March 2003 Issue | Associate Professor of Medicine and Pharmacology Boston University School of Medicine

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Welcome to *Functional Medicine Update* for March 2003. We continue this month with "The Heart on Fire." We are addressing this theme in preparation for our 10th International Symposium on Functional Medicine in Tucson Arizona, May 21-25, at the La Paloma Westin Resort. We have been fortunate to have a number of Clinicians/Researchers of the Month who have made primary contributions to our evolving understanding of cardiovascular risk beyond cholesterol.

This month we continue that theme as we direct our attention to heart disease risk factors related to inflammation, with specific focus on obesity-related factors. This often-overlooked area is difficult to manage clinically. We recognize its importance but frequently ignore it because we are not sure how to manage it. We know how difficult management of body composition and obesity is in clinical practice and we recognize the failure rate of virtually every treatment modality undertaken in the last few decades to achieve long-term management of body composition.

Research and clinical literature in this area, however, are undergoing rapid change from the perspective of how obesity relates to cardiovascular disease. We now recognize that obesity may originate in the interaction of genes and environment. This recognition points toward a new way to manage it.

Weight Management Based on First Law of Thermodynamics
For many years we assumed that obesity results from poor eating habits and uncontrolled behavior, with the deposition of extra calories as fat in the adipocytes. That model was built on the first law of thermodynamics. That law states that energy-in must be balanced with energy-out for weight to be maintained. The assumption would be that a person gained weight as a result of consuming too many calories and engaging in too little activity to burn them off. The treatment of choice was to find new, convenient ways of restricting or limiting calorie intake, or putting people on exercise programs to increase their resting energy expenditure and activity.

Both of those concepts are certainly viable and important to keep in mind. We do not want to throw the baby out with the bath water and suggest that neither calories nor exercise is important. Instead, I want to point out that our basic assumptions on the subject of weight gain, body mass change, and the relationship to vascular disease are undergoing a change. We now understand these conditions are related to alterations in the neuroendocrine immune system. Rather than simply looking at the body as a calorimeter—a device that burns calories to produce heat—we now see it as a complex metabolic network controlled by a variety of important physiological mechanisms related to the status and function of the

neuroendocrine immune system.

Signals for Weight Gain

The new understanding imposed on the old thermodynamic model of weight will lead us to new avenues for improvement. We are not members of a slothful, hedonistic society that is destined to be obese. Instead, signals from our environment may be altering our appetite satiety mechanisms, our energy regulatory mechanisms, and shunting calories away from effective use in energy production for physiological activity, neurological activity, immune healing, and so forth. These signals may be shunting that energy into a storage form called triglycerides for rainy days that never come. We will speak about that transition in this month's *FMU*.

I would not be so presumptuous as to suggest that we have all the answers and can simply implement the new rules and never again have to worry about body composition and weight. Instead, I would like to share with you a trajectory of increased understanding, an evolution of the relationship of our environment to our body composition. Today we can implement some new mechanisms to improve efficacy and success in managing body composition and lowering recidivism rate. I begin this month's *FMU* on that optimistic note. We will try to improve our understanding of the correlation between body composition and cardiovascular risk factors. Understanding these new mechanisms based on the neuroendocrine immune function of the body might help us modify body composition.

Weight Change and Quality of Life in Women

A recent issue of the Journal of the American Medical Association contained a prospective paper examining weight change and health-related quality of life in women.2 When we think of obesity, we have often focused almost all of our attention on specific obesity-related diseases such as diabetes and high blood pressure and their relationship to heart disease and cerebral vascular disease, gall bladder disease, and even some forms of cancer. We have made those the focus of our concern in considering the histopathology that might be produced after years of accumulation of fat and visceral adipose tissue.

This paper in JAMA raises a new question. Do alterations that we call functional changes occur well before the onset of a diagnosable disease from altered body composition? I believe this is another example of the movement of the perspective in medicine away from histopathology associated with endpoint disease to the earlier warning features of functionality, which are precursor markers for later disease.

Weight Change and Function

In this prospective study, the investigators looked at weight change and its relationship to functional changes that would alter the individual's perceived quality of life. The researchers administered the Medical Outcomes Study Survey, Short-Form 36 to patients with different body mass index (BMI). They were able to identify whether there were in fact any transitions or changes in functionality or lifestyle variables associated with quality of life correlated with BMI. They divided study participants into two groups: women aged less than 65 years and those older than 65 years.

The results were remarkable, although perhaps not unexpected. In women less than 65 years of age, all quality-of-life indicators, as evaluated by the Medical Outcomes Study Short-Form 36, were decreased with increased weight. Some of the most remarkably declining indicators seen with increasing BMI

included physical function and bodily pain. I emphasize bodily pain. Pain is often associated with upregulation of the inflammatory cascade. Joint pain, headache pain, and muscle pain are all conditions related to inflammation. People may start taking a nonsteroidal antiinflammatory medication (NSAID) in their 20s to modulate symptoms that later become progressive.

Bodily Pain and BMI
The study found that bodily pain of unknown origin was strongly correlated with increased BMI, or the accumulation of body fat. Individuals who did not accumulate body fat with age had far less bodily pain and physical dysfunction. Therefore, there is a suggested correlation between body fat accumulation and bodily pain and discomfort. You might say: Well, of course, if you are overweight, you are less comfortable. But at a mechanistic level, what does this mean? Is it connected to the inflammation association I described relative to heart disease, diabetes, cancer, and events associated with non-cholesterol-related cardiovascular risk factors?

C-Reactive Protein and Obesity

In 1999, the Journal of the American Medical Association contained a paper by Visser, et al. titled "Elevated C-Reactive Protein Levels in Overweight and Obese Adults."3,4 The authors showed a strong correlation between BMI and the elevation of high-sensitivity CRP or inflammatory protein levels. CRP is a classic acute-phase reactant, plasma levels of which can increase as much as 10,000-fold in response to tissue injury and infection. Clinicians have normally thought of CRP as an acute-phase reaction in conditions like rheumatoid arthritis. However, there has been increasing interest in the possible relevance of low-grade inflammatory process to cardiovascular disease and vascular risk factors.

