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A. Berg et al.

24,25-Dihydroxyvitamin D3 and Vitamin D Status of Community-Dwelling Black and White Americans.

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Guest:

Dr. Anders Berg is Assistant Director of Clinical Chemistry at Beth Israel Deaconess Medical Center in Boston, and an Assistant Professor of Pathology at the Harvard Medical School.

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.

Vitamin D insufficiency has been widely associated with numerous negative health outcomes, including higher all-cause mortality, although no mechanism has been formally established for such observations.

But are researchers in laboratories assessing the correct forms of vitamin D in such studies? Most commonly, 25-hydroxy-vitamin D is measured but the implications of having low serum concentrations of this form of vitamin D maybe ambiguous. For example, African-Americans have lower serum concentrations of 25-hydroxy-vitamin D than whites, yet they also have paradoxically higher bone mineral density and a lower risk of osteoporosis and fragility fractures than do whites.

In the June 2015 issue of *Clinical Chemistry*, a paper examines vitamin D status in community-dwelling black and white Americans to further study this matter. The lead author of that paper is Dr. Anders Berg, Assistant Director of Clinical Chemistry at Beth Israel Deaconess Medical Center in Boston, and an Assistant Professor of Pathology at the Harvard Medical School. He joins us in this podcast.

Dr. Berg, your paper in *Clinical Chemistry* is on the topic of serum 24, 25-dihydroxy-vitamin D concentrations measured in black and white Americans. Can you give our listeners some background on why you are interested in investigating racial differences in vitamin D?

Dr. Anders Berg:

Yes, demand for 25-dihydroxy-vitamin D testing, which I will refer here simply as 25-D, has grown exponentially over the past decade. This is largely due to a wide array of studies that suggest that vitamin D deficiency is prevalent throughout the US and may be associated with a wide variety of diseases such as bone disorders and cardiovascular disease.

Based upon data from the 2006 National Health and Nutrition Examination Survey, it was estimated that 82% of blacks in the US had 25-dihydroxy-vitamin D levels below 20 ng/mL, which is the threshold at which the Institute of Medicine used to define vitamin D deficiency.

It has been known for decades that blacks have lower concentrations of 25-D compared to whites. The controversy surrounding these numbers, however, is that despite the fact that the majority of blacks meet the criteria for vitamin D deficiency, this population has a significantly lower risk of osteoporosis and bone fracture.

Bob Barrett: How do you reconcile this paradox between lower vitamin D, yet lower risk of bone fracture and osteoporosis?

Dr. Anders Berg: My collaborators and I believe that this may be explained by the free hormone hypothesis. Similar to thyroid hormone, the majority of circulating 25-D is tightly bound to a plasma of binding protein, the vitamin D binding protein. This high intensity of the binding interaction has been shown to inhibit the bioavailability of 25-D and analogous to free thyroid hormone, we believe that concentrations of bioavailable 25-D maybe a better indicator of vitamin D sufficiency than are total concentrations of 25-D.

In 2013 we published a study that found that concentrations of vitamin D binding protein were significantly lower in US blacks and that because of this, concentrations of bioavailable vitamin D may actually be equivalent between blacks and whites, despite their significant differences in concentrations of total 25-D.

Bob Barrett: If the differences in bioavailable vitamin D are the explanation for why blacks have lower total vitamin D levels, why did you decide to specifically study differences in 24, 25-dihydroxy form of vitamin D?

Dr. Anders Berg: Analytical issues surrounding the calculated method that we use for estimating bioavailable 25-D make it incompatible with clinical use; instead a direct assay for bioavailable vitamin D is urgently needed, and although we are actively pursuing development of a direct assay, we also decided to look for other alternative indicators of vitamin D adequacy, to test for differences in vitamin D adequacy between blacks and whites.

When considering this question we came across a number of recent papers on a vitamin D metabolite, 24, 25-dihydroxy-vitamin D, which I will refer to simply as 24, 25-D. The most abundant form of vitamin D in its circulation is 25-hydroxy-

vitamin D which is converted into the kidney by 1-hydroxylase to its active form 1, 25-dihydroxyvitamin D.

Not all 25-D is converted to its active form, however, and when 25-D is present in excess it may instead be metabolized by 24-hydroxylase enzyme which converts into 24, 25-D. 24, 25-D is not believed to be physiologically active, but it's instead an intermediate in metabolism and excretion.

Although 24, 25-D is not active, the reason that there is a growing interest in measurement of this metabolite is because of evidence that concentrations of 24, 25-D and the ratio of 24, 25-D compared to 25-D may be alternative indicators of vitamin D adequacy.

First of all, concentrations of 24, 25-D are correlated with 25-D. This makes sense biochemically considering that synthesis of 24, 25-D is kinetically proportional to its precursor 25-D.

Bob Barrett: Well since as you say concentrations of the 24, 25-dihydroxyvitamin D are so strongly correlated with 25-hydroxy-vitamin D, aren't they merely redundant measures of vitamin D status? Does measurement of this metabolite provide us with any new information?

Dr. Anders Berg: Although concentrations of these two metabolites are usually correlated, the ratio, which we referred to in the paper as the vitamin D metabolite ratio, is not a constant, and there is evidence that the ratio may be decreased in vitamin D deficiency.

Expression of the 24 hydroxylase has been shown to be controlled by both parathyroid hormone signaling, and vitamin D receptor signaling, suggesting that conversion of 25 D to 24, 25 D is an actively regulated process. This means that in patients with functional vitamin D deficiency, 24 hydroxylase should be down-regulated, and the vitamin D metabolite ratio will be disproportionately decreased.

Bob Barrett: Your previous work suggests that serum vitamin D binding protein influences concentrations of 25-hydroxy-vitamin D and may complicate the interpretation of 25-hydroxy-vitamin D measurements. Would you predict that the effects of vitamin D binding protein would also confound use of 24, 25-dihydroxyvitamin as a biomarker?

Dr. Anders Berg: The majority of both 24, 25 D and 25 D are tightly bound to vitamin D binding protein in the circulation. We would predict therefore that the effects of vitamin D binding protein will influence the concentrations of both metabolites. But this should be balanced and thus it will not change the

vitamin D metabolite ratio. This characteristic is also part of the reason we chose to investigate the vitamin D metabolite ratio, because in theory it should solve the confounding effects of vitamin D binding protein on 25 D concentrations since the ratio should not be influenced by race, genetics or individual differences in concentrations of vitamin D binding protein.

Bob Barrett: Let's talk about some of the findings during the new study that was published in the June 2015 issue of *Clinical Chemistry*.

Dr. Anders Berg: In this new study we measured serum 24, 25-dihydroxy-vitamin D3 and 25-hydroxy-vitamin D3 by mass spectrometry in a random cohort of healthy US adults in order to compare concentrations of these metabolites and their ratio between blacks and whites. Similar to previous reports, we found that concentrations of the two metabolites were generally correlated. As predicted, blacks had lower concentrations of both 25 D and 24, 25 D compared to whites. Although blacks had lower concentrations of the metabolites compared to whites, the vitamin D metabolite ratios were similar in both races.

Furthermore, vitamin D metabolite ratios were correlated with parathyroid hormone concentrations, in both blacks and whites. This last observation was particularly significant to the study because it demonstrated an independent association between vitamin D metabolite ratios and a physiologic indicator of vitamin D sufficiency. The fact that vitamin D metabolite ratios did not differ between blacks and whites also corroborated our suggestion that blacks may not actually be suffering from vitamin D deficiency.

Bob Barrett: What other studies support the association between vitamin D metabolite ratios and vitamin D deficiency?

Dr. Anders Berg: Our study is just the most recent paper to support the hypothesis that the vitamin D metabolite ratio may be an independent indicator of vitamin D sufficiency.

In 2011 Reinhold Vieth and colleagues measured vitamin D metabolites in healthy patients during and after six weeks of treatment with vitamin D supplements. They observed that concentrations of both 25 D and 24, 25 D rose in response to vitamin D supplementation. But more interestingly, they found that the subjects' vitamin D metabolite ratios before treatment, were strongly correlated to their response to supplements. In other words, the patients with lower vitamin D metabolite ratios at baseline increase their 25 D levels more when given supplements suggesting that a low metabolite ratio is a predictor of responsiveness and predictor of true vitamin D deficiency.

In 2012 and 2014 Ian DeBeer and colleagues published studies of measurements of 24, 25 D in patients with chronic kidney disease. This patient population suffers from functional vitamin D deficiency because their kidney disease prevents them from converting 25-hydroxy-vitamin D to the active 1, 25-dihydroxy form.

They observe that concentrations of 24, 25 D and the vitamin D metabolite ratio decreased in association with worsening kidney function. Even more interestingly, they found that concentrations of 24, 25 D were more strongly correlated with plasma parathyroid hormone concentrations than were the concentrations of 25 D or 1, 25-dihydroxy-vitamin D.

Then in 2014, Glenfield Jones and others have published a large study of healthy white postmenopausal women, which observed that 24, 25 D concentrations drop precipitously in women with 25 D concentration that's below 20 ng/mL. This suggested down regulation of 24, 25 D synthesis when 25 D levels are deficient.

And then finally, in 2014 Jason Stubbs and colleagues published a clinical trial of vitamin D supplement therapy in subjects with chronic disease and in healthy controls.

They observed that initial 24, 25 D concentrations and vitamin D metabolite ratios were disproportionately lower in chronic kidney disease patients compared to controls.

And after vitamin D supplementation, the chronic kidney disease patients did not increase their 24, 25 D level or vitamin metabolite ratios like the healthy control subjects did. Together, these studies all corroborated that functional deficiency of vitamin D signaling results in lower vitamin D metabolite ratios and suggest that this may be a clinically meaningful indicator of vitamin D deficiency.

Bob Barrett: Can 24, 25-dihydroxy-vitamin D be measured for clinical purposes, and well, should it be?

Dr. Anders Berg: Currently the only way to measure a 24, 25- dihydroxy-vitamin D, or the vitamin D metabolite ratio, is by mass spectrometry. And although commercial calibrators and isotopic standards for these analytes are available, measurement so far have been done only for research purposes. Additional larger studies correlating concentrations of this metabolite with clinical outcomes, as well as randomized control trials testing whether it can predict who will benefit from vitamin D supplementation therapy, are needed before anyone should use this new biomarker for clinical diagnostic purposes.

Bob Barrett: Finally doctor, what conclusions have you reached based on your work and the work of other related studies?

Dr. Anders Berg: Together our studies and studies by other groups investigating the physiology of vitamin in blacks suggests that the field needs to reevaluate our current measurement of 25-hydroxy-vitamin D as an indicator of vitamin D sufficiency, and to reconsider how we use generic clinical cut-offs for interpretation of 25-hydroxy-vitamin D measurements.

As Ian Reid and others have suggested we should consider adaption of demographically specific reference intervals for interpretation of 25-hydroxy-vitamin D values. And we should be evaluating alternative biomarkers such as the vitamin D metabolite ratio, which are not confounded by the effects of race, genetics, or vitamin D binding protein concentrations.

Dr. Anders Berg: Dr. Anders Berg is Assistant Director of Clinical Chemistry at Beth Israel Deaconess Medical Center in Boston, and an Assistant Professor of Pathology at the Harvard Medical School. He has been our guest in this podcast from *Clinical Chemistry* on estimating vitamin D status.

I am Bob Barrett. Thanks for listening!