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Dietary Iron, Circadian Clock, and Hepatic Gluconeogenesis

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Circadian rhythms in energy metabolism and behavior have been examined at the molecular level with the identification of key clock genes that synchronize the endogenous rhythms in metabolism to external cues (zeitgebers), primarily day–night cycle and activity. These rhythms are temporally orchestrated and rigorously regulated by the central oscillators located in the suprachiasmatic nuclei and are synchronized with peripheral cell autonomous clocks resident in individual tissues that regulate specific functions. Among the peripheral organs, the hepatic clock is also entrained by nutrients; major metabolic processes in the liver have been found to be under circadian control (1). Disruption of the hepatic clock by change in behavior or specific nutrients could lead to metabolic disorders, such as obesity and type 2 diabetes, by affecting specific metabolic functions (2,3).

In the current issue of *Diabetes*, Simcox et al. (4) have examined the impact of dietary iron on hepatic circadian rhythms of glucose production in mice and in isolated hepatoma cells. By administering different amounts of iron in the diet, they achieved hepatic iron levels within the range observed in healthy humans under usual variations in dietary iron and without iron toxicity. In a series of elegant studies, they show that higher iron intake, and consequently higher hepatic iron, was associated with the change in the circadian rhythm of glucose and gluconeogenesis mediated by the repression of key gluconeogenic enzymes, PEPCK and glucose-6-phosphatase (G6Pase). The effects of iron were mediated by increased oxidative stress, resulting in increased peroxisome proliferator–activated receptor γ coactivator 1α (PGC-1α) transcripts and protein levels; induction of aminolevulinic acid synthase 1 (ALAS1), the first enzyme for heme synthesis; and an increase in total heme and heme B in the liver. The mechanism of the repressive effect of iron was shown to be the requirement of heme for nuclear receptor subfamily 1 group d member 1 (Rev-Erbα) to bind nuclear receptor corepressor 1 (NCOR) to form a repressor complex without any change in its protein levels, instead of increasing hepatic heme by administering aminolevulinic acid, thus bypassing ALAS1 or by feeding heme repressed gluconeogenesis.

The study by Simcox et al. (4) underscores an important feature of chronobiology. The change in dietary iron did not perturb the periodicity of the chronome and it only impacted the amplitude of expression. The periodicity of expression of transferrin receptor, ferritin, PGC-1α, ALAS1, PEPCK, or G6Pase did not change and peaked at zeitgeber time (ZT) 10 and is likely related to activity and food intake. The sequence of peak expression of ALAS1 at ZT10, followed by heme concentration at ZT12 and of heme oxygenase 1 (HO1) at ~ZT18 ensures the tight regulation of cellular concentration of heme. High dietary iron was associated with higher peak expression of PGC-1α, ALAS1, HO1, and the total heme in the liver. The antioxidants (SOD1 and catalase) peaked at ZT6. These data point to the exquisite regulation of diurnal rhythm by central oscillators. As reported by the authors, changes in dietary iron did not affect total food intake or the feeding behavior. Disruption of these temporal relationships in circadian pattern by changing the time-restricted feeding or by clock gene *Bmal1* knockout has been shown to affect intermediary metabolism in mice (2,3). These observations are consistent with the concept that the peripheral clocks, although cell autonomous, are slave to them and are entrained and synchronized by the central clock. Changes in dietary pattern and quantity of nutrients differentially influence the peripheral clocks, with little impact on the central clock (5).

The current study shows unique interactions between heme, Rev-Erbα complex, and gluconeogenesis. While low heme concentrations released the repressive effect of Rev-Erbα on gluconeogenesis, high heme and greater occupancy of the gene promoter of PEPCK and G6Pase by the repressor complex did not result in greater suppression of gluconeogenesis compared with the high normal

