YALE JOURNAL OF BIOLOGY AND MEDICINE 91 (2018), pp.197-200.

INTERVIEW

YJBM

# A New Holistic Approach to Diabetes Research: Measuring Metabolism at the Cellular Level

## An Interview with Richard Kibbey, MD, PhD

Ian Gonzalez*

*Department of Cell Biology, Yale University School of Medicine, New Haven, CT*

Richard Kibbey, MD, PHD, is an Associate Professor of Medicine (Endocrinology and Internal Medicine) and of Cellular and Molecular Physiology. After graduating from Trinity University with degrees in both Music and Biochemistry, Dr. Kibbey obtained his MD and PhD degrees from the University of Texas Southwestern Medical School. After completing his residency and fellowship in Internal Medicine and Endocrinology at Yale University School of Medicine, Dr. Kibbey stayed on as a faculty member and is currently an active physician-scientist where he is board certified in Internal Medicine and Endocrinology.

Dr. Kibbey's lab takes a unique approach to the study of metabolic disorders such as diabetes and obesity. Through his discoveries, Dr. Kibbey has pioneered the use of highly quantitative approaches that can detect metabolic flux at the cellular level. These approaches offer a new and exciting dimension to diabetes research. In our conversation, Dr. Kibbey discussed the discovery that influenced his lab's work, his unique perspective on metabolism and how this technique can change the way we evaluate, study, and treat type-2 diabetes and metabolic disorders at large.

***Can you talk about what your lab is focused on and any current exciting projects?***

Right, so I guess the background is that I'm a clinical endocrinologist, so I see patients with diabetes as well as other hormone-related diseases – where I became very interested in the connection of intracellular metabolism to whole-body metabolism. One of the key insights that led to the work that drives much of our laboratory was a rare genetic disorder that causes hypoglycemia and it is bizarre in its presentation. The children who have this disease are born completely normal. The first months to years of their life are not problematic, but then later on they start developing severe hypoglycemia, and their blood sugar goes really low. Some really clever endocrinologists figured out the hypoglycemia precipitated when their diet was moving away from breast milk, which has a nice balanced carbohydrate protein composition, to higher protein-containing meals. In particular, the abundance of amino acids in their meals as they got older provoked the hypersecretion of insulin. Insulin causes the movement of glucose from the blood into tissues, so amino acid-induced hyperinsulinemic hypoglycemia was the consequence of their mutation. Really high blood am-

*To whom all correspondence should be addressed: Ian Gonzalez, Email: i.gonzalez@yale.edu.

†Abbreviations: GDH, glutamate dehydrogenase; SCS, succinyl CoA synthetase; PEP, phosphoenolpyruvate; GTP, Guanosine Triphosphate; ATP, Adenosine Triphosphate; TCA, Tricarboxylic Acid; MIMOSA, Mass Isotopomer Multi-ordinate Spectral Analysis.

Keywords: Diabetes, Metabolism, Mitochondria, GTP

monia levels in these patients clued the researchers into a particular enzyme, glutamate dehydrogenase (GDH†), that was found to be mutated. GDH was mutated in an inhibitory site, so once you lose the normal inhibition, the enzyme can inappropriately be turned on by the amino acids.

In diabetes, there is defective insulin secretion. I was trying to understand how this mutation was connected to insulin secretion from the pancreatic beta-cell. The GDH signal seemed like it was a very different angle to learn about beta-cell function. Interestingly, the lost inhibitory site in the mitochondrially-localized GDH enzyme was where GTP normally binds. The concept of GTP regulating mitochondrial metabolism struck me as an important insight because I had always been taught that mitochondria just made ATP. I started doing a lot of research to figure out what was going on and found that the only enzyme that can make GTP in the mitochondrial matrix is one of the TCA cycle enzymes itself, not oxidative phosphorylation. That enzyme succinyl CoA synthetase (SCS) comes in two flavors, one that makes GTP (SCS-GTP) and one that makes ATP (SCS-ATP). Since both can be present in the same mitochondria, the idea was that the ratio of one relative to the other determines how much GTP is made per turn of the TCA cycle. I realized I could manipulate those ratios to see if GTP was connected to insulin secretion. Ultimately, we discovered a novel metabolic cycle that the mitochondria uses to sense how much energy is around in the cell. The faster the TCA cycle turns, the more GTP it makes. Thus, we believed that mitochondrial GTP is intrinsic to a mechanism for insulin secretion among other signals in different tissue types.

