September 2002 Issue | Jerome J. Belzer, MD,

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Welcome to Functional Medicine Update for September 2002. In this issue, we will focus on a great paradigm shift in medicine. We have an opportunity to improve the way we manage one of today's most problematic and prevalent conditions, cardiovascular disease (CV). The theories, concepts, and treatments we have held tightly are being challenged and reevaluated. We have an opportunity to make great progress in the way we approach the prevention and treatment of CV disease. Let us first trace the history and development of the functional approach to CV disease.

In the 19th and early 20th centuries the first explanations for vascular disease emerged from the work of the renowned German physiologist and medical doctor Rudolph Virchow. It was Virchow who first described the origin of atherosclerosis on a pathophysiological level. When he examined arterial walls from a pathological standpoint, he saw they appeared to be inflamed on the inside, as though they had been injured. His injury model for atherosclerosis suggested that abrasions on the arterial wall led to injury, creating an inflammatory process, and ultimately the artery was occluded as a consequence of the process of attempting to heal the inflammatory lesion.

Lipid Model of Atherosclerosis
Virchow's injury model remained an esoteric discussion point because CV disease was uncommon in the 19th century. People didn't pay much attention to it. At the start of the 20th century, the Russian physiologist Anichkov developed the cholesterol or lipid model of atherosclerosis. This model emerged from his work with rabbits. He fed them high-fat, cholesterol-laden diets and demonstrated fulminating arteriosclerosis throughout the animals given this diet. When he opened the arterial system of the animals, he observed a soft, viscous, gooey substance that was deposited on the arterial walls. It looked similar to what the animals had been fed. Fat intake results in adhesion to artery walls.

This was a model anyone could understand. From it was born the cholesterol and dietary lipid hypothesis. This hypothesis has gained credibility through lipid-lowering effects in intervention trials. The National Lipid Research Clinic Trial, for example, showed that as lipid levels in our blood rise, particularly atherogenic LDL particles, risk to coronary artery disease (CAD) rises. Every 1 percent lowering of lipids yields a 2 percent reduction in probable incidence of CV disease.

The Inflammation Model
Anichkov's model was not without value, and the development of agents and therapeutic approaches for lowering lipids through drugs or lifestyle changes have proved beneficial. Lurking in the background, though, was the Virchow model of inflammation and heart disease.

Over the past few years we have begun to see an intersection of the Virchow model with the Anichkov model of cholesterol and lipid contribution to heart disease. The two may combine to form a new understanding of the prevention and treatment of CV disease, showing they are not separate or dissimilar, but that they have common linkages.

The Role of Nitric Oxide in a Combined CV Model

This month in FMU we will look at what is emerging and where the paradigm shift is. In this connection, we will examine one of the most remarkable discoveries of recent years, the role of a small molecule that is an important vascular regulator that might connect the cholesterol story with the inflammation story. That molecule is nitric oxide (NO).

Nitrogen has an atomic weight of 14, and oxygen has an atomic weight of 16. The molecular weight of (NO) is 30. How could a molecule this small, a gas, have a significant impact on vascular, neuronal, or immunological function? We will discuss this topic throughout this issue of FMU and in our Researcher of the Month interview with Dr. Louis Ignarro, winner of the Nobel Prize in Medicine or Physiology in 1998 for his research on NO as a signaling molecule in the cardiovascular system. This issue of FMU may, in fact, lead you to create better outcome and more successful therapies for the prevention and treatment of CV

Our discussion begins with statins, the discovery of which provided a major breakthrough in technology. They turned cardiology into preventive cardiology. Suddenly, cardiologists had a tool for regulating atherogenic factors by the administration of reasonably safe and convenient agents called statins, fungal metabolites from specific types of mold. They influence hydroxymethylglutaryl coenzyme A reductase (HMG CoA reductase), the rate-limiting enzyme for cholesterol biosynthesis. Statins serve as cholesterol-lowering agents because of their ability to inhibit this rate-limiting enzyme in cholesterol biosynthesis. Recent research has revealed that, in addition to lowering atherosclerosis by inhibiting HMG CoA reductase, statins may also be antiinflammatory agents. This research begins to connect Anichkov's message to Virchow's.

A recent paper in the Lancet discussed the disparity between angiographic regression and clinical event rates with hydrophobic statins. The investigators explained that when blocking cholesterol biosynthesis and lowering LDL cholesterol levels, specific health states in some individuals may cause statins to interrupt the production of other molecules that could be of benefit in modifying cellular physiology, producing adverse reactions.[1]

Downstream Effects of Mevalonate

When mevalonate is converted into cholesterol, it produces a variety of other molecules that are important for physiology. Included among them are steroid hormones, stress hormones, and other molecules important for cardiovascular function, including coenzyme Q10. Interrupting the production of these molecules can produce untoward side effects in certain individuals. Those side effects with statins in some individuals can include neuromuscular symptoms, difficulties in mitochondrial energy function, disturbances in signal transduction, and even rhabdomyolysis, a condition of breakdown of muscle and increase of body fat content in individuals on statin drugs.

The rhabdomyolysis condition is often seen in HIV patients treated with statins and protease inhibitors or nucleoside analogs which lead somehow to a buffalo hump configuration, the accumulation of fat, and the

breakdown of muscle. These unique situations occur as a consequence of the alteration of signal transduction through HMG CoA reductase inhibitors.

Potential Adverse Effects of HMG CoA Inhibitors
We can also observe changes in myocardial contractility, electrolyte levels, and bioenergetics of the cardiac muscle through alteration of coenzyme Q10 and mitochondrial function as a consequence of HMG CoA reductase inhibition. The most common symptoms of this condition are tingling, neuromuscular symptoms, and myalgia-like symptoms related to the adverse effects on bioenergetics from the use of statins.

Statin drugs are not benign products. We need to look at them in the context of the individual's genomics and outcome related to the production of function

Not everyone who presents with an incipient cardiovascular risk has observably cholesterol-laden arteries and narrowing of coronary vessels. A recent article in the New England Journal of Medicine, titled "Abnormal Subendocardial Perfusion in Cardiac Syndrome X Detected by Cardiovascular Magnetic Resonance Imaging," discusses this topic.[2] I want to emphasize that the syndrome X in this particular study is not the same syndrome X we have spoken about with Gerald Reaven, which involves insulin resistance and hyperinsulinemia. It is unfortunate that the authors also chose to call this condition syndrome X. Now we may become confused about which X we are talking about.

The authors of the NEJM article are referring to individuals who present with angina and chest pain who, upon normal catheterization and examination of perfusion, are not seen to have significant narrowing of their coronary arteries. These individuals are atypical, and difficult to explain. If they do not have atheroma that can be seen by normal diagnostic techniques, why do they have this cardiovascular symptom that appears as angina?

Changes in Oxygen Delivery to Tissues
The authors of this paper discuss these syndrome X cardiovascular patients who, on magnetic resonance imaging, demonstrated subendocardial hypoperfusion, meaning that leads to low suboptimal oxygen delivery to the tissues during the intravenous administration of the vasodilator adenosine and is associated with intense chest pain. According to the authors, the data support the notion that chest pain may have an ischemic cause in the coronary vessel beyond atherosclerotic plaque.

This is an interesting point. Chest pain, or pain associated with ischemia in any muscle, is a consequence of altering the oxidation/reduction potential of that tissue, changing mitochondrial energy potential, proton pump activities, and potentially ATP formation (although some of these effects occur well before alteration in ATP levels). It is a membrane activity-calcium coming in, magnesium leaving. All the things we associate with altered contractility of tissue in either the cardiocyte or the myocyte are related to membrane activity.

Oxidative Stress
Events that could alter oxygenation, oxidative phosphorylation, or oxygen in stimulated metabolism could induce pain and altered metabolic pathways in the cardiac muscle that could increase the risk to cardiac event and ultimate cardiac death. This is the topic of an editorial that accompanies this particular article. Three pictures illustrate a mechanism of the coronary artery in which blockage is caused by luminal

narrowing due to plaque. In other words, in the proposed mechanism in cardiac syndrome X, there are normal epicardial coronary arteries, but inappropriately increased vascular tone of coronary microvessels that lead to myocardial ischemia.[3]

This leads to what we call oxidative stress, a term that is emerging in the cardiac research community as an important pathophysiological variable associated with CV disease. It may or may not be associated with coronary artery plaque. That is an important point of differentiation from our past understanding. In the past, we thought heart disease resulted from cholesterol; cholesterol was plaque; plaque was narrowing of the arteries; and narrowing of the arteries increased the risk of a myocardial infarction (MI).

A New Model for CV Disease

Now we are talking about events beyond traditional plaque that may contribute to that as well. According to the authors of this editorial, in up to 20 percent of patients with angina chest pain, the coronary angiogram usually obtained because of a positive finding on one or more other noninvasive tests, does not show clinically significant narrowing of the coronary vessel lumen.

What, then, causes the problem? Is it something else related to oxygenation of the tissue and the appropriate support of aerobic metabolism? The authors call this condition cardiac syndrome X. It may be an unfortunate use of the term, but it differentiates it from the traditional mechanism called the pathopneumonic event in CV disease.

CV Symptoms beyond Cholesterol

What are the variable factors beyond cholesterol that may contribute to CV symptoms and ultimate disease risk? The list is fairly long. I will cover the areas that represent the tip of the iceberg, but there is probably a lot more beneath the surface of that particular iceberg. One important cholesterol-independent risk factor is glucose metabolism and insulin sensitivity. Here is where cardiac syndrome X meets glucose syndrome X, the Gerald Reaven model we have discussed in previous editions of FMU. Insulin resistance/hyperinsulinemia is increasingly acknowledged as a cholesterol-independent risk factor to CV disease

The authors of a recent paper in the Lancet discuss glucose metabolism in patients with acute MI and no previous diagnosis of diabetes. They summarize a prospective study in which investigators observed that previously undiagnosed diabetes and impaired glucose tolerance were common in patients with acute MI, and that this was a cholesterol-independent risk factor.[4] These abnormalities can be detected early in the post-infarction period.

The results suggest that fasting and post-challenged hyperglycemia in the early phase of an acute MI could be used as a marker for high-risk individuals. We may not be adequately examining these glucose-related dysfunctions with CV disease, and the best way to do so is through a challenge. We used to call this the oral glucose tolerance test. You challenge a person with a glucose load, look at postprandial glucose levels and possibly postprandial insulin levels, and mark how the patient's insulin levels respond to a glucose challenge.

CHD Risk in Glucose Intolerance

As the author of the editorial that follows this article in the Lancet points out, the increased risk of coronary heart disease in type 2 diabetes, and even in milder states of glucose intolerance, is a public

health problem.[5] The risk likely precedes a diagnosis of maturity-onset or type 2 diabetes. The precursor markers of hyperinsulinemia/insulin resistance are public health problems because they increase the risk of coronary atherosclerosis. This risk exists even for patients who are not hypercholesterolemic.

Some people advocate the regular use of the oral glucose tolerance test to screen patients who have this particular risk. Opinion varies widely on this subject. The dominant opinion, which is shared by the American Diabetic Association, is that this test is not a cost-effective approach. Not enough patients would show positives, and it would be over-utilization of medical services. The routine use of the oral glucose tolerance test, therefore, according to the ADA, is uncalled for and would be wasteful.

Indicators for Use of Oral Glucose Tolerance Test
An argument might be made for using the oral glucose tolerance test in dealing with patients with identified high risk factors. Those risk factors include a high waist-to-hip ratio, altered triglyceride-to-glucose level, low HDL level, hypertension, and a relevant family history. One might use a number of variables to screen a patient for later confirmation with the use of the oral glucose tolerance and postprandial insulin tests.

Some cases do exist in which a patient would be best served by the oral glucose tolerance and postprandial insulin tests. First, however, one should identify the individual's relative risk factors and other variables to make the precision of the test and its validity or necessity much more cost-effective.

