

April 2006 Issue | Eleanor Groniger Rogan Eppley Institute

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Welcome to *Functional Medicine Update* for April 2006. The focus of this month's FMU will be on estrogen-estrogen-related dysfunction and estrogen-related functional capabilities in physiology. I think you will be pleased to hear from our Researcher of the Month, who is coming back for a second time after a two-and-a-half-year absence-Dr. Eleanor Rogan from the Eppley Institute at the University of Nebraska Medical Center. She will talk about her extraordinary pioneering work on estrogen metabolism.

The Women's Health Initiative (WHI)

Before we get to that, let me speak briefly about the WHI. You probably recall that the WHI was an extraordinary study of over 160,000 women, age 50 through 79 years. This multi-million dollar, 15-year project was focused on trying to understand the relative impact of diet, lifestyle, and other factors on postmenopausal women's health. In this study, hormone therapy, calcium and vitamin D supplementation, and dietary and lifestyle patterns were all examined in association with heart disease, cancer, and osteoporosis, respectively. We owe Dr. Bernadette Healy, the previous director of the National Institutes of Health, a debt of gratitude for her advocacy to do this extraordinary bit of work to try to better understand the gender-specific relationships between risk factors and age-related disease in women. In particular, this project focused on the peri- and postmenopausal times in women's lives that are associated with increasing incidence of breast cancer, heart disease, bone loss, bone fractures, and osteoporosis.

Low-Fat Diets and Weight Loss

This extraordinary work is now starting to be analyzed and the data, which is massive, is being examined from multiple perspectives. We have seen things like the relationship between low-fat diets and weight change, which is one of the more recently published parts of the WHI. The outcome of that part of the study is quite fascinating. There has been a trend over the last several years in thinking that carbohydrate somehow increases weight gain, and is the scourge of our population. As a consequence of too much carbohydrate intake, we are seeing insulin resistance, type 2 diabetes, cardiovascular disease, and rampant obesity. This speaks in opposition to the previous concept that a high complex carbohydrate, high-fiber, lower-fat diet actually achieved weight loss, lowered insulin, and promoted better glycemic control and lipid patterns. It seems paradoxical that we have swung from the high-carbohydrate to the low-carbohydrate regime as being a favorable diet. The low-fat diet connection to heart disease in postmenopausal women, therefore, is part of trying to understand the dietary connection to disease risk.

Low-fat Dietary Pattern and Weight Change

A paper was published earlier this year in the *Journal of the American Medical Association*, titled "Low-fat pattern and weight change over 7 years: the Women's Health Initiative Dietary Modification Trial."¹

Initially, the investigators had targeted trying to get the total fat calorie percent in the intervention group down to 20 or lower. In actual fact, this was not achieved. The women did not comply with the extent of the low-fat diet. They were able to lower the average fat intake by about 8 calorie percent over the standard American diet for the control group, so it was a modestly restricted carbohydrate diet. Therefore, the results of the study would probably be considered intermediary, or somewhat compromised, because the fat restriction was not achieved with all that was proposed.

The outcome of the trial, even with that limitation, indicated that there was a marginal weight loss, but it was a loss and not a weight gain in the individuals on the lower-fat, higher-carbohydrate diet. The concept that a carbohydrate-rich diet increases body fat does not appear to be true, based on this study.

Types of Fat, Carbohydrates, and Protein

There are a number of variables that should be taken into account when examining this topic. One of the most important, which we often forget, is what *type* of fat, what *type* of carbohydrate, and what *type* of protein is involved? It is becoming more apparent that, although we have spent much of our time focusing on the calorie percent of each of these macronutrients, the actual composition of those families is as important, or more important, than the number of calorie percent they represent. If we talk about carbohydrate as refined (sugars and starches)-the highly white type of diet-they will have a different physiological effect than a diet with the same number of calorie percent carbohydrates that come as minimally processed grains, rich in fiber, rather than rich in bleached starch. As a consequence, the glucose molecules from carbohydrate are digested and absorbed differently, but there is also the association in the minimally-processed diet with literally hundreds (probably thousands) of different phytochemicals that influence physiology through their individual signaling properties to gene expression patterns. When one looks at carbohydrate in the Pridikin or Ornish approaches, it is a different kind than what we are talking about-increased white carbohydrate (starch and flour products) in a refined state.

We often get caught up in the carbohydrate/fat/protein argument, and we are missing the focus, which should be on the composition of the diet in its total nature. Many other nutrients-soluble fibers, insoluble fibers, lignans, isoflavones, polyphenols, carotenoids, xanthophylls-as well as all the other bioactive molecules in a minimally processed diet, influence function. That was not controlled for in the WHI dietary intervention trial. We saw that by decreasing fat by about nine calorie percent (meaning an increase in percent carbohydrate calories), there was no increase in body weight. Actually, there was a statistically significant, though marginal, lowering of body weight.

It comes down to how we can arrest the epidemic of obesity. The individuals who wrote the editorial on the *JAMA* article discussing the WHI low-fat diet hit upon some very important information.² It was written by Michael Dansinger and Ernst Schaefer, the co-authors of a previous study published in *JAMA* about a year ago that we reviewed in FMU, comparing the Atkins, Ornish, Weight Watchers, and Zone diets for weight loss and heart disease risk reduction.³ These authors, who also wrote the editorial to the 2006 paper, ask: "Is it time to admit defeat? Is US society doomed to be one in which few individuals maintain body weight and one third of the adults are obese?" The recent low-fat diet in the WHI does little to reassure skeptics, and some see no hope on the horizon.

"Many believe humankind does not have the self-control to counterbalance the forces that create a predictable wave of obesity in technologically advancing societies. Some believe national governments will never enact the bold policy changes that could make a dent in the obesity rates, such as substantially

altering food advertising practices and creating economic incentives for vigorous adherence to lifestyle recommendations. Even modest steps, such as limiting advertising of unhealthy food during children's television programming, or placing small taxes on unhealthy foods are met with seemingly insurmountable resistance from the food industry and others. With a government and society that seemingly reject the pursuit of economic and other reforms that could make a real difference, the burden of obesity treatment and prevention continues to rest on the shoulders of 'individual responsibility'

That is an interesting perspective, and one certainly worthy of our attention. If we are constantly feeding our children and adults molecules that create a different message that translates into storage rather than metabolic utilization, we are going to see a continued increase in the obesity epidemic, with what might be considered very severe consequences as our children grow into adulthood, with regard to coronary artery disease, stroke, and perhaps even certain cancers associated with hyperinsulinemia.

We have to start asking questions that are different from those that have been asked in the past about just restricting calories and changing protein/carbohydrate ratios in diets. We need to look at the complex nature of the diet that creates the signaling in cells that regulates appetite, physiology, and metabolism, so that usable energy is available for physiology, and storage is less likely to occur-storage for the rainy day that never comes called adipocyte hypertrophy, where we start storing lipids in our fat cells, producing central adiposity, insulin resistance, and adipocytokines that induce inflammation. We need to come up with a better model. The concept of calorie restriction, weight-loss diets, and eating less of the same foods we have available in commerce, which send the wrong messages to our genes and produce an outcome called dysfunction and inflammation, is a model that is not working. We need to find something different than the first law of thermodynamics to try to address this problem of obesity.

That is my takeaway from the article on the WHI. Yes, there was a minor weight loss seen in these individuals by restricting some degree of fat in their diet, but it was not significant enough to deal with the major nature of the sea change that is occurring in obesity. We need to find a different model, one that relates to finding out how to promote proper nutrigenomic signaling to the cells, producing an outcome related to proper physiology and appetite regulation through the gut, the liver, the pancreas, the muscles, the adipocyte cells, and the brain, all working cooperatively to regulate what has evolutionarily been built into our genes. We have overridden it.

We know how to eat. Our genes know how to control these aspects of physiology, but we have been sending toxic molecular signals to the cells through toxic diets that create outcomes of gluttony and lack of self-control. People do not elect to eat gluttonously. It is the number of things that we are signaling to this regulatory system that create the outcome. It is not as simple as simply cutting calories. If we cut calories, and we are still delivering these false and toxic messages to that person, it is likely we are going to have the same outcome. That is my takeaway from the WHI weight management, lower-fat diet study.

There was a paper that appeared in the 2006 issue of *JAMA* on page 58 that is another interesting example of how a nutrient component can have an effect on cellular signaling in physiology.⁴ I am talking about an amino acid found in protein-L-arginine, which has been frequently discussed in the news over the last nearly 15 years. In the mid 1990s, nitric oxide (NO) was chosen as "Molecule of the Year" by the editors of the journal, *Science*. NO is formed in the body by the conversion of arginine into citrulline. The identification of NO and the work identifying its synthesis pathway won the Nobel Prize in Physiology and Medicine for three researchers in 1998. One of those researchers was Dr. Louis Ignarro, a

distinguished professor of pharmacology at the University of California at Los Angeles.

It has been suggested that L-arginine could be useful for increasing endothelial NO, which would lead to improved vascular compliance, lowered blood pressure, and an overall improved vascular endothelial outcome and function in individuals with damaged endothelia. This was a model we were fortunate to have discussed with Dr. John Cooke on FMU in November 2002. Dr. Cooke is a cardiology researcher from Stanford University, and he talked about his clinical intervention trials using oral arginine supplements in individuals (first in primates, and later in humans) with either hypercholesterolemia or vascular endothelial injury. He found that supplemental L-arginine appeared to improve vascular and endothelial functions.

The paper in JAMA that I am now discussing is titled "L-Arginine therapy in acute myocardial infarction," and contains a description of the randomized clinical trial called "The Vascular Interaction With Age in Myocardial Infarction," the so-called VINTAGE MI trial. In this intervention trial study, which was performed from February 2002 to June 2004 at Johns Hopkins by Steven Schulman and his colleagues, the amino acid, L-arginine was used as a supplement in a single-centered, randomized, double-blind, placebo-controlled trial. The trial involved 153 patients following a first ST-segment elevation myocardial infarction; 77 patients were 60 years or older. These were post-infarction patients who were all quite sick. In this trial, patients were randomized to receive either L-arginine (the goal was to get them to take 3 grams of arginine three times a day, or 9 grams total) or matching placebo for six months. The investigators examined change in gated blood pool-derived ejection fraction over six months. The results showed that baseline characteristics, vascular stiffness measurements, and left ventricular function were similar between participants randomized to receive placebo or L-arginine. At the end of the trial, it was found that there was no significant change from baseline to six months in the vascular stiffness measurements or left ventricular ejection fraction between the two groups, including those 60 years or older. It was pointed out that in the L-arginine group, six participants, or 8.6{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36}, died during the first six months of the study versus no deaths in the placebo group. The researchers go on to suggest that L-arginine may be contraindicated in this patient population.

Although not directly assessed in the study, this raises questions about what happens if you put a person on a high vegetable-based diet, which is rich in L-arginine, and they have vascular defects. Might you induce some injury? Recall that arginine is found in high quantities in proteins, particularly in some vegetable-based proteins, and might this imply that dietary proteins high in L-arginine, given to people with post-infarction injury could pose a risk to more serious outcomes?

That does not appear to play out with the data that has been previously published, in which it has been suggested that vegetable-based diets decrease the relative risk to secondary heart attacks. This suggests that one would want to recommend diets in which proteins were probably richer by nature in arginine. Some of these things just do not seem to line up. Is there a difference between L-arginine given as a supplement to post-MI patients versus arginine in the diet? Again, there are many questions, because we would again assume that L-arginine from dietary protein would be delivered into the blood. Once the protein had been digested, the amino acid would be absorbed into the blood and produce a postprandial elevation of L-arginine, similar to what one would get when supplementing with equivalent amounts of purified or isolated L-arginine.

This is a swirling debate. I asked Dr. Louis J. Ignarro, the Distinguished Professor of Molecular & Medical Pharmacology at UCLA, if he would like to offer his opinion about this particular trial. Work in pharmacology has shown us that all substances are toxic at some level, even air and water. The question is: What is the level of potential toxicity and relative risk of L-arginine, if at all, in individuals with specific types of cardiovascular defects? With this in mind, Dr. Ignarro pointed out the following concerns he has about the study published in JAMA:"

1. Patient Selection- This clinical study was performed with very sick patients (dying patients) suffering from advanced cardiovascular disease. They were receiving several different types of potent drugs to treat their condition. We know that these individuals have very brittle physiological function that is very sensitive to interaction with any additional bioactive agent. L-arginine is not a drug, but rather an amino acid nutrient, which when administered therapeutically to patients with serious cardiovascular disease might have numerous influences on vascular biology just as any dramatic dietary change could have. Generally, in order to test the potential therapeutic benefit of any dietary supplement or functional food in humans, it seems prudent to design the clinical study carefully and to use as a measurable endpoint a parameter that is known to have the potential to respond to the given treatment."

2. Study Design and Endpoints of the Study- The VINTAGE MI clinical trial was designed to test whether the addition of L-arginine to standard post infarction therapy in patients following a first STEMI over a 6-month period "would decrease vascular stiffness and improve left ventricular function." There is a serious flaw in this design and the expected therapeutic response to L-arginine. That is, the nitric oxide generated from L-arginine is a vascular smooth muscle relaxant and inhibitor of platelet function. There is no evidence, however, that nitric oxide decreases vascular stiffness. …Increasing the production of nitric oxide would NOT be expected to alter vascular stiffness. Therefore, the design and rationale for the clinical study are ill conceived."

3. Sample Size and Outcome- The patient sample size was small and the statistical evaluation was marginally significant. It is clear that the control patient group itself (not receiving L-arginine) experienced a mortality rate of greater than 10{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} as would be expected in a group of patients this ill. The percentage of patients taking L-arginine who died during the study was well less than 10{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36}. This means that the deaths of the patients taking L-arginine were likely attributed to chance. Indeed, the authors themselves indicate this as a likely possibility. Therefore, a second and larger clinical study might reveal no significant response to L-arginine in a similar subset of patients. Accordingly, it is premature, unscientific, and unethical to conclude that the patient deaths in this study were attributed to the ingestion of L-arginine."

4. Lack of Proof of a Cause and Effect Relationship- This small clinical trial does not provide any evidence for a cause and effect relationship between L-arginine administration and death in sick patients with cardiovascular disease. There is no evidence that L-arginine was responsible for the patient deaths. Moreover, there is no scientific or statistical evidence for any association between L-arginine administration and patient death."

5. Flaw with Proposed Mechanism of Action- When a beneficial or detrimental response to a substance ingested by humans is reported in the literature, the authors are obliged to provide a clear rationale for the

study and a viable hypothesis to explain the response. Neither was done in this study. The hypothesis forwarded to explain the potentially harmful action of L-arginine in this patient population is flawed and scientifically unsound. The authors concluded that tetrahydrobiopterin (BH4) levels were deficient in these sick patients, and that this condition resulted in more superoxide anion production than normal, and at the expense of nitric oxide. That is, L-arginine is normally converted by NO synthase to nitric oxide plus L-citrulline in the presence of adequate amounts of BH4. But when the BH4 is deficient, more superoxide anion than nitric oxide is formed. Since superoxide anion is an oxygen radical, the thinking is that this excessive production of superoxide anion can cause local tissue injury. There are many flaws in the application of this hypothesis to the current findings. First, no measurements of tissue levels of BH4 were made. Second, the quantity of NO synthase in the affected tissues in these patients was not determined and there would have to be very high levels of NO synthase to make enough superoxide anion to be of any concern. Third, it seems that the authors were not familiar with the biochemistry of nitric oxide. It is well known that when NO synthase is saturated with L-arginine substrate, the enzyme produces exclusively nitric oxide and no superoxide anion. This can be demonstrated clearly in vitro. As L-arginine concentration increases, no superoxide anion production occurs. The cell would have to be deficient in L-arginine for the production of any appreciable quantities of superoxide anion to occur. As these patients were given relatively large doses of L-arginine, it is highly likely from many past studies that their cells were NOT deficient in L-arginine. Indeed, the likely presence of saturating concentrations of L-arginine around NO synthase would assure only little or no production of superoxide anion. Therefore, the authors incorrectly conclude that tissue BH4 deficiency might have caused increased production of superoxide anion, which in turn might have contributed to the death in the patients."

6. The inappropriate mechanistic connection of arginine to atherosclerosis-The authors claim that an increase in inducible NO synthase (iNOS) can increase in patients with atherosclerosis. There is a well recognized relationship between inflammatory markers and atherosclerosis. However, iNOS is a marker of inflammation rather than a cause of it. Indeed, the iNOS goes up in order to produce more nitric oxide to act as an anti-atherogenic agent in the body. The authors claim that the increased nitric oxide can react with the superoxide anion that might be formed to produce peroxynitrite, a potentially dangerous substance that can cause tissue injury. This is theoretically possible but there has been no evidence for this in vivo. The current belief is that insufficient peroxynitrite would likely be produced to elicit any deleterious effect in tissues. Moreover, the nitric oxide and the superoxide anion would have to be generated very rapidly in precisely the same spot in order to allow the two to react chemically to form peroxynitrite. As discussed above, the presence of such a large concentration of L-arginine in these patients who ingested L-arginine would make it highly unlikely that any superoxide anion was being generated."

7. The inappropriate connection of arginine to homocysteine-The authors make a statement that L-arginine administration causes an increase in plasma homocysteine levels in humans and that this could increase the risk of heart failure. Again, this statement is incorrect as it is well known that one of the most important physiological roles for nitric oxide in the body is to function as an antioxidant to lower the levels of homocysteine and, thereby, protect against atherogenesis and atherosclerosis."

8. The lack of balance in describing the influence of L-arginine on vascular function- The authors have chosen to ignore the extensive literature documenting that L-arginine administration to animals and humans with atherosclerosis causes a provocative reversal of inflammation and disease state. L-arginine administration in animals also increases the expression of endothelial NO synthase, which serves to allow

the production of more nitric oxide in the blood vessels, thereby slowing the progression of atherosclerosis." ⁵

Editorial Bias and Philosophy in the Literature

With regard to Dr. Ignarro's comments on this paper, one might ask how it ever got published in *JAMA*, when there are so many papers that vie for publication in this primary, distinguished, peer-review journal. It begs the question as to why the editors decided to accept it for publication, and why it did not end up in a secondary or tertiary journal instead, where it could be seen as an "n of one" type study requiring a fuller evaluation to demonstrate proof of the model. These are interesting questions that come up in this field. It is my observation that a number of published studies related to nutrient impact on physiology that have a negative spin end up in primary journals, whether or not the methodologies are flawed or the trials of limited size. Therefore, they fall prey to the same criticism that is often levied against nutrition studies that show a positive effect. That is, they are not done with a large enough study group. They do not have a high enough statistical significance. They deal with mechanisms that are not completely understood. When that criticism is levied against something that comes out positive, it often will not appear in the journal; it will be rejected. When the criticism is levied against something that shows a negative outcome, it seems to somehow end up being published. It raises some interesting questions about editorial philosophy and bias that we need to take into account as we evaluate the published literature.

That leads into a discussion of the estrogen connection to autoimmune disease. Almost 90 percent of autoimmune disease disorders of the most common forms-rheumatoid arthritis and systemic lupus-are primarily found in females. In March 2005, there was a remarkable report published by the U.S. Department of Health & Human Services through the National Institutes of Health (NIH), titled "Progress in autoimmune disease research" (this can be found on the Internet).⁶ It is the summary of a report to Congress on the progress on autoimmune disease, and it contains some remarkable statistics.

Autoimmune disease is much more prevalent than previously recognized because historically, it has been broken up into 80 or more clinical conditions that have never been grouped as an aggregate so as to examine the total number of people affected. For reasons we are still trying to understand, approximately 23.5 million people in this country have autoimmune disease, and the prevalence is rising. In fact, the number of people affected rivals that of other major disease indicators, including dyslipidemia, insulin resistance, and hypertension. The autoimmune family of disorders, which is on the rise, is certainly a significant problem.

Another thing that is pointed out in this report, which I think is very interesting, is that the conditions-although they have been segmented as independent diseases under respective diagnostic categories-are now being found to be associated with changes in various immunological mechanisms. Our concept of this disease category is that the individual diseases are distinctly different from each other. For example, we try to completely segregate myasthenia gravis from systemic lupus erythematosus, multiple sclerosis, and ulcerated colitis. This concept of differentiation is starting to change; we are starting to look at similar mechanisms of immunological imbalance and dysregulation of the thymus dependent 1(Th1) and thymus dependent 2 (Th2) lymphocyte system to help us better understand the etiology and the potential origin of these diseases. That leads to what I would call a functional medicine approach-one that is focused more on the underlying mechanisms and less on the medical taxonomy of a disease. Medical taxonomy is no longer the "sine quo non" for understanding these diseases.

As we begin to examine the mechanisms that connect these diseases one to the other, estrogen

metabolism seems to be one of the metabolic variables associated with these conditions that may help us to better understand why they are more common in women than men. In fact, in this nicely written and lengthy report published by the NIH, the authors discuss the metabolic, environmental, and genetic factors associated with autoimmune disease. The first thing the authors point out is that there is no single gene allele connected to individual autoimmune diseases. No one has "a gene" for thyroiditis or multiple sclerosis, but rather there are families of genes that interrelate immunological susceptibility, or sensitivity, to environmental triggers that can weave together to give rise to the outcome we call autoimmunity, or immunological imbalance.

Genetic Relationship to Autoimmune Disease

It is now being said that about one third of these autoimmune disorders has a genetic relationship, which implies that two thirds of the disorders are related to environmental factors, both internal and external, that weave together to give rise to the expression of the disease in the phenotype. When we talk about something that is two thirds related to the environment (67 percent), it strongly addresses the hypothesis that we should be spending more time trying to understand environmental relationships, rather than trying to focus on genetic propensity. Part of prognostic screening would be to understand genes of risk, but beyond that, we would want to look at the environmental modifiers that give rise to the potential outcome of expression of the immune system that we diagnose as "immune disease," or "autoimmunity." This is an interesting part of the emerging story-beginning to look at key factors that can modulate the expression of autoimmune susceptibility. These are reducible, or modifiable factors, and we now understand that they have potential for being altered by modifying the environment.

In the NIH report, it is stated that although approximately one third of the risk of developing an autoimmune disease can be attributed to hereditary factors, the remainder of risk is thought to be associated with non-inherited events. These include things like mercury in the environment, and other toxic heavy metals now being seen as potential immune-activating substances in sensitive individuals. It also includes exposure to certain chemicals and xenobiotics, such as halogenated hydrocarbons, polynuclear aromatic hydrocarbons, and polychlorinated biphenyls. All of these have potential xenobiotic risk to the immunological system in susceptible individuals.

We know about drug-induced systemic lupus erythematosus. This is a variant form associated with increased levels of single-stranded DNA auto-antibodies. As a consequence, these individuals may have a unique reaction to exposure to specific chemicals that can induce an autoantibody against their tissues. Now, we start to piece together that this could be a "total load" effect. Layer upon layer is added to the immunological system until auto-antibodies are expressed (depending on the individual's genetic uniqueness). This is the immune system reacting with itself, but perhaps it is reacting with things that have come as a consequence of injury to self.

Infectious Agents and Autoimmune Disease

Infectious agents are the most often-cited environmental factors implicated as triggers of autoimmune disease. We know about the classic example-the Group A beta-hemolytic streptococcus in the development of rheumatic heart disease. Acute Guillian Barre syndrome has been associated with a number of bacterial and viral infections. Even reactive arthritis has been linked to a variety of intestinal infections. We begin to see focal infections, which could be a root canal, dysbiosis of the gut, or sinusitis, any of which may result in continued leakage of bacterial and viral products that initiate immune imbalance.

Lifestyle and Dietary Factors in Autoimmune Disease

Lifestyle and dietary factors are now being seen as much more important issues in autoimmune disease. Diets very high in total calories and fat calories induce injury to DNA and produce "funny" DNA. The immune system may recognize these as "foreigners" and begin to form antibodies against DNA called "anti-DNA autoantibodies." It is actually against a foreign DNA that has resulted as a consequence of oxidative injury to native DNA. Diet is beginning to be seen as a potential additional modifying factor in the expression of autoimmune disease.

Food Allergy and Autoimmune Disease

We know that food allergy and food sensitivity may modify function via the immunological changes that occur. Gluten sensitivity stands out in conditions such as thyroiditis, ulcerative colitis, and Crohn's disease. We are beginning to understand how the food of one may be the poison of another, as it relates to the immune system. When I read through this NIH document, I recognized that we are entering into a dramatic period of change related to how we view the origin of autoimmune disease.

Estrogen and Autoimmunity

Estrogen is another factor in autoimmunity. It used to be thought that 17-beta-estradiol was strongly correlated with autoimmune disease. Now, there is more and more evidence indicating that estrogen metabolites, possibly the 16-hydroxyestrogens, and perhaps the 4-hydroxylated estrogens, may be precipitating metabolites for immunological imbalance, inducing immunological activation of inflammation. We will talk more about estrogen metabolites with our Researcher of the Month, Dr. Eleanor Rogan.

Autoimmune Disease in Females Compared to Males

I want to mention the prevalence of autoimmune disease in females as compared to males and how that may relate to androgen/estrogen balance and estrogen metabolism. Some therapies for arthritis have to do with increasing androgen, such as administering DHEA at high levels with systemic lupus erythematosus patients, or giving an aromatase inhibitor, which has shown to be of some benefit. Taking women off birth control pills to lower estrogen exposure, or giving Tamoxifen to block estrogen signaling have been found to decrease some of the signs and symptoms observed in autoimmune disease.

Clearly, there is an estrogen metabolism component in autoimmunity. What is interesting is that the exacerbation of signs and symptoms in autoimmunity does not appear to be directly correlated with the plasma levels of 17-beta-estradiol, but more related to estrogen metabolites, the 16-hydroxyestrogens in particular. This was discussed in an article published in the journal *Lupus* in 2002, which examined the relationship between 16-hydroxyestrogen and the appearance of signs, symptoms, and severity in systemic lupus erythematosus patients. There is a correlation between a variety of environmental factors and dysregulation of the immune system. Modulation of estrogen metabolism is certainly on the list of factors we need to keep in mind that may help in autoimmune disease.

