December 2001 Issue | Thomas Klug, MD

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Welcome to *FMU* for December 2001. Throughout this year we have been working to improve our understanding of the etiology and mechanisms that contribute to age-related chronic degenerative diseases. Part of our anti-senescence program has been to understand the possibility of ameliorating the course of events we see as aging. We frequently view disease as a natural part of aging. When one searches the literature, however, few articles appear that indicate disease is an inevitable consequence of aging. In fact, contrary to our usual assumption, the literature suggests that from mid-life on, the major causes of morbidity, which are the chronic degenerative diseases, result from a complex interaction of our genes with our environment to give rise to our phenotype.

The outcomes we call disease are, in fact, modifiable based on the environment. This results in a much more flexible, plastic, or modifiable relationship between age and disease than the deterministic model. That earlier model may be a legacy from the Mendelian period of genetics, which indicates that once you've got it in your genes, your phenotype is fixed and there's little you can do about it. We now recognize that although medicine is built on this deterministic model, it doesn't match contemporary thought about the etiology of chronic age-related degenerative diseases.

This month we will continue our focus on the promotion of healthy aging through the modulation of the neuroendocrine/immune system. We might call this area functional endocrinology. No single textbook pulls all this information together. Throughout the past 12 months we have been assembling this information, and through the voices of our Clinicians and Researchers of the Month, we have learned more about functional neuroendocrine immunology. A lot of this field depends on our understanding of functional genomics and functional proteomics. These two constructs, which were not even part of our vocabulary until very recently, are becoming important tools in the way we personalize medicine to the individual patient.

For those of you who are unfamiliar with functional genomics and functional proteomics, I will provide brief definitions. Functional genomics refers to the way our genes and the pluripotential messages locked into our 23 pairs of chromosomes are expressed as a consequence of the constitutive and inducible factors that are regulated and translated into ultimate messages that control physiology and cellular function. Functional genomics is another term for biochemical individuality. It has to do with the way the genes are expressed under different conditions. Diet, lifestyle, environment, stress, toxins, trauma, and ischemia are all factors that modify gene expression and the phenotype. This is the genotype/environment influencing phenotype connection.

Once the gene has been transcribed and translated, functional proteomics refers to the way it ends up as

active protein, or something that influences cellular function. We should not assume all DNA in our genes that is being transcribed is ultimately translated into active protein. Evidence suggests that only about 50 percent of the genes that are transcribed into messenger RNA ultimately end up in active protein. Translation of messenger RNA into protein depends on ribosomal protein synthesis, which depends on a variety of environmental considerations. Proteomics, then, includes the post-translational modification of those proteins after they are synthesized on the ribosome. The modification includes, among other things, phosphorylation, sulfation, glycation, and oxidation. These post-translational effects influence the ultimate folding or functional personalities of the protein.

Glycosylated Hemoglobin

Hemoglobin A1C or glycated hemoglobin is an example. We know that glycation is a post-translational modification of the protein hemoglobin resulting from the nonenzymatic reaction of glucose with the epsilon amino group of lysyl residues on the beta chain of hemoglobin. Thus you get a glycated protein, a modified protein, with attached sugars that change its personality and its function. That's an example of a non-genetic-modification of protein.

Proteomics is a measurement of both the transcription and translation processes and also the epigenetic effects that influence protein or enzyme activity. The combination of functional genomics and functional proteomics is essentially the study of cellular phenotype—tissue, organ, and organ system function. Many variables, mind/body connections, influence the genotype/phenotype relationship. Environmental toxins, chronic infection, inflammatory agents, drugs and medication, and alcohol all influence specific relationships within the genotype/phenotype connection and contribute to our understanding of functional genomics and functional proteomics

In previous issues of *FMU* I've said we should not assume that the way we learn genetics is the way it actually operates in human systems over decades of living.

What most of us learned was what is often called Mendelian genetics. Gregor Mendel elucidated recessive and dominant traits and the inheritance factors we receive from the sperm meeting the egg. His research described a deterministic view of characteristics that are hard-wired into our phenotype. He determined we can do nothing to escape from these recessive or dominant characteristics. If they affect critical genes they can be very serious, leading to premature death or at least premature illness.

We know this from work that has been done on genetic metabolism diseases or disorders of infancy, such as Tay-Sachs, Wilson's, Gaucher's, Fabry's homocysteinemia, and phenylketonuria. These are classic examples of gene mutations that relate to potentially lethal characteristics in the infant or child. Other such examples are Werner's and Huntington's disease. Researchers have identified more than 5000 of these metabolic disorders that are related to single gene mutation(s) that are so severe that the life expectancy, or at least the viability of the organism in its younger years, is jeopardized.

Multifactorial Inheritance Characteristics

Most of the conditions Americans die from these days, however, are not caused by single defects in the

genes that create life-threatening events in infancy or youth. They are conditions that relate to the production of dysfunction over decades of living, which produce coronary heart disease, stroke, diabetes, cancer, various inflammatory disorders, metastatic processes, and type 2 diabetes. These are not single-gene diseases. A recent commentary in *Science*, titled "The Land between Mendelian and Multifactorial Inheritance," discusses this topic.[i] The authors point out that we generally divide genetic disorders into Mendelian and multifactorial characteristics. In the classical Mendelian inheritance, a change in observable features (phenotype) arises as a consequence of mutations in one (dominant) or both (recessive) copies of a gene.

In contrast, multifactorial diseases like diabetes, asthma, heart disease, cancer, and arthritis seem to be caused by more than one gene and with an implied contribution from environmental factors. It is the weaving together of the environment with a number of genes that gives rise to different expression patterns, i.e., functional genomics and functional proteomics, and results in diseases unique to the individual. We lump these disorders together under broad ICD9 descriptors called diagnoses. Coronary heart disease, cerebral vascular disease, or cancer may be a broad descriptor for individual functional changes that have occurred by the interaction of genes with environment to give rise to an individual's unique type of dysfunction, which, for the sake of simplicity, is then called a specific disease.

Severed Trust

In his recent book *Severed Trust*, Dr. George Lundberg discusses the nature of medicine today. The importance of diagnosis has declined significantly in the past decade as our understanding of the mechanisms of disease that come from the interaction of genes and environment has improved.[ii]

Medicine of the future will focus more on mechanisms and function than on diagnosis. That is an interesting insight into the current transition in our medical paradigm.

Diagnosis is still important, but it is only a part of understanding what led a patient to a specific point in his dysfunction and how it might be remedied. We need to look at the unique interaction of the person's genotype with his environment and the expression through the whole cycle into functional proteomics. The genotype/phenotype discussion in this article is a profound marker along the landscape of this change in thinking that we are seeing in the field of medicine.

[i] Burghes AH, Vaessin HE, de la Chapelle A. The land between Mendelian and multifactorial inheritance. *Science.* 2001;293:2213-2214.

[ii] Lundberg G. *Severed Trust. Why American Medicine Hasn't Been Fixed.* New York, NY: Basic Books; 2000.

One example of this changed view is the relationship between smoking and heart disease. Because not every smoker gets heart disease, one might believe, mistakenly, that it is the luck of the draw. It is not just the luck of the draw. It is the interaction of specific features in the smoke and specific aspects of the smoker's genetic susceptibility and the post-translational influences that smoking can have on proteomics. A recent paper in the *Lancet*, titled "Apolipoprotein E4 and Coronary Heart Disease in Middle-Aged Men Who Smoke: a Prospective Study,"[i] described this phenomenon. The authors evaluated the genotypes that are more susceptible to the adverse effects of oxidants resulting from cigarette smoking. They found that the apoE4 apoE4 genotype, in either its homozygous or heterozygous

form, increases the relative susceptibility to oxidant initiators, or proinflammatory agents.

In this study, cigarette smoke appeared to be one of those initiators, and apoE4-the apoE4 allele had significantly greater risk of heart disease than the apoE2 or E3 alleles. That does not mean that those possessing apoE2 or E3 have no risk; it just means their risk is lower than the risk of more oxidant- or inflammation-prone apoE4 individuals.

