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This is the podcast from *Clinical Chemistry*. I'm Bob Barrett.

Vitamin D has recently become the shining star of the nutritional universe. For decades, vitamin D deficiency was thought a thing of the distant past. But recent evidence has associated low vitamin D levels with a number of diseases and conditions. A large number of papers were devoted to vitamin D at the 2008 meeting of the AACC in Washington and in April of that year the journal *Clinical Chemistry* published a perspective article entitled "Vitamin D Reinvented" by Dr. Graham Beastall. Dr. Beastall is the current President of the International Federation of Clinical Chemistry and Laboratory Medicine and serves as a part-time advisor to the UK Government's Department of Health.

Dr. Beastall, tell us, how is the vitamin D status of a person checked?

Dr. Graham Beastall:

Vitamin D is actually a family of fat-soluble compounds derived either from cholesterol or ergosterol, and we can get vitamin D from two sources. We get it from our diet, and we can make it when our skin is exposed to ultraviolet radiation found in sunlight. The vitamin D that we make ourselves and that which we get from animal food sources is vitamin D₃, or cholecalciferol, which is made from cholesterol. The vitamin D that we get from invertebrates and plants is vitamin D₂, which is made from ergosterol.

Now, both vitamin D₂ and vitamin D₃ are available in synthetic form; so we do see both of them in blood from human beings. Once absorbed into the body, the vitamin D is rapidly hydroxylated by the liver. As a liver enzyme, it produces 25-hydroxy vitamin D, and it is this 25-hydroxy vitamin D, which is transported in blood; it is bound to plasma proteins, and it is really the measurement of this plasma or serum 25-hydroxy vitamin D that represents a really good measure of the total vitamin D status of an individual.

Host:

So, why is there so much recent interest in the role of vitamin D in calcium metabolism and bone disease? We've known about this for years, right?

Dr. Graham Beastall:

Yeah, we have. We've known about vitamin D and calcium and bone material for a long time. Classically, the deficiency of vitamin D has been responsible for rickets in children and osteomalacia in older adults, and perhaps a causative factor in osteoporosis.

More recently, however, there have been some really fascinating epidemiological studies that have shown an association between latitude, which is taken as a surrogate measure for sunlight exposure, and a number of really quite common chronic diseases. This raises a sort of fascinating anthropological theory that as man has moved over many thousands of years from equatorial regions to more northerly climates, he has been exposed to less sunlight, and to compound this lack of sunlight exposure, of course, man has also covered up more of the skin with clothes in order to keep warm. And in northerly climates, of course, the skins become paler, which does make it more efficient in synthesizing vitamin D, but taken all together, there is a growing view that relative vitamin D deficiency could indeed be a causative factor in the development of these chronic diseases.

And of course there is an added irony here, which won't be lost on many people, and that is that because of the known association between excessive sunlight exposure and malignant melanoma, especially in pale-skinned peoples, there is now a popular view that we should actually protect even further our sensitive skin from the sun.

Host: Well, which conditions are now being associated with the relative deficiency of vitamin D?

Dr. Graham Beastall: The list is impressive, and it is growing. The strongest epidemiological evidence for these non-calcium-related effects of vitamin D actually comes from investigations of its role in protection against cancer. There have been many studies that have suggested that sunlight and, by presumption, vitamin D may have a protective effect against really quite a wide range of common cancers. These include breast cancer, prostate, ovary, and pancreatic cancer.

Other epidemiological studies have suggested that relative vitamin D deficiency may also be involved in some of, you know, the really big diseases, such as heart disease, type I diabetes, multiple sclerosis, rheumatoid arthritis, and asthma. And there is even some evidence that suggests increased risk in tuberculosis, pneumonia, poor cognitive function, periodontal disease, and reduced muscle tone.

So it's very wide-ranging, and collectively these conditions account for more than 60% of all deaths in the Western world. So it is hardly surprising that the popular media, as well as the scientific media, have

latched onto these findings and taken an interest in them.

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Host:

What's the possible scientific basis of this association?

Dr. Graham Beastall:

This is where it gets a little bit more difficult. The scientific basis for vitamin D as a protective agent against these chronic diseases and their development isn't terribly well-established, but as you can imagine, there is a lot of work going on at the moment, and there is certainly some growing evidence.

To give you just two perhaps illustrations, quite a few of the diseases that I listed have an immunological basis, and certainly vitamin D is known to modulate autoimmunity. And then when you look at cancer and the anticancer effect could be due to the activation of vitamin D receptors on some key target cells, and this of course is a sort of cellular differentiation and so inhibits proliferation of tumor cells, invasiveness, angiogenesis, and metastatic potential. So lots of ideas, but still a lot of work to do.