That interest led Paul Ridker and others to develop the high-sensitivity CRP test, which measures CRP at low levels, and correlates it with some chronic illnesses. Its plasma level is determined mainly by the synthesis rate in the liver, and closely reflects inflammatory activity. This is hepatic synthesis of CRP in response to a purported or presumed inflammatory message.

CRP and Obesity
The study I just described was the first to demonstrate convincingly that plasma CRP levels are substantially higher in obese and overweight people than in leaner people. It suggests a correlation between these bodily pain syndromes and the sense of lowered physical function as seen in increasing BMI and a possible relationship to later-stage heart disease and other vascular complications as a consequence of this inflammatory process. As an historical aside, this would connect with what Rudolph Virchow proposed as the origin of heart disease in the mid-1800s.

We previously thought the principal purpose of statin drugs was to lower the risk of heart disease by lowering blood cholesterol levels. We now understand that long-term use of statins may actually produce sustained reductions in plasma CRP levels that appear unrelated to their cholesterol-lowering activity. This is a new view of the role of statins. The inflammatory process, at a pathophysiological level, has something to do with the vessel wall or endothelial activities that give rise to oxidized LDL and ultimately to atherogenesis. We have been discussing this process with our Clinicians and Researchers of the Month in the past several months.

Chronic Systemic Inflammation Associated with Obesity

Systemic inflammation associated with obesity may involve more than physical disability, pain, and loss of bodily function. It may also represent an early risk factor for much more serious, in fact life-threatening conditions. That risk was the subject of a recent article in the*Journal of the American Medical Association.*[5] In this article, authors Alexandros Vgontzas and Edward Bixler, from the Pennsylvania State University College of Medicine, reported that patients with elevated BMI demonstrated a positive association between obesity and plasma IL-6 levels. Levels of interleukin-6, which were significantly increased in middle-aged individuals, were also positively associated with sleep apnea, a common symptom in individuals with increased BMI.

The origin of sleep apnea is unknown. Its treatment frequently requires detailed sleep studies and certain kinds of medications or surgery. Vgontzas and Bixler found a strong correlation between increased IL-6 in individuals with increased BMI and sleep apnea. It is interesting to hypothesize that this inflammatory cytokine may play an important role in mediating sleepiness and fatigue in these subjects. Therefore, not only CRP and TNF-a, but also IL-6 may be associated with symptoms such as sleep disturbances and sleep apnea.

Is Obesity an Inflammatory Condition?

We are beginning to develop a model that describes the role of body fat in a variety of functional changes. The fact of a person's getting fat is not the sole contributor to heart disease. Contributing functional changes occur over years, according to the authors of a recent article in the *Journal of Nutrition,* titled "Is Obesity an Inflammatory Condition?"[6] The authors state that obesity may be considered a low-grade systemic inflammatory disorder. It interacts with a variety of neuroendocrine immune hormones ranging from neurotransmitters to immune modulators.

We used to think of body fat as benign storage tissue. We now recognize it is tissue that is generating its own messages by upregulating gene expression within the adipocyte, the fat cell. Inflammatory mechanisms and messages are being produced that interact with dopamine, serotonin, and neuropeptide-Y. The adipocyte also produces the hormone leptin, which influences acetylcholine and melanocyte-stimulating hormone, the cytokines and nitric oxide, and insulin and insulin receptors.

Fat and Function
This complex neuroendocrine/immune relationship gives rise to a different physiological state of the individual. It is not just that fat causes diabetes or heart disease. Fat changes the body's function by regulating genes to produce new, different messengers. The drugs that have been used recently for modifying diabetes or heart disease risk may help us understand the mechanisms that underlie these inflammatory-mediated processes associated with chronic disease—the statins or HMG Co-A reductase inhibitors, the PPAR inhibitors, the NSAID drugs.

We may understand the effects of all of these drugs if we look at the central mechanisms and connect them to changes in body composition. Fat plays a role in modifying the orchestration of our body talk, and these different mediators are influencing function at the neuroendocrine and immune system levels.

Waist Circumference as a Predictor of Cardiovascular Risk

It is not just body fat in general that appears to cause difficulty. Some regions of fat deposition may

increase the risk of these adverse or inflammatory messages. Concern has evolved from body fat to body VAT. Visceral adipose tissue (VAT) is interabdominal fat, not subcutaneous fat. Wrapped around organs, it has direct effect on organ system physiology. A recently published series of papers show that waist circumference is a better indicator of relative disease risk than BMI, which is height-to-weight ratio. Waist circumference is a better measurement of where body fat is being deposited in terms of its relative risk.[7] VAT, which is associated with waist circumference, or waist-to-hip ratio, seems to be associated with the greater risk for altering cellular and physiological function and increasing the risk for all obesity-related diseases, including heart disease.

The *American Journal of Clinical Nutrition* contains an article titled "Waist Circumference and Obesity-Associated Risk Factors among Whites in the Third National Health and Nutrition Examination Survey: Clinical Action Thresholds." [8] The authors found waist circumference was more closely linked to cardiovascular disease than BMI alone. When a man's waist measurement exceeded 90 or 100 centimeters, he almost always had increased risk factors for cardiovascular disease.

Waist Circumference and Cardiovascular Disease
The consistent conclusion from a large body of literature is that waist circumference is a predictor of cardiovascular risk. Disease risk depends on where fat is deposited and the role it plays through the elaboration of its own mediators, such as leptin, and the elaboration of inflammatory mediators like CRP.