Genotype-Environment-Phenotype Connection

The editorial that accompanies this article amplifies the genotype-environment-phenotype connection we have been describing over the last four or five years in *FMU*.[ii] According to the authors, our understanding of the genotype-specific effects of smoking on the risk of coronary heart disease is improving. These have to do with transcriptional, translational, and post-translational effects on functional gene expression.

The term "functional" is used more and more frequently in reference to the direction in which medicine is headed. It shows the transition of medicine from a deterministic Mendelian view of disease to this wide array of expression patterns that create dysfunction in the tissue or organ that ultimately arrives at disease at the functional level. Cigarette smoking is interrelated with apoE4 and increased risk of oxidation of LDL, and alteration in endothelial nitric oxide synthase activity in the apoE4 versus the apoE2 or apoE3 individual. We are beginning to recognize these mechanisms and their relationship to detoxification of various substances in smoke that have to do with the cytochrome P450 family and their unique differences in detoxification effects in the different apoE genotypes.

Genetic Risk Factor Analysis

We are beginning to use genetics to analyze risk factors. This ability leads us toward personalized medicine. When the apoE4 genotypes were first discussed, the literature clearly stated that one could do nothing to modify an inherited apoE4 genetic characteristic. With that view in mind, many people may determine they don't want to know if they have the apoE4 genotype. They choose simply to live their lives and hope for the best. It is becoming clear, however, that apoE4 genotype is just a susceptibility factor. It is when the apoE4 genotype is plunged into a "harmful environment" (harmful in terms unique to those characteristics) that the expression into the phenotype becomes a significant risk for disease. The risk associated with apoE4 is not just for heart disease. It is for cancer and the dementia of Alzheimer's as well. Therefore, alcohol, high-fat diets, and cigarette smoking are environmental stressors that increase the risk of these diseases in susceptible genotypes, of which apoE4 is one.

Can you see the new medicine emerging from this type of analysis?

In the 1950s Roger Williams described this as the genetotrophic theory of disease and biochemical individuality. Linus Pauling, in the late 1940s, called it molecular medicine. We are beginning to call it functional genomics and proteomics. It is the basis of functional medicine. It is not just a characteristic, but a characteristic related to the unique genotype of the individual that may translate into the phenotype of disease.

[i] Humphries SE, Talmud PJ, Hawe E, Bolla M, Day IN, Miller GJ. Apoliprotein E4 and coronary heart disease in middle-aged men who smoke: a prospective study. *Lancet.*2001;358:115-119.

[ii] Wang XL, Mahaney MC. Genotype-specific effects of smoking on risk of CHD. *Lancet.* 2001;358:87-88.

Another example is heart disease risk beyond LDL cholesterol evaluation. For several years in *FMU*, we have discussed Paul Ridker's work. He is a cardiology research professor at Harvard Medical School who has been talking about extended risk factors for cardiovascular disease, including the apoA family of lipoproteins, fibrinogen, homocysteine, and high sensitivity C-reactive protein, as biomarkers for heart disease beyond traditional HDL/LDL/total cholesterol relationship. Just as the apoE4 is related to heart disease risk through precipitating factors like cigarette smoking and other oxidant initiators, it is also linked to heart disease through these proinflammatory mediators.

It appears that Rudolf Virchow, the German physiologist, was right on the mark in the 19th century when he said that heart disease, or what now we call arterial sclerosis or atherosclerosis, was an inflammatory condition, not a cholesterol condition. In fact, he was convinced that if you looked at the atheroma or the lesion, it was an inflammatory type of lesion. Only in its later stage did it become associated with cholesterol deposition and calcification; the early state was inflammation.

Predicting Heart Disease

That theme is discussed in a recent *Lancet* article titled "Soluble Adhesion Molecules and Prediction of Coronary Heart Disease: a Prospective Study and Meta-Analysis."[i] This study and meta-analysis of previously published data indicates that elevated measurements adhesion molecules, intracellular adhesion molecule-1, or ICAM-1, and vascular-associated adhesion molecule VCAM-1, (E-selectin and P-selectin), fail to predict the future risk of coronary heart disease.

The editorial that follows this article includes a table that shows that while the adhesion molecule, sVCAM-1, is of little prognostic value, a number of other molecules serves this function well. In particular, the high sensitivity assay for C-reactive protein, a well-accepted inflammatory marker, has a relative risk prognostic value almost twofold that of LDL cholesterol.[ii] If you look only at cholesterol and not at inflammatory mediators, you may miss a risk factor that is twice as predictive of heart disease as cholesterol. These extended risk factors are mediators produced as the individual's genotype responds to his or her environment.

Measuring ICAM-1 or VCAM-1

When we talk about increasing adhesion molecules (ICAM-1, VCAM-1,

E-selectin, P-selectin) or C-reactive protein, we are discussing the production of various inflammatory mediating molecules that are responding to a specific series of stressors in the environment and their interaction with a specific genotype. We are utilizing the tools of functional genomics and proteomics at the applied clinical level. Translation of what may seem to be esoteric variables related to functional genomics and proteomics provides assessment and treatment protocols that will personalize medicine on a functional basis for the individual.

The Hypothalamus/Pituitary Axes

At what level does the modulation of function occur? Where is the interaction between the environment and the organism reflected in the messages that alter gene expression? The emerging answer to those questions is that one site of the mind/body interaction is through the hypothalamus/pituitary axes. These axes are responsible for the interrelationship between neuroendocrine function and the secondary components of the immune and endocrine messaging system that regulate function of every cell in the body.

The outside world consists of sights, sounds, smells, tastes, and things to touch, traumas, electromagnetic radiation in the visible and non-visible spectrums, antigens, and toxins. One way the body senses these phenomena is through the parasympathetic and sympathetic nervous systems. The hypothalamus/pituitary axes, the pineal gland, the amygdala, and the deep structures of our central nervous system interpret these messages and initiate a response. The cerebral cortex is the highly adept higher learning center that supposedly separates us from lower animals.

Creating Homeostasis

Humans have a frontal lobe, and the cortex components of the brain hold these messages, make patterns out of recognized information, and translate it into electrical and chemical signals that control the outcome of function. The translation and sorting of all of these variables through this receiving system ultimately create our individual response to our environment. They influence the expression of our genes in unique ways by up- and downregulating their expression, creating in the process what could be called a new phenotype. This phenotype may reflect a homeostasis of disease. We often think of homeostasis as a word that defines the maintenance of health. It is possible to be in a steady state of disease, or “dis-ease,” as well, however, in which the gene expression patterns are shifted into a state of alarm that stays with an individual, not for an hour, a day, a week or a month, but for years. These shifted patterns become new homeostatic resting points of dis-ease. I call them functional disabilities because they start collecting damage over time and ultimately lead to a chronic related illness that was modulated through the activation or effective changes in the functional state of the hypothalamus/pituitary axis and their response in terms of messenger molecules.
[i] Malik I, Danesh J, Whincup P, et al. Soluble adhesion molecules and prediction of coronary heart disease: a prospective study and meta-analysis. *Lancet.* 2001;358:971-975.[ii] Ridker PM. Role of inflammatory biomarkers in prediction of coronary heart disease. *Lancet.* 2001;358;946-948

Type 2 diabetes, the most common form of diabetes in our culture, is an example of a functional disability. More than 30 years ago, when I was in my post-graduate training, I was taught that diabetes was a disorder associated with the insufficiency of the peptide, or protein hormone, insulin. The beta cells of the pancreas were unable to secrete enough insulin to meet the needs of the individual. Blood sugar became elevated, driving too much sugar into insulin-insensitive tissues of the nerves, kidneys, and eyes. This process activated secondary metabolic pathways called the sorbitol pathway or the aldose reductase pathway, which created cataracts in the lens of the eyes, and nephropathy, or peripheral neuropathy through glucose-mediated dysfunctions on these insulin-dependent secondary tissues. I also learned that diabetes was closely tied to genes, that people had the genetics because it occurred in families.

What I have just described is the type 1 form of diabetes, which constitutes about 5 percent of cases of diabetes. The percentage of a population with type-1 diabetes has remained relatively constant over time. It is even constant in other populations. What has changed is the prevalence of type-2 diabetes. We used

to call it maturity-onset diabetes, but we have to change that term because a rising number of those who are getting type-2 diabetes are adolescents, not adults. We now call it type-2 or insulin-resistant diabetes, which is often associated not with low levels of insulin, but with high levels of insulin, or hyperinsulinemia.

Explaining the Increase in Type 2 Diabetes

In the last several years we have often spoken in *FMU* about diabetes, including Dr. Gerald Reaven's work on syndrome X, and the compensated and non-compensated forms of insulin resistance. What we are starting to recognize is that the most prevalent form of this rising pandemic called diabetes is type-2 insulin-resistance, sometimes hyperinsulinemic diabetes, with glucose abnormalities.

Researchers have worked to find an explanation for the increasing prevalence of this disease. It seems unreasonable to blame it on “bad genes” that are suddenly showing up. It seems more likely that we are witnessing an interaction between genes and a changing environment that alters gene expression and produces unique outcomes. We might suspect that variables associated with the environment/genotype connection (i.e., diet and lifestyle) may be regulating, or at least influencing, the prevalence of type-2 diabetes.

That longstanding research challenge has lacked a definitive answer, but a step toward finding that answer appeared recently in the *New England Journal of Medicine.* This article, which described the work of Walter Willett and Meir Stampfer's group at Harvard, was titled "Diet, Lifestyle, and the Risk of Type 2 Diabetes Mellitus in Women."[i] It resulted from a study of 84,941 female nurses from 1980 to 1996. These women were free from diagnosed cardiovascular disease, diabetes, or cancer at the baseline, and then followed prospectively over many years. The researchers found that the majority of cases of type 2 diabetes in this group of nurses could, in fact, be related to diet and lifestyle. The differences affected the expression of multiple genes and resulted in a phenotype which we call diabetes for lack of an easier definition, meaning insulin dysfunction and dysglycemia.

Diabetes and Lifestyle: Signs of a Paradigm Shift

Willett, Stampfer, et al. conclude there is strong support for the hypothesis that the majority of cases of type-2 diabetes could be prevented by adopting a healthier lifestyle. That is a profound clinical conclusion. We are really talking about the mismatch in the sand pile of genotypes in the U.S. population, with the environment. When a particular genotype is matched with our common environment—diet, lifestyle, and exercise patterns—the result can be a dysfunctional phenotype. In this case the phenotype, for the sake of convenience, is called "diabetic." That is an important part of our evolving model of medicine.

Diabetes is not a disease in search of a drug. It is a functional condition in search of the appropriate environment to create a gene expression profile that relates not to dysinsulinism, but to normal insulin modulation of cellular function. Insulin does not influence only blood sugar. It also influences gene expression—protein tyrosine kinases and other types of transcription factors that are modulated at both the genomic and post-genomic levels. The symphonic orchestration of these mediators influenced by environmental factors then creates a shift in metabolism that we later define as disease.

I hope you can appreciate the power of this emerging model. It represents what Thomas Kuhn called a

scientific paradigm shift in the structure of scientific revolutions. We are witnessing a profound era of transition in thinking in medicine. The medicine we will practice tomorrow as a consequence of the application of this model will be vastly different from that built on the deterministic Mendelian model of single gene mutation producing disease, which then requires single drugs to treat single outcomes. When I say a single drug, I mean a single molecule to create a single outcome to treat a single disease.

[i]
Hu FB, Stampfer MJ, Willett WC, et al. Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. *N Engl J Med.* 2001;345(11):790-797.
Chronic fatigue syndrome (CFS) is a good example of the types of disorders we are seeing in this multigene interrelationship with a multifactorial environment to produce a phenotypic change in outcome. Paul Cheney and his colleagues first identified the post-viral fatigue syndrome that appeared after a bad flu season in Incline Village, Nevada, in the 1970s. Since that time we have seen an explosion of articles about etiological contributors to this post-viral fatigue syndrome, myalgic encephalitis or CFS, with or without fibromyalgia syndrome.

Some common factors have emerged through this multitude of published papers. CFS could easily be related to the activation of the hypothalamus/pituitary axis. There is a neuroendocrine/immune component. This upregulation and initiation can result from a variety of contributors. It may be related to total load of stressors, not to the viral infection by itself. It may be a viral infection on top of chronic stress on top of a trauma on top of a toxic exposure on top of a poor-quality diet. We load the messenger system of the body with more and more perturbing factors until eventually it shifts over to a different steady state, a different harmonic vibration of gene expression patterns we call the HPA disactivated system, the hypothalamus/pituitary/adrenal/thyroid disactivated system.

Many Causes, Many Solutions

Many factors can cause dysregulation and alter gene expression. Therefore, many factors may be associated with its remediation, breaking the link of the cycle. A number of drugs have been tried in an attempt to intervene at the different symptom levels that appear in the CFS patient. One might wonder what the perfect drug could be. If we think of CFS as a polygenic phenomenon, no one molecule, no one drug is likely to solve the problem. It has multiple components that may require multifactorial therapeutic intervention. It may have to be personalized to the individual. That appears to be what we have learned from a recent paper that appeared in the *Journal of the American Medical Association* titled, "Interventions for the Treatment and Management of Chronic Fatigue Syndrome."[i]

This meta-analysis is from the National Health Service Centre at the University of York, in England and the Evidence-Based Practice Center, Department of Medicine, University of Texas Health Science Center at San Antonio. The researchers considered a variety of therapies, which included a total of 2801 participants in 44 trials. Across the studies, they evaluated 38 different outcomes using about 130 different scales or types of measurements. They broke down the therapies into six categories—behavioral (graded exercise therapy and cognitive behavioral therapy), immunological, pharmacological, supplements, complementary/alternative, and other interventions. The researchers concluded that the interventions demonstrated mixed results in

terms of effectiveness. All conclusions about effectiveness should be considered along with the methodological inadequacies of the various studies. There was no set standard of evaluation.

Breaking the Link of Expression

Interventions that showed promising results include cognitive behavioral therapy and graded exercise therapy. What role do those have on link-breaking alterations in expression of the hypothalamus/pituitary/adrenal/thyroid axis? In other words, do these neuroendocrine/immune therapies help establish a different setpoint of gene expression in individuals who have activated and dysregulated HPA/HPT axes? Those are good questions. We know we can use the mind to alter body function. In fact, by thinking through imagery, meditation, relaxation therapy, music, and exercise, we can change the messenger molecules produced in the hypothalamus and pituitary that are sent to the rest of the body, and which ultimately change the cellular phenotype. We might expect the mind to have an influence over the body in a condition that was caused by dysregulation of the HPA axis, and in fact that seems to be what the data in the *JAMA* study suggest.

This research does not suggest that metabolic or other types of interventions are useless. What it indicates is that the answers to these complex syndromes may come, not from single molecules for single conditions, but from integrated therapies that consider the interaction of an individual's genetic pluripotentiality with the environment to produce a better outcome by modulating that environment.

The Mind/Body Connection

An editorial that follows this *JAMA* article is titled "Chronic Fatigue Syndrome—Trials and Tribulations." Its author discusses how this information relates to differing influences of the mind on the body, and how lifestyle variables can play a significant role.[ii] If you believe the word psychosomatic means mind/body, every disease may be a mind/body or psychosomatic disease. In the negative connotation of psychosomatic, as if it was "all in the mind," I don't think we can jump to that conclusion at all. The body is the mind, and the mind is the body. These hormonal messenger molecules we are describing have receptor sites on all cells of the body, as Candice Pert told us in her book, *Molecules of Emotion*.

