 200 prescription medications in rank order of sales tells us what kind of drugs people use most in this aging Baby Boomer culture of rising health expectations. It tells us something about consumer perceptions and attitudes related to medicine.

The number one drug on the list is Premarin (hormone replacement therapy) for women going through menopause. Second is Synthroid, thyroid replacement for individuals with endocrine dysfunction, low energy, and fatigue-related conditions. Next is Lipitor, a cholesterol-managing product. It is followed by Prilosec, an acid-controlling agent that is used in what might be considered lifestyle disorders, such as bacterial infection with *Helicobacter pylori* or immune defense issues that are not crisis illnesses.

None of these top four drugs is used to combat a crisis disease. Their application is in functional conditions of endocrine imbalance, cholesterol management in heart disease risk, or acid formation in gastrointestinal function.

Drugs for Mismatched Genes and Environment

The number five drug is Hydrocodone for pain and inflammation. It is followed by Albuterol for asthma, environmental toxins, and respiratory immunological function. Next is Norvasc, a hormone-related modulator. Claritin, eighth on the list, is for allergy and is a rapidly rising blockbuster drug because of people’s increasing sensitivities to their environment and the immunological cross-matching or dysfunction in our culture. With the next drug, Prozac, we get into SSRIs for depressive and affective disorders. Last on the top 12 list is Glucophage (metformin), used principally for type 2 diabetes, which is certainly a lifestyle/gene-connection disorder related to diet, sedentary lifestyles, and premature biological aging.

All of the top 12 drugs are functional medications to modify disorders associated with the mismatch of genes and environment. They represent a medicalization approach to manage conditions that are largely remediable or alterable in outcome in the phenotype by the way we treat the genotype. That message frames this month's *FMU* discussion.

In October I participated in a National Institutes of Aging conference called "Biomarkers of Aging." The participants were there to develop a NIH position white paper describing the biomarkers of aging. How can we track functional characteristics and capabilities throughout a person's life? We know some individuals age prematurely, faster than their genes would have them do, based on mismatched circumstances or lifestyle, environment, or health condition. We met for two-and-a-half days with an esteemed group of scientists. We'll hear from one of the participants later in this month's *FMU.*

We learned that no documented, agreed-upon biomarkers of aging exist. Some substantive biomarkers, related to anthropometric variables like muscle mass, body composition, basal metabolic rate, hearing, vision, and sense of peripheral and neurological sensations, do exist, however, and these whole-organism characteristics may be candidate biomarkers for aging.

Tissue or Cellular Biomarkers of Aging

Some tissue-specific and cellular-specific biomarkers also may be related to gene expression patterns. These particular molecules, which can be analyzed in various fluids, might indicate accelerated biological aging processes. These biomarkers have to be considered more speculative at this point, but taken as a whole they produce patterns that help us understand a person's progression toward senescence, illness, loss of function, or perhaps even premature death. Responsible researchers and the clinical community are now trying to understand the connection between our genes and our environment and how they translate into functional health after mid-life.

This work applies the Human Genome Project to health and disease. In a recent article, Dr. Ken Gray, from the American Association of Clinical Endocrinologists 9th Annual Meeting and Clinical Congress, describes the role of the Human Genome Project. Now that we are moving to a complete sequence of the code of the human genome and the 26 pairs of chromosomes, we recognize there are markers that seem to code for relative risk of disorders. They don't cause the disease by themselves, but they are clustered in multi-gene families that give rise to the expression potential for diseases like Alzheimer's, heart disease, inflammatory bowel disease, cancer, type 2 diabetes, and dementia.

Medicine in an Age of Transformation

Understanding of these multi-gene families is leading us to recognize that medicine is in an age of transformation. We have seen nothing like this in the last 100 years, ever since the origin of the vector disease model of past years. We are seeing a sea change in the way health is defined and in the way doctors will treat and evaluate patients over the course of their lives to improve their health span.

What, exactly, is this anti-aging medicine? In a recent paper from the journal *Geriatrics,* interviewees talk about partners and putting into practice evolutionary theory that is emerging from this field of geriatric and cell biological research related to aging. I find this very interesting, because it may not be anti-aging. There may be a process tied into our genes that we do can little about related to the biological mechanisms of aging and ultimate death. What seems to emerge, however, is the understanding that we can influence senescence, which is the overlaying of our genes with accelerated processes of dysfunction associated with premature aging and disease. Anti-senescence programs will probably emerge as being scientifically grounded and workable.

The New Genetics in Clinical Practice

The question of whether we can prevent aging and extend life expectancy, I think, is open to further question. It may be a very complex process related to pattern recognition and multi-gene expression in different individuals across a wide range of heterogeneity. It may, therefore, resist our ability to define a single mechanism.

This is, in part, the topic discussed in the journal, *Geriatrics*, in relation to anti-aging versus senescence versus improving health span. In an editorial in the *British Medical Journal*, Dr. John Bell describes the new genetics in clinical practice. He believes this human genome message will not translate into genetic screening and counseling, but into personalized medicine. It will involve evaluating the clusters of characteristics expressed through the genes that can be modified by altering lifestyle, environment, nutrition, and various factors that influence inducible gene expression patterns to produce a phenotype of long life and good health. That, according to Bell, is the way the new genetics will be employed in clinical practice.

The New Genetics and the Doctor/Patient Relationship

An article titled "Gene Tests and Tradition: Emerging Gene Sciences Will

Reunite Patient and Doctor," by Dr. David Shaywitz and Dennis Ausiello, appeared recently in *Hippocrates* magazine. That concept contradicts what many doctors thought, which was that the human genome and molecular biology, when absorbed into medicine, would dehumanize medicine and create more distance between doctor and patient. Instead, according to these authors, the information from the Human Genome Project is reuniting patients and doctors in ways that can personalize medicine. Nearly 60 years ago Dr. Roger Williams described a genetotrophic theory of disease, in which we modify and personalize an individual’s environment to maximize his or her genetic expression for long life and good health. Realization of that theory may be on the horizon for the new medicine. We will describe this anti-senescence program in relation to the practice of the new medicine.

Some notable examples of the application of these concepts in anti-senescence medicine include cardiovascular disease, insulin resistance, oxidative stress and its relation to modified proteins and immunological responses, autoimmune disorders and how they relate to central nervous system dysfunctions associated with aging, such as dementia, Parkinson's or Alzheimer's disease. We will also look at this in relation to autoimmunity or one’s becoming allergic to him/herself as he or she grows older. Those are some examples of the application of anti-senescence medicine.

Dysinsulinism and dysglycemia are associated with premature senescence not only in humans, but in animal as well. Forty years ago, Dr. Gerald Reaven, an endocrinologist at Stanford University School of Medicine, made some fundamental discoveries about the role of insulin in glucose management and glucose economy, particularly in what was later to be called type 2, or maturity-onset diabetes. In these individuals the problem is not insulin insufficiency, but insulin resistance and hyperinsulinemia. We have been discussing this topic for some time in *FMU*. Hyperinsulinemia/insulin resistance is associated with premature age-related disease risk, including cardiovascular disease, stroke, and possibly colon cancer and certain other soft-tissue endocrine-related cancers. It also relates to autoimmune disorders. It seems to cut across a range of ICD9 diagnostic codes and is a fundamental process of biological aging.

Dr. Reaven explained the hallmarks of insulin resistance, from a clinical assessment perspective, are elevated triglycerides, reduced HDL, increased levels of LDL (atherogenic particles), increased blood pressure, increased visceral adiposity (related to elevated waist-to-hip ratio), and increased plasma uric acid. Uric acid has for years been identified as a cardiovascular risk factor, as well as a risk factor to gout. No one, however, really understood the relationship between uric acid and cardiovascular disease until Dr. Reaven helped us appreciate the role of hyperinsulinemia/insulin resistance in uric acid metabolism and renal excretion.

Importance of Elevated Serum Uric Acid Measurement

A recent issue of the *Journal of the American Medical Association* contained a report from the NHANES I Epidemiological Study (1971-1992 follow-up data), showing that elevated serum uric acid is very closely associated with cardiovascular mortality. The data suggest that increased serum uric acid is an independent and significant risk factor in cardiovascular mortality. The mechanism by which this increased risk occurs could be linked to the hyperinsulinemic/insulin resistance situation Dr. Reaven described. We know the cholesterol-independent risk factor that most defines heart disease risk is high insulin and insulin resistance, even before one experiences type II diabetes. Only 10 percent of those who have hyperinsulinemia go on to develop what is diagnosed as type II diabetes. In the majority of people, insulin resistance serves as a "silent marker" of increasing risk to premature aging, much like elevated blood pressure that has not been measured. One does not know if that person is at risk for stroke or heart attack until the measurement is made. So it is with insulin resistance.

Individuals with increased insulin levels and peripheral insulin resistance also have increased oxidative stress. This emerging story seems to be associated with insulin’s role at the mitochondrion and the uncoupling of function of the mitochondrion, the alteration of its function, such producing increased oxidants at the mitochondrion. Peripheral blood mononuclear cells, for instance, isolated from patients with diabetic nephropathy, show activated oxidative stress-responsive NF-k B and oxidative processes. Therefore, there is a suggestion that oxidative stress, induced by either type I or II diabetes, is tied to risk of heart disease and other disorders associated with oxidative injury to organelles or cellular materials. We will talk more about this in relation to oxidative stress.

Improving Insulin Sensitivity

When we talk about the insulin signaling pathway, we talk about agents that can improve insulin sensitivity. Those agents include regular exercise, a diet low in sugars and balanced with protein and complex unrefined carbohydrate, and the appropriate amount and type of fats. (Those would be more polyunsaturated fats and low levels of saturated fat, increased omega 3 fatty acids, alpha-linolenic acid and the omega 3 oils like EPA, which are able to help improve cell membrane function and structure, and increased insulin sensitivity and glucose transport.) A variety of accessory nutrients also seem to improve insulin sensitivity and glucose transport. These accessory nutrients include chromium and vanadium, vitamin E, and antioxidants like lipoic acid.

Considerable literature published recently shows how insulin-sensitive pathways and glucose transport can be improved by lipoic acid supplementation. These are studies both in cell cultures with adipocytes and in animal models or even in humans. A study that appeared recently in *Diabetologia* looked at insulin-sensitive pathways in cell culture work with adipocytes. The observation that 2-deoxyglucose transport is

sensitive to protein kinase inhibitors, even in the presence of lipoic acid, indicates a direct effect of lipoic acid on the insulin receptor.

Effects of Lipoic Acid on Glucose Metabolism

We can look at the differential effects of lipoic acid on glucose metabolism in insulin resistant skeletal muscle. An animal study recently published in the *American Journal of Physiology*showed the role of lipoic acid in improving insulin sensitivity and glucose transport in muscle. Another study described the effect of insulin and lipoic acid in lean and obese animals, It showed that obese animals were more insulin resistant. Lipoic acid exhibited greater efficacy on lean muscle.

Lipoic acid plays a role in improving mitochondrial function and reducing mitochondrial-induced oxidative stress. Its role may be one of multiple functions of lipoic acid in insulin sensitization, at the cell membrane-binding site for insulin, the post receptor site for glucose transport, and the mitochondria related to oxidative signals. Work from Lester Packer's laboratory at Berkeley shows that cytosolic and mitochondrial systems respond to lipoic acid very strikingly, and lipoic acid is a central redox-active nutrient for protection of mitochondrial function. In human studies, lipoic acid supplementation improves glucose transport and insulin sensitivity. Blood levels exhibit a dose/response. Lipoic acid supplementation in individuals with elevated proinflammatory cytokine levels increases cytokine-induced glucose uptake. Since cytokines are also known to increase insulin resistance, lipoic acid acts as an insulin mimetic under these conditions.' Lipoate, therefore, along with an insulin-sensitizing diet and regular exercise, has widely ranging value.

Prevalence of Insulin Resistance

As Dr. Reaven explained, 20 percent or more of the nondiabetic population—the normal, apparently healthy population—carry some degree of insulin resistance. These individuals have increased risk for premature aging and age-related diseases associated with insulin resistance such as heart disease, heart attack, stroke, certain forms of cancer, and arthritis. "Insulin Resistance—A Secret Killer?" is the title of an editorial published in the *New England Journal of Medicine.* The author discusses the role of insulin resistance in a variety of age-related diseases.

The disorder called syndrome X, which Dr. Reaven proposed, is the low HDL/elevated triglyceride, central adiposity, and elevated blood pressure/uric acid syndrome. It is a condition of gene expression, modified as a consequence of diet and lifestyle. Syndrome X is a modifiable, age-related condition that is not just waiting for a drug to cure it. In fact, the reason it may not be amenable to single-drug treatment is that the control of insulin and glucose dynamics is dependent on the interaction of many gene products and does not necessarily reflect the failure of a single enzyme. Therefore, one drug that might influence one gene process would not necessarily cover the bases. A lifestyle, diet, and environmental control program, on the other hand, can influence multiple elements and many genes, giving rise to the phenotype of improved function. That process is described in a review titled, "Syndrome X: Medical Nutrition Therapy."

Single-point interventions may not be successful in multi-gene conditions of mid-life. Lifestyle, nutrition, and environmental approaches that are multifactorial and give rise to multi-gene activities in terms of expression patterns are preferable. This model differs from the pharmacological model of single agent for

single endpoint, in which most doctors were trained. Now we are talking about multiple interventions for multiple gene expression patterns to give rise to an overall phenotype associated with improved health and longevity.

Oxidative stress is a second modifiable risk factor for biological aging. Not everyone would agree that oxidative stress is a modifiable risk factor. Some may feel it is preprogrammed into the genes of certain individuals. This is not so likely, however, because you can find varying levels of oxidative stress in individuals, based upon their diets, lifestyles, and activity patterns. Within a certain range, each individual has variable oxidative patterns, depending upon what his or her genes are being exposed to, or how the genes are being expressed based on the person's environment.

Molecular gerontologists suggest the mitochondria are the seat of aging. I don't want to imply that understanding mitochondrial genomes and their expression will unlock the secret of aging. Certainly, death-signal integrators have been identified, which are associated with mitochondrial oxidative functions. The mitochondrion, the cell's Pandora's Box, contains potentially harmful proteins that it keeps hidden away. Activation of these proteins sets in motion the programmed cell death we call apoptosis. This process can produce early cell senescence and mortality, the results of which, on the tissue and organ level, are obvious.

Effects of Mitochondrial Membrane Permeability

In many of these pathways, permeability of the mitochondrial membrane is a critical event that results in release of various molecules crucial for this apoptotic cell death process. Such molecules include cytochrome c, and other apoptotic-inducing factors that are often signaled by oxidative shifts within the cell, or in the mitochondrion. I refer to recent work published in*Science* magazine on cytochrome c release and apoptosis induced by mitochondrial targeting of a nuclear orphan receptor for the thyroid hormone T3 molecule. A relationship exists between T3 sensitivity, or thyroid function, mitochondrial function, and the signals delivered to the mitochondria which result in the release of various messengers associated with cell suicide of the mitochondria, the so-called apoptotic cell death. The process is a means of protecting tissues from mitochondrial free radical generation. The enhancement of oxidative stress within the cells, damages cellular membrane constituents (like oxidation of the unsaturated tails of fatty acids within membranes of phospholipid components), DNA strand breaks, and DNA oxidation. It is a general shift in cellular function. It is useful, at least teleologically, to eliminate cells which have accumulated such damage.

One might ask if it requires alterations in triiodothyronine (T3) for this to occur, or if other variables can also increase mitochondrial oxidative stress. The answer is that many other variables have been identified. They include dysinsulinism, radiation-induced factors, heavy metals, including such toxic metals as free iron, which induce oxidative reactions. Other variables are various kinds of chemical pollutants. The cytochrome P450 families associated with mitochondrial function increase oxidative release when one is exposed to xenobiotics. The mitochondria are exposed to many variables that could create an increased shift of oxidative machinery toward apoptotic cell death. Mitochondrial free radical generation, oxidative stress, and aging, therefore, are tightly interrelated, according to an exhaustive body of literature published over the last few years. A review of this topic appears in *Free Radical Biology & Medicine.* It is titled "Mitochondrial Free Radical Generation, Oxidative Stress, and Aging."

Effects of Uncoupling Mitochondrial Oxidative Phosphorylation

Uncoupling of mitochondrial oxidative phosphorylation can be initiated by nitric oxide and the result is the formation of peroxynitrite ($O_{2+NO\ ONOO}^{-}$). Peroxynitrite is associated with chondrocyte matrix defects and poor mineralization of bone, and this plays a role in joint degeneration seen in osteoarthritis. Therefore, in addition to heart disease or cancer, cross-organ effects of oxidative stress can be related to changes in connective tissue composition, resulting in changes in bone formation and bone remineralization. These processes that uncouple or modify mitochondrial function have a number of consequences, all of which, in the whole organism or human, may be associated with increased risk of biological aging.

We can assess oxidative stress associated with mitochondrial dysfunction or mitochondrial shifts in oxidative chemistry in a variety of ways. One common way is the thiobarbituric acid method for evaluating remnants of oxidized lipids like malondialdehyde (MDA), or 5-hydroxynonenal. The tests are fairly nonspecific, but they provide a gross measure of whole-body oxidative chemistry. I emphasize that you are not looking at the mechanism or the early warning sensitization, but residuals of high levels of these markers of oxidative stress in plasma certainly indicate something is going on that is worthy of attention.

Indices of Lipid Peroxidation

Drs. Meagher and FitzGerald reviewed the strengths and weaknesses of indices of lipid peroxide and peroxidation *in vivo*. They discuss oxidant stress measurements by this technique and warn us to be cautious about overinterpreting urine lipid peroxides or serum lipid peroxides. Although peroxide measurements are reasonably noninvasive, peroxides can be generated in a number of ways that may not be specific to a disease. False negatives may appear, and one may still have organ-specific or tissue-specific oxidative stress without having elevated lipid peroxides. As a screening tool, if they are done correctly in the laboratory, however, these tests can have clinical value.

We can also measure DNA damage in lymphocytes by measuring 8-OXO-deoxyguanosine, a DNA base that is sensitive to oxidative stress. Oxidative stress accounts for about 50 percent of the 8-OXO-DG levels in plasma. The central nervous system is very highly oxygenated and does not have a highly evolved antioxidant protection system. Therefore, oxidative stress is a major contributor to the overall burden of these oxidized DNA remnants, 8-OXO-DG. Using new and more sensitive techniques, laboratories can now measure the 8-OXO-DG level with reasonable reproducibility.

Measuring Oxidative Damage

Methodology was recently published for a simple, more robust means of analyzing 8-OHO-DG in DNA. Dr. Bruce Ames at the University of California at Berkeley has been studying this for many years and has worked to develop new methodologies and more sensitive and reproducible laboratory techniques. One needs to be cautious about the laboratory he or she uses in measuring the oxidized forms of guanine. The sensitivity of many labs was not good enough in the past, nor were the results reliably reproducible to give the data clinical specificity. New methods of measuring 8-OHDG are improving our understanding of DNA damage as a consequence of oxidative stress. This damage ties back to mitochondrial uncoupling and oxidants produced principally through mitochondrial oxidative processes.

New methods of evaluating unsaturated fatty acids that have been oxidized by nonenzymatic processes have also been developed. These isoprostanes may be a sensitive indicator of various oxidant stress-related diseases like asthma. A recent paper in the journal *Lipids*discusses the use of plasma isoprostanes for measuring severity in mild asthma. With appropriate treatment, the patient's isoprostane levels go down. These prostaglandin-like molecules, formed by nonenzymatic reactions with oxidants in the cell, cause fatty acids to form these prostanoid-like structures indicative of this oxidative stress process, or "cells on fire." This is another technique for measuring aspects of oxidative stress that has now been clinically correlated with a variety of diseases like asthma, heart disease, and arthritis. Therefore, in addition to serum and urinary lipid peroxides and 8-OHDG in plasma, we can now measure isoprostane levels in the plasma.

Measuring Protein Carbonyls

We can also look at protein carbonyls, which result from oxidant damage to proteins. They can be studied in the plasma as well. A number of investigators have studied them to determine if they are a useful clinical marker of antioxidant barrier impairment in the plasma. In one recent paper, investigators found a very close correlation between clinical indices of juvenile chronic arthritis and the amount of protein carbonyl present in children's plasma. These are remnants or byproducts of oxidatively damaged proteins.

We have lipid peroxides for oxidatively damaged fatty acids. We have isoprostanes, which are a measurement of oxidative damage to arachidonic acid; we have the 8-OXO-DG measures for the oxidative damage to DNA; and we have protein carbonyl analysis for measuring damage to proteins. By using different techniques, we can get a mosaic pattern of oxidative stress associated with a variety of age-related dysfunctions.

Measuring Antioxidant Status

This pattern of oxidative stress is indirectly related to antioxidant status. Oxidative stress depletes antioxidants. Techniques have been developed to measure antioxidant-buffering capacity in the blood. One assessment of antioxidant status and its resistance to oxidative stress is the ORAC test (oxygen-reducing absorbence capacity). This test exposes plasma, *ex-vivo,* to an oxidant in the laboratory. One can then look at the resistance of the plasma to that defined oxidative stress. It is an indirect measurement of redox capacity of the plasma, which is related to antioxidant status. The ORAC test and other measures of antioxidant status were recently reviewed in the journal *Nutrition.* You can measure damage that has occurred from oxidants, with tests like the 8-OHDG analysis, the protein carbonyls, the lipid peroxides, or the isoprostanes, or you can measure the resistance to damage using the ORAC test.