Have you ever thought that your body fat is producing inflammatory substances that are telling the rest of the body that you are on fire? I hope that will become part of your thinking when you are dealing with a patient with an increased waist-to-hip ratio and increased BMI. In that patient you are dealing with someone who has a different series of messenger molecules that are increasing functional disability, increased bodily pain and later-stage increased risk for these degenerative diseases.

Thigh Adipose Tissue Distribution and Insulin Resistance in Obesity and Type 2 Diabetes

Intramuscular fat, such as thigh adipose tissue distribution, is strongly associated with later-stage conditions like type 2 diabetes or coronary artery disease. Authors of a recent paper in the *American Journal of Clinical Nutrition* discussed intramuscular adipose tissue deposition, such as thigh adipose tissue deposition, in which there was an increased accumulation of triglycerides within this interim muscular area. They found this "marbled" fat had a very strong association with the onset of type 2 diabetes.[9]

Body composition, body compartmentalization, physiological messaging and its relationship ultimately to cardiovascular disease are at the cutting edge of the new research. You might wonder if these transitions that occur with aging, when our body loses muscle and gains fat, are naturally locked into our genes. Are we raising alarm about mechanisms over which we have no control?

I think the evidence indicates the answer is no. We are not creating alarm without possibility of action or alternatives. It is better to know about these transitions and understand that they are not necessarily locked into the hard wiring of our genes. Losing muscle and gaining body fat as we age may be the normal thing that happens, particularly in Western society, eating the foods, living the lifestyle, and being exposed to the stress that we are. But it is not required.

Basal Amino Acid Kinetics and Protein Synthesis in Healthy Young and Older Men

Why do I say that? A study published in the *Journal of the American Medical Association*started us thinking about the loss of muscle and the gain of body fat from a different perspective. This new perspective is related more to metabolic balance, cell signaling and transformation, the biotransformational steps in our body that regulate cell phenotype. That study is titled "Basal Amino Acid Kinetics and Protein Synthesis in Healthy Young and Older Men."[10] The researchers compared 20-year-old to 60-year-old men, using a very sophisticated double labeling methodology with amino acids.

The study sought to determine if the difference in muscle mass between young and older men was a consequence of the decreasing ability to convert dietary amino acids into muscle protein. If that were the case, it would indicate that biosynthesis rates decline with age, and as one ages the body cannot effectively replace muscle protein, and body fat percentage increases as lean muscle mass decreases.

The traditional theme in physiology textbooks was that we lose muscle mass as we grow older because our biosynthetic rate for the production of muscle protein goes down. At the same time, our biosynthetic rate for putting fat into adipose tissue remains the same or may increase as it is stimulated by extra calories.

Results of Test Comparing Muscle Mass
The results of this study changed the old perceptions and perhaps set up the opportunity for a paradigm shift. In the summary, the authors wrote the following:

"Net protein balance was similar in both groups. Small differences were found in mean (SE) muscle protein synthesis in comparisons of older vs. younger men: arteriovenous balance. Small differences were also found in mean (SE) muscle protein breakdown. Differences in basal muscle protein turnover between older and younger men do not appear to explain muscle loss that occurs with age."

If it is not locked into the genes, one might wonder what does explain the loss of muscle protein with age, because it is apparent in most older men and women in comparison to younger individuals.

According to the authors of this study, sarcopenia (the loss of muscle) is not due to inadequate basal (fasting) protein synthesis in older individuals. Instead, aging muscle fails to respond to stimuli (e.g., diet and exercise) that are anabolic to young muscle. The evidence suggests older individuals still have the ability to make the muscle protein, but they are not getting the right signals. The reason older people are less responsive to these stimuli is that hormonal or immunological changes that occur with age no longer favor anabolism, or the building up of new protein. They favor catabolism, the breaking down of protein. These catabolic signals include the inflammatory cytokines we talked about earlier—IL-6, TNF-a, and chemokines like CRP.

Cell Signals for Catabolism
This process may sound similar to a dog chasing its tail. You gain body fat; the fat sends out messages; the messages are catabolic to muscle; muscle breaks down; fat is higher in percentage composition in the tissue; and around you go. More fat produces more signals that produce more catabolic messages to muscle. It was not determined in the genes that a person would lose muscle and gain fat with age. It was as a consequence of waves of signals over the cells, creating a different phenotype.

These studies implicate insulin resistance and immune factors such as catabolic cytokines, acting primarily in the postprandial state, as an important cause of sarcopenia. I emphasize postprandial. After eating, we flood our bodies with messages, not just insulin, but thousands of chemicals that are released from messenger systems to create downstream effects on cell regulation that reshape our bodies and our function over time. You develop bodily pain conditions and lowered physical function; you get foggy brain syndrome. This is a neuro-immuno-endocrine interrelationship. We can try, using diet and exercise, to modify the signals that create downstream effects on protein synthesis or sarcopenia.

At the Functional Medicine Research Center we are studying this process and working to develop a better understanding. What signals do we send from our selection habits each day? We send signals from our diet, our lifestyle, our exercise patterns, and our environment. We tell the receptor sites how the body will be reshaped and what messages then travel in our blood to create downstream effects at distant sites in our bodies. The accumulation of visceral adipose tissue creates different messages that signal for a different body. The effects are not just localized; they occur throughout the body.

These effects are clearly related to body composition, the relationship of muscle, fat, bone, and water to overall body activity or physiological function. We often neglect an important tool in medicine, which is the way body composition is disposed in an individual. What are the relative amounts of body fat, muscle, and extra- and intracellular water?

Answers to these questions tell about the trajectory of physiological status of that patient. We can measure it serially, not just once but yearly or every other year, to assess the trajectory of body composition measurements. Is body fatness increasing? Is compartmentalization of electrolytes decreasing so phase angle and bioimpedance analysis are changing? These physiology meters tell us something about the regulated process of these chemokines, cytokines, and inflammatory mediators.