A relationship exists between molecular events that occur and thought beliefs, behaviors, and attitudes. This study also suggests, however, that real things in the environment (I call it molecular personification of materialisms), can be toxin molecules. Other factors can produce toxic events in the body. You could have toxic thoughts, chemicals, endotoxins, exotoxins, inflammatory mediators. All of these variables we have been describing create a different outcome that can lock a person into a chronic fatigue/fibromyalgia condition for years, making a complex therapy more likely to throw the net over these variables in a positive way.

[i] Whiting P, Bagnall AM, Sowden AJ, Cornell JE, Mulrow CD, Ramirez G. Interventions for the treatment and management of chronic fatigue syndrome. *JAMA.* 2001;286(11):1360-1368.[ii] Wessely S. Chronic fatigue syndrome--trials and tribulations. *JAMA.* 2001;286(11):1378.

Diet can help manipulate these variables. Specific dietary considerations can help lower the body

load of some of these toxins. Some foods contain nutrients that modulate gene expression of the detoxification enzyme systems. One extensively studied family of foods with these properties is the family of cruciferous vegetables—broccoli, Brussels sprouts, cauliflower, and cabbage. We have talked a lot about the role of glucosinolates and how they are broken down by the enzyme myrosinase found in the plant cell. Myrosinase can convert the glucosinolates into secondary chemicals like indole-3 carbinol and phenylisothiocyanate and 2-hydroxy-3-butane and sulforophane. These secondary chemicals influence gene expression in specific ways to upregulate the expression and activity of specific types of either phase I or phase II detoxification enzymes.

A paper describing this process in a human study appeared recently in the journal *Carcinogenesis.* It showed that individuals who consume increased crucifers in their diet and eat charbroiled meats will have increased detoxification of the heterocyclic aromatic amines, the potential carcinogens associated with charbroiled meat. [i]Individuals can activate the detoxification of these carcinogens by consuming broccoli with their steak. The way we have eaten traditionally is by consuming both dietary carcinogens and anti-carcinogens. Nearly 10 years ago in *Science* magazine, Dr. Bruce Ames discussed the balance between dietary carcinogens and anti-carcinogens.

Endogenous Estrogens

On side II of this month's *FMU*, our Researcher of the Month, Dr. Thomas Klug, will talk about estrogen metabolism from the 2- and the 16-hydroxyestrogen metabolic perspective. He will explain that the 2-hydroxyestrogens tend to be anti-estrogenic and the estrogen breaks on mitotic activity and cell replication and cell cycling, whereas the 16-hydroxyestrogen is a highly estrogenic cell mitotic stimulator. We might ask if it is possible to bring about improvement in women whose estrogens running away with them, with increasing 16- and lowered 2-, by improving their 2-hydroxylation patterns. The answer is yes. Dietary variables--soy isoflavones, lignans from various plant foods, and substances found in the cruciferous vegetables specifically indole-3-carbinol and one of its acid condensation products, diindolylmethane-- influence the 2- and the 16-hydroxylation patterns.

Metabolizing Estrogens

A recent paper, again published in *Carcinogenesis,* showed that these catechol estrogen metabolites, the 2-hydroxyestrogens, and their glutathione, glucuronic acid and sulfate conjugates, were related to the reduced risk of mammary tumors.[ii] If you prevented the animal from being able to manufacture 2-hydroxyestrogens, you actually increased the risk of mammary tumor initiation. Again, it indicates that the way we metabolize estrogens may play a significant role in modifying gene expression patterns because these hormonal metabolites are gene expression modulators.

The same thing was found true in a teratogenecity and mutagenicity study on the catechol estrogens, looking at the difference between the 16- versus the 2- and 4-hydroxy estrogens. This study also appeared in *Carcinogenesis*.[iii] This paper showed that 17 b estradiol and estrone are minor stimulators of DNA damage, whereas if you produce more of the 4-hydroxyestrogen or the 2-hydroxyestrogen, you modify the actual production of these mutagenic agents. The

4-hydroxyestrone appeared to be the most powerful DNA-transforming substance.

Changing Gene Expression

This estrogen metabolite increases the risk of mitogenic, cell transforming, and carcinogenic activity. These changes are related to gene expression shifts, the modification of detoxification enzyme expression, which then create differing families of estrogen metabolites. The result depends on which cytochrome is upregulated, cytochrome P450 1A2 or cytochrome P450 1B1, which more converts estrogen into the 16- and the 4-hydroxylated families.

How do you modify this activity? You can't change your genes, but you can change the exposure of genes to certain kinds of communicating agents. Indole-3-carbinol from crucifers, for example, selectively upregulate the expression of the 2-hydroxylating enzymes of the cytochrome P450 family, so it lowers 16-hydroxylation at the expense of increasing 2-hydroxylation. It lowers the 4-hydroxylation at the expense of increasing the 2-hydroxylation. Thus crucifers have a positive effect on normalizing estrogen metabolism.
[i] Murray S, Lake BG, Gray S, et al. Effect of cruciferous vegetable consumption on heterocyclic aromatic amine metabolism in man. *Carcinogenesis.* 2001;22(9):1413-1420.[ii] Devanesan P, Santen RJ, Bocchinfuso WP, Korach KS, Rogan EG, Cavalieri E. Catechol estrogen metabolites and conjugates in mammary tumors and hyperplastic tissue from estrogen receptor-a knock-out (ERKO)/Wnt-1 mice: implications for initiation of mammary
tumors. *Carcinogenesis.* 2001;22(9):1573-1576.

[iii] Yagi E, Barrett JC, Tsutsui T. The ability of four catechol estrogens of 17-estradiol and estrone to induce DNA adducts in Syrian hamster embryo
fibroblasts.*Carcinogenesis.* 2001;22(9):1505-1510.

Androgen receptors and androgen metabolites are associated with human prostate cancer risk. Estrogen metabolism plays a role in prostate cancer, as it does with breast and endometrial cancer risk in women. Recent research indicates that the herbal product silymarin, which comes from milk thistle concentrate, inhibits the function of the androgen receptor by reducing nuclear localization of this receptor and modifying detoxification in such a way as to normalize androgen receptor activity.[i]

We might consider, at least from this study, that silymarin has a positive impact on the activity of androgens or their receptors in the prostate gland. I emphasize this is a human prostate cell line, so this is an *in vitro* study, but it certainly gets us thinking about agents we know that influence hepatic detoxification, i.e., silymarin, exhibit other effects on genetic expression.

The Importance of Color in the Diet

Many of these molecules are part of the flavonoid family. A review in the *American Journal of Clinical Nutrition* discusses flavonoids and modification of genetic expression.[ii]Approximately 4000 varieties of flavonoids have been identified in various foods. These flavonoids are responsible for the attractive colors of flowers, fruits, and leaves. Eating color in our diet, therefore, is important for getting adequate levels of flavonoids. The all-white diet is not so good.

We want to get more flavones, flavonones, catechins, and anthocyanins into our diet. The authors of this article explain how the colored red, yellow, orange, blue, and violet plant foods, both fruits and vegetables, positively influence immunochemical function, reduce cellular proliferation in cell cycling, have antioxidant effects, lower cholesterol, and chelate metals.

We are witnessing the emergence of a new medicine in which the modulators of function will, to a great extent, be things we do to ourselves every day—how we think, how we act, where we live, what we are exposed to, what we eat, whether we exercise. Those factors translate into the outcome called our phenotype. That is exactly what Dr. Klug will be talking about as our representative on side II of *FMU*.

[i] Zhu W, Zhang JS, Young CY. Silymarin inhibits function of the androgen receptor by reducing nuclear localization of the receptor in the human prostate cancer cell line LNCaP. *Carcinogenesis.* 2001;22(9):1399-1403.