Stress and Autoimmunity

Stress also plays a large role in autoimmunity, as well. I want to thank Dr. Peter Madill for calling an interesting paper to my attention. He is one of our long-standing supporters and a physician in Sebastopol, CA. In a paper published in *The Journal of Neuroscience* in 2005, the authors discuss maternal programming of stress responses, and how activation of the hypothalamus/pituitary/adrenal axis can initiate various types of messenger molecule changes, including inflammatory cytokines and other cell

regulating substances that have to do with growth hormone and gene expression patterns. This interaction can create a feed-forward cycle that can facilitate and activate stress responses that we see as inflammation, and encourage and feed into the inflammatory pathway.² This paper is also remarkable because it demonstrates (in animals) that one epigenetic response can be epigenetically reversed. That is, the epigenetic programming thought to be static after birth was reversed with respect to stress-related marker genes. This reversal was accomplished by infusion of methyl donor nutrients in these adult animals' brains, and demonstrated a lowering of some of the stress responses.

These are epigenetic types of phenomena that occur, not from modifying the genetic code directly, but the environment in which the genes are expressed, leading to changes in the messenger RNA and subsequent proteins. This is a huge, new advancing concept in medicine. We used to think that everything was linked to our genes and there was little we could do about it. Now, we recognize that the environment actually influences not only how our genes are expressed, but how biomolecules are expressed from the genes finally evolve into the phenotype and alter metabolism, cell signaling, and what we call the phenotypic component of the cell. People with many inflammatory conditions have a lot of dysregulation in their immune systems. Things can be done posttranslationally, such as improving methylation patterns with folate, B6, and B12, as well as reducing stress. With that in mind, let us move to our Clinician/Researcher of the Month interview.

INTERVIEW TRANSCRIPT

Clinician/Researcher of the Month

Eleanor Groniger Rogan

Eppley Institute

8210 Bowie Drive

Omaha, Nebraska 68114

JB: It's time for our Clinician/Researcher of the Month. Those of you who attended the 13th International Symposium on Functional Medicine were privileged, as I was, to hear Dr. Eleanor Rogan, one of our premier plenary lecturers, talk about the extraordinary work she has done over the years on estrogen metabolism. This is such an important area, and one that we previously introduced on FMU during our first interview with Dr. Rogan in November 2003. We thought it would be important to update our listeners on the significance of Dr. Rogan's work.

Just to review her background, Dr. Rogan works at the Eppley Institute at the University of Nebraska Medical Center. She is an elegant researcher, developer, and discoverer of both the mechanisms and influences of estrogen metabolites-a very sophisticated area of chemistry. We're not talking about things that are produced at very high levels. Therefore, methodologies have to be developed to analyze them, as well as their reaction products. Many of these products have high reactivity so they don't remain in biological specimens very long after the sample has been taken. Analytical ways of demonstrating their presence have to be found, either by surrogate markers or direct chemistry analysis. This is a very complicated portion of the "cellular soup" we see in animals. Dr. Rogan and her colleagues have been able to sort out these complex puzzles with great precision. We're very fortunate to have her with us again to update us on the latest information in this emerging area, which has tremendous clinical implications for breast cancer, as well as prostate cancer. Once again, Dr. Rogan, welcome to FMU and thank you for being willing to share your extraordinary work, which has led to publishing more than 160 papers over

the years. You're certainly the person in the field.

ER: Thank you, Dr. Bland.

JB: For our listeners who may not have heard our first interview in 2003, and for those who did not attend the 13th symposium, would you tell us how you got into the area of estrogen metabolism that led to your discoveries on the 2- and 4-hydroxyestrogens?

Aromatic Hydrocarbons Lead to Estrogens

ER: We've always been interested in the estrogens, but I began 30 some years ago studying another class of carcinogens called aromatic hydrocarbons that are involved in pollutants in any kind of smoke. I studied them for 20 some years, understanding the kinds of DNA adducts that they form. When they react in cells, it initiates the process leading to cancer. In the early 1990s, we were able to move from the hydrocarbons over into the estrogens. We were able to apply what we'd learned from the hydrocarbons to estrogens very quickly. What we already knew from the hydrocarbons was that the reactive forms that attack DNA and attach themselves on the purine bases-the adenines and guanines-cause those bases to fall out with the carcinogen attached, leaving behind gaps in the DNA message called apurinic sites. These are the important adducts that relate to cancer-causing mutations. We also knew, from other people's work that there are two catecholestrogens, depending on whether the second or third OH group is on the 2 position or the 4 position. We knew that the 2 catecholestrogens, with the OH on the 2 position, were, at best, very weak carcinogens, whereas the 4- catecholestrogens are stronger. We immediately discovered that it's the reactive form of the 4-catecholestrogens, the so-called catecholesterogen quinones, that formed the so-called depurinating adducts that fall out of DNA, leaving behind the apurinic sites, whereas the 2-catecholestrogens quinones form the so-called stable adducts that are in a different position in DNA, and they stay in the DNA unless removed during normal repair. They actually form the depurinating adducts, but at a much, much lower number than the 4-catecholestrogens. We were able to apply all that. We also found that the mutations that come from these depurinating adducts come from the 4-catecholestrogens. All of this fit together. Now, we are applying that to humans.

JB: This is a very exciting evolution of the story. I recall from reading your work and discussing it with you in 2003, that you talked about the different isoforms of cytochrome P450 (CYP) that were involved in the formation of these different hydroxylated products, the difference between CYP 1A1/2, CYP 1B1, and CYP 3A4 (each one of those being inducible), and that there may be inducers that would drive each one differently. Am I summarizing it correctly?

Isoforms of CYP Involved in Hydroxylated Products

ER: Yes, that's correct. None of it is an absolute, but certainly some of the inducers will induce more of, let's say, the 1A family. For example, TCDD, particularly dioxin, induces the CYP 1B1, which predominantly forms the 4-catecholestrogens, whereas the 1A1 or 1A2 predominantly form the 2-catecholestrogens. These two forms are the ones that are primarily present in extra-hepatic tissues. The 3A4 is highly concentrated in the liver.

JB: Let's move from the point you made about the inducing properties of CYP 1A1 and 1B1. You made the very important point that CYP 1B1 and A1 are in extra-hepatic tissues at high levels, where 3A4 is primarily in hepatic tissues. That obviously directs us toward the breast or the prostate. Are there any data that have come out of those tissues related to the activities of these transforming enzymes?

EG: They're certainly present. The 1B1 and 1A1 are both present in the breast. We haven't published that, but 1A1 is present. We've also seen them in the prostates of animals. I would say that these enzymes are clearly present in breast tissue, both 1A1 and 1B1.

JB: What you've said would suggest that in tissues where there is high potential activity of 1B1, one might have higher risk to these apurinic bases adducts that come as a consequence of the 3-4 quinone estrogens from 4-hydroxylation.