COMMENTARY

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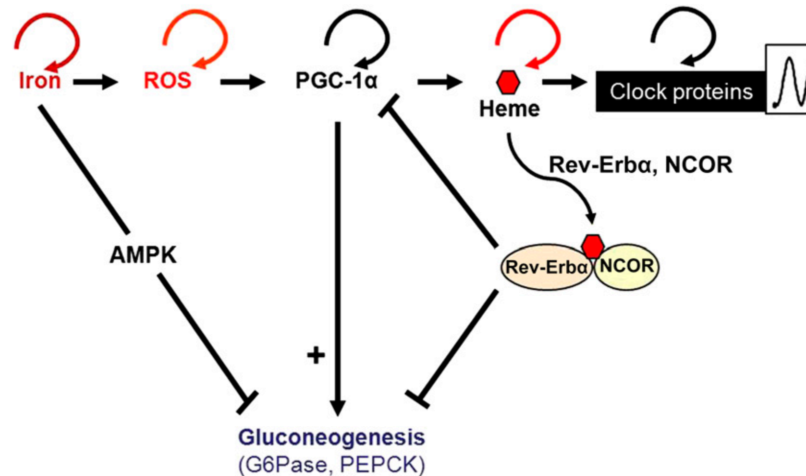

Figure 1—Schematic representation of the complex regulation of gluconeogenesis by changes in dietary iron and the self-regulated feedback loops that rigorously control the pathway. PGC-1α and AMPK are critical regulators of gluconeogenesis in the liver. As shown, dietary iron can suppress gluconeogenesis by activating AMPK and also can increase gluconeogenesis via increased expression of PGC-1α. Iron can also suppress gluconeogenesis by increasing heme, which by binding to Rev-Erbα recruits the NCOR to downregulate the genes for hepatic gluconeogenesis. The interaction of these and other interacting signaling pathways results in maintenance of hepatic gluconeogenesis within the physiological range throughout the day. ROS, reactive oxygen species.

dietary iron. Rev-Erbα, a nuclear receptor, plays a critical role in the regulation of metabolism as a potent transcriptional repressor of circadian behavior (6). Heme binding to Rev-Erbα recruits the NCOR to downregulate the genes for hepatic gluconeogenesis (7,8). Heme is bound to the receptor in its oxidized state as ferric heme, such that higher amounts of iron are unlikely to perturb its redox state but would shift the equilibrium in favor of the heme-bound Rev-Erbα. The intracellular concentration of heme is tightly regulated by control of its synthesis and degradation. Rev-Erbα controls the levels of heme by repressing the transcription of PGC-1α (9,10). Heme controls its synthesis by feedback inhibition of transcription, translation, mitochondrial import, and degradation of ALAS1 (11–13). Heme regulates its own degradation by inducing HO1 (14). The temporal relationship of peaks in diurnal rhythms of ALAS1 transcripts, heme, and the expression of HO1 in the current study are consistent with these data. How these complex, interacting feedback systems of regulation ultimately impact the repressor effects of the heme–Rev-Erbα complex remain to be delineated.

Hepatic gluconeogenesis is regulated within a narrow range by a number of hormonal signals translated into intracellular molecular signals (15). Among these, PGC-1α and AMPK are critical regulators of gluconeogenesis. PGC-1α, a transcriptional coactivator, is involved in a number of biological processes and also stimulates hepatic gluconeogenesis by coordinating the expression of PEPCK, fructose-1,6-bisphosphatase, and G6Pase (16–19). PGC-1α via heme synthesis and Rev-Erbα has a repressive effect on gluconeogenesis. In addition, high dietary iron has been shown to suppress gluconeogenesis by activating AMPK (20). Thus, the effect of dietary iron on hepatic gluconeogenesis in vivo is a net effect of a number of interacting signaling pathways resulting in the maintenance of gluconeogenesis within a physiological range. The repression of gluconeogenesis by Rev-Erbα may be the buffering effect on various positive signals.

The study by Simcox et al. (4) underscores how several self-regulated processes are integrated in vivo and are reset by exogenous disruptors such as dietary iron. Iron and heme homeostasis and the clock network of genes are autoregulated by intricate feedback-signaling loops with input from other mediators (Fig. 1). How these pathways, investigated individually using powerful reductionist tools, are integrated in vivo and are reset to a different physiological state by single or multiple interventions continue to be the challenge for the future. Circadian rhythms and their temporal relationship to the whole-body metabolism present intriguing, complex interactions and challenges to be addressed by systems biology approaches.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

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