[ii] Nijveldt RJ, van Nood E, van Hoorn EC, Boelens PG, van Norren K, van Leeuwen PA. Flavonoids: a review of probably mechanisms of action and potential applications. *Am J Clin Nutr.* 2001;74:418-425

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JB: This month, in keeping with our custom of alternating researchers and clinicians, we have a Researcher of the Month, Dr. Thomas Klug. Dr. Klug, an expert in estrogen metabolism, has done collaborative work with Dr. Leon Bradlow, whom we heard speak on aspects of estrogen metabolism at our Eighth International Symposium on Functional Medicine. Dr. Klug has studied hypothalamus/pituitary/adrenal-related functional status in animals. He has published work on methods of analyzing 2- and 16-hydroxyestrogen metabolites and has studied the physiological effects of these types of compounds as messenger molecules. Dr. Klug will help us understand what is happening in the evolving understanding of the estrogen family, particularly the downstream metabolites and their role in female and male health.

Analyzing Estrogen Metabolites

Dr. Klug, welcome to FMU. As a principal in Immuna Care Corporation in Bethlehem, Pennsylvania, you have focused attention on developing methods of analyzing estrogen metabolites. How did you get interested in this area of research?

TK: My background was in developing immunodiagnostics for cancer research. As a new company, we at Immuna Care wanted to do innovative things in the area of women's health. We noted there were virtually no diagnostic tests that could determine a woman's risk for breast cancer and other estrogen-related diseases. We did to exhaustive research in the literature to get a handle on what kind of tests could be evolved to determine a woman's risk for estrogen-related cancers and also how to manage that risk. This led us into the area of estrogen metabolism.

We noted that several lifestyle factors, diet and exercise, influenced whether or not a woman developed estrogen-related cancers. We discovered, through looking at the literature, that there was a group at the Rockefeller University working on this very question of how estrogen metabolism was modulated by a woman's lifestyle and other factors. Working collaboratively with that group, we developed diagnostic tests for key metabolites of estrogen that are related to a woman's risk factors. These estrogens, and their levels, are modulated by such factors as a woman's diet, how she exercises, whether or not she smokes, and so forth.

We think this has developed into a new paradigm for managing breast cancer, not only its risk. Once a woman has breast cancer, it determines what changes she needs to make in her lifestyle and diet to affect the likely outcome in the course of the disease.

Evolving Understanding of Estrogen Metabolites

JB: Over the years, we have evolved beyond the early assumption that we knew enough about estrogen to treat women successfully through menopause with hormone replacement therapy (HRT). As research has progressed, we have discovered that all sorts of other metabolites, such as the 16-hydroxyestrone and estradiol, may have equal or greater influence on estrogen in female physiology compared to the estrone and estradiol molecules. Has the medical community accepted the information this new research about estrogen metabolites reveals? Are doctors in obstetrics, gynecology, and endocrinology areas beginning to understand its importance?

TK: As with any new finding, there are always people who adapt readily to new information. In general, physicians in the alternative medicine field have been more willing to try new things. Mainstream physicians generally are followers of what they read and are unlikely to use new things. We find people in what we call the alternative medicine field are more likely to be advocates of new things. We do see that mainstream physicians are beginning to use this, but the growing use is primarily in alternative medicine, where people recognize the importance of diet.

It was only when these tests became available that people could use them. It's been known for years that these metabolites exist, and there has even been some recognition that they are important in the disease process. Only when the tests became broadly available in easy formats, which is what our corporation did, did they become tools in cancer management. I appreciate speaking in a forum like this, because perhaps it will accelerate that process.

A History of Estrogen Research

JB: Would you give our listeners a brief history of estrogen research? How long ago were these 2- and 16-hydroxyestrogen metabolites discovered and recognized to be components of the physiological

function of estrogens?

JK: The key work probably took place at the Rockefeller by Drs. Bradlow and Fishman in the early 1960s. They were generally looking at the question of what happened to estrogen in a woman's body. The government has spent a lot of money in this area because it was so exciting. Like anything else, however, there is a crest of the wave and there has to be a conjunction of interests.

We have only recently learned there are ways to modulate metabolism, such as the use of indole carbinol, soy in the diet, and so forth. This conjunction of new natural treatments for breast cancer, combined with the new tests, has exploded the interest in these tests. The actual involvement in recognition of the importance of estrogen in breast cancer goes back more than 100 years. Beatson knew about it when he removed women’s ovaries and discovered their breast cancer went into remission (published in The Lancet in 1896).

Since that time, however, very little progress has been made in taking this information about how estrogens are involved in cancer and bringing it into a useful paradigm.

Soy and Indole-3-carbinol

JB: It's interesting that the number of years you talked about, a period of 40 to 50 years, seems to be a standard period of latency between the discovery of some major new potential advance in medicine and its final incorporation into practice. A number of years ago I published a paper in which I evaluated 10 medical technologies, showing the length of time from their discovery to their acceptance was about 50 years. Perhaps that's a general rule in medicine. You'd think, with today’s rapid rate of information transfer, we could compress that time, but it appears to remain constant for some reason.

I was recently in Finland at the University of Helsinki visiting with Dr. Herman Adlercreutz, another contributor in this area who has published with Dr. Bradlow a couple of papers on indole-3-carbinol. Is the soy and indole-3-carbinol connection to metabolism of estrogen something you see as a rapidly advancing understanding, or are we following the 40-50 year rule on that as well?

The Importance of Metabolites

TK: No. Although it is not yet a standard of care, it is becoming a routine method of treatment. Some people, women in particular because they are generally more interested in their health than men, are beginning to incorporate into their diets vegetables that contain indole carbinol. They know these foods will indeed lower the risk for estrogen-dependent diseases.

One point I want to make is that breast cancer is not the only estrogen-dependent disease. Using the tests for the 2- and 16-, we've shown that estrogen metabolism is involved in head and neck cancer, cervical cancer, and probably colon cancer. The importance of metabolism is what I want to emphasize, beyond estrogen metabolism. Metabolism is an important factor to consider in all disease processes. We've studied in particular how this involves estrogen. The federal government is sponsoring clinical studies looking at indole carbinol, so even the august agencies of our government understand the importance of these compounds in reducing disease risk.

Estrogen Metabolism in Male Health

JB: We don't often consider the importance of estrogen in males. Some strong evidence, however, indicates that prostate cancer risk may be related to imperfections or alterations in estrogen metabolism. What are your thoughts about this evolving concept?

TK: Historically, initial studies regarding the association between altered metabolism and breast cancer were done in 1968 and 1970 in males. Thus it was first noted in males. When males do have breast cancer, their metabolism is drastically altered, even more than in women with breast cancer. This is unpublished information, but a recent study has confirmed the link between prostate cancer and estrogen metabolism.

One problem we have in communicating the importance of this information is the lag between getting the results of clinical studies and having them published. The lag can often be as long as two years. Of course, one cannot talk about these results without putting at risk the publication of the data, but I feel it is very important. I will not mention the specific study, but there is one showing that men with prostate cancer have altered metabolism in exactly the same direction as women with breast cancer. That study will probably be published in the coming year. Yes, estrogen metabolism is important in men. It is equally if not more important than in women, because some preliminary evidence also connects it with heart disease.

2- and 16-Hydroxyestrogen Tests

JB: This information indicates how functional components of our physiology tie together across genders, ages, and even diagnostic codes. That is one of the principles of functional medicine. We are less tied into diagnosis and more interested in understanding mechanisms. The mechanism of estrogen metabolism appears to have a very wide range of potential applications in clinical medicine. Regarding the 2- and 16-hydroxyestrogen tests, what difficulties occurred in the past, and what advances has Immuna Care accomplished in making the diagnosis or assessment more specific?

TK: Dr. Herman Adlercreutz is really the pioneer in this field. In 1980 and 1981, he published the seminal articles about detecting these metabolites using what is called gas chromatography mass spectroscopy. This test is very expensive and time-consuming, however. Only two or three laboratories in the entire world can do it.