Oxidant Stress and Cardiovascular Disease

Once you understand if a patient is under oxidative stress, you can determine the relation of that oxidative stress to his or her clinical condition. In relation to cardiovascular disease, an increasing body of literature discusses the interrelationship between cardiac oxidative stress and cardiac disease. A good review in *The American Journal of Medicine* discussed the relationship of oxidative stress and cardiac disease. Ischemic conditions are closely associated with increased oxidative stress. It seems paradoxical that low oxygen delivery to the tissues increases oxidative stress, but that is exactly what happens. Low oxygen tension in

a tissue produces an allosteric transformation of an enzyme, xanthine dehydrogenase, which becomes xanthine oxidase. It increases the production of superoxide, which, in the presence of iron, is dismuted into hydroxyl radical. Along with hydrogen peroxide, hydroxyl radical is a very powerful oxidant that can induce LDL oxidation and is associated with atherogenesis.

According to Dr. Steinberg at the University of California, San Diego School of Medicine, along with many other investigators, oxidative modification of lipoproteins has a powerful effect on increasing atherogenic risk. A review paper from Dr. Steinberg and his colleague Dr. Chisolm appeared in *Free Radical Biology & Medicine.* They discuss this LDL oxidation concept, which was at one time considered "theory" but is now considered a fundamental mechanism related to the initiation of atherosclerosis.

Oxidant Stress in an Animal Model

In an animal model, researchers reduced antioxidant capability. By creating Knockout mice for the formation of apo E and exposing them to oxidant stress, they caused the mice to have a much higher incidence of heart disease. That information appeared in a series of recently published papers. Researchers studied Knockout mice with increased oxidative stress potential. They found supplemental levels of the antioxidant coenzyme Q10 could ameliorate the gene effects from these Knockout mice that would have induced early-stage atherosclerosis. Although this animal trial doesn’t prove anything in humans, the mechanisms shared between the marker animal in humans are similar, if not identical. One can at least make a strong assumption that what we observe in the whole human through epidemiological trials can be tracked back to the mechanism we see in these animal models.

We derive a range of natural antioxidants from foods we eat in a complex diet. In addition to vitamin E, CoQ10, lipoic acid, and acetyl cysteine, we ingest a range of flavonoid and carotenoid molecules. Unprocessed foods are generally colorful foods. Processing produces primarily "white" foods from which the beneficial nutrients have been refined away as mere "flotsam and jetsam." Whole, unrefined fresh fruits and vegetables and whole grains contain an array of bioactive molecules and redox-active substances that preserve biological activity of the endothelium and help us resist oxidative stress and LDL oxidation. They also reduce peroxynitrate-induced damage, the nitric oxide-mediated effects that may be associated with oxidation. Dr. Balz Frei and his colleagues at the Linus Pauling Institute and in Corvallis are conducting research on the role of these natural antioxidants and preserving biological activity of the endothelium. This work is described in an article titled "The Role of Natural Antioxidants in Preserving the Biological Activity of Endothelium-Derived Nitric Oxide," published in *Free Radical Biology & Medicine*.

Oxidized LDL is an unusual protein. Because the immune system is not familiar with this protein, an immunological reaction occurs. That reaction to oxidized proteins brings into play the immune and inflammatory mechanisms of heart disease. A web of interacting variables beyond cholesterol may be associated with the early stages of premature aging of the vasculature and ultimate premature mortality. Immunological responses to oxidized LDL induce an autoantibody or an antigen/antibody reaction. The immune system is activated. Cell-signaling molecules, such as intracellular adhesion molecule 1(ICAM-1) or vascular cell adhesion molecule (VCAM-1) are released from white cells. These surrogate markers for angiogenesis and atherosclerosis are associated with risk of both heart disease and cancer. We can cut across a variety of disease entities from similar processes associated with premature senescence, or biological aging.

An article that appeared in the *Journal of the National Cancer Institute* showed that serum-soluble VCAM-1 is a surrogate marker of angiogenesis in cancer. It is also associated with increased risk of heart disease through the transmigration of leukocytes across the vascular endothelium process I described.

Chronic Inflammation in Premature Aging

Chronic inflammation is another fundamental process in premature biological aging. A few years ago in *FMU* we discussed a *Journal of Neurology* paper that pointed out that patients who regularly took ibuprofen to manage arthritis had a lower statistical incidence of Alzheimer's disease (1996;47:425-432). That study started people asking about the role of nonsteroidal antiinflammatory drugs (NSAIDs)in preventing Alzheimer's disease. It appeared to be through this inflammatory pathway occurring in the brain associated with the formation of neurofibrillary tangles and amyloid plaques, but the mechanism was not clear. The mechanism still has not been fully described, but we are starting to see that NSAIDs may be useful in reducing Alzheimer's risk by altering glial cell inflammatory processes. Microglial cells are the brain's immune system and can be upregulated in inflammation by signaling molecules.

This is related not just to heart disease and cancer, but also to neuronal death or perhaps even dementia and Alzheimer's, as another possibly modifiable factor in premature aging. Once we understand that a person has chronic inflammation, we can modify the expression of those inflammatory mediators through lifestyle, diet, and specifically tailored interventions. An article in *Nature Medicine*, titled "Ibuprofen, Inflammation and Alzheimer Disease," tracks the status of this field since the first report from an epidemiological study appeared in *Neurology*.

Modifying Inflammation With Fatty Acids

Inflammatory mediators, the second-signal messengers of inflammation, the leukotrienes and the proinflammatory prostanoids such as the 2-series prostaglandins, can also be modified by the kind of fats we eat in our diet. The omega 3 fatty acids, alpha-linolenic acid and eicosapentaenoic acid (EPA), can prevent arachidonic acid accumulation and reduce the potential for proinflammatory formation of these second-signal messengers. This topic is discussed in a paper in the *Journal of Nutrition*, titled "Addition of Eicosapentaenoic Acid to g -Linolenic Acid-Supplemented Diets Prevents Serum Arachidonic Acid Accumulation in Humans." The authors show that arachidonic acid accumulation in humans can be prevented by supplementation of 3 grams per day of EPA and omega-6 gamma linolenic acid (GLA), from primrose oil, borage, and back current seed oil. A combination of EPA and DHA blocked accumulation of arachidonic acid and blunted the inflammatory response of these second-signal messengers.

Chronic infection upregulates the genetic expression of these inflammatory mediators. Infectious agents could be *Chlamydia pneumoniae*, *Helicobacter pylori*, or gut infection associated with dysbiosis. Individuals with certain HLA genotypes like HLA-B27, are more sensitive to enteric bacteria in their gut that can upregulate the formation of proinflammatory cytokines and be associated with spondylarthropathies.

Functional Gastroenterology and the Aging Process

Gut ecology, the functional gastroenterological barrier of defense, and gut mucosal integrity play a role in

the aging process. Sixty percent of the immune system is clustered in the gut-associated lymphoid tissue (GALT). Gastrointestinal function is another modifiable factor of aging. The 4R™ Program, which stands for Remove, Reinoculate, Repair and Replace, gets rid of the foreign invaders that upregulate immune function, adds back the friendly, symbiotic bacteria, aids digestive function, and helps repair the gastrointestinal mucosa.

Antioxidants play a role in preservation of gut function. Animal studies indicate that resveratrol, an antioxidant found in red wine, grapes, and peanuts, helps protect against aberrant crypt formation in the colon as a consequence of exposure to carcinogens. This conclusion again shows the complex functions of antioxidants and oxidants and their relationship to age-related disorders.

Diet and Brain Function

Cognitive impairment is one of the most significant problems associated with premature senescence. Diet has a lot to do with cognitive function and preservation of central and peripheral nervous system function in aging individuals. A paper in the *American Journal of Clinical Nutrition* in 1997 discussed dietary intake and cognitive function in a group of older-age individuals. It showed that diet does play an important role in protecting against the loss of mental acuity.

Dr. John Lindenbaum, a neurologist at the Columbia School of Medicine and former *FMU*Clinician of the Month, has published a number of papers on neuropsychiatric disorders related to insufficiencies of cobalamin and folic acid in the absence of anemia, or macrocytosis. In one of these papers, published in the *New England Journal of Medicine*, he showed that a variety of neuropsychiatric disorders in older individuals, which might even be called Alzheimer's-like, were really related to vitamin B12 and folate insufficiency. B12 plasma levels in these patients were within normal range, and the patients did not have pernicious anemia or macrocytosis. On supplementation, their clinical neurological conditions improved markedly. The best markers, according to this research, are plasma homocysteine or methylmalonic acid levels, which are much more sensitive indicators of activities of folate and B12 than macrocytosis.

Cobalamin

Cobalamin, vitamin B12, is poorly absorbed as a consequence of loss of parietal cell function, which is common in older individuals. Various disorders are related to parietal cell atrophy. One common disorder, atrophic gastritis type B, causes a loss of both of stomach acid secretory ability and intrinsic acid secreting ability. Sufferers may have difficulty absorbing B12, which may be further complicated if they are taking acid-blocking drugs like H2 blockers, which further decrease the ability to absorb vitamin B12. These individuals, therefore, have a functional B12 deficiency even though dietary evaluation indicates B12 adequacy. This information is contained in a paper in the *American Journal of Clinical Nutrition,* titled "Cobalamin, the Stomach, and Aging."

Insufficiencies of vitamin B12, folate, and B6 also translate not only into dementia but also into alterations in DNA methylation patterns. These alterations can alter gene epigenetic signaling, turning on or turning off different genes that need to be properly methylated. The inappropriate turning-on or -off of these genes may increase the risk of certain gene-response difficulties we call malignancy. A recent paper in the *American Journal of Clinical Nutrition* is titled "Genomic DNA Methylation Decreases in Response to Moderate Folate Depletion in Elderly Women." It explains how that modulation in gene

expression influences increasing cancer risk in women and men—colon cancer, breast cancer, and perhaps even prostate cancer. It is a nature/nurture interrelationship with folate and B12, seen with elevated homocysteine and methylmalonic acid levels.

Polymorphism in the Use of Folate

Polymorphism in the use of folate, the so-called C677T methylenetetrahydrofolate reductase polymorphism, is quite common. About 20 percent of the population has this polymorphism. These individuals may not be able to use folate effectively at levels that are acceptable for others. A recent paper in the *Journal of Nutrition* shows that one person's diet may be insufficient for another, increasing age-related dysfunctions. This localized folate and B12 deficiency can also relate to squamous cell changes associated with lung cancer and alterations in global DNA hypomethylation defects. This is discussed in an article in *Nutrition and Cancer*.

To conclude, we can do something to affect functional aging. Functional genomics is the frontier of medicine. The possibility of improving functional capability throughout the biological life expectancy of the individual is real. Using the information properly and putting it in the right context, looking for patterns of multi-gene expression will lead to the greatest benefit for patients.

INTERVIEW TRANSCRIPT

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Bioimpedence Measurement as a Patient Management Tool

JB: This month in *Functional Medicine Update*, we have been focusing on biomarkers of aging and ways to reduce senescence or biological age. Our Clinician of the Month, Dr. Donald Hayes, has integrated these concepts into clinical practice. Dr. Hayes has traveled a great deal over the last several years, speaking to and educating doctors about implementing nutritional medicine with his Innovative Practice Solutions programs. Dr. Hayes graduated from the University of Oregon with a BS in Science, was awarded his Doctor of Chiropractic from Western States Chiropractic College, and has been in practice for about 20 years. His practice management concepts have gained national prominence. He brings the concept of healthy aging into his practice through evaluation of biomarkers.

Don, the first question I'd like to ask is how you have integrated the construct of healthy aging into practice through the evaluation of biomarkers and the use of bioimpedence as one method of getting information about the patient's body composition.

DH: The use of bioimpedence analysis in the process of measuring biomarkers is significant. Healthcare

consumers today are quite interested in aging and how they can reduce it or slow it down. They are approaching healthcare providers of all disciplines with questions. Most consumers want to see some objective indication of where they are in the aging process and how to measure it. Bioimpendence analysis, or BIA, is an objective, noninvasive way to measure a patient, to demonstrate to where he or she is, and provide a guideline for continued monitoring of the patient. When you, as a practitioner, ask a patient to do something, or make a lifestyle or nutritional change, you have an objective device that can monitor the patient's improvement or lack thereof.

Bioimpedence and Biomarkers of Aging

JB: In their book, *Biomarkers of Aging*, Dr. Bill Evans and Dr. Irving Rosenberg talk about sarcopenia, loss of muscle mass, as one principle of aging. I think they even coined the term, sarcopenia. BIA tells us something about body composition. How do you weave that information into the concept of biomarkers of aging?

DH: The device itself can measure a couple of key areas. Body composition is critical, of course, and it measures things like fat-free mass, fat mass, fluid indexes, and toxicity status. It also includes other measurements, such as phase angle.

Quite a bit of research today demonstrates that phase angle is a direct measurement of cellular health and cellular function, independent of a patient's body fat. In one of his papers, Ott said he felt phase angle was the single best predictor of prognosis. A lot is being discovered about BIA today. You're right about the book by Rosenberg and Evans, which indicates there is a great misconception about people feeling that aging is synonymous with illness. They have demonstrated it's clearly not that way. Some people with energy loss don't necessarily have a diagnosed disease. Rosenberg and Evans created this category called sarcopenia, and they feel it's important to measure and monitor it. They listed 10 key biomarkers of aging in order of importance and called the top four "the decisive tetrad." A BIA machine can measure three of the top four in the decisive tetrad. I think it's a device that practitioners of functional and nutritional medicine today would benefit from using.

The Top four Biomarkers of Aging

JB: I'm sure our listeners are wondering what the top four biomarkers of the decisive tetrad are. Would you tell us?

DH: The number one biomarker agent, according to Rosenberg and Evans, is muscle or fat-free mass—lean body mass. We certainly need a device that will measure that. The second biomarker they list is strength. The BIA machine will not measure strength, but we can use a hand dynomometer or old-fashioned push-up as an effective way to measure and monitor strength. The third biomarker is basal metabolic rate (BMR), which we know is a direct indicator of lean body mass. The fourth biomarker is fat mass. Those are the top four biomarkers in order of importance, according to Rosenberg and Evans.