Presence of CYP 1B1 in Women With and Without Breast Cancer

ER: That's exactly correct. When you have higher levels of 1B1, you are more likely to get formation of the 4-catecholestrogens, and you get higher levels of the DNA adducts formed. We have begun analyzing that in breast fluid from women with and without breast cancer, but I don't have any results to share yet. We think we see differences, but the work is too preliminary to share. I can tell you that we have published a very small preliminary study, comparing breast tissue from women with breast cancer (non-tumor tissue) and women without breast cancer (excess tissue from reduction mammoplasty). What we find is that the level of P450 1B1 is significantly higher in the breast tissue from women with breast cancer than in the women without breast cancer. We looked at four different enzymes, and found that the two we consider estrogen-activating are higher in the women with breast cancer and lower in the women who don't have breast cancer. On the other hand, two enzymes that we consider protective against activation of estrogen are higher in women with healthy breasts compared to women who have breast cancer.

JB: That's fascinating.

ER: That fits right in with what we had hoped to find.

Impact of Bioactives from Cruciferous Vegetables on Estrogen Transforming Enzymes

JB: We've talked about the fact that these are inducible enzymes, so the inducers of some of them may be different, one from the other. We talk about the cruciferous vegetable family and its relationship to purported epidemiological breast cancer risk reduction. Would you help tell us a little bit about the bioactives in crucifers and the impact they have on these estrogen transforming enzymes?

ER: I think they have a variety of effects, one of which is that they have some compounds that will scavenge the reactive estrogen metabolites so that they're not reacting with DNA. That's one of several effects they have. One of the most important effects of cruciferous vegetables is that they contain compounds that induce a very important enzyme called quinone reductase. That takes the reactive form of estrogen back to catecholestrogens, which are not reactive. We're trying to collect data to support the idea that induction of quinone reductase is a very important component of what the cruciferous vegetables do. I know that they also affect a variety of other anti-tumor cell processes. In addition to inducing the metabolic enzymes, I think they also have some effects on the repair of DNA damage and on some signaling pathways.

JB: That leads to looking at some of the individual glucosinolate bioactive secondary metabolites, such as indole-3-carbinol (I3C) and some of its acid polymers like 3,3'-indolymethane, or what has been called diindolymethane (DIM). As you know, in our field, there is a fairly large controversy going on right now as to whether there is preference for one of those bioactives over the other in favorably

affecting estrogen metabolism. Would you tell us a little bit about the 4-hydroxylation and the aromatic hydrocarbon receptor activation of I3C versus DIM, as you've reviewed the literature?

ER: I certainly have reviewed the literature to try to sort out these differences. One of the confounding effects is that when you look, for example, in animals, or even in people (because the I3C is metabolized to the so-called DIM), it's hard to sort out what's doing what. You treat with I3C, but you don't know what the active component is. That confuses the whole issue. However, when I reviewed this literature, I didn't see a great difference between what I3C was doing and what DIM was doing. There were a few differences in the effects of these two compounds, but it didn't seem to me that people had demonstrated that there are significant differences.

JB: We were told at a recent meeting that there was a very significant difference in 4-hydroxylation patterns between the I3C and DIM. Does that seem to be true from what you've seen in the published literature?

ER: I have seen that opinion stated, and I've seen it purported in citations, but when I read the papers in the literature, I don't see that that's been demonstrated. I see that people have made some assumptions (for example, on P450 1B1), but I have not seen anybody actually demonstrate that. I've also read that administration of I3C significantly increases the amount of P450 1B1 and consequently the 4-catecholestrogens, but again, the only data I find in the literature shows a small increase that is far from being statistically significant.

JB: In looking at some of the purported adverse effects of either I3C or DIM, people have talked about cross reactivity with the aromatic hydrocarbon receptor, or the formation of 6-alpha-hydroxyestrogens (which you probably know a lot more about than I do), and implied that perhaps there's some difference that could be seen in either hormone receptor or in 6-alpha-hydroxyestrogens. Have you seen anything relating to that?

JB: I have seen a study in women where DIM was administered, and the 2-hydroxylation to form the 2-catecholestrogens increased significantly. That increased the ratio of the 2-catecholestrogens to the 16-alpha-hydroxyestrogens, which would be a good thing.

Again, that difference was not quite statistically significant by general standards. It seemed to be going in the right direction, but it was not a conclusive study. Unfortunately, since that study, there hasn't been anything published specifically on the effects of I3C or DIM on 4-hydroxylation of estrogens in humans.

JB: How about the effects in humans of I3C with formation of the 2-hydroxy versus the 16-hydroxy? Has that been adequately demonstrated?

ER: Yes. That improves the ratio of the 2-catecholestrogens to the 16-alpha-hydroxyestrogens. Whether that is important has not been demonstrated. In my view of the world on this whole subject, this is not a significant difference, but I don't really want to pretend to be an expert in terms of human medical issues. The data are pretty marginal.

JB: Let's talk about the intermediates, which I know you've done quite a bit of work on-the 2,3 and the 3,4 catechols (the quinines)-and their roles in physiology. I am now alluding to the methoxylation pathways.

Would you tell us a little bit about where these intermediates travel and what happens to them?

Action of Quinones in Physiology

ER: These are pretty reactive compounds and so they're going to react with something in the cell. (They won't travel very far in a cell.) They don't just react with water in the cell; they react with glutathione, which is a scavenger for many kinds of oxidized products. That's a good thing, because that takes them to being excreted. They can also react with DNA (a bad thing) to form these DNA adducts that can lead to the apurinic sites that seem to be the source of the initiation of cancer. The catecholesterogen 3,4 quinones are much more reactive than the catecholesterogen 2,3 quinones, just because of their chemistry. The formation of these DNA adducts by the 2,3 quinones is very small compared to the 3,4 quinones, and I think the major danger comes from the catecholesterogen 3,4 quinones. That's what seems to happen with them. They either react with DNA or they react with glutathione. They probably react with proteins, too, although I don't think anyone has shown that particularly. But I don't think that would be a damaging thing; it would just get rid of them. In the worst possible case, if one molecule of an enzyme gets screwed up, that's not a big deal

JB: One of the things you point out in the excellent review paper that was published in the journal *In Vivo* last month, titled "The natural chemopreventive compound indole-3-carbinol: state of the science,"⁸ is that the 2-hydroxylation patterns, facilitated by upregulating the enzymes associated with I3C, go on to principally form the methoxylated derivatives, which can be trapped, versus the 16-hydroxy compounds that don't get methylated, so they may be more promiscuous types of molecules. I think that's a very interesting concept-that there's an exit opportunity for the 4- and the 2-, but that doesn't seem to be the case with the 16-.

ER: Right. The 16s can continue to have effects on various cellular processes-biochemical processes, signaling processes, and things like that.

JB: Do you feel that the ratio of 2- to 16-hydroxylated estrogens measured by laboratories is more of a surrogate marker for relative risk, and that what we really should be measuring is the 4-hydroxylation pattern, or do you feel that there is something of concern relative to the increased 16-hydroxylation?

ER: I think the 4-catecholestrogens are the important ones to be measuring. I don't think it's been significantly shown that the ratio of the 2- to the 16-alpha is a particularly useful characteristic to measure. It's the 4-catecholestrogens that are going to do the damage and start the cell down the road to being cancerous, and that's what we should be measuring.

JB: If I interpret your review paper correctly, you are suggesting that to date, there is no statistically significant data that show that either DIM or I3C significantly elevate 4-hydroxyestrogens.

ER: Correct. The only paper I have seen that dealt with the 4-catecholestrogens was published in 1997. There, the difference, for example, the 4-hydroxyestrone, the so-called P value, which normally you would want to be at 5 percent or less, was only 16 percent. That's not statistically significant and you can't rely on that. Normally, you would feel comfortable if the statistics indicate that the difference you're seeing occurs 95 percent of the time, let's say in terms of the 4- catecholestrogens being induced. At 95 percent of the time, at least you'd almost always see that result. In the case of the P values indicating a 16 percent significance, you would only see the difference perhaps 85 percent of the time, which is not really

good enough to consider it a reliable difference.

JB: It sounds as if there's still room for a lot of additional work in this area, and that there are other variables influencing this, You mentioned methylation patterns and other things that participate, perhaps away from the 2- to 16 ratio, that have to do with how crucifers modulate cancer risk.

ER: I think that's very true. I also think that maybe next year, we'll probably publish a lot of data from humans that will put to rest a lot of these different controversies, because the technologies for doing these analyses have improved by orders of magnitude. The sophisticated kinds of analyses that you can do now, you couldn't do even five years ago, with the same level of sensitivity and the ability to separate all of the very similar compounds chemically or chromatographically.

JB: Being a researcher, a woman, a mother, and a person of the universe, based on what you've seen, would you recommend women not eat crucifers or foods containing either I3C or those that would be converted to DIM? Is there any evidence at this point that those foods could be hazardous?

ER: Oh, absolutely not. Everything I have seen has indicated that eating crucifers is an excellent thing to do. That food group, to me, is the most likely one to help prevent cancer.

JB: It sounds like we are at the threshold of some very exciting abilities, using the methodologies that you're developing. Also, from what you just said, I interpret that some of the past published data probably is somewhat questionable, just on methodological grounds, because we didn't have the sensitivity to address some of these questions about 4-hydroxylation patterns that we're now asking. It seems that we're on the threshold of being able to answer some very important questions about how the fractionated components of crucifers-phenyl isothiocyanates, I3C, DIM, or sulfurofane-influence these individual pathways.

ER: That's absolutely true. We are looking at the levels in urine, serum, and different body fluids in tissues, but I think that other laboratories that have access to much of this same improved analytical technology are going to be doing studies to see what happens when people ingest crucifer components. For example, there was a paper published about six months ago from a lab at the University of Kansas and analytically, it was the nicest and most advanced paper I've seen. Unfortunately, they didn't look at the 4-catecholestrogens, but they were already using much better technology than was available six, eight, or 10 years ago.

JB: As we look at where we are today, do you feel that we are seeing the emergence of a new field of chemoprevention that is going to be focused on personalized diets based upon metabolic characteristics of estrogen metabolism?

ER: I absolutely do. For me, and for our research group, this is really the goal-to use dietary supplements from natural chemicals, tailored to different people's basic metabolic profiles to contain the estrogens so that these reactive metabolites either aren't formed or are scavenged, so they are much less likely to damage DNA and start the process leading to cancer. I think this whole field is going to make tremendous strides forward in the next few years.

JB: At press and in the literature, have you seen a greater prevalence of human clinical work with I3C

versus DIM, or is there about the same ratio between the work on these two chemicals?

ER: There's more on I3C.

JB: The next couple of years in this field will be quite extraordinary for you, your colleagues, and your associates, in helping us to piece this all together and move us toward personalized medicine.

ER: I think so. I'm very confident about that.

JB: Thank you very much for all your diligent work. We're going to save a lot of unnecessary misery and suffering as a consequence of the application of your discoveries.

ER: I hope so. That is certainly the goal.

JB: Thank you again, Dr. Rogan. Our best to you, and we'll talk with you as the data start coming out.

Modification of Estrogen with Nutrient Management

I would like to spend the last few minutes of this issue of FMU reviewing the takeaways of what we have been talking about. We talked about estrogen and dietary intervention, and the relationship of low-fat diets to women's health. What I did not mention, which I think is clearly apparent to most of you who have been in this field for some time, is that moving to a more vegetable-based, minimally-processed diet modifies estrogen production and metabolism quite dramatically. It has an effect on the outcome from estrogen states of modified function. In women on vegetarian diets, there is altered estrogen level, improved androgen/estrogen balance, and increased metabolism and alteration in sex hormone globulin levels so that what we ultimately end up with is a better regulated endocrine system. I am talking about the work published by Joanna Dwyer and others over the years.

We also recognize from what I discussed earlier concerning the reversal of stress responses in maternal programming, that the immediate environment of the individual can influence aspects of hormone balance. We talked about the neuroendocrine, or the psychoneuroendocrine environment playing an important role in modulating some of these factors. We also talked about dietary factors that influence estrogen metabolism directly by hydroxylation (the wonderful work of Dr. Rogan), and how these intermediary metabolites that come through the regulation of the cytochrome P450s that metabolize estradiol and estrone into their various hydroxylated derivatives, can be encouraged to eliminate these estrogens in a non-toxic form through methylation and enzymes that go through S-adenosylmethionine (SAM). These are the catecholmethyltransferase enzymes, and they can be facilitated with proper levels of substrates of the SAMs by proper B6, folate, B12, and betaine nutriture. Many women who are on marginal diets relative to these methylating nutrients could benefit significantly by increasing those four nutrients in their diets, so as to promote proper methylation of their hydroxyestrogens.

We also talked about the use of the crucifers and their glucosinolate breakdown products-indole-3-carbinol (I3C) and diindolymethane (DIM)-to facilitate improved formation of the 2-hydroxylated estrogens at the expense of the 16.⁹ I said that both the 2- and 4-hydroxyestrogens can be trapped as the methylated derivatives, whereas the 16-hydroxy cannot. We talk about one to two portion sizes of cruciferous vegetables a day, or somewhere in the range of 200 to 300 mg of I3C equivalent that

comes from the crucifer glucosinolates, as the level that has been demonstrated in clinical trials to have positive effects on function in women. These are hormone-driven functions, such as papillomas, that have been demonstrated to improve with I3C supplementation. What I am starting to see emerge is the recognition that many of these women's health issues that occur postmenopausally are more than just modifying protein, carbohydrate, and fat levels in the diet. There is a rich library of phytochemicals that are present in a minimally processed diet with color that have influence on function.