What we did was to take modern immunodiagnostic techniques, that is, we used monoclonal antibodies, to make very specific immunodiagnostic reagents to each of these metabolites. We then took these antibodies and converted them into diagnostic tests that could be done quickly and relatively inexpensively. The tests of metabolites in urine, for example, can be done in three hours, and tests for metabolites in serum can be done overnight. The cost of these tests, instead of costing hundreds of dollars, is now in the range of tens of dollars. Essentially, what we did was to convert this into simple, easy-to-use, easy-to-diagnose tests that can be done in any diagnostic laboratory.

Plasma and Urine Samples in Testing

JB: Are the urine and plasma samples used to perform these tests routinely acquired?

TK: Yes. There's nothing easier to get than urine, which is why we like the test for urine. However, as with any type of diagnostic fluid, there are always optimum conditions of collection and preservation of the urine and/or serum. The metabolites in the medium are very stable. Women routinely can collect a urine specimen at home and bring it that morning to the physician for shipment to the laboratory. Or the physician can gather the serum very easily.

Menstrual Cycle and Serum Ratio

JB: Do variables like menstrual cycle or menopausal status alter hormones and influence the 2- to 16-ratio?

TK: There is an effect of menstrual cycle in the serum ratio in women. The effect is small, however, relative to the difference one sees between women with breast cancer as opposed to women without it, or women with benign breast disease. There is, however, a portion of the menstrual cycle that is the recommended time for collecting the samples. In premenopausal women, for both urine and serum, we recommend collection at ovulation or within a week after, during what is called the luteal phase. In postmenopausal women, we simply recommend collecting the first morning urine, because concentrations of the metabolites are highest in the urine in that first urinary void. Postmenopausal women have lower levels, so they are essentially less easy to measure. However, if the urine is collected in the morning, the levels are higher and they can be measured very accurately.

Dietary Variables and Metabolite Measurements

JB: What about the dietary variables? If a woman had a soy beverage the day before the test, but she normally did not consume soy, might it create a significant alteration in her test results?

JK: It depends on the amount she had and also the timing. Within 24 hours, one would expect to see no change. One would not expect to see a change in the metabolite ratio if one simply had a soy drink. The amount of soy required to change metabolism is in the range of 100-200 mg a day for several days. A single change in diet, whether it is cabbage or broccoli, would not affect the metabolism. However, if one had several large portions of these over a period of several days, it might have an effect.

Soy Isoflavones

JB: When you say 100-200 mg of soy, are you referring to total isoflavones?

JK: Yes, total isoflavones. But this amounts to probably 100-200 grams of soy protein, which is a lot of soy. So, one ordinarily takes the soy isoflavones if one wants to change one's ratio. The important thing to realize is that a woman needs to determine what her current metabolite ratio is. After that point, she needs to determine whether or not she needs to change her diet either to increase the ratio or, in some women, actually to decrease the ratio. Having too high a level of what we call the "good estrogens" or the 2-hydroxyestrogens is not good, either. Women with very high ratios are more in an anti-estrogen situation and as a consequence, studies reveal they may experience bone loss. A woman needs to look at factors in her lifestyle and in her diet, to reduce the amount of 2-hydroxylation.

Optimal Estrogen Ratio

JB: Again, we use this as a kind of parabolic curve of optimal range of the 2- to 16- ratio. Too low a ratio of 2- to -16 is not good; too high a ratio is not good. What is the mid-range for optimal estrogen metabolism, according to your work?

JK: A dozen studies, in both the United States and Asia, have indicated (depending on a woman's individual physiological makeup) that one should strive for a ratio of around 2 in the urine test, and about .5 to .6 in the serum test. The ratios are different because one of the metabolites, the 16-hydroxyestrone, binds very tightly to components in the serum, so it clears from the serum less rapidly. This is why, relative to the other metabolites, it is a higher concentration, which leads simply to a lower ratio. However, the ratios do correlate between the urine and the serum if the urine is collected appropriately.

Detecting Increased Risk

JB: Would we conclude that ratios of the 2- to 16-hydroxlated estrogens below 2, as we went down to say 1.6, would be indicative of increasing risk or relative concerns about the highly estrogenic 16s in relationship to the lower estrogenic 2s?

JK:Yes. What is happening physiologically is that the 2-hydroxyestrogens, what we call the "good estrogens," the catecholestrogens, are easily changed, either increased or decreased, by simple changes in diet and lifestyle. The 16-hydroxyestrogens are constitutive. It's more difficult to change them. There are some dietary ways to change the 16, perhaps fish oils, etc., but generally speaking, they are hard to change. What one really wants to look at is the level of the catecholestrogens relative to the 16. They're in the bottom range of ratios, which are probably appropriate, and a woman shouldn't worry is she's in the 2.5 or 1.8 range.

When we look at studies, we speak of them broadly because these are studies done with large numbers of women. This is why it is important to look at individual women and their overall health picture. It is not so much to just say that a woman has a ratio of 1.6. It's important to look, for example, at whether or not that low ratio could be due to the fact that the woman has a high body fat percentage. The ratio is not only a benchmark, but it also gives you a tool to make changes in an individual woman's lifestyle. For example, we might find that if a woman with a 1.6 were to lose 10 pounds, her ratio may go to 2.

Revealing the Larger Health Picture

The ratio tells you not only about estrogens per se, but it also reveals information about the overall medical picture of the woman, her physiology, and her medical history. You might find, for example, that the woman is taking anti-depressants and she has a low ratio. Immediately that sends up a red flag. Is it the anti-depressant that has changed the ratio? If so, let's take the woman off anti-depressants. We know, for example, that some anti-depressants would lower the ratio. Other anti-depressants, for example things like Prozac, seem to raise the ratio. One needs to look at the entire context of the medication the woman is taking, her percent body fat, and her diet, to determine how we should change that ratio if we need to change it, and what factors may be changing that ratio.

The power of this paradigm is that it is all encompassing. It gives a woman a benchmark to make positive changes in her life. After she has made those changes, she can return to the doctor and find out how it has affected her ratio. Is she in better shape now?

Importance of Estrogen Ratio in Breast Cancer

The importance of this is brought home in another unpublished study showing that women with breast cancer, who had a ratio above 2, had a mean survival of greater than 10 years. Women with breast cancer with a low ratio below 2 had a mean survival of much less than five years. So even women with disease need to look at that ratio. It's not only a marker for risk, but for health in general.

Exactly why this is we don't know. This ratio may be a surrogate marker for a lot of other metabolic things that are going on. It may even be a surrogate marker for oxidant/antioxidant balance. I'm not sure, but as we go forward, we're finding that absolutely every risk marker for disease is affected positively within the 2- to 16- ratio. You name any risk factor for breast cancer. It changes that ratio in the appropriate direction. Smoking and alcohol consumption, for example, affect the ratio and are correlated with disease risk appropriately.

Hydroxyestrogen Ratio in Males

JB: Does this ratio you describe in urine or serum of the 2- to the 16- hydroxyestrogens also relate to males? Is that a similar ratio you would see in males, the 2:1 ratio in urine, and the .5:1 ratio in serum?

TK:The mo st potent inducer of the ratio is estrogen itself, as you might expect. When a woman is given ERT, for example, the amount of her natural anti-estrogens, that is the 2- estrogens, increases dramatically. Men, who have less estrogen, tend to have somewhat lower ratios in blood and serum. This probably indicates they need less normal anti-estrogen. What is happening is that the body has a natural mechanism to control the activity of its own estrogen. As the amount of estrogen in the body increases, more is converted to the anti-estrogen, the anti-hormone. As the percent of estrogen in the body decreases, to keep the amount of bioavailable estrogen constant, the body decreases the 2-hydroxyestrogen. We also see higher levels of androgen in men. We believe that androgen, per se, lowers the amount of 2-hydroxylation and the catecholestrogen.

Catecholestrogens

JB: Let's talk about the catecholestrogens. We have been focusing on the 2-hydroxyestrogen, catecholestrogen family, but there is increasing discussion about the 4-hydroxylated estrogens and their production of catecholes that go on to become the quinones. Do you feel the 4-hydroxys are going to prove to be hazardous relative to their apurinic potential in causing DNA damage?

TK: We are in the midst of developing tests for all the metabolites, including 4-hydroxyestrone. However, we have looked at all the literature correlating enzymes that increase 4-hydroxylation and indeed, this probably is an important factor, but it does not seem to be as strongly linked to risk for estrogen-dependent diseases as the 2- and the 16-measurements.

I should say, however, that the enzymes that produce the 4-hydroxyestrone are very similar to that which produces the 16-. It could be that when you measure the 16, indirectly you're measuring the amount of the 4-hydroxy. They tend to go together. The enzymes that produce these, called cytochromes, are very similar. There are specific cytochromes that produce the 4-hydroxy, but these are not found at high levels. The real answer to your question is, we don't know. However, the epidemiological and biochemical

evidence at this time does not point to a strong link between 4-hydroxylation and risk for cancer and other estrogen-dependent diseases.

Estriol

JB: Let's also look at the estriol part of the story. Many doctors in the field of functional endocrinology utilize estriol as part of the replacement therapy, and that relates to the 16-hydroxylation pattern. Would you tell us about that connection?

TK: Regarding the biochemical connection, it appears that the 16-hydroxyestrone is an extremely potent estrogen. It binds to the estrogen receptor that turns on the estrogen receptor inside the cell, and it leaves it on. It is known that 16-hydroxyestrone, which is a normal metabolite of all estrogens in the body, that is estradiol and estrone, is a potent estrogen. However, the metabolite that occurs after the production of 16-hydroxyestrone is estriol. They can be interconverted, but generally, the pattern is that the 16 goes to estriol. The thought has been that if I give estriol, it should not be metabolized into the bad estrogen of the 6-hydroxyestrogen. Indeed, this does make sense biochemically.

There is some evidence, in countries where they use estriol as ERT, that there is a lower risk of breast cancer. However, it's uncertain whether or not this is because they're using estriol, because these countries indeed may have a lower risk of breast cancer, or that the women taking it are in different parts of their life cycle. I would say it certainly is probably somewhat safer to use estriols for ERT. However, there's probably an increased risk for breast cancer in women who are taking estriol, as there is in women who are taking estrone sulfate or estradiol.

Estrogen Replacement Therapy

I would like to make a slight diversion into the estrogen metabolism story, in terms of ERT. We now believe that, using our tests, you can identify women who should not take ERT. We think that women who are on ERT who will go on to develop breast cancer do so because they have an inappropriate metabolism of the exogenous estrogen. We have done some studies that indicate that only 10 percent of women taking ERT are at increased risk for breast cancer, but it is very substantially increased risk. Ninety percent of women on ERT are actually reducing the risk for breast cancer by taking estrogen. Among all women taking ERT, there is roughly a 30 percent increased risk of developing breast cancer at some time in their lives. However, if one removes those women with the excess risk of 10-fold increased risk, the risk in the women taking ERT, the 90 percent, is probably decreased two- or threefold. This is borne out by other studies that looked at using estrogen in treating women with breast cancer.

It's interesting that the best therapy ever developed to treat metastatic breast cancer was high-dose estrogen. They don't do this any longer, because some toxic side effects occurred and some women died from that treatment. However, the majority of the women had total or partial remission of their metastatic disease. We believe this occurred because women with breast cancer need the 2-hydroxyestrogen. The modern dogma is to take away estrogen from women with breast cancer. Give them anti-estrogens. This is also what's happened 40 years later. We find out we've been doing exactly the wrong thing. Women with breast cancer need estrogen, but they need the right kind of estrogen.

Good Estrogen/Bad Estrogen, Good Cholesterol/Bad Cholesterol

This is no different from the good cholesterol/bad cholesterol story. For years, we thought high cholesterol was bad. You had to reduce your cholesterol. We now find that even if you can have a cholesterol level of 350, if you have a good HDL level, the total level doesn't matter. Total cholesterol doesn't matter within the context of knowing the amount of high versus low density.

It's exactly the same in this situation. The total amount of estrogen you have is not as important as what you're doing with it. Do you have the good estrogen, which is the 2-hydroxy, or do you have the bad, the 16-hydroxy? It's really important to recognize that this new paradigm is probably as important as the breakthrough that followed the recognition that there are different types of cholesterol.

The Immuna Care Test

JB: That is a very powerful statement and a great summary of the paradigm shift that is occurring in endocrinology. We are moving into assessment of what one might call the functional metabolic consequences and their influence, over time, on gene expression patterns and metabolic outcome that later are revealed as healthy or unhealthy aging.

This test is now commercially available so doctors can use urine and serum to analyze the 2- to 16- ratio with the antibody test you've developed. I presume people can contact you directly at Immuna Care to learn more about the test?

TK: Yes, they can.

Preventing Premature Disease

JB: That address and phone number will appear on this month's summary cards so our listeners can follow up. Thank you for the precise way you've described your work and its importance. I think it will save people from premature disease and give them options they otherwise did not have.

TK: As you and I know, Jeff, that's really what we're all about. The bottom line is helping people. Thank you for this opportunity. Perhaps some time in the future we will have more information and can go into more detail regarding the genetics of estrogen metabolism.[i][ii][iii][iv][v][vi][vii][viii][ix][x][xi][xii][xiii][xiv]

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Dr. Klug presented a provocative and informative discussion of estrogen metabolites and their important role in our assessment, in both women and men, of a trajectory of the metabolome of the individual toward healthy or unhealthy aging. That assessment relates well to our continuing discussion of the hypothalamus/pituitary axis and its influence on gene expression through the secondary modification of these mediators.

Dr. Klug focused on the hydroxylation patterns of estrogen, the 2- or 16-hydroxylation patterns, and by association, the 4-hydroxylation patterns that follow the 16-hydroxylation patterns. Dr. Klug described the 2-hydroxyestrogen as the "good estrogen." Using terms like good and bad can sometimes present a problem, because anything that is too good can become bad if we get too much.

The breaking molecule of estrogen-mediated cellular activity is not 2-hydroxylation estrogen, but the 2-methoxylated estrogens. You have to first hydroxylate. Then you have to methylate in order to form the appropriate estrogen break. If we think of the estrogen accelerator as the 16-hydroxyestrogens, then we might think of the estrogen break as the 2-methoxylated estrogens. This is an important part of the story.

SAM and COMT

By the way, the 4-hydroxylated estrogens also are methylated to these "non-toxic" estrogens. The

2-methoxyestradiol, or 2-methoxyestrone is formed from the 2-hydroxylated compound by a methylation reaction catalyzed by catechol O-methyl transferase. Catechol O-methyl transferase or COMT plays the role of taking a methyl group from S-adenosyl-methionine (SAM) and transferring it over to the point where methyl groups are needed, to a variety of bioactive catechols such as epinephrine.

SAM conversions play important roles in the presence of COMT. The COMT enzyme is polymorphic. Some people have very sluggish COMT and the methylation reaction is relatively slow. One might wish to consider the need in such individuals of increasing SAM, in order to promote the proper detoxification of their 2- or 4-hydroxylated estrogens.[i]

Beyond Indole-3-Carbinol in Improving Estrogen Metabolism

We shouldn't stop with indole-3-carbinol, soy isoflavones, or lignans as the endpoint for improving estrogen metabolism. We should also talk about those agents that promote methylation.It is worthwhile to consider where the methyl groups come from—the tetrahydrofolate cycle. That means vitamin B12, folic acid, vitamin B6, betaine, serine, or other methyl donor compounds. These substances become an important part of that cycle. They work together to create an active available methyl as SAM. If there are metabolic disturbances in the folate cycle, as in methylenetetrahydrofolate reductase (MTHFR) polymorphisms, the individual may be less able to use his or her dietary folate in converting it to the active methylating derivative, which is 5-methyltetrahydrofolate.