HIV as a Model of Accelerated Biological Aging

JB: You mentioned the 1995 paper by Michael Ott, which appeared in the *Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology.* He talked about bioelectrical impedance analysis as a

predictor of survival in patients with HIV. I found that fascinating. We can view the model of HIV as an accelerated biological aging process that causes cachexia, wasting, and loss of muscle mass at an accelerated rate. If you compare that model to the things people do through poor nutrition and lifestyle in later age, there does appear to be something important to be taken from this article as it relates to the power of BIA.

DH: I agree. Ott showed that phase angle was the single best predictive factor of long-term survival among 12 other nutritional parameters. He included things like serum tests of cholesterol, triglycerides, and albumen. That article concluded that phase angle could be used as a new objective criterion of prognosis. In a practical sense, patients want to know why you're asking them to make such incredible lifestyle changes, like getting off certain foods and onto other proper foods, and macronutritionally balancing their life. It helps them a great deal when they can objectively see, through the use of a device like BIA, that they are improving. We know it to be that they're going anabolic on a regular basis. If we can demonstrate this improvement to them by way of a simple tear-off sheet from the BIA machine, it helps them. We all know it's important if we can help patients to see improvement, even before they feel it.

Measuring Improvement and Encouraging Patient Compliance

JB: How fast will patients show a change in BIA when you put them on a nutrition and health improvement program? Is the result reproducible, and do the majority of patients stick with the program?

DH: That's a very good question. We all know that each patient is different and will change at his or her own speed. Some clinicians using BIA have noted substantial changes very quickly. Getting a patient to change his dietary habits of eating no breakfast, skipping meals, and eating donuts and sugar-laden foods, to having some type of a proper breakfast at 10AM and having some kind of macronutrional mini-meal at 2PM, can substantially change the fluid readings, the toxicity reading levels.

Since phase angle is a measure of overall improvement of general cellular health, that can help, too. Adding products such as omega 3 oils has increased the cellular membrane permeability fairly quickly. It can take weeks to effect a substantial change, but in some patients it can change quickly. We don't want to focus just on body fat, because as Evans says, society as a whole isn't over-fat; it’s undermuscled. Focusing on the number one biomarker, lean body mass, and then coaching patients on ways to save lean body mass and, better yet, to add some, creates positive changes. It depends on their commitment level, and whether or not the doctor is set up to counsel patients in that direction, which I think this is the future of health care.

Putting BIA Measurement into Practice

JB: This is something that every practitioner can do in his or her practice. We're not talking about a very significant or traumatic invasion into a patient's life, or even about a high expense. It's applicable to all types of health promotion and disease prevention work. You create an environment in which there is almost no excuse not to use it. People should be involved with this as part of their routine evaluation. If practitioners haven't been doing it in the past, how can they integrate this successfully into their practices and make it work so it delivers improved patient outcome? That's what you've been doing at Innovative Practice Solutions, and your wisdom and expertise in that area can guide us. What have you learned about

the successful application of BIA measurement into a of a program for reducing biological aging?

DH: It begins with a mentor of mine, Mr. Michael Gerber**,** author of *The E-Myth*. He is an organizational consultant and a fan of operating systems. He says success in business is much like a three-legged stool. To organize a business for success, the owner needs to have an equal amount of knowledge and skill in three distinct areas. You need to have technical skills, business skills, and marketing skills. Doctors will be quick to tell us all, and I know myself from experience, that we're probably a little heavy in the area of technical skills. Gerber says that if they focus on just one skill 96 percent of all businesses will not be as successful as they could be.

With that in mind, we need to consider what areas in our practice we need to strengthen. Certainly, the BIA is very scientifically and technically oriented, so it's important how we weave that in to make it work. I feel that the inconsistency in practices is what creates a lot of the stress. We need to have better-trained staff to support doctors better, and certainly knowing how to use the BIA will make it better. I have broken this process down into seven key areas, which, if they are addressed in an operating system format can be integrated into a practice quickly and easily.

Innovative Practice Solutions

JB: This sounds like the seven key points in your Innovative Practice Solutions program. Would you tell our listeners what those seven key areas are?

DH: It begins with a basic growth philosophy in practice, which is to meet patients where they are and lead them where they need to go. Most patients don't come to doctors looking for nutritional medicine, and they certainly are not looking for a BIA. Consumer researchers suggest that most people seek out doctors because they want to get rid of pain. According to a recent article in the *Lancet*, back pain is the second most frequent reason for visits to a physician. I'm sure it's the number one reason for visits to a chiropractor.

When we suggest that doctors consider meeting patients where they are and leading them where they need to go, we're just suggesting the obvious. Step 1 in our program is the suggestion that doctors promote pain relief. In working to relieve pain they should use a natural and nutritional approach when possible, but not shy away from what the patient wants. That's the first part of the component, meet the patients where they are.

Comprehensive, Patient-Directed Care

Step 2 is that in reporting your findings, or in your case presentation, you and your staff need to learn how to offer comprehensive care. That's the other part of the component of leading them to where they need to go. The better able you are to explain comprehensive care, what it entails, its benefits, and how you would incorporate the BIA, the more likely you will be to have patients who want those services. We need to keep in mind what they came for.

Step 3 is encouraging the practice to be patient-directed. By that, we mean don't get pushy about selling the comprehensive care, because patients do need to understand it. If you find patients who don't want to do these things, it means that they just don't understand. You need to have some additional tools, or

patient education materials available to help them understand.

Improving Available Services and Practice Efficiency

Step 4 is making sure you increase your selection of available services. It never ceases to amaze me that doctors want to practice nutritional medicine, but they haven't really posted a menu of things that are available. If you don't apprise patients of all the different services available, it's not likely you'll sell additional services, so you do want to have that selection there, and perhaps you need to get some additional technical training to add these things to your menu.

Step 5, which is a big step, is to improve what we call practice efficiency. We need to know how to see more patients during what I call peak demand times. We are all excited about putting patients in at times when the practice is slow, but research shows that people want to come in at a certain time. You need to deliver consistent product and increase and improve the ability to deliver that service during those peak demand times.

Providing Acknowledgment for Patients and Staff

Step 6 is an important one. Learn how to increase patient satisfaction. We need to make sure we're in the consumer business. We have to give consumers what they want. They want things like risk reversal. If something doesn't work and they're not happy, they can let me know and we'll make it right. We need to acknowledge patients, not just for referring people, but also for when they improve. An example would be seeing that BIA marker move one slight amount. A small movement in phase angle or a small increase in lean body mass is a significant improvement. Acknowledging that helps patients understand that you're a different type of practitioner. They will be excited about it and tell their friends because of that.

Step 7, which might be the most important step of all, is creating some kind of staff-powered bonus system. Offer the staff incentives to help the practice grow. Bonuses and commissions don't seem to work, but if we can build an incentive program around training staff properly, helping them get involved in the practice, they literally think about the practice as their own business. When they do that, it grows dramatically and they feel more of a part of this as a paraprofessional. That's a very integral part.

Implementing the Seven Steps in Innovative Practice Solutions

JB: How does the Innovative Practice Solutions (IPS) program assist doctors in getting the training necessary to reinforce and implement these concepts?

DH: There are several ways. IPS has a series of two-day, staff-powered, team training camps on fundamentals, foundation, and future type care, using the antiaging approach. These camps are scheduled for next year in a number of major cities throughout the U.S. and Canada. We sit down with the doctor and staff person together in the same room, go over these issues using role-playing and scripting.

It's very important if we're going to teach staff to become what we like to call LEAs, lifestyle education assistants, and learn to bill out their services. That includes simple things like explaining macronutritional balancing and exercise, something the doctor doesn't need to do. With this kind of training, staff can become a tremendous asset, not an expense. If that appeals to listeners, they can call IPS and we can tell

them when the next training session would be taking place in their area. In addition, IPS has a 32-page catalogue of specific patient education tools, various practice operating systems, for doctors who are interested in organizing a successful nutritional medicine practice. All they need to do is call IPS. We'd be happy to send them one of those catalogues.

Doctor Reactions to IPM Training

JB: What sort of comments and responses have you had from doctors going through the training program? Has it been an "aha!" experience for participants?

DH: Typically, it has been just that, a big "aha!". I practiced for 18 years and was introduced to the Gerber Method in the early 1980s. I believed strongly in reorganizing my practice and, within months, I went from operating one successful practice to owning and operating five additional practices. I was able to run six offices with less stress and more organization than most doctors who were running one. I know the impact of what organization can do. I know the importance of getting staff involved. We cannot do these things alone. We want to reach more people. We're frustrated as healthcare providers.

Managed Care is asking us to take less and less, and we can't let our staff sit around as bystanders. They need to be part of the solution. Doctors need to empower staff and make them paraprofessionals. Many doctors think they need to hire expensive, high-quality staff. We don't find this is true. They need to find people with big hearts who love to help people. Then they need to take the responsibility of training them. When they do, they don't have to get out of practice. They can utilize the staff to support more people. They get more time out of the practice, which gives them time to renew their interest and commitment to what they want to do. It makes all the difference in the world in how you practice when you have good, high-quality, trained staff working in one direction. The training has been received very well. As doctors we love what we do. That's why we get into health care, but no doctor has ever been accused of being a good businessman or businesswoman. That's not where our hearts are. It's in the area of technical skills. If they are weak in the business areas, I encourage practitioners to seek more of the other skills. Put more legs under the stool with the technical leg. They will see a huge difference in being able to deliver healthcare to the patient and increase outcomes at the same time.

The Future of Antiaging Medicine

JB: Do you think that the antiaging, or reduced biological aging emphasis, is here to stay? Do you think the interface between the new type of patient and the new type of doctor will create this different relationship as we move into 21st century medicine?

DH: From everything I've read, that is clearly the future. Gerald Celente, president of the Trends Research Institute, has written a great book, titled *Trends 2000.* In it he says that Baby Boomers are more interested in antiaging than anything else today. They represent, I think he said, one third of the population, but they have two thirds of the disposal income. They buy anything they want. It may not necessarily be what they need, but they buy anything they want. He feels they want to slow down and reverse the aging process.

Physicians like Rosenberg and Evans in their work state the same thing. In a perfect world, they feel, there would be a national health policy to combat sarcopenia, this wasting process of aging, just like there

is for osteoporosis. They feel there should be a nationwide education campaign aimed at it, a program to treat patients suffering from it, and what they call special sarcopenia clinics, available on a daily basis, to reverse the condition. There are not nearly enough of us out there doing this to fill the demand. As we focus in that direction, I think we will find we're not only very busy, but very satisfied with the outcomes we get for our patients. I believe it's the future, but we can't do it alone as practitioners. We need highly trained, skilled, motivated and incentivized staff to support us to make it a wonderful experience and a wonderful journey.

Contacting Innovative Practice Solutions and Dr. Hayes

JB: Dr. Hayes, I want to thank you. You have provided affirmation and energy as we look to the future. The Innovative Practice Solutions toll-free number is (800) 761-0011. I wish you the very best in your continued education efforts and helping in this transition in functional and nutritional medicine.

DH: Thank you, Jeff. I would also like to tell your listeners that if they have some particular issues they don't feel will work for them, they can call our 800 number and talk to one of my staff about setting up a consultation with me. I travel and speak a lot, but I have set time aside to do that because a lot of doctors feel this won't work for them, or that they're too far buried in their way of practicing. I'd like to talk to them if they feel that would help. I'll make myself available that way, too.

JB: Here in the studio, we have a colleague and friend, and a person whose science I very much admire, Dr. Richard Weindruch, from the University of Wisconsin School of Medicine. Dr. Weindruch is a leader in the field of nutrient modulation of gene expression, specifically on calorie restriction and its impact on gene expression. You have heard me speak of Dr. Weindruch's work on previous editions of *FMU*. Specifically, his papers in the *Scientific American* and *New England Journal of Medicine* made us think about oxidative stress relationships to calories and their processing.'

Calorie Restriction and Gene Expression

Most recently, he published a landmark paper in *Science* magazine the effects of calorie restriction on post-mitotic tissues, the muscle cells in animals, and the influence on gene expression. The calorie-restricted animals did not show the up- or down-regulation in their gene expression associated with those animals that ate *ad lib.* The results suggest they maintain the same kind of gene expression patterns, at least based on the markers that were studied, of the 6347 genes that were more indicative of younger animals. It is a remarkable study and a neat technology using gene arrays and the gene chip concepts that are starting to help us understand how environment influences gene expression over the process of chronological aging.

Rick, welcome to *FMU* and our physician network. What brought you into this area of calorie restriction and its effect on gene modulation?

RW: I have been studying the relationship between caloric intake and aging for my entire 25 years in science. That seed was planted by my mentor, Dr. Roy Walford, in 1975 when I embarked on my PhD studies at the University of California in Los Angeles, where I earned my PhD in experimental pathology. I've continued to study caloric intake and aging, specifically how reduced caloric intake retards aging and disease processes. Two main questions now face investigators in this area. One question is being

addressed in laboratory rodents such as mice and rats, where we know that caloric restriction retards the aging process. The question concerns the underlying mechanisms. That sort of investigation was the type we did, using the gene chip technology you talked about. This work has been done in collaboration with Tom Prolla, a geneticist at the University of Wisconsin in Madison. Our labs have teamed up to look at gene expression in aging and its modulation by caloric intake and other nutrients that we are screening as well.

The other big question in caloric restriction concerns whether or not it will retard the aging process in humans or other primates. To that end, we are carrying out a large study in Rhesus monkeys at the Wisconsin Regional Primate Research Center. We are asking the question, does a 30 percent lowering of caloric intake retard aging in a species very closely related to us?

The Value of Animal Studies

JB: Human experimental biologists or doctors often wonder if there is a similarity between a rodent's physiology and that of a human, and if animal studies have any value in human medicine. I have learned from you that these studies are quite valuable in helping us understand some of these processes. Why animals, what target tissues in animals, and why can we get information that could be of use to the human?

