November 2012 Issue | Walt Gall, PhD Metabolon

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Welcome to *Functional Medicine Update* for November 2012. As you know, we've been progressing over the last couple of years with a model of functional medicine that relates to manipulation of very fundamental processes that relate to signaling in the body that ultimately controls either function or dysfunction under metabolically modified conditions. It's this interrelationship between the environment of the individual and their genes that then controls, ultimately, the signaling that relates to how these genes are expressed and how cell biology, tissue, organ, organ systems, and whole-organism response is manifest. That's the tenet that really underlies the functional medicine model (this systems biology approach). There's really no better example of that than our focus today on the conditions associated with type 2 diabetes.

The State of the Science: Type 2 Diabetes

The central question that has arisen over the last few years is whether type 2 diabetes is, in and of itself, a disease, or whether it is really a definition of a collection of metabolic disturbances that occur as a consequence of unique interactions between certain genetic characteristics and the environment to produce an outcome that we call disturbed metabolism, or dysglycemia, or dysinsulinism that later gets defined as a singular disease: diabetes. Meaning, are there many paths to a single definition of a name (a medical taxonomic name)—diabetes? Or does diabetes stand kind of as a singular entity that is the same among all patients with that diagnosis? I think the answer to that question is fairly obvious to those of us that have been following this field for some time, and that is that the more we learn about the pathogenesis of type 2 diabetes, the more we recognize that there are multiple paths, at a physiological level, that give rise to the exigencies of blood sugar and insulin that we later call diabetes. That then takes us down the path to understand better what the individual characteristics of that person's dysfunction and how that relates to what later is to be called, in their diagnosis, type 2 diabetes.

When we take that from the abstract level of theory down to the reality of fact in the clinic, in the exam room with that patient, and ask the question, "What does this really mean?," it takes us to an understanding that the concept of dysinsulinism and dysglycemia is more than just that of elevated blood sugar. It's even more than that of longstanding elevations hemoglobin A1c or glycosylated hemoglobin, which we consider to kind of be the sentinel biomarker for evaluating proper glucose control. I don't want to suggest that there is no value clinically to the biomarker hemoglobin A1c, nor do I want to suggest that there is no value in understanding elevated fasting blood sugar as an indicator of dysinsulinism and dysglycemia. But what I really want to suggest is that as the emergent model of this

condition that we call type 2 diabetes and the companion disease adjacencies that are associated with it, like cognitive dysfunction, Alzheimer's disease, cardiometabolic disease, polycystic ovary conditions, conditions of non-alcoholic fatty liver disease, things related to sleep apnea, things related to gout—all of these conditions which are associated illnesses or diagnoses associated with type 2 diabetes are a manifestation of slight modifications of the metabolic control of complex regulatory pathways associated with bioenergetics, of which the molecule glucose plays a principal role (that's the principal metabolic fuel to produce energy in eukaryotic organisms). And so when we start really looking at this condition that we call type 2 diabetes and its companion illnesses as more of a bioenergetic dysfunction, it really takes us into a slightly different way of evaluating the patient and possibly even how we manage the patient using variables other than solely just blood sugar or hemoglobin A1c.

This topic was very nicely discussed in a recent series of papers that appeared in *The Lancet*medical magazine back in June of 2012. I did allude to these in a previous issue of *Functional Medicine Update* in which we talked a little bit about the concept that diabetes quality improvement is beyond glucose control itself. This was an editorial that actually appeared on page 2218 in the 2012 issue of *The Lancet*.[1] The authors say that quality improvement strategies in diabetes—as we look at 94 randomized controlled trials, with the findings from 48 cluster randomized trials—that it is found that there is a growing recognition that blood glucose control alone is not adequate to prevent both the microvascular and macrovascular complications of diabetes. We now recognize diabetes care is no longer glucose-centric. It is crucial to understand that other efforts that sustain a broader view—a systems biology view—must be employed if we're going to really reduce the overall burden of the companion illnesses associated with dysinsulinism. I think it is very, very important to indicate that at present only one person in eight with diabetes has their disease controlled to the representative goals of hemoglobin A1c, LDL cholesterol, and blood pressure. What we start recognizing is that even with very tight control of blood sugar and A1c, in clinical trials the results appear disappointing for cardiovascular outcomes because we still see very significant incidence of cardiometabolic disease or cardiac complications that are associated even with tightly controlled cases of hemoglobin A1c, who have this metabolic disturbance that we call dysinsulinism.

Bioenergetic Conversion of Glucose: A Complex Metabolic System

So you'll notice there is—by this discussion—much more to the concern of metabolic disarrangement of bioenergetics associated with glucose than just diabetes itself. With that, let's go back and explore for a moment what people are talking about as it relates to the control points for this complex metabolic system that relates to the regulation of bioenergetic conversion of glucose into things like ATP and NADPH and FADH2, these high-energy-carrying intermediates that really power up our body. We recognize that one of the control points that has gotten a lot of fashion over the past ten years are so-called nuclear orphan receptor signaling components. These would be the things like peroxisome proliferated activator receptor alpha and peroxisome proliferated activator receptor gamma, so these are PPAR? and PPAR?.

The Challenges Related to Thiazolidinedione (TZD) Drugs

We have seen a lot of note in the medical literature on these because these are targets for drugs to improve their function or modify the function of these nuclear orphan receptors, which signal messages from the cytosol of the cell into the nucleus of the cell to turn on specific genes that regulate insulin and glucose metabolic control points. Drugs like Actos and Aredia have been formulated and approved that have very high ligand activity specificity for PPAR?, so we call these the PPAR? agonist drugs that are there to enhance the activity of PPAR? and enhance then the regulatory effects of these transcription factors on the control of insulin response of genes. You know, the clinical trials for these medications showed very significant improvements in things like hemoglobin A1c and blood sugar in individuals who have the diagnosis of type 2 diabetes.

The challenge, however, as you probably recognize, is that these medications that are called thiazolidinediones, or TZD drugs, are pleiotropic. They don't just influence PPAR? alone, although they have very high affinity for that ligand. It appears as if the influence that they have on PPAR? as transcription factor modulators is different than the kind of natural modulation of PPAR? that occurs through things like prostaglandin J2 and some other natural agonists that regulate PPAR? in normal cellular physiology. What we call this is slightly different PPAR? binding activities than the natural ligands, which means that they do things like what? They increase fat deposition, so we see that the PPAR? agonist drugs cause weight gain, which is generally kind of counterproductive for individuals that are trying to treat diabetes and trying to lose weight.

Secondly, we've seen recently that the stronger these TZD drugs are in influencing certain activities of the PPAR?, the more they seem to have cardiometabolic adverse outcomes (meaning, adverse cardiac side effects). And there is also some suggestion that they might have adverse effects on increasing risk to certain cancers as well. This kind of risk/benefit trade-off that is now being recognized with the TZDs have put them in some question, and as you know, one of the members of this family—the stronger of the two drugs—has actually been disallowed in Europe and a black box warning put on it in the United States, so now we're starting to see the adverse potential effects of these synthetic PPAR? ligand drugs being much more well recognized and prominent.