Now, in addition to being present in the mitochondrial matrix, GTP is also found in the cytosol where it's made principally by a number of different enzymes transferring phosphates from ATP to GTP. Interestingly, the GTP pool in the mitochondria is isolated from the cytosol – that is, there's no transport or connection between GTP in the cytosol and the mitochondria. This raised the question of how the mitochondrial GTP signal is transmitted to the events in the cytosol connected to insulin secretion. By following the metabolism of GTP consumption in the mitochondria, we found that it was connected to the highest energy metabolite in the cell, phosphoenolpyruvate (PEP). So the efforts of our lab are towards understanding how the mitochondria senses how much energy is present in the cell, how to connect it to amino acid metabolism, and how PEP production work together to signal an event as important as insulin secretion. And just remember, you have enough insulin in your pancreas to kill yourself hundreds of times over if you release it all at once inappropriately. So, it's a very tightly controlled metabolic signal. As such we developed all sorts of tools both highly analytical as well as *in vivo* models to be able to address all this.

Richard Kibbey, MD, PhD, Yale University School of Medicine and Yale Health

### *How do you think your lab approaches these questions uniquely?*

I think what we try to do is use whatever approach will best get to the solution. I came to the lab trying to solve a tough clinical problem related to metabolism. Since there were not tools that could make the metabolic measurements we needed, we had to build them. That is how we got into stable isotope mass spectroscopy in order to more holistically and quantitatively understand discrete intracellular metabolism. For instance, you obtain glucose, which normally contains 99 percent carbon-12, where instead each carbon atom in the molecule is carbon-13; if you add it to a living cell the labeled glucose will enter metabolism and you can follow it using mass spectroscopy. We call this technology MIMOSA, which stands for Mass Isotopomer Multi-ordinate Spectral Analysis. MIMOSA allows us to follow the carbon-13 label as it moves carbon by carbon through metabolic pathways such as glycolysis and the TCA cycle. Presently, we're getting one of the most accurate and complete views of mitochondrial metabolism within a cell. This platform is highly analytical and computationally demanding, sometimes taking months to analyze a data set. Now we are also developing software solutions and have actually spun off a company to speed the process from months to hours.

***Have you already seen this research form clinical applications or change the way you think in the clinic?***

Yeah absolutely. What I would say is before we started this investigation there was a very dogmatic understanding of metabolism in the beta cell, for instance, and it was very oxidative phosphorylation, ATP-centric, and prior pharmacologic approaches to change these aspects in the beta cell – they can secrete insulin, but they don't work long-term because they kill the beta cell by changing metabolism too much. By identifying this new metabolic sensing cycle we could zero in and identify a clear molecular target that we could go after. And we found that there was a drug that had already been created to go after this target (incidentally a drug made because of a mistake in understanding of metabolism in cancer that failed as a cancer drug). We think its potentially a great, completely new category of diabetes drug, that not only can stimulate insulin secretion, but it does so without hurting the beta cell (like other drugs do) but maybe even can protect these precious cells.

We have begun to align these investigations with the pharmaceutical industry. So starting with our studies of a rare genetic disease, combined with our novel MIMOSA platform measurements, we may have repurposed a drug to treat diabetes – a disease of epidemic proportions. And along the same lines, we think as our computational workflow gets sped up a little bit, this becomes a great drug screening platform. Using these high resolution measurements of metabolism, not only to understand mechanism, but to understand off-target effects will also be really important for developing new human therapeutics.

We think of metabolism like a highway or the road systems. If there's a crash on one side of the highway, it's not only affecting that lane. Even the lane that's oncoming that may be even across the divider is slowed down because there are people looking at each other. When traffic flow is impaired, cars choose different roads and create other backups scattered throughout the traffic network. That's what happens in disease states or with pharmacologic treatments. If you only look very narrowly where the crash is (or where a drug is acting), you're missing everything else and you may not be able to identify the best route to get from point A to point B. So, we're also taking a more of a holistic view of metabolism and how things feed forward and feed back. Along those lines, we have been trying to understand how to improve a cancer drug that has some toxicity. Using MIMOSA we think we've found more the direct action of where the compound's working and we can retool and target that.

***How long do you think it will be before a normal part of going to the doctor could include personalized genomic and metabolomic screens?***

I think we're there in some cases. It may be reasonable to get whole exome sequencing for particular disorders and you need to figure it out. Not for everyone, but the technology is getting cheap. The question is how often is that information going to be helpful? How connected is the genome with at least the metabolome that we're interested in? Those real studies haven't been done.

Let me just bring up another point about metabolism that we like to think about a lot, and it relates back to this traffic analogy. If you look at the number of cars on a road, that does not tell you how fast the traffic is moving. You could have a lot of cars there because there's a lot of motion or because there is a collision and things are backed up. So, just looking at a metabolite or even a group of metabolites and their concentrations, that could be because the production rate is higher, the clearance rate is lower or both, it's just a snapshot. So, you need more kinetic information.

That's where the flux metabolism that we're trying to do may be helpful and to do that you have to have some kind of label to be able to distinguish one thing from another and that's where stable isotopes come in. They're inexpensive, they are not dangerous, they're not radioactive and mass spectrometers have gotten really good and really fast at being able to measure these. It is possible to easily distinguish an experimentally labeled substrate like carbon-13 labeled glucose from what's normally there. So, in principle this type of approach may eventually show up in clinical practice, but it'll likely take decades before we're there. Nevertheless, that is the direction that we're going in.

***On a different note, personally, how do you balance the role of being an investigator while also being a clinician? How do those roles inform one another?***

The whole concept of physician-scientists was that you can take information that you learn at the bedside, bring it to the lab and then hopefully go back to the bedside. I think that's what we've tried to do. We've started with a genetic disease that may be rare but relates to a much more common disease like diabetes. We've used that to identify mechanisms, targets, and compounds and we're trying to go back. We're hoping that in the not too distant future we'll actually be doing these studies in humans as opposed to rodent models. So, there's a very clear foundation for that type of interaction and I think it's really important – that's why I went into it.

I will say now that the clinical stuff has become

more and more demanding and burdensome. There's such a high burnout rate even among physicians who aren't doing research. It is becoming harder and harder to stay clinically active and maintain a research presence. Physician-scientists compete for the same grants as full-time scientists. It wasn't as big a deal when the NIH had a lot of money, but now it is. I'm not sure what the solution is, but I hope that there is one coming along the way because it's going to be harder for the next generations to keep up.

### *What do you think is the biggest obstacle in your field from both a research perspective and maybe also from a public health perspective?*

Diabetes is a world-wide epidemic with over four hundred million people worldwide with the disease and an upwards slope that doesn't show signs of tailing off anytime soon [1]. So, that's a real problem. Part of what is driving that is that we're really successful as economies. We're able to generate excess nutrition that is relatively inexpensive for large numbers of populations and our brains are kind of wired to try to take in as much as you want, so you have this over-nutrition, obesity, and insulin resistance all feeding into this. And again you might say that these were successes of a society, but now we're starting to pay the price. So, that will be I think the greatest challenge and we're just on the edge of being able to have really effective treatments for obesity. I mean we have surgery and have about four medications that all work okay, at least for the short term, but they come with liabilities. We're a long ways away from being able to reverse those trends.

### *We've talked a lot of diabetes and obesity, but do you think the techniques you're using could be amenable to any kind of metabolic disorder?*

Every cell has metabolism, right? A metabolic disorder is not the primary cause of many diseases, but the response may be. If you have an immunologic response to something, you have immuno-metabolism that is going to impact that disease state. If you have a cancer, you have cancer metabolism that is going to be either the same or different than the host. Those are real clear connections, but then also you have these unexpected side effects of treatments that you may not appreciate until you have a broader view of the whole metabolic traffic pattern – metaphorically the "Google Maps" of what's going on in the organ or tissue. It may be those off-target effects that really limit your ability to go in and help someone out. I think the possibilities are extensive – it even goes to drug screening. If you have a thousand compounds, you want to choose the ten that may not have the best binding characteristics but the ones that have the most on-target effects with limited off-target liabilities. If you think of drugs that are for long-term treatment like cardiovascular disease or diabetes, the biggest toxicity you have to worry about is cardiotoxicity typically related to mitochondrial metabolism. If you can measure that then you are in a much better place. I think there are lots of possibilities – again, that's why we have started a company to try to make this much more widely available and higher throughput to be able to really broaden our impact.

### *Do you have one big goal you'd like to reach by the end of your career?*

I'm really eager to see if we can push this particular therapeutic, because I think a lot of the diabetes drugs that are out there are just treating the symptoms, they're not helping the root cause of the failure – in type II diabetes in particular. There's so many people who are really suffering from this disease and if we have a new way of going out there and solving that problem for them it would really be exciting. The whole idea of starting with a rare disease and translating that into a therapy of a human disease is the physician-scientist's dream. So that's where I'd really like to go and along the way we're making tools that may be helpful for other processes, but I'd like to make an impact on health.

### *Any other last things you want our readers to know about you or your lab?*

Just that it's the extreme curiosity that is driving all of this. When things don't make sense, there's often an explanation that if you dig down into far enough, things will start to make sense and that's what's been really fun about this exploration into metabolism. We had a view that when we came into it was 180 degrees in the opposite direction of what was actually going on and it wasn't until we started trying to understand that, that we started making lots of interesting discoveries about how metabolism was working itself. As we progressed it really coalesced into a believable working model. Pursue that curiosity and make sure you're satisfied with the questions that are in your mind and don't just accept something because it's published or someone told you. Our lab is always very interested in attracting the most creative curious minds who don't accept the status quo as the final word.

## REFERENCES

1. WHO. Global Report on Diabetes. 2016. Available from: www.who.int/diabetes/global-report/en/