Folic Acid

This may have clinical relevance. If a person has a genetic defect in the MTHFR, he or she may require enhanced amounts of folic acid. The best way to provide increased folic acid is by giving a folate supplement, not dietary folate. Dietary folates are polyglutamyl folates, which have to be broken down by glutaminase enzymes in the GI mucosa to liberate bioavailable folic acid. The supplemental forms of folic acid are actually are more bioavailable than the food forms of folate. (This is one of the few cases where it is true.) Therefore, folic acid as a supplement may be required for optimal function of the methyl delivery system. Bypassing the block and giving 5-methyltetrahydrofolate (which has just become commercially available) can also enhance the folic acid metabolism. So one might use 5-methyl-THF to get around that genetic polymorphism.

You may ask how frequently that genetic polymorphism occurs. Evidence suggests that, in the heterozygous form, 20 to 30 percent of the population could have alterations in their MTHFR, the so-called C677T polymorphism. If so, they may require either enhanced levels of folate in the diet, enhanced B6, B12, and/or 5-methyltetrahydrofolate. This increases SAM, which then helps drive the sluggish COMT enzyme to methylate the hydroxylated estrogen. And the 2-methoxyestrogens are the breaks of estrogen metabolism, or the breaks of the mitogenic cell replicative estrogens.

I hope you see this is a web of interacting variables, a web of genetic uniqueness, a web in which we interact with our environment in different ways. Yes, we do want to increase indole-3-carbinol from crucifers. Yes, we do want to increase soy isoflavones and lignan components from soy and flax. But we also want to help improve methylation reactions with folate, B12, B6, serine, and betaine or rather methyl donor compounds.

Flax Consumption and Estrogen Metabolism

Do things like flax consumption influence estrogen hormone concentrations in postmenopausal women? More and more published studies indicate that in clinical trials, these dietary variables we have discussed, when augmented in the diet, do influence estrogen levels, estrogen metabolism, and the relationship among the metabolites. One such paper, related to flax and its lignan concentration and the influence on endogenous hormone concentrations in postmenopausal women, appeared in *Nutrition and Cancer*.[ii]

In this study, Joanne Slavin and her colleagues at the University of Minnesota evaluated the effect of lignans like enterolactone and enterodiol on estrogen in postmenopausal women.

These secondary metabolites of lignans occur in the gut by the process of bacteria working on the lignans to increase the production of compounds like equol. This process reminds us, once again, that we live in an ecological system. We are not separated from the rest of the environment. The ecology of our gut flora plays a role in the way some phytonutrients are metabolized and the endproducts that are delivered to our neuroendocrine immune systems.

Various gut bacteria metabolize lignans in different ways. Different types of bacteria create different messages to the receptor sites of these endocrine modulators, which then produce a different gene expression pattern and a different phenotype. These are powerful examples of ecology-based functional medicine as contrasted to the medicine of single agents to produce a single disease in need of a single molecule for their treatment. I hope you understand this methylation component. Don't just stop at the 2-hydroxylated estrogens. Think also about the methoxylation component.

[i] Mannisto PT, Ulmanen I, Lundstrom K, et al. Characteristics of catechol O-methyl-transferase (COMT) and properties of selective COMT inhibitors. *Prog Drug Res.*1992;39:291-350.

[ii] Hutchins AM, Martini MC, Olson BA, Thomas W, Slavin JL. Flaxseed consumption influences endogenous hormone concentrations in postmenopausal women. *Nutr Cancer.* 2001;39(1):58-65.

We close this month's *FMU* with a broad-based study that puts all of this information together into an interesting and provocative form. It is a pilot study looking at specific dietary supplements with stage IIIB and IV non-small cell lung cancer patients, most of whom would be considered terminal.[i] Many of these patients have secondary tumors in the brain and other tissues. Most have gone through traditional therapy and are at the end of their course. Their mean average life expectancy from that point on is less than four months by statistical estimation. This is an end-of-the-line type of study.

This study was done in 18 human subjects who had elected by informed consent to be involved in a nutritional intervention trial using complex, plant-based materials to modulate the outcome of gene expression patterns. The patients were from a variety of places, including Mt. Sinai Hospital, New York; Kumamoto University Hospital, Japan; Tri-Service General Hospital, Taipei; North Shore University Hospital, Cornell University Medical College, New York; Alta Bates Medical Center, Berkeley; University of Washington School of Medicine, Seattle; Palo Alto Medical Clinic, California; Brigham and Women's Hospital, Boston; Memorial Sloan-Kettering Cancer Center, New York; and Massachusetts General Hospital, Boston. The study dealt with well-defined cancer in patients whose history is well known, who have been treated by high-quality traditional treatments in major cancer treatment centers

around the world and who now are at the end of the success of those therapies.

Plant Food Concentrates against Cancer

The investigators used a concentrate of a complex mixture of plant foods, all of which had been identified by epidemiological, animal, or human observational studies as associated with lowered incidence of cancer. These plant foods included soybeans, shiitake mushrooms, mung beans, red dates, scallions, garlic, lentils, leeks, Hawthorn fruit, onions, ginger, angelica root, licorice, dandelion root, senegal root, ginger, olives, sesame seeds, and parsley. This was a concentrate of these vegetable products with a wide array of phytochemicals with different effects on gene expression. They used different combinations of these vegetable concentrates and the whole vegetable concentrate, first in animals that had a controlled type of tumor, to see if they could influence the effects on survival of these tumor-bearing animals.

The results in animals were quite remarkable, measuring tumor size in tumor-bearing animals, so this is treating cancer with these vegetable concentrates in tumor-bearing mice. These are BALB/c mice. The combination of vegetable concentrates produced a synergistic positive effect, more than just additive in the individual effects, which decreased tumor size or growth by a factor of more than fourfold compared to control animals. A powerful reduction in progression was seen in these animals that were tumor-bearing, after administering these vegetables as 10 percent of their diet.

Vegetable Concentrates and Human Cancer

That information is interesting, but in and of itself it does not answer the question. What happens when these vegetable concentrates were administered to humans, with or without adjunctive therapy, who have these serious terminal cancer situations? The patients were qualified in the study, and a table defines their characteristics. Many had metastatic lesions identified by CT scanning. One was a patient whose brain tumor was followed over a period of about a year after introducing the vegetable concentrates. The principal site of the brain tumor completely resolved in just over a year during which the vegetable concentrate was given. You actually see three different lesions completely resolving through CT scans over that period of time.

Another patient was an individual with metastasis to bone, and the bone lesions resolved over a period of a year and a half on the complex vegetable concentrate supplement. The researchers discuss the relative survival of these patients compared to the projected mean survival one might expect from traditional response after therapy at the state at which they began the therapy. It is interesting to note that the mean response showed these patients dying, on average, within four months. Many of the patients in the trial were still alive more than 40 months later. Demonstrable increases occurred in survival of these patients, improved quality of life, and reduced new tumor formation.

Encouraging New View of Disease

This obviously does not unequivocally explain whether modulation of cell communication occurs in cancer patients by giving vegetable concentrates. It is very encouraging, however, because it indicates that even in very severe states of dysfunction, such as metastatic disease, giving different messages to the genes and different post-translational effects can produce remarkable differences in cellular outcome. This means that disease is not locked in stone. We do not have deterministically defined cancer, heart

disease, or diabetes in most cases. By changing the experiment of life at any time we might be able to turn back some of these expression patterns.

With this optimistic and uplifting message, we move ahead into 2002. It gives us a reason for looking at the science, a reason for continuing our vigilant studies, a reason for continuing to be warriors and champions of the new medicine. I can see the dawn of this functional genome-based medicine. It gives us tremendous opportunities to be better at helping people achieve their objectives of long, healthy, productive lives.

Thanks for being with us in 2001. We'll look forward to seeing you next year.

[i]Sun AS, Yeh H-C, Wang LH, et al. Pilot study of a specific dietary supplement in tumor-bearing mice and in stage IIIB and IV non-small cell lung cancer patients. *Nutr Cancer.* 2001;39(1):85-95.

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