Clinical Chemistry

Circulating Human Hepcidin-25 Concentrations Display a Diurnal Rhythm, Increase with Prolonged Fasting, and Are Reduced by Growth Hormone Administration

Bob Barrett: This is the podcast from *Clinical Chemistry*. I am Bob Barrett. The discovery of peptide hepcidin-25 and the elucidation of its unique properties have substantially affected our thinking with regard to the treatment of both iron overload and anemias. In the August 2012 issue of *Clinical Chemistry*, Dr. Robert Konrad and coworkers from the U.S. and Sweden have found that hepcidin-25 concentrations in serum display much more dynamic and rapid variations than expected. Dr. Konrad is our guest in this podcast. Doctor, did the relatively large diurnal variation of hepcidin come as a surprise and what drives this diurnal variation?

Dr. Robert Konrad: So it did come as a bit of a surprise to us. Historically, if you look at what has been reported for iron levels, there has been reports of a diurnal variation not particularly strong but somewhat the opposite pattern of hepcidin. So we didn't quite know what to except when we performed these experiments. When we saw that the hepcidin diurnal variation was the opposite of what's been reported historically for serum iron, it made us wonder is it that hepcidin is driving the variation in the iron levels, or is it vice-versa, that there's something else that's controlling the variation in iron and that's feeding back somehow and the iron status is being sensed by the liver and that's driving the diurnal variation of hepcidin. So at this point, we don't know for sure what the mechanism is.

Bob Barrett: Well, during prolonged fasting, hepcidin concentrations increase dramatically. What's going on? What's responsible for that?

Dr. Robert Konrad: So this was a big surprise for us. When we originally did those experiments, we thought that under fasting conditions, the body would be starved for iron and as a result, it would try and decrease hepcidin levels to maximize the potential for any iron absorption. But as I often say, there is nothing like data to ruin a good hypothesis. In reality, it was the exact opposite as people went through a period of prolonged fasting, the hepcidin levels actually increased dramatically. And when we started to think about this data, what we realized is that probably the mechanism for this is that during a period of prolonged fasting, the body has no access to iron and it's forced to make a choice. And it has to essentially choose between either maintaining tissue iron levels or erythropoiesis to make more red blood cells.

So red blood cells have a relatively long life span, about 120 days or so. So the body, essentially, when it gets into a period of prolonged fasting, we believe what happens is the erythropoiesis is shut down in order to preserve tissue iron levels because you need tissue iron in order to have oxidative respiration, the mitochondria, and all sorts of

other really important cellular processes. So we think what happens is that the erythropoiesis gets shut down by the prolonged fasting and the decrease in erythropoiesis is sensed by a mechanism that we don't fully understand what it is, but somehow there is a mechanism in the bone marrow to sense that decreased erythropoiesis and that's relayed to the liver. And that, we believe, is responsible for increasing the hepcidin levels.

Bob Barrett: So after prolonged fasting, followed by re-feeding, hepcidin concentrations seemed to overcorrect and are actually lower than baseline. Is this overcorrection real and if so, what accounts for that?

Dr. Robert Konrad: Yes, we do believe it's real. It was certainly highly significant when we looked at the data. Again, unexpected, as is the best kind of data that you get. And what we believe happened is that during the prolonged fasting period, there was suppression of erythropoiesis. After the approximate three days of fasting, when iron becomes available in the diet again, the body is now actually, we believe, turns erythropoiesis back on and it's probably a compensatory increase in erythropoiesis to make up for having shut the erythropoiesis down for several days.

So again, by an unknown mechanism, the erythropoiesis that's going is sensed somehow by the liver and then the liver actually lowers the synthesis and secretion of hepcidin in order to be able to let more iron in, to be able to accommodate the compensatory increased erythropoiesis.

Bob Barrett: So what's the most likely mechanism for growth hormone administration to cause such marked decrease as in circulating hepcidin concentrations?

Dr. Robert Konrad: So there are many possibilities for this. Our leading hypothesis is that growth hormone directly stimulates erythropoiesis. So when we saw the growth hormone data, we were again a little bit surprised by them. After looking at what have been reported in the literature, it is fairly clear there is not a tremendous amount on it but there are, I think, some good papers out there, dating back to the '70s, showing that growth hormone actually stimulates erythropoiesis.

So we believe what happens is when the subjects were administered growth hormone over a period of a few weeks, that caused increased erythropoiesis in the bone marrow and again, by that same mechanism that we don't understand what exactly what it is that increased erythropoiesis is sensed by the liver and then the liver decreases the amount of hepcidin secreted in order to be

able to let the body absorb more iron to accommodate that increased erythropoiesis.

Bob Barrett: Doctor, what do the fasting data and growth hormone data tells us about the relationship between erythropoiesis and regulation of hepcidin synthesis and secretion?

Dr. Robert Konrad: So to us, what it clearly tells us is that erythropoiesis in the bone marrow is tightly linked to hepcidin synthesis and secretion in the liver. So under periods where erythropoiesis is increased, the liver somehow knows to secrete less hepcidin in order to get more iron on board. With erythropoiesis being decreased, just the opposite occurs. So clearly, I think, all the data together tell us that there's a very tight link here. Well, we're still trying to understand it. What we don't understand yet is what is the actual mechanism of that link, what is the nature of the signal by which the erythropoietic activity that's going on in the growth marrow is somehow sensed by the liver so that the liver is able to adjust how much hepcidin is synthesized and secreted.

Bob Barrett: Do we know that? Do we know what goes on in the bone marrow with regard to erythropoiesis to get sensed by the liver?

Dr. Robert Konrad: We have several hypotheses but they are all at a very early stage and we have no definitive data to know exactly what the link is.

Bob Barrett: Well, based on this data, what are the major implications as far as measuring hepcidin concentrations in the clinic?

Dr. Robert Konrad: I think from a practical standpoint, the large diurnal variation in hepcidin and certainly the variation with prolonged fasting means that when measuring hepcidin level in the clinic, it's important to try and control for that. So I think we would recommend, if possible, a fasting serum blood draw first thing in the morning. And that if you're going to compare hepcidin levels over time in the same patient, you would want to have a standardized blood draw process. That would be, I think, our overall recommendation. And then obviously, in addition to that, because of the important role that hepcidin is being shown to play in, essentially as the master regulator of iron metabolism, we increasingly believe that there will be a strong need for a clinical hepcidin assay to be part of an iron panel.

Bob Barrett: Dr. Robert Konrad is a researcher at Lilly Research Laboratories in Indianapolis. He has been our guest in this podcast from *Clinical Chemistry*. I am Bob Barrett. Thanks for listening.