Lifestyle Intervention
The author of this editorial suggests that people with impaired glucose tolerance do not currently require pharmacological intervention. Instead, all patients should be offered lifestyle intervention, including a diet and exercise program. The American Diabetes Prevention Program, which we discussed in an earlier issue of FMU, indicates that intensive lifestyle advice without an active pharmacological agent, and without metformin, reduces the frequency of type 2 diabetes by almost 50 percent if patients comply.

The first line of defense for a patient who is insulin-resistant and hyperinsulinemic, according to this author, before introducing pharmacological therapy, would be to see if the patient would comply with an appropriate diet and lifestyle intervention program. The exercise component of that program would be as simple as walking 20 minutes every day on level ground. The diet would be balanced in protein and carbohydrate and lower in refined carbohydrates and simple sugars. It would contain increased omega-3 oils and antioxidant-rich unrefined grains, fruits, and vegetables. It would contain increased levels of trace minerals, including magnesium, zinc, calcium, chromium, and selenium; and increased antioxidants from natural sources including vitamin E, vitamin C, and flavonoids. This would be a desirable approach to pursue before consigning the patient to more expensive and potentially adverse side effect-inducing pharmacological therapy.

Another useful blood parameter, other than the glucose tolerance and postprandial insulin test for evaluating risk, might be glycosylated hemoglobin, or hemoglobin A1C. This routine test can be done reasonably inexpensively. It looks at the amount of glycosylation of the heme protein, knowing that this is a running record of the approximately 120-day life of a red cell and how it has been exposed to non-enzymatic glycosylation reactions through the changes in glucose concentrations.

As glucose in the plasma rises temporally, protein can become glycosylated through the combination of

the lysyl residues of protein with the aldose form of glucose to form the shift basis, or what are called glycosylation residues. The more glycosylation that occurs, the more glucose reactions have occurred. Even with normal fasting blood sugar levels, a person may have elevated glycosylated hemoglobin, because the red cell collects all the things that go on 24 hours a day.

In measurements of fasting blood sugar, on the other hand, the person has not eaten and his or her blood sugar is low. If you catch the patient at another time of day, however, his or her blood sugar might be high following consumption of two candy bars, a doughnut, and a soft drink. With that model, the use of glycosylated hemoglobin might be a useful tool for early screening of some of the aspects of glucose intolerance or insulin resistance. Some evidence suggests that.

Variations in Glycosylated Hemoglobin
A recent paper in Clinical Chemistry reviewed the variation of glycosylated hemoglobin in individuals and points out an interesting thing.[6] According to the authors:

> "We (also) note that glycosylated hemoglobin is a more comprehensive measure of mean glycemia than fasting blood glucose, as evidenced by recent studies showing that, in diabetic individuals, postmeal plasma glucose correlates better with glycohemoglobin than it does with fasting sugar."

It is useful as a screening tool if you look at the glycosylated hemoglobin elevations. When you get up above 6 or 6.5 percent, although it may still be in the normal range, it may not be what we consider optimal relative to glucodynamics

A significant paper appeared in the July 4 issue of the New England Journal of Medicine this year.[7] Titled "Widespread Coronary Inflammation in Unstable Angina," it reminds us once again of the important role of inflammation in a variety of cardiovascular functions. We realize that Virchow was taking us down the right path 100 years ago. We just became excessively focused on unidirectional approaches based on lipids.

According to the author of an editorial following this paper, "The realization that atherosclerosis is, morphologically, an inflammatory disease was originally derived from studies of animal models."[8] Our understanding is not based simply on the observational studies of Virchow, but on extensive work that has taken place since then. There is an initiation as a consequence of an atherogenic diet. (This is where the Anitchkov concept comes in of high-fat, high-cholesterol diets.) The initiation factor from dietary triggering leads to monocytes that adhere to the vascular endothelium and accumulate in the lesion-prone arterial sites.

Adhesion Molecules
The adherence of monocytes to the arterial surface is facilitated by the endothelial expression of surface proteins known collectively as adhesion molecules. These adhesion molecules include intracellular adhesion molecule-1 (ICAM-1), or vascular-associated adhesion molecule-1 (VCAM-1). These adherent monocytes are enticed into the arterial intima and differentiate into macrophages, eventually become foam cells, and can be engaged in the oxidation of LDL and initiate monoclonal hyperplasia and

atheroma. Here the inflammation story meets with the lipid story and the physiological story in the pathogenesis of arterial disease.

During this inflammatory process, higher circulating levels of markers occur, which include high-sensitivity C-reactive protein. I emphasize the importance of measuring high-sensitivity C-reactive protein, because the normal C-reactive protein measured in the lab, for example in a rheumatoid arthritis patient, is not sensitive enough to detect lower levels of variation. You want to use high-sensitivity C-reactive protein and also serum amyloid A protein, or SAA protein, for evaluating inflammatory potential in cardiovascular patients with unstable coronary disease.

Inflammatory Stress
Researchers in the Framingham Study found elevated levels of fibrinogen, an acute-phase reactant, were independently associated with future coronary events. Other markers of inflammation, including inflammatory cytokines such as IL-1, IL-6 and TNF-α , as well as ICAM-1 and VCAM-1, and C-reactive and fibrinogen, have been identified prospectively in association with coronary vascular disease. Thus inflammation, the Virchow model, converges with the Anichkov lipid model.

We would call this inflammatory stress, a term used in an editorial in the New England Journal of Medicine titled "The Value of Inflammation for Predicting Unstable Angina."[8]Inflammatory stress identifies people at risk to future cardiovascular events. These are extended cardiovascular risk factors that have significant potential implication beyond cholesterol in the production of vascular disease. An anti-inflammation approach, as well as an anti-cholesterol, or anti-lipid approach, would be the approach of choice based on this model.

Neutrophil Activation in Unstable Angina
The authors of the July 4 New England Journal of Medicine paper state there is widespread activation of neutrophils across the coronary vascular bed in patients with unstable angina, regardless of the location of the culprit stenosis. This challenges the concept of a single vulnerable plaque in unstable coronary syndromes and suggests a generalized systemic disorder, an inflammatory disorder across the whole vascular tree.