The fat cell itself, the adipocyte, reveals quite a bit of information. The adipocyte is not, as we previously thought, a benign or inert cell that stores fat. Well before fat storage occurs, the adipocyte is creating a different message to the body through upregulation of its genes. It is producing hormones like leptin, CRP, and other inflammatory cytokines like TNF-a and IL-6, which have effects throughout the body, as well as in the central nervous system, to create different affect and physiognomy.

Adipocyte physiology is fascinating because it is a fairly new theme in medicine. A recent paper in *Trends in Molecular Medicine* discusses adipose tissue. We now understand it plays an active role in metabolic regulation, secreting a variety of metabolic hormones actively functioning to prevent deleterious lipid accumulation in other tissues.[11] When a breakdown occurs in the fat cell physiology signaling mechanism, you start to get a pushback by the body, and risk factors arise. It is as though the body fat cell has decided it has had enough and needs to send a message. “Listen to what I am saying. I am going to produce symptoms of pain, dysfunction, and declining physical activity, and you need to take notice.”

We are beginning to understand the function of hormones like leptin, which is produced by the adipocyte. Intrinsic sensitivity to leptin in the extracellular, extra-adipocyte area is very important. It is not just an appetite-controlling hormone that works in the hypothalamus of the brain. It also appears to affect many other tissues, such as liver, bone, and muscle, where it can signal different physiological activities. You can develop leptin resistance, just as you can have insulin resistance, in which this message gets blunted

and the body may be tricked into thinking it is suffering from a leptin deficiency.

Leptin deficiency creates a new physiological effect of deprivation, which is associated with the alterations in eating behavior and food intake we often see as hyperphasia. A *Nutrition Reviews* article reviewed new research on leptin physiology and the role of leptin in controlling body weight and distribution.[12,13,14] Breakthroughs are occurring in our understanding of leptin's action as an adipocyte hormone, its effect on appetite and extra-central nervous system influence in angiogenesis, wound healing, blood pressure, and homeostasis, beyond that of satiety. Regulation of leptin, neuropeptide Y, insulin, and the inflammatory cytokines is involved in the understanding of visceral adipose tissue physiology and its effects on vascular disease.

Studies of Leptin in Animals
Animal studies have recently revealed that knocking out stearoyl-CoA desaturase-1, one of the enzymes involved with leptin-mediated function, has an influence on a component of metabolic actions associated with leptin.[15] This enzyme is involved in formation of the monounsaturates like oleic acid found in olive oil. It suggests that certain fatty acids in the diet, monounsaturates, may have different effects on leptin physiology, thermogenesis, and appetite. Other fatty acids, such as omega 3 fatty acids, the fish oils like eicosapentaenoic acid (EPA), may influence another class of obesity-related hormones associated with the PPARs. (We will discuss PPARs in more detail on side 2.) Increased EPA levels in the diet may help regulate PPARs, assisting with insulin control, appetite regulation, and obesity regulation.

Supplementation of animals with N-acetyl-carnitine improves leptin activity and reduces leptin resistance. Carnitine may have some value as a nutrient, as well. This topic is on the frontier of exploration. According to a paper published in the *Journal of Nutrition*, N-acetyl-carnitine supplementation positively influenced leptin sensitivity in older animals, and improved glucose transport and insulin regulation as well.[16]

Let's turn to side 2 and talk about antioxidants and their relationship to vascular disease. We will finish up with a discussion of the relation of adipocyte physiology to our increasing understanding of cardiovascular risk management.

INTERVIEW TRANSCRIPT

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JB: Once again, it's time for our Clinician/Researcher of the Month interview. We are privileged to have Dr. John Keaney, Jr. as our guest this month. Dr. Keaney is associate professor of medicine and pharmacology at Boston University School of Medicine. He completed his undergraduate work at Harvard College, his medical training at Yale, and was an intern in medicine and a resident at Brigham & Women's Hospital. He is now in academic research and a clinical physician in cardiology at Boston

University.

Nitric Oxide and Cardiovascular Function

Because of this year's FMU focus on cardiology and modifiable factors beyond cholesterol, we have had some remarkable interviews with Dr. Louis Ignarro, Nobel Prize winner in Medicine & Physiology in 1998, and Dr. John Cooke from Stanford. Dr. Keaney will add another dimension to our understanding of this emerging field of cardiology and endothelial function. It is with great pleasure that we welcome Dr. Keaney to FMU. John, I noticed that one of your first publications was with Dr. Jeremiah Stamler, looking at the relationship of nitric oxide to cardiovascular function.17 Would you tell us how you got into this field?

JK: Sure. It's a pleasure to be here. When I started out in my training, I was interested in how blood vessels work. My training coincided with a number of observations in cardiology that tended to suggest the physical presence of a blockage in the artery provided an incomplete explanation for the development of vascular events such as heart attacks and strokes.

In my search for investigator training, I came across an individual named Joseph Loscalzo, who was doing research at Brigham & Women's Hospital. My first foray into research had to do with understanding how different compounds may affect the function of blood vessel walls. That's how I became interested in that line of investigation. That was when I was training in research and assisting in a larger investigation. It opened my eyes to the idea that certain compounds made in the vascular wall have an important effect on how the vascular wall functions and reacts to its environment. That was the first part of my research experience and my interest in how the vascular wall functions.

Homocysteine/Nitric Oxide

JB: I notice that work led into a collaboration with Dr. Balz Frei, to whom we have often referred in FMU during the past 10 years. You looked at the interrelationship of homocysteine, nitric oxide, and redox potential. This takes us back to the discovery by Kilmer McCulley, another FMU contributor. How has the homocysteine/nitric oxide story unfolded?

JK: It continues to unfold. I think it's unequivocal that homocysteine is an independent risk factor for vascular disease, although we don't yet understand the precise cause. There are several competing theories, not all of them mutually exclusive. One involves some effect of homocysteine on the redox state, or the oxidative stress within cells. Another component concerns the way homocysteine may modify proteins or other molecules or particles in the vascular wall and make them more prone to facilitate the atherosclerotic process. I don't think we've completely determined which of these two contemporary theories is more prominent. They are not necessarily in competition.

Oxidative Stress

JB: You first used the words "oxidative stress" in your publications in 1995, looking at vascular oxidative stress and dietary probucol. Was that a start down the path of looking at agents that modified or influenced intimal cellular redox?

JK: Yes. When I started doing research, we were interested in compounds like nitric oxide, which was discovered by Lou Ignarro. We wanted to know how it affects the vascular wall. We became very interested in the notion that the nitric oxide system was defective in the setting of both vascular disease and risk factors for vascular disease. For this reason, we began to investigate mechanisms whereby that might be true. One prominent theory of atherosclerosis at the time was that, in order for LDL to contribute to the atherosclerotic process, it needed to be modified into a form that could be taken up into

macrophages, start the inflammatory process, generate foam cells, and then beget the entire spectrum of atherosclerosis.

That modification process involved oxidation of the LDL lipids. That is where we began to investigate how antioxidant compounds, compounds that might interrupt the oxidation of either LDL or adjacent cells, might affect the way blood vessels behave. We began to explore the relationship between oxidative stress and the nitric oxide system in the vascular wall.

The Vitamin C Connection

JB: In a 1996 paper in Circulation, you looked at the role of vitamin C/ascorbic acid and endothelial dysfunction in patients with coronary artery disease. Since then you have been involved in a number of studies related to ascorbic acid and other dietary antioxidants.18 What has been your takeaway to date on the vitamin C connection?

JK: We became interested in vitamin C through our collaboration with Balz Frei, who reminded us that not all antioxidants are lipid soluble. We became interested in how the vitamin C status of cells could affect the nitric oxide pathway. The paper you referred to involved administering some oral ascorbic acid to patients with established coronary disease. We were able to observe that their nitric oxide-mediated vascular dilation, a surrogate for the function of the endothelium and the vascular wall, was vastly improved.

At the time, there was a theory in regard to the vascular wall. All abnormalities of nitric oxide that related to oxidative stress were believed to be due to a simple chemical interaction between nitric oxide, which is a radical species, and superoxide, which was one of the better known oxidants produced in the vascular wall during processes such as atherosclerosis and diabetes. We thought that explanation might be overly simplistic as it pertains to vitamin C, due to kinetic constraints that we won’t go into here. So we began to investigate other effects of vitamin C in the endothelium.

Tetrahydrobiopterin

Probably our most important discovery was that of a very important cofactor for the enzyme that makes nitric oxide synthase, called tetrahydrobiopterin. We found the vitamin C status of cells is an important determinant of how much tetrahydrobiopterin is available in endothelial cells to support the production of nitric oxide. I think now it’s generally well established in the field that administration of vitamin C that leads to improvement in vascular function is predominantly mediated through its effect on tetrahydrobiopterin, a cofactor for nitric oxide synthesis.

Heterogeneity of Ascorbate Needs

JB: Has there been enough work in humans to determine the heterogeneity of needs for ascorbate on that process with tetrahydrobiopterin? Do we know what percentage of the population achieves saturation of plasma levels under normal dietary intake?

JK: I think there really has not been enough research. Mark Levine at the NIH has attempted to address some of this. He has done a series of very elegant studies involving admitting patients to a monitored ward, depriving them of vitamin C, repleting them slowly but surely, and then looking at some of the physiologic parameters that might go hand in hand with vitamin C status. He has made some important observations, the most notable of which is that the amount required to prevent scurvy is probably lower than the tissue limits with implications for other effects of vitamin C, such as vascular function and a number of other biologic processes. Based on the RDA, we may actually be under-treating a significant

group of the population.

Alpha Tocopherol

JB: In your work, I notice you started looking at other antioxidants and their role in the redox relationship to endothelial function. A 1996 publication had to do with alpha-tocopherol and inhibiting the aggregation of human platelets.19 There seems to be a transition into looking at the tocopherol family. What has your work indicated about the importance of vitamin E in this regard?

JK: We tried to look at some of the effects of vitamin E that weren't distinctly related to changes in LDL oxidation. We initially investigated the importance of vitamin E status of both endothelial cells and platelets in events that are germane to atherosclerosis and cardiovascular disease. We found that endothelial cell concentrations of vitamin E have a profound impact on how robustly the endothelial cells can defend against the insults that are common to atherosclerosis, such as oxidized lipids and cytokines.

With respect to platelets, we demonstrated that vitamin E has an important effect in inhibiting the aggregation of platelets, particularly the aggregation of platelets due to certain agonists known to be relevant in atherosclerotic vascular disease.

Vitamin C in Prevention of Nitric Oxide/Superoxide Interaction

JB: In a later paper you discussed high concentrations of vitamin C in preventing the interaction of nitric oxide and superoxide, suggesting above-normal physiological levels.20

JK: It was an investigation in which we tried to explain whether observations with vitamin C had to do with preventing the interaction of nitric oxide and superoxide. To make a long story short, one needs very high levels of vitamin C to effect that. I would point out, however, that those levels are not achievable through anything short of pharmacologic manipulation, i.e., infusion of high doses of vitamin C. It is not clear to me that level of dosing is actually needed. On the other hand, robust oral dosing—1/2 gram to 1 or 2 grams per day—appears to replete the tissue levels sufficiently to effect improvement in vascular function.

Vitamin E/Vitamin C Relationship

JB: Have you found a relationship, either positive or negative, between vitamin E and vitamin C in some of these processes, the peroxynitrite-generating processes, or other dynamics related to nitric oxide?

JK: That is really the $64,000 question. A lot of people have demonstrated in vitro evidence for a cooperation between vitamin E and vitamin C. Thus far, the ability to demonstrate that in vivo has been elusive. In our case, we haven't found any important cooperative activity, but to be honest, we haven't looked for it very intensely.