RW: Laboratory rodents provide outstanding animal models that mimic a broad spectrum of human aging changes. We have been focusing our investigations on what were called post-mitotic tissues, such as neuronal tissue. The brain would be largely a post-mitotic tissue, as would the heart and skeletal muscle. The predominant cells in all of these organs share the properties of having very limited repair capacities while utilizing abundant amounts of oxygen for energy metabolism. Therefore, they produce relatively high, steady-state levels of reactive oxygen species, or free radicals.

It's no coincidence that these tissues comprise some of the most serious and stubborn sites of geriatric dysfunction and damage. Loss of cardiac myocytes contributes to heart failure. Loss of skeletal muscle fibers contributes to physical frailty in old age and all of the negative sequelae from that event. Damage and dysfunction to cells within our brain have been linked to problems as severe as Alzheimer's and Parkinson's disease. Dr. Prolla and I have opted, for our initial studies using the gene chips, to profile gene expression. To look at these tissues initially, we are now expanding our inquiry into other sites, including kidney, liver, and other major organs.

Impact of Calorie Restriction on Neuronal Tissue

JB: One of your recent papers looks at neuronal tissue and breaks it down into three tissue types, looking at the impact of calorie restriction. Can you tell us about that as it relates to the nervous system and the observed results?

RW: We were fortunate to have a manuscript published by the journal *Nature Genetics*, last July. In this study we compared the cerebellum and neocortex, two brain regions, from mice. To learn about the effects of aging, we compared 5-month-old versus 30-month-old, normally fed mice. A 5-month-old is a young animal in the prime of its life, and a 30-month-old animal is, I would say, equivalent to a 75- or 80-year-old person, based on the survival characteristics of the mouse strain we were looking at.

To learn about the effects of caloric restriction, we compared the 30-month-old normally fed mice to 30-month-old mice that had been subjected to a 26 percent reduction in calorie intake since the age of two months. One of the real highlights of this study was that genes we classified as being involved in the inflammatory response were markedly upregulated, or activated, as a consequence of aging in both of the brain regions. Further caloric restriction was able to oppose the development of these changes in both brain regions.

We also saw higher levels of messenger RNA, i.e., higher gene activity, or upregulation, for transcripts that Dr. Prolla and I classified as being involved in what's known as the stress responses. Basically, these are genes that are upregulated in response to damaged molecules, mostly proteins, and include some very ancient, highly conserved genes such as heat shock proteins. This same class, incidentally, was also upregulated in the old muscle. In both tissues, brain and skeletal muscle, we observed transcriptional evidence for increased macromolecular damage, and we think these upregulations are consistent with a state of increased oxidative stress, causing macromolecular damage. In other words, the transcriptional findings we are seeing lend support to a far more extensive number of papers describing biochemical changes of this sort.

Calorie Restriction Defined

JB: Caloric restriction, to the uninitiated, might suggest you are subjecting these animals to malnutrition. Could you explain what you mean by calorie restriction in these studies?

RW: That's a very important point. For anything beneficial to occur with caloric reduction, and the reduction is usually in the zone of 30 or 40 percent below some control level of intake, there can be no malnutrition. In my laboratory, we feed animals that are eating the reduced caloric intake a special diet that is enriched in content of protein, vitamins, and minerals. If you imagine a mouse eating for a week's period of time, the animal eating the nutrient-enriched diet, but on a lower calorie intake, eats the same number of grams per mouse per week as the control animal eating the nonenriched diet. We strive to create a situation of undernutrition without malnutrition. With malnutrition, nothing good happens, and I wouldn't be talking to you today.

Hormone Effects on Genes and Aging

JB: Some papers, including one in *Diabetologica* in 1999, and others on development and mechanisms of aging, have discussed the potential role of hormonal flux in gene expression and aging through induction of mitotic changes, perhaps influencing apoptosis. Is there any suggestion from your work of hormone-like effects, say insulin-mediated effects, that have downstream regulatory effects on some of these genes?

RW: I think our data are consistent with the possibility that insulin signaling and insulin-related pathways may be very important in the aging process. This general concept has been quite strongly supported by genetic manipulation of very simple organisms like roundworms, *C. elegans*. There have been some very high-profile studies basically involving genetic engineering of these worms, involving their insulin-signaling pathways such that in most cases, hypometabolic states are achieved and associated with increased longevity.

There is certainly a hypometabolic element to caloric restriction, as well. The mice that are on fairly severe caloric restriction regimens show decreases in body temperature and thyroid hormone T3 levels, which I think can only be viewed as hypometabolic.

Nutrients and Gene Expression

JB: In studying the impact of caloric restriction on gene expression and aging, with the endpoint marker of increasing the animal's life span, do you feel that specific nutritional principles, such as vitamins, minerals, or other accessory nutrients, also influence gene expression?

RW: I definitely think so. We are currently doing experiments that support that suggestion. The important thing coming out of our work with gene expression profiling is that Dr. Prolla and I think we are on the path to having a better assay for aging. Currently, the gold standard to judge if an intervention retards the aging process is to conduct a longevity study. Caloric restriction is viewed as the best tool we have to retard aging because it extends maximum life span. That's obviously an important observation, but that assay of longevity to measure aging is not ideal because of several factors. Most notably, it takes three to four-and-a-half years to do the study. It's very expensive. It occupies a significant fraction of an investigator's productive research life span. Perhaps most worrisome, it doesn't really provide information on an organ-specific basis, about the rate of aging.

Dr. Prolla and I are actually commercializing this effort and starting a company known as LifeGen Technologies. Our goal is to amass a large database that will tell us in individual tissues, such as heart, different regions of the brain, different skeletal muscles, and kidney, what are the largest changes in gene expression that occur with aging. What are the pathways of changes that are occurring if we look at animals at five-month intervals to get an idea of which changes may be causing the other ones at later ages?

With this database of age-associated changes in gene expression, we are becoming positioned to screen the effect of other interventions and nutrients on the aging process on a tissue-specific basis. We are also becoming poised by doing the sequential and careful examination of gene expression changes, say, over five-month intervals in the life span of a mouse. The goal is to pick out potentially critically important genes, the causal genes. Once we can identify these genes, they could be used as targets for drug development by colleagues in the pharmaceutical companies.

Gene Expression Studies and Functional Capacity

JB: One of the difficulties in using death as the endpoint, or life span as the endpoint for these studies, is that many people are concerned about quality as well as quantity of life. As you pointed out, the ultimate cause of death in many of these strains of mice is cancer, so you may be looking at the specific impact of nutrient or caloric restriction on cancer versus its effect on life processes and health span. Do you think gene expression studies will give us more information about functional capacity over time?

RW: I think they will. I think that gene expression profiles will be very informative about the health of an organ and its functional status. These data are derived at the molecular level and are really far upstream, if one considers how cells operate. Indeed, we are talking about transcription process, which precedes translation, which then creates the protein, and then proteins are subject to various modifications and

degradations. What we are probing is really a molecular event that is quite upstream.

Dr. Weindruch as Symposium Keynote Speaker

JB: I want to thank you for spending time with us. Dr. Weindruch will be a keynote speaker at the Eighth International Symposium on Functional Medicine, to be held in Vancouver, British Columbia, in May 2001, over the Memorial Day Weekend. He will educate and enrich us further on the contribution of functional genomics in the development of functional aging and a health span improvement program. Rick, thanks for being with us. We look forward to seeing you next May.

RW: Thank you for this invitation and that one, too

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