This is an important point. Too often, we think disease is localized where we can see it. If we can see a lesion, we say that is the point of the disease. We treat that lesion and the patient is well. However, as these authors point out, atherosclerosis is a state of function that relates to systemic inflammation in the vascular system, not just a localized lesion that may be weakest point in the system. You remove the lesion, but you may still have to treat the underlying situation or you may have another re-occlusion and another need to take something else out

Many other modulators of inflammation have also been identified as risk factor markers. One that has recently been discussed, which has a strong predictive correlation, is another of the inflammatory cascade modulators called macrophage inhibitory cytokine-1 or MIC-1. MIC-1 is part of the TGF-β superfamily of cell transduction growth factors.

In a study of women recently published in the Lancet, investigators found that women who had higher levels of MIC-1 at baseline had more frequent cardiovascular events.[9] According to the authors this effect was independent of traditional cardiovascular risk factors and is at least additive to C-reactive protein. We

may be able to evaluate many different factors that are representative of alterations in the inflammatory cascade associated with increased vascular risk

If statins work not simply by limiting cholesterol biosynthesis, but also through anti-inflammation, we might suspect that administering statins and other agents that lower inflammatory potential would result in lowered levels of these markers of atherosclerosis, i.e. high-sensitivity C-reactive protein. Papers on this topic are now appearing in the literature.

One such study looked at the effect of a statin and fish oil, omega-3 EPA, on plasma high-sensitivity C-reactive protein concentrations in individuals with high risk to coronary disease. These individuals had significant apple body shape with increased BMI and visceral obesity, which means their waist-to-hip ratio was increased.[10] When these individuals were given a statin drug along with fish oil, their C-reactive protein levels went down, suggesting that inflammatory mediation was lowered and the statins may participate as both anti-inflammatories and as cholesterol-lowering agents. When statins were given alone, C-reactive protein went down. When statins were given with fish oil, an additive effect was achieved with increased reduction of C-reactive protein, suggesting that nutritional antiinflammatory agents can participate in lowering the risk to coronary disease through this antiinflammatory mechanism

Growth hormone insulin-like growth factor-1 (IGF-1) also plays a role in modulating glucose sensitivity. An interesting paper in the Lancet discusses circulating IGF-1 and its interaction with IGF-1-binding protein (IGF-1BP). According to this paper, their interaction could be an important determinant of glucose homeostasis and provides evidence for the possible protective role of IGF-1 against development of glucose intolerance.[13]

The interaction between hormone IGF-1 and its binding protein IGF-1BP, IGF-1 binding protein-sex steroid hormone, estrogen- testosterone- progesterone-, and insulin combination is a symphonic orchestration. It may relate back to our understanding of how to regulate and manage patients who may have CV risk based on hormone imbalances

A recently published paper, titled "Endothelial Function and Oxidative Stress in Renovascular Hypertension", provides a breakthrough in understanding."[14] The investigators conducted a study looking at renal vascular hypertension, which is known to activate the renin angiotensin system and lead to increased oxidative stress. Angioplasty decreases systolic and diastolic blood pressure. They found it also decreased the production of 8-hydroxy-2'-deoxyguanosine, or 8OHDG, which is an oxidative marker for injury to DNA as a consequence of increased free radical oxidative stress. They also found angioplasty reduced the production of malondialdehyde-modified LDL in the blood, meaning it lowered lipid oxidation.

This is an interesting study. Increasing oxygen delivery to tissues lowered the oxidative injury and was associated with reduced blood pressure in these individuals, and lowered the activation of the renin-angiotensin system. This means it was almost like giving a natural angiotensin-2 inhibitor. Here is the key for those of us who have been following this field for some time. It was Dr. Linus Pauling and others whose advocacy got us to think this way.

The Role of Vitamin C in Angioplasty

As it turns out, these patients were also administered vitamin C by infusion therapy. Ascorbic acid, 24 mg per minute, was administered to patients before, and in some patients after, angioplasty. Remarkably, they found that when patients were infused with vitamin C before angioplasty, and their blood flow was stimulated by acetylcholine stimulation, there was an extraordinary reduction in oxidative stress and increased perfusion, and their blood pressure was lowered. There was lower blood pressure, increased oxygen delivery, increased perfusion of the tissue, and reduced oxidative stress.

The author of an editorial that follows this study points out that the patients with unilateral renovascular hypertension who had impaired endothelial-dependent vasodilation of the brachial artery improved in their function when they were administered intravenous vitamin C.[15] An accumulation of evidence indicates that angiotensin-2 increases oxidative vascular stress as a consequence of the activation of the NADH/NADPH oxidase system that induces and releases oxidants, like superoxide anion.

Vitamin C and the Redox Concept
When superoxide is released along with another small molecule, nitric oxide, peroxynitrite can be formed. Peroxynitrite may be a serious pathophysiology-inducing agent. Therefore, vitamin C, which uncouples the production of peroxynitrite and serves as an antioxidant, may help prevent renovascular activation of angiotensin-renin system.

Basically, vitamin C serves as a natural angiotensin inhibitor. This is powerful support for the antioxidant concept, the redox concept, that Dr. Pauling introduced 30 years ago, and how it applies to medicine.

With that in mind, we turn to the interview with Nobel Prize-winning laureate, Dr. Louis Ignarro, on side II of this tape. He will provide important new insights about both the prevention and treatment of vascular disease

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INTERVIEW TRANSCRIPT

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JB: We are fortunate this month to have as our guest Dr. Louis Ignarro, whose name is familiar to many of you. I have cited his work several times in FMU in recent years. Dr. Ignarro was a 1998 recipient of the Nobel Prize in Medicine or Physiology, an honor he shared with Dr. Robert Furchgott and Dr. Ferid Murad in their discovery concerning nitric oxide (NO) as a signaling molecule in the cardiovascular system.

NO was the molecule of the year in 1991. We have discussed it extensively in FMU during the last 10 years. We have explored the peroxynitrite/superoxide/oxidative stress/nitration question, and the endothelial, neuronal and inducible immune form of NO synthase in relation to physiological function.

Dr. Ignarro, welcome to FMU.

LI: Thanks very much, Jeff.

Dr. Ignarro's Background

JB: As I understand it, you began on Long Island thinking you might go into architecture or something related to engineering. You wound up getting your PhD in pharmacy at Columbia University, a physiology minor at the University of Minnesota, and then a NIH post-doctoral fellowship. You went to UCLA where, in 1998, you were one of the recipients of the Nobel Prize in Medicine or Physiology. With that bit of background, perhaps you could tell us how you arrived at the nitric oxide discovery.

LI: What you said is fairly accurate. Initially, I was interested in mechanical engineering, perhaps chemical engineering, but even before that, I was interested in chemistry. I was probably one of the youngest kids on the block to have a chemistry set. I used that to do the usual things kids like to do, like trying to make firecrackers, bombs, rocket fuel, and so on. I did all that fairly successfully, and caused a little bit of damage to the house, as well, but luckily not to myself.

I also maintained that interest. I used to race cars, as well. I liked the mechanical part of it, not so much the actual driving of the cars, but building the engine, tuning it up, modifying the cars, and so on. I had an aptitude for mechanical as well as chemical projects. Then I decided to take the route of chemistry. Some neighbors who were pharmacists influenced me to use that knowledge and go into pharmacy so I could develop a nice profession, open up a drugstore, and so on. I did my pharmacy training and worked in pharmacies for a couple of summers and realized that I didn't want to fill prescriptions.

Origin of Interest in Nitric Oxide

What I wanted to do was explore and do research. After getting my pharmacy degree, I decided to go on and get my PhD in pharmacology, the study of the effects of drugs on the body and effects of the body on drugs. My interest in nitric oxide came in the late 1970s when, as a young pharmacologist, I decided I was going to elucidate the mechanism of action of nitroglycerin. I remembered nitroglycerin from when I was a kid, actually trying to make it. (Thank God I was unsuccessful in synthesizing nitroglycerin!)

I understood it had been used in people to treat heart disease and angina for well over 100 years, but the mechanism was not understood. I tackled that problem, and we were able to show that nitroglycerin works by first being metabolized to nitric oxide in smooth muscle cells. Then we developed the pharmacology of nitric oxide. It is a vasodilator, inhibits platelet aggregation, and so on. Being expert in the area of NO, we were able to recognize that vascular cells could actually make NO and that was an important finding we made in the mid-1980s. The rest is history, as it were.

Clinical Relevance of Nitric Oxide

JB: It was an extraordinary process that resulted in a paradigm shift in medicine-a small molecule such as NO having the dramatic range of effects it has as a neurotransmitter, a modulator, and a cell signal transduction modifier. That may sounds to many people like an esoteric, albeit important, part of physiology. Apart from Viagra, they might wonder, does it translate into anything of importance in clinical medicine?

LI: We don't want to play down Viagra. That was an important development, but I understand what you're

saying. When one spends one's entire career doing research, it certainly feels at times like an esoteric pursuit. When we were working out the pharmacology of nitric oxide, which is a gas, it was difficult to publish those papers. It was difficult to get NIH grants to fund that work because the question came up-of what physiological significance is this? NO is a component of polluted air. Big deal. So it mediates the effects of nitroglycerin, but that's one drug, and this is probably not important physiologically.

This is what drove me to determine whether or not NO could exist in the body. I figured if we had receptors that could respond to such incredibly low concentrations of nitroglycerin and NO, then why would we have those receptors in the body? There was only one reason. The body must make something like NO or nitroglycerin. That's why we have receptors for it. We looked and looked, and we found it.

NO as a Neurotransmitter: Viagra and Beyond

It turns out that NO, as you pointed out earlier, is an extremely important, diverse, widespread signaling molecule, and its importance extends beyond its role as a signaling molecule in normal physiology and biochemistry. That understanding has profound clinical implications. One of them was the development of Viagra. I like to use that as an example because in 1990, the neurotransmitter in the nerve activating the erectile tissue to cause erectile function was unknown. We were in the right place at the right time, and we discovered that those nerves released NO, believe it or not, as the neurotransmitter.

Now we know that many nerves release NO as a neurotransmitter. But knowing the neurotransmitter and knowing that it works through cyclic GMP and so on, thereby enabled others to utilize that information to develop the first orally useful drug to treat erectile dysfunction. This problem affects almost 10 percent of the male population of the world. That little bit of esoteric information led to the development of an incredible drug. There are dozens and dozens of additional examples in other areas where this knowledge of NO is now leading biotech companies, pharmaceutical companies, big pharma companies to develop new drugs that are in clinical trial. That's the most exciting part of all of the research.

Inducible and Constitutive NO Synthases

JB: Through your collaborative work we now understand that NO synthases are enzymes that convert arginine into NO. NO synthases appear to be in two families, one that has been described as constitutive, which suggests it doesn't up- and downregulate very significantly, and the other as the inducible form. Do you believe this differentiation between inducible and constitutive is real or synthetic, or are there targets you can employ on both sides of the equation?

LI: Unfortunately, to some people "constitutive" means it is present. It's there, so you don't have to signal its formation. Inducible NOS, that NO synthase, is not there. The message needs to be induced, and it's translated to protein. That is the enzyme protein, and now it makes NO.

Constitutive means endothelial and neuronal NOS are there, but of course, we know now that those constitutive forms can still be up- and downregulated. That is, the amount of protein can actually go up and down. It's always there, but it's not necessarily there in the same amount. In fact, controlling the up- and downregulation is very important in normal physiology in the control of various functions.

Individual Variations in NO Production

JB: That leads to a follow-up question. With the deciphering of the human genome, we now know variations in these polymorphisms exist around certain loci, and your research has addressed this topic.

What is the variation in NO production among individuals based upon genetic constitution?

LI: We are still learning a lot about that. What is clear is that in a number of different disorders, such as hypertension, atherosclerosis, and perhaps even complicated forms of diabetes, there seems to be a downregulation of the endothelial NO synthase. Less NO seems to be formed. This may be due to a downregulation of NO synthase. There may be cofactor deficiencies as well.

Not all the answers are in yet, but there is a difference. Several hundred studies indicate that these disorders, as well as others, are at least characterized by deficiencies in NO production. Of course, that raises the question, what happens if you increase the amount of NO production to normal or above? Can you restore normal processes and treat or even prevent the disease in suitable animal models? All of this seems to be the case. Where you see NO deficiency you can take measures to restore NO production. The evidence looks quite good that one is able to treat or lower the incidence of many of these disorders.

Potential Problems of Excessive NO Production
JB: We have also been told that some disorders are associated with the production of high levels of NO. In some neuronal disorders, for example, it is suggested that high levels of NO in the neuron can uncouple mitochondrial oxidative phosphorylation and encourage apoptotic changes in neuronal cells. Some people have a view that NO is bad.

LI: There are many more examples of the protective effects than detrimental effects of NO. In certain places, where NO can be generated in fairly large amounts, there is the potential for indirect harmful effects. When endothelial cells make NO, they don't make enough to be harmful. Certain nerves (not all nerves) can release quite a bit of NO by virtue of the neuronal NO synthase and other pathways that feed into those nerves to stimulate even further NO release.

The NO is toxic, most likely, by virtue of the presence of oxygen radicals. In other words, if NO were present by itself, without any oxygen radicals like superoxide, hydrogen peroxide, and so on, I doubt if the NO would be toxic. But NO is a radical. It's not a terribly reactive free radical, but by definition, it is a radical because it has one unshared electron. So NO, as a radical, can react with other radicals like oxygen radicals in a chemical reaction to form other products. Some of these other products, as you know, like peroxynitrite, could be toxic to tissues, provided they are generated in large enough quantity. That may be causing some of the problems in certain parts of the brain when there is excessive NO release during excitotoxicity, when a lot of glutamate is released and so on. It's a complicated situation, but you're right. Under certain conditions, excess NO can lead to problems.

A Clinical Look at NO Production
JB: Let us look clinically at that difference between the endothelial and neuronal NO production. If you upregulate NO production, do the regulatory mechanisms feed first into endothelial production? Some clinicians might worry that by manipulating NO they might do good in one place and harm in another.

LI: NO is very, very selective. Not all NO synthases are the same. Endothelial NO synthase is completely different. Even though it catalyzes the same reaction, endothelial NOS is regulated very differently from neuronal NOS, which is regulated entirely differently from inducible NOS.

If you're doing something to the endothelial NOS, you're not going to touch the neuronal NOS as long as

what you're doing is selective. By virtue of the very differences among these three isoforms of NO synthase, it should be very easily possible to design therapeutic measures that are highly selective.

NO in Respiratory Therapy

JB: One interesting clinical application of your discovery is the use of low levels of NO gas in respiratory therapy for persistent pulmonary hypertension in newborns or in individuals with high altitude-induced difficulties. Based on our former understanding, this use may appear counterintuitive.

LI: This was an amazing story. It really points out for all of us biologists, including physicians and pharmacologists, that we really need to understand chemistry. Dr. Warren Szabo from Massachusetts General Hospital came to see me at UCLA in the late 1980s and told me he had an idea. He wanted to use inhaled NO, mix it with air or oxygen, and give it to newborns, babies who have fatal persistent pulmonary hypertension.

He described how these infants have to be placed on extra corporeal membrane oxygenation (ECMO) to rid them of this condition. He told me how invasive it was and that many times it didn't work, and the babies died.

Neonatal NO Therapy

He said that maybe all we have to do is allow the babies to breathe in the NO, and it will dilate the pulmonary vascular bed and the vasoconstriction will go away, the hypertension will go away, and everything will be fine. I told him he was crazy and that he was going to kill these babies because the NO is oxidized by oxygen to NO2, nitrogen dioxide, which is an incredible poison. It will kill you instantly, so you can't do that. So he left.

He's a physician, so my chemistry background is better than his. He didn't have a very strong chemistry background, but apparently he talked to some chemists. A few months later he told me that at low concentrations, NO should not react that much with oxygen and it shouldn't get much NO2 formation. It is only when you have very highly concentrated NO that it reacts rapidly with oxygen to form lots of NO2. I remembered reading or learning about that a long time ago in chemistry.

Pulmonary Vasodilator Effect of NO

I went back and did my homework, and I could see he was absolutely right. At the low concentrations he was talking about, less than 100 parts per million, let's say, mixed in air, the rate of reaction between NO and oxygen would be so slow that you would get negligible NO2 formation.

He went on to do the animal studies, and they worked. Finally, they tested their first human patients, neonates, and they found that it provided a remarkable pulmonary vasodilator effect. It eliminated, or cured if you will, the persistent pulmonary hypertension without using ECMO, and these infants could go home and lead perfectly normal lives. That's a long story, but I think it teaches an important lesson.

The Nutritional Component of NO

JB: It is good for our listeners to learn how these discoveries are made. By taking a fundamental idea that may appear esoteric and extrapolating it into different areas based on an individual's expertise, unexpected magic can occur.

Let's shift to the modulation of NO through nutrition. Some individuals believe nutrients have no impact on NO, and others say it does play a role. Vernon Young at MIT made some interesting discoveries in the early 1970s. He collected urine samples from individuals who had the flu and various viral infections and showed that their urinary nitrate levels went up quite remarkably under these circumstances.. He wondered why urinary nitrate levels go up when people have the flu even though the nitrate concentrations of their diets remain constant. We can now explain this phenomenon in light of your work. Would you tell us about the nutritional component of NO?

LI: There certainly is one. Four or five years ago, when people would ask me if there was a nutritional component to this area and if one could take nutritional products to enhance NO production or make NO work better, I would laugh. I was not a vitamin person. I thought if you ate a good healthy diet, it was fine.

I realized about a year later that I was completely incorrect. I had been really ignorant about what was going on around me and about that aspect of the literature. The whole time it was right before my eyes. The research community in the NO field realized that the most important way in which the actions of NO in the body are terminated is by reactivity with oxygen radicals. NO reacts very slowly with oxygen. Low concentrations of NO in the body, low physiological amounts of NO, react very slowly with oxygen. But they react very quickly with oxygen radicals like superoxide. In fact, that chemical reaction has a rate that's faster than any other reaction we know about. That is the major way in which the action of NO is terminated. You have to terminate actions of NO quickly. This is true for any signaling molecule. You don't want signaling molecules to remain around for more than a few hundred milliseconds because too much signaling is not good.

Effects of NO in Oxidative Stress
In many cases, disease is associated with oxidative stress. This understanding has now become very important. People have been talking about oxidative stress for decades, but during the last five years we have come to recognize that many disease processes are actually acute inflammatory processes that are characterized by excessive oxidative stress. This means there is a lot of superoxide, which means a lot of destruction of NO. All of that translates to deficient quantities of NO in the tissues.

NO is extremely protective. I could talk for two hours on the protective effects of NO in virtually every cell in the body. When you remove that NO, you lose the protection, and then the oxidative stress fully manifests itself. That is going to cause lipid oxidation. There will be destruction of various components of tissues.

Antioxidants and NO
How do you fight oxidative stress? Everybody has heard of antioxidants-vitamin C, vitamin E, coenzyme Q10, folic acid, and so on. These antioxidants are present in all kinds of vegetables, fruits, grains, and cereals, and so on, and you can take higher doses in the form of supplements. All of those antioxidants are very important. People pooh-poohed antioxidants before they understood how they worked. Why were they important?

One view today, in which I'm a very firm believer because we did many of the experiments ourselves, is that antioxidants work, at least in part, by increasing the levels of NO, by protecting against the destruction of NO. Depending on conditions, it's very important to engage in taking compounds, whether

they be natural products or not, that contain antioxidants.

Production of NO in the Body
By the same token, we have to keep in mind how NO is made in the body. NO is a gas, but we don't breathe it in and store it in our bodies in that way. NO is synthesized from arginine in a rather complicated biochemical reaction involving NO synthase. The point I want to make is that arginine, the basic semi-essential amino acid, is the precursor, and the only precursor, for NO. Taking more arginine has been shown in animal and human studies actually to lead to the production of more NO.

So we have two ways to increase NO. We can take arginine, which is present in every protein, and we can take antioxidants. By two different mechanisms of action, you get a synergistic effect and you can raise those levels of NO and get a better protective effect of the NO.

NO-Potentiating Effects of Drugs
JB: It strikes me that perhaps drugs like Probucol might work through some of these NO-potentiating effects as cardioprotective agents.

LI: More work needs to go on in that area, but that's certainly a possibility. This research is enabling people who are experts in their respective areas to consider that NO may play a role in physiology or drug effects or therapeutic effects in their particular areas. So many people are working in this field that there is a continual explosion of literature on NO. People are demonstrating that NO plays a significant role in the effects of those other drugs, which is very interesting and almost unbelievable.

NO and Redox Potential
JB: Your discovery that this redox potential, or reduction/oxidation potential in cells modulates the rate-limiting reaction between NO and superoxide, thereby lowering the production of peroxynitrite, is a fascinating example of why understanding something about chemistry can have profound path-finding opportunities in physiology and medicine.

LI: Yes, absolutely. When you are guessing and you make descriptive observations, that may enable you to develop better drugs, but it's going to take a long time. If you understand the mechanisms, then you can just cut to the chase and design more effective and more logical therapeutic measures and go so much further and so much faster.

Biopterin
JB: The coenzyme biopterin, or tetrahydropterin, has been discussed recently as a nutrition-related agent for modulation of NO. If you measure plasma or urine in individuals who have upregulated immune systems, you'll see more neopterin. It is somehow an indirect measure of activation of NO synthase. Do you feel biopterin is an important part of the overall understanding of the pharmacology of modulating NO?

LI: I think you're absolutely right. We know more about the other cofactors and about how the enzyme actually works to convert arginine to NO than we do about the precise role of tetrahydrobiopterin. It's interesting.

NO synthase is a heme protein. Like hemoglobin and myoglobin, it contains heme iron to bind oxygen.

But it's also a flavoprotein in that it has FMN and is FMN-bound. NO is one of very few proteins in the body that is both a heme protein and a flavoprotein. And it is the only protein in the body that has heme and flavins and is regulated by tetrahydrobiopterin. So it is very important for us to know what the tetrahydrobiopterin is doing.

Most people think tetrahydrobiopterin does not play much of a role in catalysis, but it plays an important role in stabilizing the enzyme protein, holding it together so that it can do its thing to make NO. This research has led others to unravel deficiencies in tetrahydrobiopterin. How can folic acid deficiency lead to deficiencies in tetrahydrobiopterin formation? Can analogs of tetrahydrobiopterin replace it, the cofactor? A lot of these experiments are going on now, but one thing is very clear. Everyone in the field recognizes the importance of tetrahydrobiopterin and that deficiencies in its presence or formation could definitely lead to impaired NO production.

Educating Clinicians about a Difficult Topic
JB: Dr. Ignarro, as the Jerome J. Belzer Distinguished Professor of Pharmacology in the Department of Molecular Pharmacology, UCLA School of Medicine, we know how busy you are, and we appreciate your spending time with us. You demonstrated your ability as an educator in the half hour you just spent with us, taking a topic that is for most of us pretty daunting, and making it approachable.

LI: Thanks very much, Jeff. It was really a great pleasure. I'll be happy to talk to you again

I would like to add a few comments to Dr. Ignarro's extraordinary contribution. My comments are related to genetic polymorphisms and the variation of nitric oxide (NO) response, a topic Dr. Ignarro's pioneering research has helped to open up. The authors of a recent article in Clinical Chemistry discuss independent risk factors for moderate-to-severe internal carotid artery stenosis, looking at alleles of the endothelial NO synthase gene.[16]

The investigators found that homozygosity for the T786C allele of the endothelial NO synthase gene is an independent risk factor for moderate-to-severe internal carotid artery stenosis because of underproduction of NO. This is exactly what Dr. Ignarro shared with us. Too little production of NO is a vascular risk, in this case with respect to carotid artery disease. The nutritional modulation of NO with the B vitamins known to help promote and support proper NO production may be important in individuals who have specific genetic polymorphisms and higher levels of arginine and biopterin.

Myeloperoxidase
Myeloperoxidase is a leukocyte-derived enzyme. It is the Klebanoff enzyme, involved with cell-mediated defense that produces hypochloride, which becomes dismutated and converted into hydroxyl ion. It becomes the microbiocidal killing agent of white cells against bacteria when those cells are activated. Myeloperoxidase has recently been identified as a vascular NO oxidase. Therefore, it plays a role in balancing NO and directly modulates vascular inflammatory responses by regulating NO bioavailability.

This is a fascinating part of the emerging story that connects the immune system to the inflammatory model of atherosclerosis and the NO story. NO is a principal player in modulating the immune signaling process throughout the body and the vasculature. Dr. Ignarro and his colleagues have been instrumental in helping us understand this connection. The authors of a recent paper in Science magazine discuss how myeloperoxidase, a white-cell-derived microbiocidal enzyme, modulates NO production and activity.[17]

Xanthine Oxidase

Xanthine oxidase is an enzyme that produces peroxynitrite, a caustic chemical that is the problem child of the NO/superoxide story. You know about gout and hyperuricemia and the role it plays in inflammation. Hyperuricemia is produced at higher levels as a consequence of activation of xanthine oxidase. Allopurinol, the drug that blocks xanthine oxidase activity, has been used in animals to prevent certain types of oxidative stress-induced disorders. It undoubtedly does so by reducing peroxynitrite-induced nitrosation of various proteins and injury to various tissues. It may be an interesting additional part of the oxidative stress model in those with hyperuricemia and increased xanthine oxidase activity. This is the topic of a recent paper in Redox Report.[18]

There are many variables that influence the immune system, including NO modulation, insulin and glucose tolerance, and hormone balancing. All of these regulate aspects of vascular dynamics, the connection between the Virchow and the Anichkov view of atherosclerosis.

Finally, we should consider the role of exercise. As exercise tolerance increase, perfusion increases, reducing oxidative stress and reducing inflammatory mediation. That is an interesting process associated with habitual, regular, properly designed exercise.

What happens if you engage in an eccentric program involving high levels of exercise only periodically? For example, a couch potato may suddenly decide to run a marathon or ride a bicycle 100 miles, or exercise intensively at high altitude with oxygen deprivation without the appropriate training and conditioning. What influence does that have on the system? We know that everything, even exercise, has a parabolic dose/response curve. Too little is not good, but too much may not be good either. We want to be in the zone of optimal self-regulation.

Biomarkers of Exercise Training and Cardiac Function

What do we know about biomarkers of exercise training and cardiac function? The authors of a recent paper in Clinical Chemistry looked at characteristics of cardiac biomarkers in marathon runners.[19] The investigators used the albumin cobalt binding tester (ACB), which evaluates the amount of ischemic-modified albumin. Oxidative events can injure serum plasma proteins, producing altered albumin, which has a different cobalt-binding pattern. This is a provocative test for evaluating ischemic injury or oxidative injury that has occurred to the major plasma protein, albumin.

The researchers found that intensely exercising individuals who were well trained did not appear to have significant positive results from the ACB test, meaning they did not have evidence of high albumin damage. Those individuals who engaged in eccentric exercise and overdid it without proper training, demonstrated signs of stress markers and increased oxidative injury to muscle cells that correlated with muscle soreness.

Long-Term Effects of Inappropriate Exercise

A companion paper, which appeared in Medicine & Science in Sports & Exercise, considers what happens if a person overdoes it. The authors discuss how that activity can influence oxidative injury to cells as a consequence of ischemic events and other free radical oxidative-induced events that may participate in injurious long-term effects if they are not properly regulated.[20]

Leukocyte mitochondria are also altered after heavy aerobic exercise.[21] Therefore, increased oxidant release from mitochondria in high-intensity exercise can result in a cumulative effect on mitochondria.

Appropriate Exercise Training Combined with Diet

We are starting to see everything in balance once again. It is the functional medicine approach-finding the right balancing properties for interventions to attain the zone of the genomic and proteomic regulation of the patient. We are talking about personalized medicine. Obviously, we do not start a patient's exercise regimen with a marathon training program. Ideally, he or she begins with a regular walking program and a strength and conditioning program. Bill Evans described this type of program when we talked to him on FMU in the March 2001 issue. He talked about a balance of resistance and aerobic training exercise to build strength and endurance.

In addition to an exercise program, the clinician should design an appropriate diet. You try to normalize insulin. You try to balance the sex steroid and stress hormones. You try to intervene with antiinflammatory substances, the flavonoids, antioxidants, and NO modulators.

A New Approach to CV Disease Management

This is a new approach to CV disease prevention and, potentially, to treatment. It is not as simple as intervening to reduce a single molecule, cholesterol. This approach examines the complex orchestration of events that lead to CV function. The pioneering NO research of Dr. Ignarro and his colleagues has opened up a new therapeutic opportunity. I believe we will see this approach cut across CV disease to include neurological degenerative disorders, autoimmune disorders, and inflammatory disorders of the joints and muscle. It will provide a more generalized approach for managing many chronic illnesses for which the best therapy we had in the past was simply to treat the symptom and hope for the best. Now we are looking at the pathophysiological underlying mechanisms of disorders for which, by using inducible modulators for their remediation, can lead to the correction of the problems. We are not just treating symptoms. This is an exciting chapter in the evolution of functional medicine.

We will see you in October.

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