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Edward Yu, et al.

Association of Tryptophan Metabolites with Incident Type 2 Diabetes in the PREDIMED Trial: A Case–Cohort Study.

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Guest: Edward Yu is a doctoral candidate at the Harvard T.H. Chan School of Public Health in the Departments of Nutrition and Epidemiology.

Bob Barrett:

This is a podcast from *Clinical Chemistry*, sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I am Bob Barrett.

According to the World Health Organization, type 2 diabetes comprises the majority of the more than 400 million people with diabetes around the world. Type 2 diabetes occurs when the body becomes resistant to insulin or it doesn't make enough insulin, largely because of excess body weight and lack of physical activity.

In efforts to better understand the disease, metabolomics measurements have identified tryptophan metabolites as potential biological mediators in the development of type 2 diabetes. There is a need to further investigate these findings in larger studies, like that possible when sampling subjects enrolled in the PREDIMED trial.

The PREDIMED trial was a multi-center trial of Mediterranean-style diets where the primary prevention of cardiovascular events that also included type 2 diabetes as a secondary end point. An original research article published in the August 2018 issue of *Clinical Chemistry* studies whether tryptophan metabolites in PREDIMED participants were associated with development of type 2 diabetes and insulin resistance. The authors also examined the effects of the Mediterranean diet on unfavorable metabolite profiles.

We are joined in this podcast by Edward Yu, who is the lead author. Edward Yu is a doctoral candidate at the Harvard T.H. Chan School of Public Health in the Departments of Nutrition and Epidemiology. His interests lie in the intersection of diet, lifestyle, metabolites, and cardio metabolic disease. And the first question is, what is the tryptophan kynurenone pathway and why did you decide to study this pathway?

Edward Yu:

So, tryptophan is an essential amino acid. It's an important protein building block. If it's not used in protein synthesis about 5% of tryptophan is used in melatonin and serotonin synthesis, the rest of it, 95% of it, ends up going through

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this pathway, tryptophan kynurenine. So, it's important to know that in the first and late limiting step, tryptophan is converted to kynurenine by an enzyme called indoleamine 2 3-dioxygenase or IDO.

And eventually, kynurenine can be converted into other downstream metabolites like the one we're going to study. Kynurenic acid, fulminic acid, 3-hydroxyanthranilic acid, and eventually to NAD, which is an important coenzyme, pretty much every cell. So the point of this pathway is to convert tryptophan to NAD.

Not much is known about this pathway in type 2 diabetes pathophysiology, so a few small-scale studies have suggested that these intermediates are involved in insulin resistance. So, our aim is to look at whether these five metabolites that I mentioned were related to type 2 diabetes in a longitudinal setting and with insulin resistance and we used data from a large randomized trial to conduct this study.

Bob Barrett: So, what were the key findings of that study?

Edward Yu: So, when we looked at just baseline metabolites, we found that baseline tryptophan was positively associated with incident type 2 diabetes. Now, it's important to differentiate between baseline and one year change. When you look at one year change, we found that changes in quinolinic acid are positively associated with diabetes.

So these findings were mostly confirmed in our analysis for insulin resistance. Most importantly is that the baseline ratio of kynurenine to tryptophan which is a good proxy for IDO activity and just where the equilibrium lies in this pathway, was significantly decreased in relation to insulin resistance at baseline, but that one year change it was increased with the relation to insulin resistance.

Bob Barrett: Doctor, in an earlier publication, you reported that one year changes in tryptophan were inversely associated with myocardial infarction. Do these results contradict each other?

Edward Yu: So, this is an excellent question. So yes, we published earlier last year that one year changes in tryptophan were inversely associated with later cardiovascular disease, specifically myocardial infarction or heart attack. So this implies that those who had the biggest increases in tryptophan or the least degradation had the lower heart attack risk.

At the same time, in this study, those with high tryptophan at a single point in time had higher type 2 diabetes risk,

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implying that high tryptophan levels are bad, right? So, how could tryptophan be good for cardiovascular disease and bad for type 2 diabetes?

So, actually I think that these results fit together quite nicely. And again, it's important to distinguish between baseline and one-year changes. So, if we look at the insulin resistance data, we see that before diabetes diagnoses and pre-diabetes, high tryptophan and IDO activity defined by the ratio of kynureanine to tryptophan predicted significant positive one-year changes in insulin resistance.

However, when we looked at the one-year change in these metabolites, we found that the biggest increases in downstream metabolites in high kynureanine to tryptophan ratio directly predicted changes in insulin resistance, so that's a little confusing. But what this is telling us is that the equilibrium of this pathway, the tryptophan kynureanine pathway, lies upstream when IDO activity is low at early diabetes onset. And then, as you progress in diabetes severity, it shifts downstream. So tryptophan has been depleted and IDO activity goes up.

And if we look at other studies of tryptophan in type 2 diabetes, we know that every single study of baseline tryptophan in incident type 2 diabetes report a positive association, including ours. But if we look at cross-sectional studies, so case control studies, of tryptophan among patients with prevalent type 2 diabetes versus controlled, we see that tryptophan is either neutral or negatively associated with the condition. We also see this kind of initial compensatory increase and then later decrease with things like beta-cell mass, insulin secretion, and kidney function, which this gives us even more pre-instance explanation.

And so, I think this is a very powerful interpretation and this has precedence in other physiological processes related to diabetes, and this explains both the seemingly contradictory cardiovascular in type 2 diabetes data as well as conflicting longitudinal and cross-sectional findings.

Bob Barrett:

Well finally, what are the clinical implications of this? For example, could interventions that modulate tryptophan availability be used for primary prevention of cardio metabolic diseases?

Edward Yu:

Well, it's a little bit too early to make causal conclusions about whether tryptophan related therapies could be used to alleviate this risk. For example, we know that IDO, the rate limiting enzymes activated by systemic inflammation, which underlies both cardiovascular disease and type 2 diabetes

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etiology, so it may turn out that these changes simply reflect things like obesity.

However, it has been hypothesized that downstream metabolites of tryptophan pathways like kynurenic acid and xanthurenic acid may play a role in blunting influence sensitivities. This was done in small scale studies. So it would be a big finding if this were really shown to be the case because it would imply that we could pharmacologically inhibit IDO activity to help prevent worsening diabetic states.

One last nuance is that, I mentioned earlier that about 5% of tryptophan is used to synthesize the neurotransmitter serotonin and then the hormone melatonin, which regulates mood and sleeping patterns. An imbalance of these molecules can lead to mood and sleeping disorders. We also know that type 2 diabetes, depression, and sleep disorders are all comorbid conditions. So, they occur together.

So an interesting question we can pose is, could depletion of tryptophan via the IDO activation, be the link between these comorbidities? So, you know that's just a hypothesis, we don't have an answer to that, but it's certainly an interesting thing to look at in the near future. Overall I think that tryptophan metabolism is largely understudied in cardio metabolic diseases and I think we will see exciting developments in the future.

Bob Barrett:

Edward Yu is a doctoral candidate at the Harvard T.H. Chan School of Public Health. He has been our guest in this podcast from *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening.