Hepatic Peroxisome Proliferator-Activated Receptor γ Coactivator 1α and Hepcidin Are Coregulated in Fasted/Refed States in Mice

To the Editor:

Hepcidin plays a central role in iron homeostasis and contributes to the pathogenesis of several disorders. Consequently, development of a clear understanding of hepcidin regulation in various pathophysiological conditions has been the subject of intensive research. Technical limitations, however, have limited the wide availability of assays for measuring this iron-regulatory hormone in human serum. Recently, Troutt et al. developed a specific and robust sandwich immunoassay for hepcidin-25 and measured it in the serum of healthy volunteers. They demonstrated that hepcidin-25 concentrations in the circulation (1) vary diurnally in its concentrations in the circulation (1). In particular, the authors demonstrated that hepcidin-25 concentrations were significantly increased after 3 days of fasting, an intriguing result considering the hyposideremia of hepcidin. The authors hypothesized that this increase in hepcidin could be caused by suppressed erythropoiesis to maintain tissue iron concentrations.

To investigate the molecular mechanisms responsible for hepcidin regulation by fasting, we fasted C57BL/6j mice for 24 h and then separated them into 2 groups. The first group was fasted for an additional 12-h period (“fasted” conditions); the second group was refed during the 12-h period with a high-carbohydrate diet (“refed” conditions). Interestingly, mouse hepcidin antimicrobial peptide 1 (hepcidin1)1 mRNA concentrations were increased 4.2-fold with fasting [mean (SD) relative concentration, 1 (0.1) for the refed mice (n = 6) vs 4.2 (0.1) for the fasted mice (n = 6); P < 0.0001], with a significant 25% decrease of plasma iron. These findings confirm the results of Troutt et al. and suggest that an increase in hepcidin-25 in human serum most likely reflects the direct upregulation of HAMP2 (hepcidin antimicrobial peptide) gene expression in response to fasting.

To determine whether this regulation could be explained by systemic and/or cell autonomous pathways, we studied mouse Hamp1 (hepcidin antimicrobial peptide 1) gene expression in isolated hepatocytes maintained under culture conditions that mimic the fasting/glucoregulatory state. To achieve these conditions, we deprived isolated mouse hepatocytes of serum for 16 h and then treated them for 8 h with dibutyryl-cAMP (to mimic the effect of increased glucagon when blood glucose concentrations are low). These gluconeogenic conditions per se induced Hamp1 gene expression 2-fold [mean relative concentration, 1 (0.1) for controls (n = 3) vs 2.1 (0.1) in dibutyryl-cAMP–treated hepatocytes (n = 3); P < 0.0001]. Given the ability of peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) to activate components of the fasting response in the liver, we investigated the role of this transcriptional factor by infecting mouse primary hepatocytes for 24 h at a multiplicity of infection of 25 with adenovirus producing either PGC-1α or green fluorescent protein as a control (2). Forced production of PGC-1α increased Hamp1 gene expression 2-fold [mean relative concentration, 1 (0.1) in control hepatocytes infected with green fluorescent protein–producing adenovirus (n = 3) vs 2.0 (0.1) in hepatocytes infected with PGC-1α–producing adenovirus (n = 3); P < 0.0001]. Also increased were glucose-6-phosphatase and phosphoenolpyruvate carboxykinase, 2 well-known PGC-1α targets involved in gluconeogenesis. In addition, we found a significant correlation between relative concentrations of PGC-1α and hepcidin1 in the livers of fasted/refed animals (Fig. 1).

Fig. 1. Correlation between the expression of the genes encoding hepatic hepcidin1 and PGC-1α (as normalized to expression of the gene encoding cyclophilin).

Measurements were made by quantitative reverse-transcription PCR of RNA isolated from the liver of fasted mice (n = 6) and refed mice (n = 6).

1 Nonstandard abbreviations: hepcidin1, mouse hepcidin antimicrobial peptide 1; PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1α; HNF4α, hepatocyte nuclear factor 4α; FOXO1, forkhead box 01 protein.

2 Genes: HAMP, hepcidin antimicrobial peptide; Mus musculus gene Hamp1, hepcidin antimicrobial peptide 1.
PGC-1α has recently emerged as an important regulator of several liver functions. In the hepatic response to fasting, PGC-1α has been shown to coordinate the induction of gluconeogenic enzymes by coactivating such liver-enriched transcription factors as hepatocyte nuclear factor 4α (HNF4α) and forkhead box O1 protein (FOXO1), as well as the glucocorticoid receptor (3). Whether these regulators are involved in the control of hepcidin gene expression by fasting awaits further investigations.

Our results suggest that activation of the expression of the gene encoding hepcidin during fasting could be due not only to systemic regulation (such as decreased erythropoiesis, as suggested by Troutt et al.) but also to PGC-1α–dependent cell autonomous activation. This increase of hepcidin1 by fasting can explain the results of Conrad et al. (4), who were the first to show that 5 days of starvation in rats led to decreased absorption of iron administered orally. This observed increase in hepcidin with fasting also is in agreement with the recent report by Papillard-Marechal et al. (5), who found increased serum hepcidin concentrations in patients with anorexia nervosa, the increased hepcidin concentrations in their study being independent of inflammation and iron overload.

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