Host:

Well, in your opinion, how do we know that the causative factor is vitamin D and not some other component of sunlight?

Dr. Graham Beastall:

Yeah. I mean, this is clearly a key point. The evidence really comes from vitamin D supplementation studies. In other words, giving people vitamin D, does this actually reproduce the protective effect? Many of these supplementation studies have been reported, but actually they're quite difficult to understand because studies have used different dose regimes and different populations, looking at different ranges of disease conditions. So individual studies are difficult to interpret, and we need to rely on high-level evidence such as we might get from meta-analysis.

Just to give you one example, in one meta-analysis of 18 different randomized controlled supplementation trials, there was a 7% reduction in total mortality from any cause for patients taking vitamin D compared to controls, and the interesting thing here is that in these studies most of the patients were taking really very modest supplements of vitamin D in the sort of level of 400 to 800 International Units per day.

So there is clearly a need to try and achieve better standardization in these supplementation studies, and there have been some recent recommendations that suggest that a minimum daily dose of at least 1000 International Units should be used each day. In fact,

vitamin D is pretty safe as a replacement drug, and you'd need very much higher levels than this before you risked vitamin D toxicity.

Host:

Now, what does this mean in terms of our understanding of a normal range for 25-hydroxy vitamin D in plasma?

Dr. Graham Beastall:

Classically, the normal or, probably better, reference range for any biomarker, we derive that by measuring the concentration of it in a large number of disease-free subjects, who we define in terms of gender and age and ethnicity and a number of other factors, and then you do some statistics on it and usually you include the middle 95% of results.

Using this definition of a reference range for vitamin D, then most studies conducted in the Western world would suggest that vitamin D deficiency occurs when the serum 25-hydroxy concentration is below about 25 nanomoles per liter; that's 10 micrograms per liter. And this is the cutoff that is being used for management of vitamin D deficiency in relation to calcium metabolism and bone disease.

However, we now know that many of this so-called normal population are at risk of developing the chronic diseases in later life. Furthermore, that supplementation studies that I referred to suggest that the maximum protective effect of vitamin D comes when the serum 25-hydroxy concentration exceeds 75 nanomoles per liter, or 30 micrograms per liter. This figure is in the upper half of the reference range for most northern populations, so it rather begs the interesting question as to whether half the population in northern climates is suffering from relative vitamin D deficiency. There is no clear answer to that at the moment, but at this stage we should probably try and distinguish between a reference range derived in the classical way and perhaps target treatment levels in supplementation studies.

Host:

Okay. Well, with this in mind, what are the implications for clinical chemistry of this recent interest in vitamin D?

Dr. Graham Beastall:

Yeah, it's a good question, and from what I've already said, it's clear that clinical chemistry laboratory has a key role in helping us to understand this relative vitamin D deficiency and perhaps to provide some consistent and transferable results for the comparison of these supplementation studies.

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Unfortunately, the measurement of 25-hydroxy vitamin D in plasma or serum isn't easy. And we know from external quality-assessment schemes that there is considerable variability — I mean maybe typically 20% or so — between laboratories measuring the same sample. At present, most of the methods that we use for measuring 25-hydroxy vitamin D rely on immunoassay, but there is no international standard for calibration, and there are differences in antibody specificity between different methods.

One illustration perhaps is the marked differences that one method would have for measuring vitamin D₂ and vitamin D₃. Some methods measure one or the other; some methods measure both, and of course both may be present in plasma.

So there is now a strong trend to try and move away from immunoassay methods to more sophisticated liquid chromatography linked to tandem mass spectrometry. Such methods can certainly measure the 25-hydroxylated forms of both vitamin D₃ and D₂, and we can add the results together to produce a total serum 25-hydroxy vitamin D level. The problem is that these methods are technically demanding, they are difficult to automate, and the primary problem of calibration still remains.

So, I think perhaps there is a big role for clinical chemists working with the diagnostics industry to come up with quality standards and performance targets for the measurement of 25-hydroxy vitamin D, and that will help us, I think, over the next few years get to the bottom of this intriguing story a little quicker.

Host:

Dr. Graham Beastall is the current President of the International Federation of Clinical Chemistry and Laboratory Medicine and our guest in this podcast from *Clinical Chemistry*. Podcast listeners may be interested to know that Graham considers his greatest claim to fame to be that he was at school in Liverpool with Paul McCartney and George Harrison.

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