Now, why is that? Let's just talk about this for a second. Does it mean that PPAR? agonism is bad? Of course it doesn't. I mean, this is a natural process in the body that helps to communicate from the outside of cells to the inside of our…what we call the book of life—our genome, the information that is important for enhancing or regulating bioenergetics through the metabolism of glucose, and so this is a very important natural part of our system of biological control. But what happens is when we start synthetically manufacturing or designing or discovering certain new-to-nature molecules that are designed and selected specifically for their very high affinity (meaning high-binding) to PPAR?? It may turn out that their binding sites to the PPAR? ligand is slightly different in its binding than that of the natural ligands, the things that normally control PPAR? function and so they could have all the effects of PPAR? natural ligands plus others because they are not binding at the same exact site. They're not having the exact same effects. That's what we call pleiotropic effects (multiple effects). And there are

many papers that have been published now over the last few years that have demonstrated that these pleiotropic effects—these off-target effects of the synthetic PPAR? ligand agonist, the so-called TZD drugs—have been proven to be correct and different than the natural ligands, like PGJ2 (prostaglandin J2).

That raises some interesting philosophical questions, doesn't it, about drug development, because you might have the most potent molecule you can find that will activate or inhibit a specific metabolic process in the body, but it may have off-target effects due to a slight difference from the natural way the body would regulate that function that are only seen after maybe several years of use of that molecule. In the general public you're selecting for certain genotypes that are more susceptible to the adverse effects and it takes a while to figure that out. Clinical trials are often limited in study size and may not be large enough to pick up those people in the cohort as a part of the whole group; their data was diluted out in the statistics of the safety trials. It is only when you get very large use of that medication in, say, millions of people that you start to see these small cluster groups over years of use starting to show these adverse effects. This is one of the problems we always have with synthetic molecules versus natural ligands--trying to better understand over time what their effects might be in specific genotypes that may have different susceptibilities or different affinities for these synthetic molecules.

As we look at the literature, we see papers being published that have titles like the following: "Insulin Resistance and Metabolic Derangements in Obese Mice Ameliorated by Novel Peroxisome Proliferated Activated Receptor Gamma Sparing Thiazolidinedione."[2] Now, what does that mean in English? That's a long bunch of words strung together. What it means is that people have gone back—medicinal chemists have gone back to the laboratory—and said, "Whoa, hold it just a minute. This generation of TZD drugs that we just put on the market that sold several billion dollars a year of product, now we're finding over the long term they have some risk, so can we find molecules that are derivatives of these TZDs that don't have some of these adverse side effects? They don't have these off-target effects that we're seeing with weight gain and with cardiac risk?" As that is being examined, it's being found that yes, there are ways of tickling—I say "tickling" to use that word kind of euphemistically—the PPAR? agonists. In other words, binding at a different site on that molecule to produce slightly different effects that might reduce the risk of these adverse effects while still maintaining the positive effects of agonizing or enhancing PPAR? activity.

Here is where the medicinal chemistry--Sherlock Holmes--goes into play, where people try to explore--like a detective novel--exactly what the structure of a molecule would be, how the pharmacophore called TZD could be modified, how its scaffold could be changed in such a way to get the favorable effects without producing the adverse effects. But that then begs the question, doesn't it, and that is: Well, what about the natural things that are controlling this all the time? Aren't we kind of overriding these natural processes with these synthetic molecules and trying to redesign the nature as best we can? And of course that is the difference between the natural processes of substances that are produced in a system of natural biology in response to a changing environment versus taking charge with a synthetic molecule. As we've started to look at this in more and more depth, we start to see that there

are certain characteristics of these synthetic molecules—these TZDs—that correlate with certain activities of PPAR?, so we can differentiate the kind of properties of PPAR? influence on function.[3] When you start doing that you find out that there are certain properties of these molecules that influence mitochondrial gene expression and have effects on mitochondrial bioenergetics and other effects of these molecules that have influence on other aspects away from mitochondrial function, things like influence on adipocytokines in the adipocyte cell, and things like beta cell activities in the insulin secreting cells in the endocrine pancreas.[4] So we start to look at tissue specific differential effects, recognizing that there are multiple effects that these molecules (TZDs) have, more than just a single hit and more than just one physiological functional change. I think we're using this as a specific example of a probably more general phenomenon. If you think about medicinal chemistry, this can be seen not just for PPAR?, but you can say it about angiotensin inhibitors, or you can talk about effects of statins on HMG-CoA-reductase, but it has effects on lots of other things that influence other targets that are influencing function. These are part of the detective work that occurs (post-market surveillance) once a drug has been approved to figure out what are the other things that are being influenced in certain people, some of which may not be all that desirable?

Now, as it then takes this one to better understanding, you might say: Is there a positive outcome of this? Well, I guess the positive outcome is we're starting to better understand who might be the best candidate for some of these drugs and who might not be a good candidate, by differentiating their unique response, but also it tells us a little bit about what we might want to be measuring in these patients as it relates to their response that goes beyond just that of glucose and hemoglobin A1c, because what we might say is, "Well, hold it. Some of these effects that we're talking about that would be considered not so good may be seen early on in certain other changes in physiological parameters or metabolites or biomarkers, so maybe we should be looking at other things in the blood that are reflective of these things that might not, in the end, be so good for certain people, other than just look only at the things that we like that are good." So we ought to be looking at a combination of different parameters, not just put all of our eggs in one basket, looking at blood sugar and hemoglobin A1c, which as you know are the standards of identity for managing diabetes.

Metabolic Profiling and Type 2 Diabetes

That leads us into what we call metabolite profiling. How do we, then, take a broader array of substances that might reflect different aspects of how intervention influences this complex control of glucose economy and bioenergetics, other than just look at glucose and hemoglobin A1c alone? I hope I'm not losing you, here. I know this sounds fairly complex, but I'm trying to get you to understand a little bit as to how medicine is changing now to go away from a pill for an ill and looking at one endpoint as the marker for whether that pill is good or bad, to a more complex systems biology approach where we are looking at multiple parameters that help us to understand how that individual is uniquely responding to that therapy, to look at both positives and negatives, to personalize their treatment, to improve outcome and reduce risk to adverse effects. So that's a different strategy that's really built on a functional medicine model.

With that in mind, I'm now talking metabolite profiling in the aspect of type 2 diabetes, and there are all sorts of very interesting papers that have been published recently in this area. Let me give you one example, a paper called "Novel Transcriptome Profiling Analyses Demonstrate that Selective Peroxisome Proliferator-Activated Receptor ? (PPAR?) Modulators Display Attenuated and Selective Gene Regulatory Activity in Comparison with PPAR? Full Agonists."[5] What this paper is really looking at is the difference in metabolite profiles in patients that have been given your kind of full PPAR? agonist drugs. This would be things like Actos and Aredia—very strong TZD modulators of PPAR?. Versus given medications that are not as strong as agonists of PPAR?, but they don't have some of these off-target effects that I've described, and asking are there differences in the way they modify metabolites (the so-called transcriptome)? The answer is yes, there are differences, and when you start patterning this you develop a profile, right? Rather than looking at one number, like glucose. Or one number, like hemoglobin A1c. You have an array of different analytes that you are examining and you look at patterns of change, like shifting sands. Rather than looking at one sand grain, you're looking at how the dunes of the sand configuration change with the changing environmental circumstances of these two different families of drugs.

Now you obviously could apply this also—this same concept—to that of lifestyle, or that of nutrition intervention, or that of exercise, or that of environmental modification, and you could examine what influence does that have on the transcriptome profiling of influence on these glucose metabolism regulatory genes and their subsequent metabolites? That's really, I think, the direction—the trajectory—that this field is going, which is making it much more obvious through this type of research the important role that lifestyle, diet, nutrition, exercise, stress management, pollution plays in altering glucose and insulin physiology and ultimately bioenergetics, because it comes back again to looking at things not only like genes that are associated with bioenergetics in a traditional way of metabolism, but also looking at mitochondrial-specific genes that regulate bioenergetics in the energy powerhouse of the cell, and so how does this all play out in different tissues, like in the muscle, or the fat cell, or the liver cell, or the heart cell, or the beta cell of the pancreas that is secreting insulin. Would there be some energy catastrophe that's occurring in these cells that causes their early death? This is called apoptosis, and that apoptosis, then, leads to diminishment of those cells being able to do their work, and things like, for instance, the beta cell. If you start having apoptosis of beta cells, you then have ultimately a loss of beta cell mass and beta cell function, which means loss of insulin secretary ability, which ultimately becomes, then, the patient who becomes the type 2 diabetic that requires insulin as a form of their therapy because they have lost their insulin secretary ability.

These are really very important kinds of conceptual breakthroughs and discoveries that are occurring in this whole field of diabetes prevention and management that is revolutionizing our thinking. And so when you start looking at early metabolic markers for the development of dysglycemia and type 2 diabetes and their physiological significance, it plays a very important role of going from just focusing on pathology to focusing on function, and how would you then intervene earlier when you see the trajectory of these metabolites moving towards dysfunction moving towards pathology, meaning the end stage of type 2

diabetes? So you can get involved with patient management much earlier, and you can also evaluate the effect of whatever therapy is being used earlier by looking at how these complex patterns are normalized or altered by this specific therapy that's personalized to that patient?

That's going to be the topic that we will share with our researcher of the month this month, Dr. Walter Gall. I think you're going to be very fascinated about the progress that is being made in the development of the multi-parametered analyte profiling that leads us to better understand early on how that person is moving towards diabetes or some of the sequelae of events that are associated with insulin resistance well before they actually get this diagnosis of diabetes, and then how to manage them in a personalized way.

Questioning the Role of Hyperinsulinemia

We're starting to see from this the development of new strategies, other than just go with hard-hitting, high-activity PPAR? agonist drugs (these TZDs), new ways that we can actually modulate insulin signaling in different tissue types and improve functioning. Barbara Corkey, who is at the Boston University Medical School, was the recipient of the 2011 Banting Award, which is considered the premier award for diabetes researchers that is given each year. She titled her 2011 award acceptance speech "Hyperinsulinemia: Cause or Consequence?"[6]That was a question. And in this lecture, she—I think—takes a very provocative position, which is well-founded on her research and that of many other colleagues, that when we start talking about hyperinsulinemia, it's really a consequence of metabolic catastrophe that has occurred by mitochondrial decline and poor bioenergetics, and this occurs in things like the beta cells and other cell types where you get this mitochondrial dysfunction, this bioenergetic dysfunction, that ultimately is associated with poor glucose economy and dyslipidemia and the sequelae of events that we associate with type 2 diabetes. In fact, there is a very nice paper that was published in the journal *Diabetes* in January 2012 that is really the transcript of her lecture that talks about the nature of this mitochondrial issue associated with the advent of the bioenergetics problems that we call dysglycemia and dysinsulinism. And that was followed up, actually, with a very, very unique and I think encompassing paper, again authored by Dr. Corkey, titled "Metabolic Master Regulators: Sharing Information Among Multiple Systems," in which she shows you can't just separate out this glucoregulatory pathway as being singularly isolated from the other pathways of the body, like fat metabolism, protein metabolism, endocrine control.[7] These are all interrelated through redox balance of the body. That's reduction oxidation (we used to call this oxidative stress). I've been talking about redox control in *Functional Medicine Update* for over 20 years, so I'm kind of feeling vindicated, here, that we've been advancing that concept that it's not just antioxidants, and it's not just oxidative stress, it's really the control of what we call the voltage of the cell. It's like the voltage in the battery of your car. Your starter motor works best when the voltage is above 12 volts in your car. Well, you'll still be able to turn the starter motor at eleven-and-a-half volts probably, unless it's really a cold day, but it's going to go "er, er, er." It's not going to start very quickly. If you get below that, even though you have voltage in your battery, you're not going to start your car. This is very similar to the mitochondrial voltage, which is the electrochemical potential called the redox potential. So you might still be producing energy in your mitochondria, but if it's below the starter motor voltage you don't feel very good. You have muscle pain. You're fatigued. You're not thinking clearly. You're not producing insulin correctly if it is in the beta

cells, and that master regulator is this redox potential in the body. It's controlled by all of these interlocking regulatory systems, one of which—or some of which—are related to PPAR?, and PPAR?, and transcription factors that regulate the genes that control things like insulin and glucose economy.

Fat Cells Have Different Personalities

When we take that to a clinical level, what it teaches us is that we need to look at what cell types would be most influenced and would most be important in regulating this, and kind of do a thought process about tissue-targeted regulation. So I've talked about the beta cells of the endocrine pancreas. That's very important for secretion of insulin. If you don't have proper bioenergetics in the mitochondria of your beta cells and they are dying rapidly, you're not going to have good secretion of insulin. You're losing beta cell mass, and you're losing secretory ability. So that's one cell type.

Next, of course, would be considered the adipocyte, what used to be considered this lowly cell that was just there to store extra calories in the form of fat and kind of was metabolically inactive, but we now recognize the adipocyte is very active as an endocrine organ, and its regulation is once again controlled by bioenergetic processes—redox processes—that are associated with the secretion from activation of certain genes that we call adipocytokines, a family of regulators that have names like adipsin, and adiponectin, and resistin, and TNF?, and various inflammatory proteins like IL-1. So this is a complex array of regulatory molecules produced by the adipocyte cell in response to environmental signals that are in part regulated by redox control, just as is the beta cell secretion of insulin. Similarly we could go to the liver cell and find that redox had a very important role to play in how it stored fat in its metabolic activities, like glycogenolysis or glycogenesis.

Thirdly, we could go to the myocyte (the muscle cell) and see the same thing: that it has very important regulatory effects through its mitochondria that are regulated by redox potential. And then, of course, lastly the cardiocyte, which, as you know, about three quarters of the cardiocyte by volume is occupied by mitochondria, so it has a very dependent role in its function to that of mitochondrial function.

All of these cell types, then, are interrelated with this overall concept of redox control, redox balance, and mitochondrial function. Now if you ask the question specific to that of adipocytes and say: "Well, are all adipocytes identical in terms of their metabolic regulatory function?" The answer is no. We now recognize that there are differential effects between the subcutaneous adipocytes and the omental adipocytes (the so-called central fat adipocytes) that are more around our organs, and the ones that are maybe not as cosmetically observable as the ones that sit under our skin.[8],[9] These central adipocytes are metabolically different than those of the subcutaneous adipocytes, and they have a more important regulatory role in controlling these adipocytokines that can have effects on insulin economy, on redox potential, and have this feedback relationship with circulating immune system, meaning macrophages and

monocytes.[10] Macrophages and monocytes sit in the adipocyte matrix in our central fat and cross talk with them about the body's status. So if there is a big immune response, an inflammatory response, then you have a bunch of angry macrophages and monocytes, and they speak with their angry voice to the adipocytes and say: "By the way, I'm kind of aggravated. You should be aggravated, too." And then the adipocytes can be aggravated by up regulating the expression of these adipocytokines that get secreted, so it's like a dog chasing its tail. It's an amplification process. Or, if the adipocytes are activated, then they can speak to the macrophages and say: "By the way, you guys should be upset. I'm fed up with the way I'm being treated." I think that's an interesting metaphor: I'm fed up and I'm not going to take it anymore. This is the Rodney Dangerfield adipocyte. So then that adipocyte says to the macrophage that is circulating within the central fat mass: "I want you to take my message out to the rest of the body and say that I'm kind of upset." And when they do that they put out a state of alarm, which then produces proinflammatory mediators like TNF?, and IL-6, and these things that we now associate with systemic inflammation.

So we recognize that there is this very complex interrelationship between fat cell types and their personality and status, and the regulation of insulin signaling and insulin secretory ability. This is a system of biology. You can't separate one out and say: "Well, we're only going to worry about the pancreas today and tomorrow we'll worry about the muscle cell, and the next day we'll worry about the heart cell, and then we'll eventually get to the lowly adipocyte." You have to think about all of these relative to the patient's specific state of uniqueness with their dysglycemia and dysinsulinism. And we now recognize that there is a metabolically active form of fat called brown fat, which we've had great controversy about for years and years—decades. It's been thought: "Well, it's only found in hibernating animals, these metabolically active forms of fat. Or it's only found in infants; it goes away in adulthood." But now it has been found that brown fat is found in adults.[11],[12] Somewhere in the subscapular area there is a few grams of brown fat that are very important as metabolic regulators. The reason they are brown is they have a lot of cytochromes in them that are in the mitochondria of these fat cells that are thermogenically responsive, so they produce heat. They undergo what is called fetal energy cycles. They uncouple energy to produce heat to keep our temperature, as warm-blooded animals, up. And we recognize that the activity of these brown fat cells is in fact partly controlled by the neuroendocrineimmune system. Signals that are sent to it through endocrine and autocrine release of various messenger substances, like norepinephrine and epinephrine, but they are also regulated in part by communication they have with proinflammatory cytokines, and things coming from the immune system. So the neuroendocrineimmune system has something to do with brown fat thermogenesis and control of heat and the relationship, then, of body energy economy.

Type 2 Diabetes: What's the Chicken and What's the Egg?

This is a whole new game, isn't it, that I'm talking about? This is a whole new way of looking at obesity and its relationship to diabetes. It raises the question: Does obesity cause diabetes, or do metabolic disturbances associated with poor energy economy cause both diabetes and obesity? Meaning, maybe it is the effect and not the cause—that these are all interrelated as a consequence of metabolic disturbances. And if so, then it begs the question: What's the chicken and what's the egg? Where did the metabolic

disturbance start? This paradox that we are talking about—can we trace it back to its origin? And when you start doing that, you find out that lo' and behold, as the story is emerging, there appears to be some very interesting (at least path-finding) direction related to what might start this process. So you might say, well what could interrupt mitochondrial function? What could produce immune dysfunction? What could produce inflammation? Because all these things seem to be connected to this shift of the sand of signals associated with insulin signaling and glucose that at later stage, downstream somewhere, we'll call type 2 diabetes, and if the beta cells completely kind of expire, then that patient becomes a type 2 diabetic that requires insulin. And so, how do you get there from here? You need to ask the question what could adversely affect these bioenergetics processes that are focused on mitochondria in these target cells (the cells that are beta cells, the cells that are in the adipocytes, the cardiocytes, the hepatocytes, the myocytes), what could influence, adversely, mitochondrial bioenergetics? And that then leads us into some very interesting territory that's fairly new that is associated with things like does autoimmunity associate itself with type 2 diabetes? Are there certain kind of immune responses that could produce inflammatory response to certain cell types that lead to altered redox and mitochondrial catastrophe, meaning such that you start losing bioenergetic capability? The answer is yes. There are certain kinds of things that can influence energetics in such a way as to lose both beta cell mass and/or insulin sensitivity, like we see gluten now being associated with certain kinds of increased risk to type 2 diabetes because of the effects on these complex bioenergetics pathways. So things that activate mast cell or macrophage that then cross talk with the adipocyte cell to produce inflammatory response, not just things like gluten itself, but you might think of many different things that could activate the immune system or cause alteration in metabolic function, like, for instance, what are called persistent organic pollutants (POPs), which we've talked extensively about in previous issues of *Functional Medicine Update*. So these things that could actually poison mitochondria could be considered insulin toxic because they have an adverse effect, then, on bioenergetics, that ultimately causes the cell types that are controlling this regulatory process—this complex process—to be diminished in function. There are more and more papers now being published that indicate that certain kinds of substances like bisphenol A, or polychlorinated biphenols, or things of that nature can have adverse effects on mitochondrial function that can then have a relationship to altered energy economy in the mitochondria, altering redox, and ultimately have effects on insulin, both secretion and insulin signaling, which we then later call either metabolic syndrome and/or type 2 diabetes.[13]

I think these examples show that once you sieve this information through a functional medicine lens you start getting maybe a different approach towards clinical therapeutics other than just regulating insulin and glycosylated hemoglobin in and of themselves. You need to take a broader array of evaluation of these metabolites that are influencing or have been influenced in such a way as to alter metabolic function to later set the trajectory toward type 2 diabetes.

Now, I've said a tremendous number of things in a fairly short period of time in this discussion. You're going to hear from Dr. Gall, who will do a much nicer job kind of taking this concept of metabolomic profiling down to a more clinically relevant level. But what I want to really get you to understand is that this field that we call type 2 diabetes and its relationship to endocrinology is changing just revolutionarily underneath our feet. The way that we thought about it for decades is in a state of tremendous flux. You've heard me speak in previous issues of *Functional Medicine Update* about the work that has

recently demonstrated that there are agonists in a natural system for PPAR? that will have all of the positive effects on regulating insulin and glucose economy without some of the adverse effects, and we recognize that we can see how these natural agonists actually differentiate themselves from the TZD drugs. We can see how lifestyle intervention, such as exercise, modulates the natural ligands that have the positive effects on PPAR? without the adverse effects. Meaning exercise doesn't cause weight gain, whereas TZD drugs that work on PPAR? do, so exercise has an effect on PPAR?, but it does so through a different association of the signal of exercise to the nuclear transcription factor PPAR? that regulates the genes that associate with insulin economy in a different way than a synthetic molecule TZD does.

What we take away from this is to be cautious when you start saying, "Well, I want to have a natural substance that is just like TZDs." You probably don't want that. What you want are effects that regulate function like the natural system does that produces this more complex pleiotropic regulatory connection without some of these off-target adverse effects. Secondly, we want to be able to recognize that there may be many environmental factors that have adverse effects upon these complex processes that ranges from everything from the food of one being the poison of another (like you wouldhave with gluten in a gluten-sensitive individual), to the exposure in a certain person to certain xenobiotic substances in their environment that poisons mitochondria in such a way as to lower their energy economy and produce insulin resistance.

To that you might say: How does our body get rid of lipophilic toxins? It does so through the activity of cytochrome P450s and phase II detoxifying enzymes. What happens if that person has a genetically modulated response, and let's say it is a diminutive response, to a petrochemical toxin? That person becomes more sensitive, then, to the adverse effect of that toxin, which is stored, ironically, because it is fat soluble, in fat, which then alters adipocytokine signaling, which then lowers mitochondria, alters redox, which then has an effect on the inflammatory pathways that subsequently influences insulin signaling, and now that person—as contrasted to their neighbor, exposed to the same substance—may then start to move their way towards type 2 diabetes in the same exact environment of another person who has genetically got better detoxifying enzyme function. So now I've broadened the profile that we have to think in an endocrinological sense about detox. Or we have to think in an endocrinological sense about immune response. Or about inflammatory signaling. We can't just go in and just modulate glucose and insulin by themselves in the absence of looking at these broader principles. So this is a very powerful reinforcing principle for the conceptual framework of functional medicine, a systems biology framework.

Does that mean that no patient ever responded to these other therapeutic molecules like TZD agonists or synthetic TZD agonists? Of course not. There can be many people that responded very well because they were in such a state that their genes responded without some of the off-target adverse effects. They're favorable response gave them a good outcome. So I don't want to throw the baby out with the bathwater and just say, "Oh, these molecules that we're using as drugs are totally in absence of value." What we're really doing is broadening our concept to say the right tool, at the right place, for the right person by asking the right questions. That is the functional medicine model. And how does it interrelate to, then, the

ability of their brown fat to do its work right? To establish proper redox? To get thermogenic response to their diet? It's more than just calories alone. It's more than just: You got the genes to be fat. No, it's the whole story that interrelates the environment to that person's genomic message, that then gives rise to their metabolic control points.[14] So what we call a disease may actually be the metabolic set point for that person's genes in response to that environment. They don't have a disease. They have the proper biological response of their genes to that environment in which they find themselves. We stigmatize it, and almost give it a form of discrimination. We say, "Well you have a disease. You're flawed." And they're maybe not flawed. Maybe they're working exactly as they should work in that environment. What they need to do is go out of the flawed environment for their condition and move into a different environment.

I've used the example of the Pima Indians concerning this for some time, saying that Pima Indians don't have diabetic genes. What they have are warrior genes that when thrust into a high-sugar, high-fat, high-alcohol environment now suddenly are maladapted to that environment and produce a dysfunctional response to that which is their metabolic disturbance that we later call diabetes. They're not diabetic genes; they're genes that are actually selected for the biggest threat they've had for their survival throughout history, which is starvation. So they have what we call thrifty genes. The thrifty genes hold on to calories much more assiduously than other people's genes, so you give them a bunch of empty calories and you get these responses that produce inflammation, altered redox, and ultimately rapid obesity and type 2 diabetes, but if you change the environment to say, "Hold it, you're selected for starvation, so we need to give you a different kind of diet that's really going to be matched to that specific biological history and your legacy." And now suddenly they're not fat, they're not diabetic, they're not suffering from inflammation, they don't have cataracts, they don't have diabetic gangrene, they don't have peripheral neuropathy, and they don't have blindness due to retinopathy, just by modifying their environment to be consistent with their own genetic history.

I think these are very, very powerful concepts that are coming out of this work and really restructuring how we think about these complex metabolic disorders. Once you open this door, then you get into a much more robust—and it also might be considered a bit more confusing—environment. For instance, now we recognize that on the surface of our intestinal lining (so-called L cells in our distal ileum) sit receptors that are picking up information from our food and actually tasting our food way down south in our intestinal tract and translating the taste—when I say taste, there are certain principals in our food that are triggering the receptors to secrete substances into our blood, and those are things like glycogen-like peptide 1 (or GLP1), and some of you know there are drugs, like Amylin's drug Byetta, that actually are there to stimulate the secretion of GLP1 to treat type 2 diabetes. But our body does that naturally by tasting the right things in our intestinal tract, and the reason I say "tasting" is that the receptors that are tasting our food in our intestines have the same chemical conformation or make-up as the taste receptors in our tongue that are the bitter taste receptors. So we're getting the same signals translated in a different way. We call it bitter; our intestines say, "Oh, that's a molecule that I need to stimulate the secretion of GLP1, which goes in the blood and regulates insulin." So diet plays a very important complex role in modulating redox potential, modulating these autocrine/endocrine and neurotrophic factors that regulate insulin and glucose control, and now we recognize—and this is a new "a-ha" that the beta cells of the

endocrine pancreas actually taste nutrients to secrete insulin. Yes, they taste nutrients. Did you hear what I just said? The beta cells. So these substances that come into our bodies through our diet—things like branched chain amino acids, and various B vitamins, and other interesting molecules derived from food are picked up by receptors on the beta cells that taste these nutrients and then regulate mitochondrial redox within the beta cells for the secretion of insulin.[15]

If you think that this was all a well-understood story ten years ago, five years ago, or one year ago, I would suggest no. We are already living in a new world in which this complex interaction of genes and environment in a functional medicine matrix perspective is changing the way we will think about assessment, diagnosis, and treatment based upon the complexity and uniqueness of that individual in their environment.

Post-Prandial Metabolic Toxemia

What happens if you then eat one high-fat meal? Well, probably nothing with one high-fat meal. But if you eat a high-fat meal every day of your life for weeks, months, years, and decades, what that has been associated with is altering your intestinal permeability. It's producing what's called post-prandial metabolic endotoxemia. So there are toxins like bacterial lipopolysaccharides that then are released into your blood. They activate your immune system, causing the production of these inflammatory mediators that then activate your fat cells, that then cause an inflammatory response and you distort your metabolism, and what's the ultimate outcome? Diabetes. So now we say, "You mean eating a high-fat meal every day could actually associate itself with diabetes as a consequence of actually poisoning mitochondrial function through these inflammatory pathways?" Yes, that's what it is saying and there are many, many papers now coming out on this. Dr. Patrice Cani, who is one of our colleagues in our research team work at Louvain Catholic University in Belgium has published a number of extraordinary papers with his colleague, who you heard on *Functional Medicine Update* years ago, Dr. Nathalie Delzenne, on the relationship of postprandial endotoxemia to type 2 diabetes.[16],[17]

It's a now very increased understanding that you've got these xenobiotic substances that can poison mitochondria. You've got food-related functions that can poison mitochondria. And so we're really talking about how do we reconsider metabolic disease in the context of this 21st century understanding of the genes and environment interaction? It's not just a disease called type 2 diabetes. It is a complex personal relationship that we each have intimately with our environment that then signals through this complex systems biology to regulate our function through adipocytes, cardiocytes, hepatocytes, beta cells, myocytes that ultimately then regulates energy economy. An exciting chapter that you're going to hear now from Dr. Walt Gall, how do you translate this down into asking the right questions, assessing the right information, and using metabolic profiling to better get an early understanding of what to do in that patient's personal program. So with that in mind, let's move to our researcher of the month, Dr. Walt Gall.

INTERVIEW TRANSCRIPT

Researcher of the Month

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Here we are once again at what I consider to be the most interesting part of our Functional Medicine Update edition each episode, and that's our clinician/researcher of the month component. As you know, I look forward to this and I know you do if you've been a long-standing FMU listener. We're very fortunate once again. I can't believe how privileged I am with the kind of expertise that we've been very fortunate to have discussions about—this whole field of emerging medical technology in 21st century—and of course we're very lucky again with Dr. Walter Gall, who will be our researcher this month and will help us understand this very important area that we've been in discussion about for the better part of the last three years: diagnostic biomarkers, early warning signs, how do you understand the trajectory of a chronic disease before it becomes so acute that it requires crisis intervention? How do you then employ, let's call it, less heroic intervention at an earlier stage to modulate disturbed metabolism and avert the necessity for very costly hospitalization and end-stage disease?

You know we've had the privilege of talking to experts on HDL physiology. We've had the opportunity to speak with individuals who are in lipid particle numbers and how that interrelates with early-stage vascular disease risk. We've had discussions on oxidized LDL and Dan Steinberg's work at the University of California, San Diego. We've done a pretty good job of laying out a landscape of areas, including Paul Ridker's work from Harvard on high sensitivity C-reactive protein (CRP) as it relates to inflammatory assessment.

So, where are we in this issue with Dr. Gall? We're at the frontier of what I consider to be probably the most important singular area of discussion as it relates to disturbances in metabolism, and that's related to insulin signaling, insulin activity, glucose transport, and this whole increasing understanding of the conundrum that's associated with dysinsulinism that translates into so many varieties of chronic age-related diseases, not just type 2 diabetes and cardiometabolic disorders, but things like polycystic ovary syndrome and endocrinological effects, and translates into end-stage renal failure, neurological disorders, dementia, certain forms of epithelial cancer, hypertensive disorders leading to stroke. This is an omnibus of discussion. So how do we—as an early warning—understand whether a person is heading on towards these kinds of end-stage problems, at a point where less heroic intervention can have a measurable and significant effect on averting pathology? That is—fortunately—the expertise that Dr. Gall brings to us.

Let me just give you a quick bio—kind of who is the person behind the voice and the message. Dr. Gall earned his PhD at Vanderbilt in what I would call kind of a very interesting translational science program that couples across chemistry, and biology, and physics, and mathematics, and informatics. He had an

undergraduate degree in chemistry—obviously a kindred soul—in organic chemistry and biochemistry. And then later, his PhD work was in looking at cellular genetics and cellular signaling processes, postdoctoral work at the University of California at Berkeley, and a variety of very important appointments that ultimately led him on into his present position as director in the diagnostic area with the group in North Carolina called Metabolon that many of you have heard about. It is a very, very interesting metabolomics company looking at high-level pattern recognition and complex metabolic profiling in the network physiology area. The whole future of health care's understanding of how pathways fit into networks is really the nature of understanding the organism.

With that as a brief introduction of the background that Dr. Gall brings to us, Walter let me introduce you and thank you so much for your participation with Functional Medicine Update.

WG: It's a pleasure to be here, Jeff, thank you.

JB: Let's start off with the first question and that is could you tell us a little bit about the challenges that we have presently in diagnostics as it relates to dysinsulinism or dysglycemia—what some of the limitations are?

The Limitations of Glycemic Diagnostics

WG: Yes, I'd be happy to. Basically, what we do today in the world of identifying high-risk individuals as it relates to insulin resistance disorders such as type 2 diabetes, cardiovascular disease, chronic kidney disease, is we're essentially working in sort of a unidimensional perspective as it relates to measuring glycemic diagnostics, whether it is fasting plasma glucose, or more recently hemoglobin A1c has moved into the diagnostic sector beyond its utility and therapeutic monitoring for diabetic patients, so now it is actually being used to detect both type 2 diabetics as well as pre-diabetics, and essentially some of the limitations that go along with that is that there is discordance between hemoglobin A1c and fasting glucose, that's one point I would make. But there is also a low sensitivity in identifying insulin resistance. In fact, it has very little predictivity as it relates to insulin resistance. So what we've been doing at Metabolon is really taking an unbiased and fresh look at this unmet medical need as it relates to identifying non-glycemic novel markers of insulin sensitivity in the early stages of the disease process that precedes diabetes and cardiovascular disease, and is a very common thread, unfortunately, in our society, as it relates to chronic diseases, as well as obesity disorders such as the ones you mentioned, but also fatty liver disease, there has also been connections made with Alzheimer's and mild cognitive impairment. It's actually fairly pervasive as far as how insulin resistance is related to so many of these chronic diseases that ail western society and is actually moving into other parts of the world with the increased westernization of the global economy—you know, the increased urbanization, the increased ethnic risk of basically exposure to these westernized societies as far as their risk for insulin resistance and diabetes. This is basically a key factor that I believe contributes to the rising epidemic of diabetes and obesity as it continues from year to year.

A New Test for Measuring Non-Glycemic Metabolites and Fasting Insulin

What we have been doing is I've led the development of a diagnostic called Quantose™, which stands for Quantitative Measure of Glucose Metabolism, and essentially this is a simple blood test—a fasted plasma test—that can be taken from a single draw, and is measuring these novel non-glycemic metabolites plus

fasting insulin. Basically an algorithm is generated to give an insulin resistance estimation of an at-risk patient so that physicians, as well as patients, can be aware that they may be asymptomatic, but they are actually in a high-risk zone, if you will, so that the physician can raise that red flag and counsel the patient accordingly with regards to lifestyle intervention or other therapeutic solutions.

JB: One of the most extraordinary parts of your profile, I think, is how you derived this algorithm. Some people might say, "Well, we just chose three analytes out of a random panel," but I think the technology you are using is quite dramatic. Could you describe it?

WG: Sure. Basically Metabolon has the world's leading metabolomics, mass spectrometry-based, discovery platform for identifying disease-based biomarkers, and we've leveraged that platform approach at going after developing a fasted blood test for insulin resistance. Essentially it's an unbiased medical profiling approach that's based on multiple mass-spectrometry platforms with regards to measuring small molecules. So this would be your cholesterols, your glucoses. But raise on an order of magnitude and that really gets into the diagnostic screening of identifying novel biomarkers, where we're measuring several hundred small molecules at a time, in a single blood sample, to then identify which small molecule markers are actually correlating above and beyond traditional risk factors, and correlating significantly with the gold standard for measuring insulin resistance, which we use the hyperinsulinemic euglycemic clamp, which has been recognized for decades to be the gold standard and has been instrumental in a lot of nested clinical studies developing into diabetic drug development.

JB: So we've heard many times that various biomarkers have been chosen, but then the question is validation. Could you tell us a little bit about how this connects to other traditional methods of evaluating glycemic response like the oral glucose tolerance test or the euglycemic insulin clamp?

WG: Sure. When carrying out the discovery and validation studies within our CLIA- and CAP-certified laboratory in North Carolina, essentially we wanted to be rigorous in verifying it and validating these biomarkers, as well as the algorithm test maintenance and resistance. We worked with a really great consortium of researchers over in Europe that represents the European Group for the Study of Insulin Resistance, working with a seminal study in the insulin sensitivity field called Relationship of Insulin Sensitivity to Cardiovascular Disease, which represented the risk population. This is the study that had both oral glucose tolerance testing as well as the euglycemic clamp methodologies employed to essentially categorize them as normal tolerant, insulin sensitive, or insulin resistant, or one of the pre-diabetic categories: impaired fasting glucose or impaired glucose tolerant.[18] Essentially what we've identified from a simple, single draw, fasted blood test of these circulating metabolites, as this index of insulin sensitivity, is that it basically—from a statistical point of view—can replace the two-hour glucose value as far as identifying insulin resistant individuals. And so we believe that will be a key clinical utility message for physicians that would actually have that same question that you offered there.

JB: I've heard you compare this—and I think it's a very good analogy—to what happened with cholesterol and cardiovascular risk assessment 25 or 30 years ago. I think that analogy to what the Quantose™ test does in the area of type 2 diabetes and cardiometabolic disease is very insightful. Could you share that with our listeners?

WG: Yes, absolutely. Traditional lipid parameters we know represents around 50 percent of the cardiovascular risk that we know of today. Essentially when looking at a disease like type 2 diabetes, we actually know just from the studies that have been done that the average time a person has actually had

type 2 diabetes by the time they are diagnosed with glycemic diagnostics, they've had the disease for several years. You add on top of that the number of years that they have chronic insulin resistance and you can imagine some of the physiological damage that may be occurring with regards to insulin resistance and the nascent beta cell dysfunction that's occurring as you move towards diabetes. We believe that this test is going to be a game-changer as it relates to identifying asymptomatic, high-risk subjects that may have certain risk features such as family history of diabetes, or maybe they are overweight and may be at risk for hypertension. These are the type of patients the physicians may be concerned about, and now they have a tool to basically quantify their level of risk—whether they are normal, intermediate, or severely insulin resistant. One of the other key features of the test that I believe is of critical importance as far as clinical utility is that we've done studies as it relates to therapeutic monitoring. We've looked at insulin sensitizing interventions such as pioglitazone, or bariatric surgery, as well as exercise--all three insulin sensitizing interventions. And we've shown that our index of insulin sensitivity tracks that with a very high correlation. You mentioned the question about validation earlier. We essentially have validated this in large IR-related outcome studies, with regards to the Botnia prospective study, which is a very well-known family history diabetes population that has been followed and exquisitely phenotyped.[19] That validation has been mirrored with the Act Now study, which is a diabetes prevention study done here in the US, where we actually looked at the correlation of rigorous measures of insulin sensitivity such as the Matsuda index, which was championed by one of our collaborators, Ralph DeFronzo in San Antonio.[20] And essentially, with the additional validation and multiple population studies, and then showing on top of that that it tracks the change in insulin sensitivity with placebo groups as well as groups that are being intervened with to improve their insulin sensitivity and the fact that it tracks that really underscores the utility of tracking improvement or lack thereof when counseling a patient.

JB: So now let's go from its clinical utility to what you learned after examining several hundred metabolites in these populations. What popped out to be really an unexpected "a-ha" for you as it pertained to these risk factor markers?

Alpha Hydroxybutyrate is a Top-Ranking Marker for Insulin Resistance

WG: Great question. Essentially what was fantastic about taking an unbiased approach was that identified, statistically, these metabolites that came up as the highest correlates to insulin sensitivity. The top-ranking marker is a marker called alpha hydroxybutyrate. This is not a ketone body. Beta hydroxybutyrate is a common metabolite that is perturbed with diabetes as far as ketoacidosis. This is a different metabolite in a different pathway, with regards to energy metabolism. This is a metabolic pathway that is right juxtaposed next to the tricarboxylic acid cycle (TCA cycle), which occurs in the mitochondria with regards to energy respiration. Conventionally speaking, we actually believe that insulin resistance may originate in the mitochondria, and so the fact that this metabolite is a reduced form of a precursor substrate that is a very common substrate to amino acid metabolism and glutathione synthesis as it relates to oxidative stress response. What was really interesting was another marker that was number two in line is a novel lipid signaling molecule—what's called a lysophospholipid—that is in the phospholipase A2 pathway in the liver, as well as lecithin-cholesterol acyltransferase in the circulation, and what's interesting about that molecule is it is actually decreased with insulin resistance, and as well as further decreased with type 2 diabetics. We believe, just from the literature done by others, that this class of lipid represents a very interesting molecule to continue to watch and monitor with patients because of its implicated action with pancreatic beta cells as far as insulin secretion, as well as its action

in the enteroendocrine system with the gut and the incretin access. So we believe that looking at these nonglycemic metabolites and characterizing a person's level of insulin resistance and their potential improvement with therapy is a critical step forward in identifying the high risk individuals and then tracking their success with improving that.

JB: I think all of our listeners who have been following Functional Medicine Update for some time, their ears probably immediately perked up when you talked about mitochondrial oxidative stress, and redox potential, and bioenergetics associated with insulin resistance and beta cell mass and beta cell function. That's a topic we've been talking around, and glutathione biochemistry and NAD-NADH ratios and so forth for the better part probably of ten years. It sounds like these clinical markers are really starting to pop out of these multivariate analyses as kind of maybe defining the hypothesis as having clinical utility, it sounds like.

WG: Yes, absolutely. We see that these markers do represent an energy imbalance. In fact, alpha hydroxybutyrate is generated when you do have this redox imbalance, where you have increased NADH reducing equivalence that's a result of high lipid oxidation events, which is characteristic of insulin resistance. So the fact that we see this metabolite as one of the key markers really validates that hypothesis that there is an energy metabolism abnormality. I would go on to further say that we've seen this metabolite have effect on insulin sensitive tissues. We're looking at the effect of these metabolites on insulin release as well as mitochondrial respiration that's important for insulin release as well as glucose uptake. We're very interested in looking at the mechanistic questions in a rigorous way to further increase our insights into these novel biomarkers.

JB: I know you were recently at the American Association for Clinical Chemistry meeting, a big international clinical chemistry meeting, and presented your new profile. What kind of response did you get both positive and negative? Anything new always has some critics and some skeptics. What types of things did you see at this large national meeting?

WG: Yes, we had a couple of scientists that are a part of our team. One collaborator at UNC presented a poster as it relates to both diabetics as well as non-diabetics, essentially looking at the utility of measuring their insulin resistance. One of the cautions was you'd have to be careful of measuring patients that are actually on insulin, so that is one limitation. We'd be really targeting this test toward non-insulin-dependent diabetics, as well as non-diabetics (that would include the high-risk, pre-diabetic group). But overall there was a lot of positive enthusiasm for having a simple blood test for measuring insulin resistance. It was very well received, and we look forward to showcasing this in subsequent meetings.

JB: I know that you've done a tremendous job of linking the development of your test and the clinical validation with real strong opinion leaders that have a long history as being leaders in the field diabetes pathophysiology and etiology. You've mentioned a few of those individuals. Tell us a little bit about you've gone about networking with these individuals in collaboration for the development of the test.

WG: Sure. When essentially calling on some of the key investigators that have done these large outcome studies, such as the RISK study, employing the euglycemic clamp, Ele Ferrannini, the former president of the European Association for Study of Diabetes has been a fantastic colleague through this clinical research effort, and I just want to acknowledge him and his team, as well as his colleagues within the European Group for the Study of Insulin Resistance, at working with us as far as clinical counsel and

basically the development of this test, looking at the utility and the hard questions that you'd like to address in developing a diagnostic. Ralph DeFronzo, who championed the clamp back in the late 70s, as well as other investigators at Joslin Diabetes Center, such as Ron Kahn, Elizabeth Patti, have been instrumental in looking at these metabolic pathways, not just in adults, but in diabetes animal model systems, so we can actually look at the origin of these metabolites, as well as looking at adolescent obesity development, and looking at the perturbations of these metabolites in that setting, which is an enormous public health medical need as far as being able to identify not just adults that are high risk, but really the pediatric segment.

JB: I can tell you the first time I heard you speak I had every neuron alive and well and totally focused on what you were saying. I think you're hitting right in to a huge unmet need that may open up the opportunity for absolute patient stratification and recognition early on of the people that we really want to spend time on counseling and getting into appropriate programs, and then to track the success of those programs—those therapeutic programs—be it either lifestyle management, nutraceutical, medical foods, and/or pharmaceuticals so that we really are tailoring and personalizing those programs to maximize outcomes. I want to really compliment you, Dr. Gall, on the way that you've approached this with your colleagues at Metabolon, and also with the way that you've described it. I think it makes this very user-friendly to the clinician, and I think also ties back to these long-standing questions of mechanism, bioenergetics, mitochondrial function, and specificity of insulin-mediated responses in different tissues. Thank you for the extraordinary work and sharing this with us.

WG: Thank you, Jeff.

Bibliography

[1] Funnell MM, Piatt GA. Diabetes quality improvement: beyond glucose control. *Lancet*. 2012;379(9833):2218-2219.

[2] Chen Z, Vigueira PA, Chambers KT, et al. Insulin resistance and metabolic derangements in obese mice are ameliorated by a novel peroxisome proliferator-activated receptor ?-sparing thiazolidinedione. *J Biol Chem*. 2012;287(28):23537-23548.

[3] Fukui Y, Masui S, Osada K, Umesono K, Motojima K. A new thiazolidinedione, NC-2100, which is a weak PPAR-gamma activator, exhibits potent antidiabetic effects and induces uncoupling protein 1 in white adipose tissue of KKAy obese mice. *Diabetes*. 2000;49(5):759-767.

[4] Bolten CW, Blanner PM, McDonald WG, et al. Insulin sensitizing pharmacology of thiazolidinediones correlates with mitochondrial gene expression rather than activation of PPAR gamma. *Gene Regul Syst Biol*. 2007;1:73-82.

[5] Tan Y, Muise ES, Dai H, et al. Novel transcriptome profiling analyses demonstrate that selective peroxisome proliferator-activated receptor ? (PPAR?) modulators display attenuated and selective gene regulatory activity in comparison with PPAR? full agonists. *Mol Pharmacol*. 2012;82(1):68-79.

[6] Corkey BE. Banting lecture 2011: hyperinsulinemia: cause or consequence? *Diabetes 2012;*61(1):4-13.

[7] Corkey BE, Shirihai O. Metabolic master regulators: sharing information among multiple systems. *Trends Endocrinol Metab*. 2012; 23(12):594-601.

[8] Hamdy O, Porramatikul S, Al-Ozairi E. Metabolic obesity: the paradox between visceral and subcutaneous fat. *Curr Diabetes Rev*. 2006; 2(4):367-373.

[9] Gil A, Olza J, Gil-Campos M, Gomez-Llorente C, Aguilera CM. Is adipose tissue metabolically different at different sites? *Int J Pediatr Obes*. 2011;6 Suppl 1:13-20.

[10] Altintas MM, Azad A, Nayer B, et al. Mast cells, macrophages, and crown-like structures distinguish subcutaneous from visceral fat in mice. *J Lipid Res*. 2011;52(3):480-488.

[11] Cinti S. Between brown and white: novel aspects of adipocyte differentiation. *Ann Med*. 2011;43(2):104-115.

[12] Boss O, Farmer SR. Recruitment of brown adipose tissue as a therapy for obesity-associated diseases. *Front Endocrinol* (Lausanne). 2012;3:14.

[13] Ruzzin J, Lee DH, Carpenter DO, Jacobs DR Jr. Reconsidering metabolic diseases: the impacts of persistent organic pollutants. *Atherosclerosis*. 2012;224(1):1-3.

[14] Lodhi IJ, Yin L, Jensen-Urstad AP, et al. Inhibiting adipose tissue lipogenesis reprograms thermogenesis and PPAR? activation to decrease diet-induced obesity. *Cell Metab*. 2012;16(2):189-201.

[15] Henquin JC. Do pancreatic ? cells “taste” nutrients to secrete insulin? *Sci Signal*. 2012;5(239):pe36.

[16] Cani PD, Bibiloni R, Knauf C, et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes*. 2008;57(6):1470-1481.

[17] Harte AL, Varma MC, Tripathi G, et al. High fat intake leads to acute postprandial exposure to circulating endotoxin in type 2 diabetic subjects. *Diabetes Care*. 2012;35(2):375-382.

[18] Gall WE, Beebe K, Lawton KA, et al. alpha-hydroxybutyrate is an early biomarker of insulin resistance and glucose intolerance in a nondiabetic population. *PLoS One*. 2010;5(5):e10883.

[19] Lyssenko V, Almgren P, Anevski D, et al. Genetic prediction for future type 2 diabetes. *PLoS Med*. 2005;2(12):e345.

[20] DeFronzo RA, Tripathy D, Schwenke DC, et al. Pioglitazone for diabetes prevention in impaired glucose tolerance. *N Engl J Med*. 2011;364(12):1104-1115.p>