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Hepcidin compared with prohepcidin: an absorbing story[1,2]

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The discovery by Nicolas et al in 2001 (1) that the liver-derived peptide hepcidin plays a central role in the regulation of body iron homeostasis was a major advance in the field, and the flurry of articles that followed has further defined the mechanism by which hepcidin regulates body iron levels. Hepcidin is synthesized in the liver as an 84–amino acid prepropeptide, which is subsequently processed into a 60- to 64-residue prohepcidin peptide and then finally into the mature and biologically active 25–amino acid hepcidin (hepcidin 25), which is secreted into the serum (2). The target for serum hepcidin is the iron exporter ferroportin 1, which is found on the plasma membrane of most body cells and notably at high concentrations on duodenal enterocytes, macrophages, and hepatocytes. The binding of hepcidin to ferroportin 1 induces the internalization and degradation of the iron exporter, thus decreasing cellular iron release. Thus, increased hepcidin production reduces serum iron concentrations because of decreased iron absorption and reduced iron release from macrophages and iron storage sites.

The physiologic importance of hepcidin is emphasized by the finding that inappropriately raised or lowered hepcidin concentrations can have significant clinical sequelae (2). For example, reduced hepcidin expression explains the excessive intestinal iron absorption that characterizes most inherited forms of iron loading (or hemochromatosis). Conversely, an increase in hepcidin production contributes to the anemia of inflammation, which is a condition that can affect the mortality and morbidity of many people with chronic disease. Because hepcidin can convey important information about pathologic states, the ability to measure hepcidin in either plasma or urine has considerable clinical application. But is it better to measure prohepcidin or hepcidin 25? And which is physiologically most relevant? Young et al (3) address these issues in their article in this issue of the Journal.

Despite its potential utility, attempts to develop a simple and robust assay to detect the mature 25–amino acid hepcidin peptide have proved to be problematic. Many studies have relied on the measurement of hepcidin mRNA concentrations in the liver, and although this correlates well with the concentration of mature hepcidin in the serum, hepatic biopsies are clinically indicated in only a limited number of situations. Several groups have developed mass spectrometry–based assays for hepcidin 25; however, these require access to specialized equipment and are not widely available (4, 5). An immunoassay is clearly the desired tool. Although antibodies to hepcidin 25 have proved to be difficult (although not impossible) to generate, prohepcidin is far more immunogenic and a prohepcidin enzyme-linked immunosorbent assay is available commercially. However, recent studies have suggested that the serum prohepcidin concentration correlates poorly with markers of iron homeostasis such as intestinal iron absorption (6, 7), and the relation between serum concentrations of prohepcidin and those of hepcidin 25 has not been investigated directly. Several groups have successfully generated antibodies to hepcidin 25 and have used these to develop assays for plasma and urinary hepcidin. Ganz et al (8) initially used their antibody to develop an assay based on immunoblotting but more recently have used it in a microtiter plate-based assay (9). Measurements obtained using both of these assays have suggested that mature hepcidin correlates much more strongly with iron homeostasis variables than does prohepcidin.

Young et al (3) make 2 important and much needed contributions to this area. First, they make a direct comparison between the concentrations of hepcidin 25 measured by using a recently developed competitive enzyme-linked immunoassay and the concentrations of prohepcidin detected by using the available commercial assay. They then took the important step of comparing these concentrations with the absorption of iron in the same subjects from food containing an ^{57}Fe ferrous sulfate tracer and from a single test dose of ferrous sulfate containing ^{58}Fe. Although the sample size was relatively small (only 18 women took part in the study), their results show that the mature form of hepcidin, but not of prohepcidin, correlates with iron absorption, confirming the suspicions of many researchers in the field that prohepcidin concentrations do not accurately reflect the concentration of the mature peptide and that the prohepcidin assay is not a useful tool in the study of iron absorption. They also extend previous animal studies (10) and confirm that hepcidin is a key regulator of intestinal iron absorption in humans.

A surprising observation made in this study (10) was that there was no correlation between serum hepcidin 25 concentrations and serum ferritin, soluble transferrin receptor, or hemoglobin concentrations, all commonly used markers of iron status. This could reflect the small sample size or a combination of sample size and the fact that the study cohort was fairly uniform and

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most subjects had normal iron stores. Further investigations are needed to extend the findings to subjects of varying iron status to confirm the relation between hepcidin and absorption. Nevertheless, the findings do suggest that hepcidin 25 is the most sensitive indicator of iron absorption and that hepcidin measurements have the potential to supplant the more traditional assays for measuring iron status. Because hepcidin concentrations are increased by inflammation, hepcidin's usefulness as a marker of iron status may be limited to noninflammatory conditions, although this is also a limitation with the most widely used tests for monitoring iron concentrations. As a marker of iron absorption, however, the hepcidin 25 concentration would appear to be reliable, irrespective of the multiple factors that alter its expression.

There has been extensive interest in the use of hepcidin as an iron status marker in recent years and the data presented in this study make it clear that clinicians, nutritionists, and researchers should concentrate on measuring hepcidin 25 concentrations for this purpose because prohepcidin measurements appear to be unreliable. Quantitative immunoassays are the measurement method of choice, but it is not yet possible to purchase an immunoassay kit for hepcidin 25. The immunoassay results reported by Young et al (3) were obtained from a company that offers an assay for hepcidin 25 measurements. Hopefully, it will not be long before such an assay is widely available for use in clinical chemistry and research laboratories. The central role played by hepcidin in the regulation of iron homeostasis makes the development of such a resource of vital importance.

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REFERENCES

1. Nicolas G, Bennoun M, Devaux I, et al. Lack of hepcidin gene expression and severe tissue iron overload in upstream stimulatory factor 2 (USF2) knockout mice. Proc Natl Acad Sci USA 2001;98:8780–5.
2. Kemna EH, Tjalsma H, Willems HL, Swinkels DW. Hepcidin: from discovery to differential diagnosis. Haematologica 2008;93:90–7.
3. Young MF, Glahn RP, Ariza-Nieto M, et al. Serum hepcidin is significantly associated with iron absorption from food and supplemental sources in healthy young women. Am J Clin Nutr 2009;89:533–8.
4. Murphy AT, Witcher DR, Luan P, Wroblewski VJ. Quantitation of hepcidin from human and mouse serum using liquid chromatography tandem mass spectrometry. Blood 2007;110:1048–54.
5. Kemna EH, Tjalsma H, Podust VN, Swinkels DW. Mass spectrometry-based hepcidin measurements in serum and urine: analytical aspects and clinical implications. Clin Chem 2007;53:620–8.
6. Hadley KB, Johnson LK, Hunt JR. Iron absorption by healthy women is not associated with either serum or urinary prohepcidin. Am J Clin Nutr 2006;84:150–5.
7. Roe MA, Spinks C, Heath AL, et al. Serum prohepcidin concentration: no association with iron absorption in healthy men; and no relationship with iron status in men carrying HFE mutations, hereditary haemochromatosis patients undergoing phlebotomy treatment, or pregnant women. Br J Nutr 2007;97:544–9.
8. Nemeth E, Rivera S, Gabayan V, et al. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. J Clin Invest 2004;113:1271–6.
9. Ganz T, Olbina G, Girelli D, Nemeth E, Westerman M. Immunoassay for human serum hepcidin. Blood 2008;112:4292–7.
10. Frazer DM, Wilkins SJ, Becker EM, et al. Hepcidin expression inversely correlates with the expression of duodenal iron transporters and iron absorption in rats. Gastroenterology 2002;123:835–44.