Estradiol and Atherosclerosis

JB: You have a series of papers, started in 1998, that deal with estradiol and its relationship in antioxidant and atherosclerosis protection, which differentiates women from men.21 What have you found in your work with regard to estrogen?

JK: We were able to demonstrate a component of antioxidant activity related to 17-b estradiol that was distinct from other forms of estrogen. This antioxidant activity appeared to be related to the ability for 17-b estradiol to be transformed into a lipid ester through the action of HDL. This effective estradiol appeared to help mediate the response of endothelial cells, but we didn't investigate any of the proinflammatory or procoagulant effects of estradiol at the time.

As you know, investigation in estradiol as a potential therapy for cardiovascular disease has been sidetracked somewhat lately because at least two of the clinical trials have demonstrated a short-term increase in cardiac events in patients who undergo the initiation of hormone replacement therapy.

Estrogen Research

JB: I want to make sure we are clear on those previous studies, the HERS Trial studies. As editorials in both JAMA and the New England Journal of Medicine pointed out, those were mixed conjugated equine estrogens. So we are not exactly sure if it was estradiol or the equilin and equilinin, or other minor constituents found in equine estrogens that are not found in normal women's estrogen. I'm not sure that we have defined estrogen, have we, as the principal cause?

JK: I couldn't agree with you more. I do think the take-home message is that blanket treatment with Premarin™, for example, in the hopes of preventing cardiovascular disease, is probably not the right strategy. We probably do need to learn more about how individual estrogens work. That's clearly fertile ground for future investigation.

Iron and Cardiovascular Disease

JB: You have done quite a bit of work looking at metal iron relationships to oxidative stress. When a doctor asks about iron status and heart disease in patients, do you have a sense of what we should tell them at this point with regard to the research?

JK: The research in iron and cardiovascular disease has in some way paralleled what we've seen in oxidative stress and cardiovascular disease. Some time ago people were very interested in iron, thinking that free iron that was available contributed to tissue damage that would occur during the course of cardiovascular disease. I think that concept has been replaced by a notion that perhaps total stores of iron, or just the relative amounts of iron, are not the entire story, but rather how available the iron is to participate in potentially deleterious processes.

I think most of us in the field are coming to a new understanding that certain transition metals in the setting of vascular disease may be more available for things like redox reactions than they would normally be. Just measuring levels of iron may be overly simplistic. We may need better tools to determine how available the iron is for redox reactions as a better reflection of how much they might contribute to pathologic processes.

The Importance of Dietary Iron

JB: If a doctor asks about iron status of the diet or iron within a nutritional supplement for a man, could you make a recommendation at this point? Should he be very concerned about dietary iron, based on this emerging understanding?

JK: I think I would be, but I can't say we have firm data right now to tell us what an RDA or a specific amount of iron should be in a man who is at risk for cardiovascular disease. I myself would be concerned about providing any extra iron based on its propensity to participate in some of these redox reactions that we're concerned about.

Exercise and Cardiovascular Function

JB: Exercise is an area of paradox or confusion for the average doctor. We know of the benefit of exercise in cardiovascular function. By the same token, some people have said, well, hold it. There is an oxidative stress component to vascular disease. We're increasing the utilization of oxygen and maybe increasing the number of oxygen-derived free radicals, so are we doing injury to the patient? You published a review

article in the New England Journal of Medicine titled “Exercise—Toning Up the Endothelium.”22 What are your thoughts on exercise and its relationship to vascular function?
JK: From my vascular-centric point of view, exercise is a good thing. It’s associated with upregulation of the enzymes that make nitric oxide and, in general, a more normal phenotype of the endothelium, a more normal behavior of the endothelium is associated with exercise. Exercise, by most measures, has a vascular protective effect. This, of course, is a bit of a conundrum when one recognizes all the available studies that show exercise does cause a measured amount of oxidative stress.

To reconcile these observations, I might suggest a certain amount of “physiologic oxidative stress” may be beneficial to an organism. On the other hand, pathophysiologic, or let’s say unregulated stress, such as that which might occur during certain disease states (diabetes and atherosclerosis, for example), would tend to be deleterious.

Tea Flavonoids
JB: One epidemiological study on diet and vascular disease pertaining to oxidative stress concerned flavonoids. It was a study published in the Lancet a number of years ago. As I recall, you’ve actually followed up looking at the influence of tea on some oxidative stress parameters. Is a tea versus coffee discussion emerging with these flavonoids that have redox potential?
JK: I think there is an emerging story. I had the pleasure of collaborating with Dr. Joe Vita, who works here at BU. Through his efforts, we did a study examining acute and chronic tea consumption in individuals and its implications for the behavior of the endothelium.23 We found tea had a strikingly beneficial effect on the endothelium, both acutely and chronically. The effect on the endothelium was to improve the nitric oxide-mediated responses; generally, events that are considered to go along with improved vascular health.

In that study, we controlled for the amount of caffeine by giving some coffee to individuals to see if there was any effect of caffeine. We didn’t observe the same beneficial effects with equivalent amounts of caffeine delivered through coffee. As I’m sure you are aware, there has been an intense effort to label coffee as a bad actor, but thus far, most of the data indicate it is relatively neutral with respect to vascular disease.

Black Tea/Green Tea
JB: Was there a difference between black and green tea in terms of effects on these physiological parameters?
JK: In our study, we used only black tea. We have yet to complete a direct comparison of one to the other. Similarly, we have not yet unequivocally linked the effect of tea with its flavonoid content. We are in the process now of doing studies in cultured endothelial cells to try to isolate the effect of flavonoids per se, but I don’t have data on those studies to discuss at this point.

Pros and Cons of Antioxidant Supplementation
JB: Your work has spanned a tremendous breadth. It is the kind of work that will help doctors who are being asked these questions every day from their patients, to give them much more definitive answers. What would you tell a doctor who is confused about antioxidants and heart disease? He or she may have read conflicting reports and believe supplementary antioxidants might be dangerous rather than helpful. How would you respond?
JK: The most common question is, how much of “blank” should I take? My answer often goes back to

the original observations that emerged from studies showing that fresh fruit and vegetable consumption is associated with a healthy lifestyle and reduced cardiovascular disease. To quote a friend and collaborator of mine, Dr. Bruce Ames: “Sometimes it turns out Mom was right. You should eat your fresh fruits and vegetables and don’t try to worry about what’s in them just yet.”

Whole, Minimally Processed Foods
JB: That is nicely associated with Walter Willett and Meir Stampfer’s article in Scientific American in January of 2003. They discuss the revision of the food pyramid and explain that if you construct a diet correctly, using the right minimally processed fresh fruits and foods with a lot of color in them, you’re likely to be better off.
JK: Absolutely.

Cross-Disciplinary Research
JB: Thank you very much, Dr. Keaney. It has been a privilege to speak with you. I commend you on your tremendous work. It’s wonderful to see the people you are collaborating with. This cross-disciplinary research will reveal the answers we have been looking for. We look forward to seeing you at the 10th International Symposium on Functional Medicine in Tucson in May.
JK: Thank you very much. It has been my pleasure.

The final portion of this month’s *FMU* is devoted to our new section, titled From the Lab to the Clinic. In this section we will consider how we can take the information that has been presented and make it useful in the clinic.

We continue the discussion from side I on obesity and its relationship to inflammatory mediators and neuroendocrine modulators and cardiovascular disease. We can think of visceral adipose tissue as not simply a fat problem but a VAT problem. Now that we have outlined the problem and understand the relationship, we are still wondering what to do about it. It is a complicated topic. Many diets and various synthetic foods exist in our society to address the problem of obesity. What should we do? We certainly do not have all the answers, but let me give you some information from research studies that you can take to your clinic.

Inflammatory Status and Insulin Resistance
First of all, we want to improve cell signaling, reduce inflammation, and improve insulin sensitivity. Inflammatory status is amplified with insulin resistance. This is clearly identified in a number of papers showing that as PPAR activity is modified and insulin resistance occurs, inflammatory relationships increase. The counterpoint is that increased inflammatory mediators cause insulin resistance. It is a push/pull mechanism. An article in *Current Opinions in Clinical Nutrition and Metabolic Care* discusses this topic.[24]

Lowering Inflammatory Potential
We want to lower inflammatory potential. We also want to improve insulin sensitivity. These are two principal clinical objectives in managing a patient with increased cardiovascular risk associated with visceral adipose tissue. You might consider a program designed to lower inflammatory potential and improve insulin sensitivity. The authors of an article in *Clinical Nutrition and Metabolic Care* believe that increased release in action of proinflammatory cytokines is responsible for the occurrence of insulin resistance in inflammatory metabolic disorders, which are obesity-linked conditions like diabetes and

coronary heart disease.[25]

We want to lower inflammatory potential. That will occur, in part, by lowering the amount of visceral adipose tissue. It may also occur through the use of various types of natural antiinflammatory materials like curcuminoids, and pentacyclic triterpenoid substances like oleanolic acid, which are known to help lower inflammatory potential. Lowering inflammatory potential also requires us to think about the type of fatty acids in the diet. We want to guide a person away from an arachidonic-rich diet and into a diet rich in omega 3 oil-and polyunsaturates. Dr. Walter Willett addressed this theme in a recent paper in *Scientific American*, in which he revised the Food Pyramid to include more unrefined, polyunsaturated oils, whole grains and enriched dark-green, orange-red, blue vegetables and fruits, all of which play a role in the antiinflammatory pathway.[26] We should work to educate patients to avoid partially hydrogenated vegetable oils and products high in saturated fats and to consume more fish and unsaturated-rich oils.

Insulin-Sensitizing Diet

That type of diet would play an important role in lowering the risk not only of coronary heart disease but also of diabetes. In a sense, we are using the insulin-sensitizing diet. Get white out of the diet. Eliminate white sugar, white flour, white oils.

Foods that contain accessory factors, the colored, textured, less refined, less processed foods, deliver a range of phytonutrients and fibers that help balance insulin and lower inflammation. A recent review on diet and risk of coronary heart disease and type 2 diabetes appeared in the *Lancet*.[27]

Changing the Glycemic Index of the Diet

One of the components of this dietary intervention will be to lower glycemic index with foods such as legumes. Beans are useful in achieving that objective, as are other foods high in soluble fiber. Higher glycemic index foods are those that tend to increase inflammatory mediator production and increase fat cell accumulation of triglycerides. Strong evidence now indicates that lower glycemic index diets, the higher protein, higher legume, higher fiber diets, are less likely to be associated with weight gain. This topic is discussed in a review paper that appeared in the *American Journal of Clinical Nutrition.* Research findings provide scientific rationale to justify intervention with low glycemic diets as a weight-control approach.[28]

A recent paper in the *American Journal of Clinical Nutrition* is titled “Relation between a Diet with a High Glycemic Load and Plasma Concentrations of High-Sensitivity C-Reactive Protein in Middle-Aged Women.”[29] This is the theme we have been talking about. Can you lower high-sensitivity CRP, meaning inflammatory mediators, by changing the glycemic index of the diet? According to the information in this paper, the answer is yes. Dietary glycemic load was significantly and positively associated with levels of plasma high-sensitivity CRP in healthy middle-aged women, independent of conventional risk factors for ischemic heart disease.

As these women ate a diet that was lower in glycemic index, their high-sensitivity CRP went down. Improving weight control and reducing inflammatory mediators may improve insulin sensitivity. This turns the dog-chasing-its-tail the other way around; it cycles back up rather than down.

Changing Adipocyte Physiology

This concept has a positive impact on adipocyte physiology. You start to balance the storage versus the utilization of dietary triglycerides. That balances angiotensinogen, angiotensin conversion, and blood pressure control. Blood pressure comes down. Normalization of blood pressure begins through this different signaling process that occurs through adipocyte physiology and the modification of inflammatory mediators. This was recently described in a paper in *Current Opinions in Clinical Nutrition and Metabolic Care.* [30]

That also has a positive influence on PPARs, particularly in the PPARg family, which helps with insulin sensitivity and lowering of the inflammatory messages. So we should advise more omega 3 fatty acids, more fish, particularly the salmon family, but not farm-raised fish.Increasing evidence indicates that farm-raised salmon is not the same as the line-caught wild salmon, particularly in regard to pesticides and toxic metals. We want to increase omega 3 fats, lower saturated fats, get away from snack foods that contain higher levels of high glycemic index products—sugar, fat, and refined carbohydrate.

Medium-Chain Oils

We also need to move into medium-chain triglyceride-rich oils (MCTs) like coconut oil. New evidence suggests that MCTs may have a salutary effect on PPARg agonistic activity. A recent paper in the *Journal of Nutrition* shows that octanoate, one of the MCTs, attenuates adipogenesis and PPARg, and enhances insulin sensitivity.[31] The type of dietary oils, as well as foods that deliver those oils, may be helpful in resensitizing many of these pathways and lowering inflammatory mediators.

Soy isoflavones have also recently proven important. This topic is discussed in a recent issue of the *American Journal of Clinical Nutrition.* Soy isoflavones help normalize insulin and improve insulin sensitivity and weight control.[32] A couple of portions of soy per day may be another important addition to the diet that will deliver moderate, not pharmacological, amounts of isoflavones. These levels may be in the range of 20 to 30 mg per day of soy isoflavones. People with soy allergy might want to use some of the non-soy isoflavone products as part of the dietary intake.

Calcium and Adipocyte Physiology

Calcium is another nutrient that has been in the news recently in relation to its positive impact on adipogenesis, adipocyte physiology, and lowering the incidence of obesity-related inflammation. Calcium is an important part of this story, especially more than modest amounts of calcium, above 1000 mg per day of calcium. Two papers have appeared on this topic, one in the *Journal of the American College of Nutrition,* and one in *FASEB.* They looked at calcium in the modulation of obesity, or the regulation of adiposity by the dietary intake of calcium and its influence on the triglyceride metabolism levels with increased calcium intake.[33,34,35]

Calcium and its companion nutrient, magnesium, appear to be important parts of the story. If a person is on a dairy-free or low-dairy diet, his or her calcium status may be compromised, and he or she may want to increase dietary calcium intake.

Supplementary Chromium

Chromium has long been known to help stimulate glucose removal and insulin sensitivity. It also has a relationship to adipocyte physiology, leptin synthesis, inflammatory cytokines, and coronary heart disease. Trivalent chromium, given in the complex of glucose tolerance factor, may have a positive

impact on helping to sensitize insulin in triglyceride management, and it may play an important role through complex signaling pathways having to do with PPARgactivity.

Yeast Extracts and Glucose Metabolism

A recently published paper emphasizes learning old things in new ways. It looked at the ability of yeast extract to stimulate glucose metabolism and affect lipolysis in adipocytes.[36] People have been thinking about this for years. When I first started into the field of nutritional biochemistry 30 years ago, we thought yeast was a useful tool for stabilizing insulin levels in managing what we then called hypoglycemia. Certain forms of yeast may have higher levels of these salutary ingredients for insulin management and glucose removal.

In this paper, investigators at Ross Products Division of Abbott Laboratories found significant improvement in insulin management and glucose removal and its relationship to lipolysis in an animal model by giving yeast extract. Within the yeast-growing organism, specific minerals are concentrated and yeast form things like glucose tolerance factor with chromium. Walter Mertz at the USDA, some 35 years ago, was the first to recognize this fact. We are learning old things in new ways—in this case about chromium and its relationship to the active glucose tolerance factor that contains chromium, and perhaps yeast extract.

N-acetyl-carnitine

N-acetyl-carnitine helps sensitize tissues to these messenger molecules, lowering some of the amplified signals of inflammation. An antiinflammatory diet, eliminating allergens in the diet, and reducing the level of toxic exposure play roles in modulating inflammatory pathways, modulating insulin sensitivity, and modulating leptin cross-talk with cytokines.

A New Therapeutic Approach

What is developing is an interesting therapeutic approach in the clinic, not just focusing on weight loss. I have not talked about calorie restriction as the principal focus here. I have not mentioned putting people on “low-calorie diets.” In fact, there may be examples where putting a person on a very low-calorie diet (VLCD) actually increases rather than decreases his or her symptoms. A particular individual’s genetic structure, like that of the Pima Indians, may cause them to feel like they are starving, and they start capturing any calories that are available, actually enhancing adipocyte triglyceride storage, even under low-calorie regimes.

Rather than being principally focused on starvation, we are talking about proper nutrition. I emphasize that—nourishing the centers that create the signals that lower the inflammatory potential, lower the blunting of insulin signaling, and increase the ability of energy to be used for function rather than storage for a rainy day, in triglycerides that appear in adipocytes.

This is a different model that has to do with mind/body interactions, stress reduction so some of the endocrine signals for storage are lowered. It has to do with exercise so you produce the signal to the adipocyte to be properly regulated, and proper insulin sensitivity. It has to do with nutrition intervention and a lower glycemic index diet, higher fiber, higher unrefined carbohydrate, proper balance of protein, inclusion of soy protein, and the use of certain micronutrients like the B vitamins and trace minerals such as chromium, magnesium, and calcium. All of these factors help to stimulate this process, moving away from storage and inflammation toward regulated energy. That is where this field is headed, and I believe

it will take us beyond calorie restriction and the first law of thermodynamics.

Thanks for being with us. We will see you in April.

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