I also reviewed some of the more recent studies that have been published about bone loss in postmenopausal women that did not seem to be effectively managed by calcium and vitamin D supplements. This was a big "aha" coming out of the WHI that you heard about recently. Calcium and vitamin D supplements did not seem to reduce fracture incidence in these women. I believe this is because osteoporosis and bone fracture is more than just a calcium and vitamin D problem. It is also an inflammatory signaling problem. The osteoclasts-the bone resorbing units-are stimulated into resorption as a consequence of inflammatory signaling. Therefore, if one eats a diet of low inflammation content that has rich gene programmed nutrients that lower inflammatory potential, that will have a better effect on reducing risk to osteoporosis than simply focusing on calcium and vitamin D alone supplemented into a "white" diet.

I keep coming back to how estrogens and androgens travel properly in a woman's body. We have to look at historical and evolutionary perspectives to better understand that, because historically, women have eaten complex diets rich in an array of all these signaling molecules in foods that have impact upon estrogen balance production and metabolism.

Last, we talked about things in the diet that may precipitate an alarm, or allergic reaction. That obviously has to do with things that in one person's diet may appear to be a very good food, but in another's diet, not such a good food, because it alerts the immune system to do battle. I am talking about things like gluten or alpha-gliadin found in some cereal grains, and things like beta-casein, a milk protein, which may initiate immunological reactions in certain individuals.

There is an ever-increasing understanding of the changing patterns of food allergy. A very nice review paper written by Dr. John Walker-Smith appeared in the journal *European Journal of Gastroenterology and Hepatology*. He talked about food allergy affecting many more people than we previously thought, and that this food allergy may really be considered food sensitivity, because some of these are not traditional allergies, but may have other types of effects by influencing things like enteric flora.¹⁰ Certain foods may then alter flora, which secondarily may have an influence on GI mucosal immune function, causing inflammation. It is both a direct effect of the food constituent and possibly an indirect effect.

The management of these types of food reactions, what we call allergies, is, in part, related to the full composition of the diet-things like components of the diet that support proper bacterial flora, flora that would be "anti-inflammatory" versus "proinflammatory." There has been some very nice work done showing that oral supplementation with probiotics can help to lower food reactions and food sensitivity. One good article was published in the *European Journal of Gastroenterology and Hepatology* last year.¹¹ Certain species of probiotic organisms, when administered orally, may have a salutary effect on food reactions and lower gut inflammatory conditions. I am talking about both localized gut inflammation seen in conditions like Crohn's disease or IBD, and perhaps systemic inflammatory conditions, as well, with arthritis-like symptoms.

As breakdown of the gut mucosal barrier occurs and the gut becomes permeable, it increases the relative risk, and one is now exposed to these antigens to the gut mucosal immune system, which can signal this inflammatory process to the rest of the body. Gut barrier immune function is very important. In fact, breakdown of gut mucosal integrity is often a hallmark of long-term, food-related allergy at the gut level. Antigens can then penetrate the mucosa and induce allergic inflammation. There is another good article that describes this process in the *European Journal of Gastroenterology and Hepatology*.¹²

Clinically, the way one often goes about understanding this is by measuring the level of calprotectin in a stool sample. Fecal calprotectin is an inflammatory response protein that is produced and shed by neutrophils in the gut. High levels of calprotectin in the stool are markers of gut inflammation. They are also tightly correlated with increased intestinal permeability that could be measured by things like the oral lactulose/mannitol challenge test, which you are probably familiar with. We have found much more clinical utility recently in using the fecal calprotectin test. We have seen patients with fecal calprotectin at a level of 200 mcg/g in stool (the normal range is less than 30 mcg/g) who have very serious breakdown of gut mucosal integrity, a lot of gut inflammation, and systemic inflammatory conditions that can be precipitated or aggravated by this immune response.

The whole system is interrelated-gut connected to immune system connected to liver connected to all the circulating white cells-and all of those influence physiological health. In this case, I am talking about estrogen, estrogen metabolites, and ultimately, female-related health problems associated with different cycles in a woman's development, including perimenopause and menopause.

Postpartum Seizures

Last, when you have increasing inflammatory signals, there is also increasing risk to mitochondrial dysfunction. One of the things that is interesting in women is a condition involving postpartum seizures. Postpartum seizures are pathophysiological and they are associated with alteration in neuroendocrine signaling, particularly allopregnenolone, pregnenolone, progesterone, and estrogen. Wide swings in the neuroactive hormones can lead to depolarization, mitochondrial uncoupling, and seizure disorders. Mitochondrial dysfunction is implicated as a contributing factor in a diverse range of acute and chronic neurological disorders, but I think it is interesting to consider how estrogen may be related to this postpartum seizure condition seen in some women. This may come as a consequence of very rapid alterations in androgen/estrogen ratio levels, leading to alteration in membrane polarization, which (I am hypothesizing here) could lead to changes in mitochondrial function

That leads us to ask what alters these things. Certainly, the stability of estrogen and progesterone signaling plays a very important role in normalizing the nervous system. I have used an extraordinary example talking about postpartum seizures, but we might think about chronic neurological dysfunctions associated with endocrine ratio changes in menstruating women as they go through the estrogenic and androgenic phases of their cycle. How does this interrelate with mitochondrial function, energy to polarization, and eventually, alterations in central nervous system and peripheral nervous system function?

There is some significant work now going on looking at the connection between the nervous, immune, and endocrine systems in women, to try to understand what the modifying or balancing factors are. The only way we can understand this is to look at the system as a web. You cannot look at any one single

agent. You need to look at it as a web of interacting variables. The nervous, immune, and endocrine systems are intimately tied together through receptors and signaling molecules. As we develop the functional medicine model, we recognize the importance in the connection of the gut and the liver, which is connected to the immune system, which is connected to the inflammation system, which is connected to the cardiovascular system, which is connected to the nervous system. The approach we have been describing in this month's issue of FMU is to look at estrogen more as a surrogate signaling molecule, how it interrelates with different cycles and functions, how that is connected to the neuroendocrine immune system, and how it ultimately connects to metabolism and excretion through hydroxylation and methylation patterns. When you get to that level, you start understanding why it is that autoimmune disease is possibly heavily centered in women, why it is that postmenopausal women have cardiovascular disease, neurological disease, and bone loss as principal features of their health histories, and why the environment plays such an important role through proper dietary and lifestyle manipulation as "the best medicine."

I hope this issue of FMU has been of some interest and help to you. We look forward to sharing with you again in May.

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