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The Vitamin D Metabolite Ratio Is Independent of Vitamin D Binding Protein Concentration

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Guest: Dr. Charles Ginsburg, nephrologist and an assistant professor at the University of California San Diego.

Bob Barrett: This is a podcast from *Clinical Chemistry* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I'm Bob Barrett. The past dozen or so years have seen dramatic increase in vitamin D testing and research. Predominant in testing is for the 25 hydroxyvitamin D form, but it may actually be a poor marker of vitamin D status. That's because most of the 25 hydroxyvitamin D is not bioavailable and is down to the vitamin D binding protein. It has been shown that there is substantial variability in serum vitamin D binding protein concentrations among individuals and that there are common genetic variants in vitamin D binding protein that may lead to different binding affinity.

So, bioavailability of 25 hydroxyvitamin D varies depending on its concentration, the concentration of its binding protein and the binding protein phenotype, the so-called vitamin D metabolite ratio is a marker of vitamin D status that has been hypothesized to be independent of variability and binding protein but has not been directly evaluated. However, a paper appearing in the February 2021 issue of *Clinical Chemistry* helps address this very question. The lead author for that study is Dr. Charles Ginsburg, he is a nephrologist and an assistant professor at the University of California San Diego. His research focuses on vitamin D metabolism and biomarkers of bone health and he is our guest in this podcast. So, first of all, Dr. Ginsberg, what inspired you to pursue research in vitamin D and bone health?

Charles Ginsburg: So, my background is in nephrology and I've been studying how kidney disease affects bone health for a long time now and what got me interested in vitamin D and bone health is I realized kind of early in my training that patients with kidney disease have really high fracture rates and we're often being prescribed vitamin D for having low vitamin D levels and when I think back to my renal physiology, I think about 25 hydroxyvitamin D is the biomarker we currently use to assess vitamin D status. But patients with kidney disease have a specific abnormality in vitamin D metabolism and that is that they don't activate vitamin D to the active hormone calcitriol. So, I kind of looked at this problem of we're measuring an inactive compound in patients with kidney disease that can't

activate it and so I was left to wonder if this was really the best way to assess vitamin D status in this population and that's kind of what led me to the projects that I'm currently working on.

Bob Barrett: And so that we get all of our listeners on the same page: What exactly is vitamin D metabolite ratio and what is your interest in that?

Charles Ginsburg: Sure. So, the vitamin D metabolite ratio is essentially a way to get at vitamin D status in kind of a different approach. So, vitamin D or the 25 vitamin D that we all ingest and can get from the sunlight it can be activated into active hormone called calcitriol and it's done primarily by the kidney which can be dysregulated in patients with kidney disease. The vitamin D metabolite ratio looks at an alternative pathway in vitamin D, which is basically 25 vitamin D if you want to get rid of it instead of activating it, you make 24,25 vitamin D. So, this ratio is the ratio of this garbage product 24,25 vitamin D to the regular 25 vitamin D that we usually measure and the reason I'm interested in this quote-unquote garbage product is because your body has a feedback system where it knows when it has enough vitamin D around and when it has enough, it starts sending extra vitamin D to the garbage, which kind of makes sense, right? If you have too much you could have too much or too little of something and your kidney knows when it -- or let's say your body knows when it has too much vitamin D and it starts making this 24,25 vitamin D garbage product. And so looking at this ratio kind of tells you, A, how much of your vitamin D is your body kind of getting rid of which can indicate potentially if you've got enough or not. But it also tells us something else that's interesting. So, vitamin D levels are highly variable dependent on how much of a certain protein is in the body called vitamin D binding protein, and that amount of protein is highly variable between people. It's also possibly different between different racial groups. And so, the issue -- one of the issues in measuring vitamin D is it may not be reflective of actual vitamin D status, it may just be reflective on how much did this protein you have. And so this may alter the way we interpret this vitamin D level. When you look at the vitamin D ratio, it's a fraction with a numerator and denominator and vitamin D binding protein affects -- we thought affects the numerator and the denominator equally. Which would mean if you do kind of go back to your basic algebra, if something is equally in the numerator and denominator of a fraction, maybe it cancels out. So, our hypothesis was that this ratio isn't affected by vitamin D binding protein because it affects the numerator and the denominator of the ratio equally and therefore, this marker of vitamin D status may be the first marker we can use which is not affected by this protein concentration, which we know is going to be very different between different people.

Bob Barrett: Doctor, can you tell us about your findings in the study just published in *Clinical Chemistry*?

Charles Ginsburg: Yeah. So, what we did in this study is as we looked at a cohort of elderly individuals living in the community to participate in a study called the Healthy Aging and Body Composition study (or Health ABC for short). And in this group of individuals, we had already had measurements of all the vitamin D metabolites including the ones that make up this vitamin D metabolite ratio. We added on was essentially measurements of vitamin D binding protein, and that way we could look at the relationship of each metabolite with vitamin D binding protein and then look at the ratio and its relationship with vitamin D binding protein. Our hypothesis was people with a high level of vitamin D binding protein for whatever reason, typically it's related to genetics or maybe age. We hypothesize that people with high binding protein levels would have high vitamin D levels (because one is so directly correlated with the other), but its ratio would not be affected. So, we looked at that and what we found was essentially, if you double your -- or if you had twice as high of a protein concentration, then your 25 vitamin D level, your kind of your standard biomarker, went up by 70%. Kind of proving what we had hypothesize that your 25D level is mostly related to not necessarily bone health or how much vitamin D you take, but it's really related to how much of this protein you make. And when we looked at the ratio, we found that the ratio had actually no relationship with vitamin D binding protein. The implication of that is that this ratio gets around vitamin D binding protein again because it's in the numerator and denominator, it canceled out and we found it was independent of it. And so, what we've shown is in this older population that this vitamin D metabolite ratio or VMR is a marker of vitamin D status that is independent of vitamin D binding protein concentration, and we've already shown in prior studies that the VMR may be a better predictor of things like fracture, kidney disease and death than traditional vitamin D markers. So, we've now been able to show that this ratio may be a better biomarker of vitamin D status with respect to looking at outcomes and now we're getting at why it might be a better biomarker is because it's independent of variability in vitamin D binding protein.

Bob Barrett: So, how will this research help clinicians in managing patients at risk for osteoporosis and fractures?

Charles Ginsburg: So, I think for people who have been paying attention to the kind of latest vitamin D trials, there have been a tremendous amount of trials coming out showing lack of benefits of vitamin D supplementation. Most recently there was a big one called the vital study which gave a large number of people

Vitamin D supplements and it didn't seem to improve things like fracture or death, which begs the question of are we -- are we accurately identifying patients who need vitamin D or not? You know, it's important question because we spend billions of dollars in this country annually on measuring vitamin D, on supplementing vitamin D and it's not clear that we're really getting at the patients that need it the most. Now, this has led some to say that vitamin D deficiency doesn't exist in the United States. That's hard to say for sure one way or another, but we know vitamin D deficiency exists as an entity, you know Ricketts exists, we've seen it, we know it's real. We just -- I think need to get better at identifying who might need vitamin D and I think a lot of these trials fail because we're using biomarkers that don't adequately identify the patients that might need supplementation this vitamin D metabolite ratio or VMR seems to be a better marker for important clinical outcomes like fracture and death. However, it has not been studied as a useful target in interventional trials. So, while I don't think that we can yet tell clinicians measure the vitamin D metabolite ratio and try to treat it by supplementing vitamin D levels when it's low, I think what we can do is start building clinical trials where that is a therapeutic target and that will validate whether we can use this marker to identify the people who really need Vitamin D supplementation instead of just giving it out to everybody or just using a biomarker that may be deeply flawed as I believe the current biomarker 25 hydroxyvitamin D.

Bob Barrett: Well finally doctor looking ahead, what are the next steps that you feel will help build on these findings?

Charles Ginsburg: So, looking ahead. I really think the next step at this point is clinical trials. Looking at the vitamin D metabolites ratios of therapeutic target. We and others have already shown that you can increase this ratio with certain medications including vitamin D supplements, but what you find is in patients who already have higher 25D levels that when you supplement, they generally are diverting that supplementation to what I've called the garbage product 24,25D instead of activating it because the body and the kidney kind of know you don't need more supplement and sometimes this even happens to people with lower 25D levels because maybe their lower 25D level doesn't necessarily reflect true quote unquote inadequacy. So, I think since we know we can affect this ratio, we know how to target it and we know this ratio is a really strong predictor of important clinical outcomes. A clinical trial where we use either standard vitamin D supplementation or calcitriol supplementation with the goal of getting to certain VMR targets and then following those patients to look for changes in bone density and fractures and things like that is really going to be I think the last or the next and final step in determining: should we use this as an alternative therapeutic target and when we try to label patients as vitamin D deficient

or not vitamin D deficient? Should we use the VMR instead of 25 vitamin D as we're currently doing?

Bob Barrett: That was Dr. Charles Ginsburg a nephrologist and an assistant professor at the University of California San Diego. He has been our guest in this podcast on the vitamin D metabolites ratio. He is a co-author of a study showing that the ratio is independent of vitamin D binding protein concentration that paper appears in the February 2021 issue of *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening.