Article:
S. Devaraj, P. Hemarajata, and J. Versalovic.
The Human Gut Microbiome and Body Metabolism: Implications for Obesity and Diabetes.
Clin Chem 2013;59:617-628.
http://www.clinchem.org/content/59/4/617.abstract

Guest:
Dr. James Versalovic is Head of the Department of Pathology and Director of the Texas Children's Microbiome Center, at Texas Children's Hospital in Houston.

Bob Barrett: This is the podcast from *Clinical Chemistry.* I am Bob Barrett. Obesity, metabolic syndrome, and diabetes are major public health challenges. Recently, interest has surged regarding the possible role of intestinal microbes as potential contributors to the increased prevalence of these three disorders.

In the April 2013 issue of *Clinical Chemistry*, Dr. James Versalovic and his colleagues reviewed the Human Intestinal Microbiome and its implications for obesity and diabetes. Dr. Versalovic is Head of the Department of Pathology and Director of the Texas Children's Microbiome Center, at Texas Children's Hospital in Houston. He is our guest in this podcast.

Dr. Versalovic, can you tell us something about the Human Microbiome Project and just how this is relevant to metabolism in humans?

Dr James Versalovic: Well, the Human Microbiome Project is a big science project that was initiated in 2007. A large scale effort that was spearheaded by the director of the National Institutes of Health and a cross-institutes effort with four genome centers across the United States and many other laboratories involved. So this is really big science first of all, and it's ushered in a new era in science, because we have taken the analogy of the human genome and advances in the human biochemistry to understand not only the human genes, but all of the microbial genes that are part of us, the part as the human beings we call the second genome.

And those genes now also encode for protein's enzyme that affect a variety of metabolic pathways and the metabolic activity of the microbiome is considered to be as or more complex than the human liver, so you can imagine, potential impact of this project in understanding not only the genes, but the enzymes, the metabolites and their pathways, and

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how they might be involved in human, or mammalian biology.

So this has been a major new development since 2007. It's important to remind ourselves, it's only been six years and we are now entering really the second phase of the project, which is moving from discovery and large scale sequencing efforts describing microbial composition in humans and other mammals to try and understand the functional relevance of this and how it might translate this knowledge to understand mechanism of disease and in particular today, disorders of body metabolism, metabolic syndrome, obesity, diabetes and a number of related conditions.

Bob Barrett: Doctor, there must be thousands upon thousands of different microbes in our intestines. Which of these and which microbial genes are considered to play major roles in human metabolism?

Dr James Versalovic: Well, it's hard to answer that question at this point in time in 2013, but certainly we can say a few things based on knowledge gathered in the past six years. We have approximately 1000 different bacteria and that's more than 90% of the content to the microbiome that is bacterial. I should mention that there are certainly are viruses and yeast or fungi that are also small part of this.

Roughly about a 1000 different bacterial organisms in the human intestine and a variety of other bacteria that are colonizing different body sites. So it's important to also mention that this human microbiome differs depending on body sites, so the intestine, versus the skin, the airways, the oral cavity, a variety of body surfaces.

The intestine is considered to be really most important at this time in terms of its effects on body metabolism and there are many genes involved. At this point in the project we have counted more than 10 million microbial genes compared to 20 to 25,000 human genes. Of the greater than 10 million genes, it's really hard to say which are most critical at this time. What's interesting is of the 1000 or so microbes in the human intestine, 20 to 25 organisms are considered to be dominant in each intestine.

And so if we multiple that by that 2000 genes per microbe, we are left with the much more manageable set of genes, so we might be considering 50,000 genes and of those genes how many are most critical and certainly some of these rare microbes maybe important biologically, so it's not simply that quantity, but also quality.

We would estimate now that there are hundreds of genes that would be considered pivotal in human metabolism. That still puts us in kind of a vague zone, but nevertheless that's the ballpark and we are now trying to understand which specific genes may have specific effects and how those effects impact human health.

Bob Barrett: How can these gut microbes mediate the effects of high levels of sugar and fat in the human diet?

Dr James Versalovic: We are now beginning to draw connections between diet, nutrition, the microbiome and human health and disease. What's particularly exciting now is that the microbiome appears to be a very important bridge between diet and human health.

And as a bridge, it contains so many genes that we just discussed, and microbes and pathways, metabolites, enzymes that may be pivotal in body metabolism, and there are a number of components in diet to consider. So as we break down diet, we mentioned sugar and fat.

Sugar of course is a carbohydrate, but gut bacteria have a number of enzymes now that we know play a role in helping us to digest the sugar or carbohydrates in the diet. We've moved rapidly in the last two decades from a rather simplistic notion of sugar to the fact that we have many different kinds of sugars in the diet and we are still trying to understand the composition of many foods frankly, so food chemistry, food science is still important.

But within the microbiome there are number of enzymes that digest these sugars, organisms such as bacteroides and fusobacterium are few examples of gut microbes that contain enzymes such as endoglycosidases that metabolize the sugars and make them available to us as nutrients, much of this activity going on in the small intestine though we do think that it's important in both the small, large intestine or colon.

The bacteria then help to break down those complex carbohydrates in the diet to simple sugars that can be absorbed and those sugars, like saccharides, then they may have a direct impact on blood glucose levels for example, and the kinds of metabolites then that make their way to the liver.

Another aspect of this is early in life; gut bacteria have the ability to metabolize the carbohydrates in breast milk. So as babies get human milk, they are in a sense feeding their microbiome, but then providing the substrate, the sugar if you will for the bacteria, the gut microbes, to then break down the sugars and stimulate gut development.

That's really an interesting connection that a few scientist have uncovered in recent years, and in this way the bacteria can provide signals, and we are not exactly clear on the nature of these signals, but they are basically carbohydrate metabolism byproducts that may impact the ability of the epithelium lining of the intestine to mature, differentiate, and form a healthy intestine.

In terms of fat, there are a number of enzymes that are involved in metabolizing fat in the diet. And we know that some of this fat is essential for life and the gut microbes can break down fat in to short chain fatty acids, things like acetate and we know acetate maybe an important metabolite for human health and butyrate is another short chain fatty acid, and it turns out that butyrate is important for the health of the intestinal epithelium; it's used as an energy source by human cell, epithelial cell.

So these are some examples of how gut microbes can effectively help to metabolize our diet, but what's really interesting is that the microbes in a sense are translating, they are converting these dietary sugars and fats and proteins also, into small molecules, metabolites, that then can be utilized by human cells. And then this affects the biology of the intestine, the ability to digest and absorb nutrients, the whole body metabolism and additionally the functioning of the immune system and the nervous system, so many different affects that go beyond effects on body metabolism.

Bob Barrett: Doctor, we have grown accustomed to hearing about the revolution of various OMICS. How do these apply to the human microbiome and specific metabolites in different body compartments?

Dr James Versalovic: Well, we are now taking the OMICS that we have been using to study mammalian and human biology to the microbiome and we have added these four letters called "meta-" and the metagenomic, metatranscriptomics and metaproteomics, metabolomics referred to the strategy of characterizing all of the various compounds or macromolecules and the microbiome, in addition to what we consider to be originating from human cells.

And we have the technologies now to approach this at the meta level and as you can imagine going from 20 to 25,000 genes to 10 million plus genes is a huge jump, and so we needed the past 20 years of technological developments with the human genome project to enable us now to pursue the human microbiome project.

What we are able to pursue now with next generation DNA sequencing technologies is the ability to characterize so many different genes and do it in a way that's relatively cost effective. We could not even imagine doing this 10-15 years ago. Now we can not only sequence thousands to millions of different microbial genes, but we have the analytical tools, the bioinformatics approaches to be able to piece together these genes into pathways, metabolic modules and be able to construct maps, metabolism maps if you will.

We have the ability to go beyond DNA sequencing to RNA sequencing and metatranscriptomics to detect which genes are being expressed actually in the human microbiome, and then to go further than that with metaproteomics to characterize the various proteins.

Now this is a field that's still relatively new, it's more difficult, that we are beginning to make advances and trying to characterize the various microbial proteins, including enzymes that may have a pivotal effect on body metabolism.

And then finally, metabinomics which takes metabolomics and takes it to the microbiome level to try and understand all the various small molecules or microbial metabolites that are being produced, the business end so to speak, where the action is, and trying to understand then how these metabolites then provide signals to the body affecting metabolism in a variety of other body processes.

And we can do this in multiple body compartments, not just using stool specimens and using intestinal content, but we can also look at urine for example. And we know now the changes in the urine maybe due to metabolism by the microbiome in the intestine.

And because of metabolites are being excreted through the urine, we can actually decipher that and discern and separate those signals from human metabolites. But they are still essentially part of human. This is the tricky part of microbiome research, is it's easy to get caught into the us versus them mentality, and even in the fact, it's really a big We, but we are now able to distinguish which metabolites are coming from the human microbiome, versus the human genome and human cells.

So these various multi-omics advances and the various OMICS and the ability to analyze large skill datasets using the latest tools of bioinformatics have been pivotal in helping us to take this field forward.

Bob Barrett: Well, let's get specific. Do you have any specific examples regarding the impact of microbial composition in the gut on body metabolism and obesity?

Dr James Versalovic: Yes, we have a number of examples in the literature. We know now for several years that individuals who are overweight or obese have differences in the bacterial composition of the microbiome, and that these individuals have different proportions of groups, large groups of bacteria for example, phyla. Different ratios of firmicutes, one phylum versus bacteroidetes of another phylum.

And it turns out that this could be replicated to some extent in mouse models, and so mouse models have been very helpful in generating conceptual breakthroughs in this field during the past decade. And as examples of this, we've known that obese mice have different proportions of bacteria and that when you introduce those bacterial populations into lean mice, they gain more weight and in fact become more effective in energy harvest, and we think this is really a pivotal aspect of the effect of the microbiome on body metabolism and weight gain, which is energy harvest and the more effective we are at energy harvest, the more weight we gain.

And so we are making these correlations between differences in bacterial composition and weight gain in mouse models and humans. I should extend this conversation and mention a few other studies.

We know that in a mouse model which has a particular defect and a toll-like receptor that predisposes these animals to metabolic syndrome, a condition we know in humans. Metabolic syndrome includes hypertension, high blood pressure, hypercholesterolemia, high cholesterol levels and insulin resistance and secondary to dysregulation of cytokine signaling.

And it turns out when the microbiomes from the mice that have this defect and this toll-like receptor are moved into a different mouse that the recipient mice actually develop many of the features of metabolic syndrome. So this goes beyond weight gain and obesity to disease, and really a clear-cut disease phenotype where the microbes actually can transfer that disease state, if you will, to another animal.

So this is of course using a mammalian model, specifically the mouse. In humans, we are beginning to understand that there are some key differences in bacterial composition and functional pathways in individuals who are obese and individuals with metabolic syndrome and in patients with diabetes.

And for example, in diabetes we also have diabetes mouse models that can substantiate this and it turns out that the pathways, when we look at the genes and the metabolic pathways in the human microbiome, that there are various differences between patients with diabetes and healthy controls.

So the next question is going to be, and the next level of exploration will be, which specific changes are most important, how might we modify that to treat or prevent disease?

Bob Barrett: Are there specific cases showing the effects of microbial function and pathways on energy balance and the pathophysiology of diabetes?

Dr James Versalovic: Yes. So the prior question really sets the stage for this. We do know that there are a number of pathways that are potentially involved. Again, it's really now plenty of hard work that needs to be invested and really trying to understand which are most important.

There are mouse models that allow us to study inflammation, we know inflammation is a part of diabetes, especially Type 1 diabetes, and it appears that there are particular defects in the immune system that may promote inflammation in animals that have a particular bacterial composition.

For example, there are changes in the liver and hepatic steatosis that may be affected by intestinal bacterial products. There are changes in inflammation, and basically increased inflammation associated with changes and pathways involved in flammasomes in animal models.

We know that patients with diabetes have increased amounts of specific toll-like receptors and this extends to patients with metabolic syndrome, as well Type I and II diabetes. And the toll-like receptor signaling, as I mentioned, may be very important in linking microbial products, metabolites to enhanced inflammation or autoimmunity, which we know is a component of diabetes.

So it appears that the increased amounts of toll-like receptors in these patients on the blood cells, monocytes of these patients, may be actually predisposing these patients to more inflammation, if they have the wrong bacterial composition in their microbiome.

So those are some examples. I should mention another one that was a separate study in which they actually looked at bacterial composition and we commented on this in the

review in *Clinical Chemistry*. And specific bacteria were either less or more abundant in diabetic patients, and one group the betaproteobacteria were significantly increased in diabetic patients compared to non-diabetic, and the abundance of those bacteria significantly correlated with plasma glucose concentrations or blood sugar.

So we're now beginning to make connections between individual microbes, not just the genes, the bacteria as well, and their relative abundance and effects on blood sugar level.

So this gets into another aspect of microbiome research, which is that the human microbiome in the intestine is not just relevant to the gut, but that the effects of the bacterial composition function, those metabolic pathways, then impact whole body metabolism at sites that are distant or away from the intestine, in the blood stream and then in various other organs. So this is just one more example of that.

Bob Barrett: Well finally doctor, let's look ahead. What's next for your work and the Human Microbiome Project?

Dr James Versalovic: We're now at the point where we want to deeply understand how the microbiome makes the signals that may impact body metabolism. That's what we referred to de novo biosynthesis, basically starting from scratch, can bacteria take a variety of chemicals, really thinking now of that human biochemistry and making, signal serving as microbial factories if you will.

The other part of this and we're still, we're trying to understand which enzymes maybe pivotal and which signals maybe made de novo. In addition to that though, and even more importantly we think that luminal conversion or the ability to convert these specific components of the human diet into signals that then affect metabolism, for example, sugar metabolism, fat metabolism, protein metabolism and the immune system and inflammation. We're trying to narrow, trying to focus on which components of the diet are converted into these signals.

And that's luminal conversion--the lumen refers to the intestine itself, the cavity if you will, where the microbes including the bacteria reside--and it's the conversion of the diet that we're ingesting. We are eating everyday, we are drinking everyday beverages, and that food is then making its way down into the intestine, and so it's basically flooding the microbiome literally with food compounds, a variety of different sugars, proteins, and lipids and those are being converted by a number of different metabolic pathways of the microbiome.

We really trying to focus on the functional aspect of this problem now, to try and understand how the microbiome is functioning and then how is that directly impacting body metabolism, and that is really a major emphasis now.

In addition to that we are really going to be focusing now on translating these discoveries of microbial composition, and function of the human microbiome to new diagnostic test. In other words, we will be using these metabolic pathways to find new biomarkers and similar to how we use blood tests to look for example, for Prostate-Specific Antigen or PSA for individuals who may be at risk for prostate cancer or we may look at the blood cholesterol level, we may look at C-reactive protein to follow patients with inflammation.

We also now are thinking about microbial biomarkers. Are there biomarkers that come from these microbes that provide signals to us that something is wrong, and can we use those in a variety of tests in the hospital or in the clinical laboratory to be able to say that this patient may be predisposed to diabetes or metabolic syndrome or a variety of other conditions, even susceptibility to cancer?

We are also thinking about these genes and how the DNA/RNA may be used from the microbiome to develop new diagnostic tests and to enable us to take a disease in which we may be lumping many patients together just to begin to stratify or separate these conditions, so that we can provide a better match between the patient and the right treatment, to enable us to have more personalized or customized medicine by combining the knowledge of the microbiome and the human genome.

And that's where we are headed and in addition to that finally we want to develop treatments, of course, it's not just about diagnosing, but can we use the knowledge of the human microbiome to supplement these microbial deficiencies by adding bacteria into the diets such as probiotics to change the diet and provide the right mix of sugars and proteins and lipids and other compounds, such as prebiotics, but even more general, dietary changes or modifications this may really impact the field of dietetics and human nutrition. And then finally, do we need to do things that are more drastic, in other words, really think about cutting back on antibiotic use that may impact the function of the microbiome may predispose us to disease or should we be replacing microbiome, such as fecal transplantation or intestine microbiome transplantation in very severe cases, in which the microbiome is involved.

There are a number of therapies now that may affect, hopefully, positively impact patients that currently have

metabolic syndrome, diabetes and a number of different conditions.

Bob Barrett: Dr. James Versalovic is Head of the Department of Pathology and Director of the Texas Children's Microbiome Center, at Texas Children's Hospital in Houston. He has been our guest in this podcast from *Clinical Chemistry.*

I am Bob Barrett. Thanks for listening!