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# Cell-Nonautonomous Control of the UPR: Mastering Energy Homeostasis

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**Endoplasmic reticulum (ER) stress and activation of the unfolded protein response (UPR) in hypothalamic neurons are common features of obesity, resulting in leptin and insulin resistance. In this issue, Williams et al. (2014) demonstrate, for the first time, cell-nonautonomous UPR signaling between brain and liver in the context of glucoregulation.**

The unfolded protein response (UPR) was discovered over 20 years ago in *S. cerevisiae* as a homeostatic mechanism to orchestrate protein folding and quality control in cells undergoing endoplasmic reticulum (ER) stress. In recent years, this conserved organelle housekeeping network has been implicated in multiple physiological functions in mammals, where it plays a predominant role in specialized secretory cells, such as plasma B cells and pancreatic β cells (Hetz, 2012). The most conserved UPR stress sensor is IRE1α, a kinase and endoribonuclease located at the ER membrane that upon activation catalyzes the processing of the mRNA encoding the transcription factor XBP1. This unconventional splicing event excises a 26 nucleotide intron that shifts the coding reading frame, leading to the expression of an active and potent transcription factor known as XBP1s (Hetz, 2012). XBP1s translocates to the nucleus and upregulates a cluster of genes involved in protein folding, quality control, lipid metabolism, and many components of the secretory pathway. In the context of metabolic regulation, XBP1s is required for the differentiation of pancreatic β cells and for insulin secretion, whereas insulin resistance caused by diet-induced obesity in responsive tissues, such as the liver and skeletal muscle, is associated with local ER stress (Hotamisligil, 2010). Analogously, a high-fat diet activates the UPR in the mouse hypothalamus, and XBP1s expression has a protective role on leptin sensitivity in this brain region (Ozcan et al., 2009). These findings uncovered the contribution of the IRE1α/XBP1 branch of the UPR as a plausible target for therapeutic intervention against diet-induced leptin resistance in the brain (Hetz and Mollereau, 2014). In this issue of *Cell Metabolism*, Williams et al. (2014) unveil a predominant role of cell-specific hypothalamic XBP1 expression in the global control of energy balance not only through local protection against ER stress and leptin/insulin sensitization, but also via the unexpected cell-nonautonomous propagation of UPR signals along the brain-liver axis.

The hypothalamus is a central processing unit where many survival signals related to appetite, sleep, and other motivational states converge to generate the corresponding behavioral outputs. The hypothalamic arcuate nucleus (ARC), in particular, harbors distinct neuronal subpopulations that control energy balance through endocrine and paracrine signaling. Pro-opiomelanocortin (POMC) neurons are generally considered anorexigenic (i.e., mediators of the central response to satiety). They receive inhibitory inputs from orexigenic agouti-related peptide (AgRP) neurons, which are potent drivers of feeding behavior. Both cell types respond to insulin, leptin, glucose, and free long-chain fatty acids, acting as sensors of nutritional status and contributing to orchestrate the behavioral, endocrine, and metabolic responses to hunger and satiety (Gao and Horvath, 2007). The complexity of these regulatory networks within hypothalamic circuits is being unraveled thanks to the development of sophisticated neurogenetic and neuromanipulation tools.

Using organotypic cultures of murine ARCs, Williams et al. (2014) found that acute, pharmacologically induced ER stress is detrimental to leptin-evoked responses in ARC-POMC neurons in mice and provided convincing evidence that this negative effect is mediated by the induction of two known leptin-signaling blockers: suppressor of cytokine signaling 3 (SOCS3) and tyrosine phosphatase 1B (PTP1B). Remarkably, artificial overexpression of XBP1s selectively in POMC neurons repressed *Socs3* and *Ptp1B* expression, through unknown mechanisms, and ablated the effects of pharmacological ER stressors on leptin resistance. This finding not only suggests that ER stress causally precedes leptin/insulin resistance in the hypothalamus, but also uncovers XBP1, and by deduction IRE1, as a promising target to ameliorate leptin resistance in the nervous system and beyond (Figure 1A). Lending physiological credence to these in vitro observations, inducible overexpression of XBP1s in POMC neurons had a marked metabolic effect in mice characterized by protection against diet-induced obesity, improved insulin sensitivity, increased energy expenditure, browning of adipose tissue, and lower endogenous glucose production by the liver. Importantly, these pleiotropic metabolic changes appear to be independent of food consumption, suggesting that XBP1 expression in ARC-POMC neurons is sufficient to generate a global metabolic "fed state"

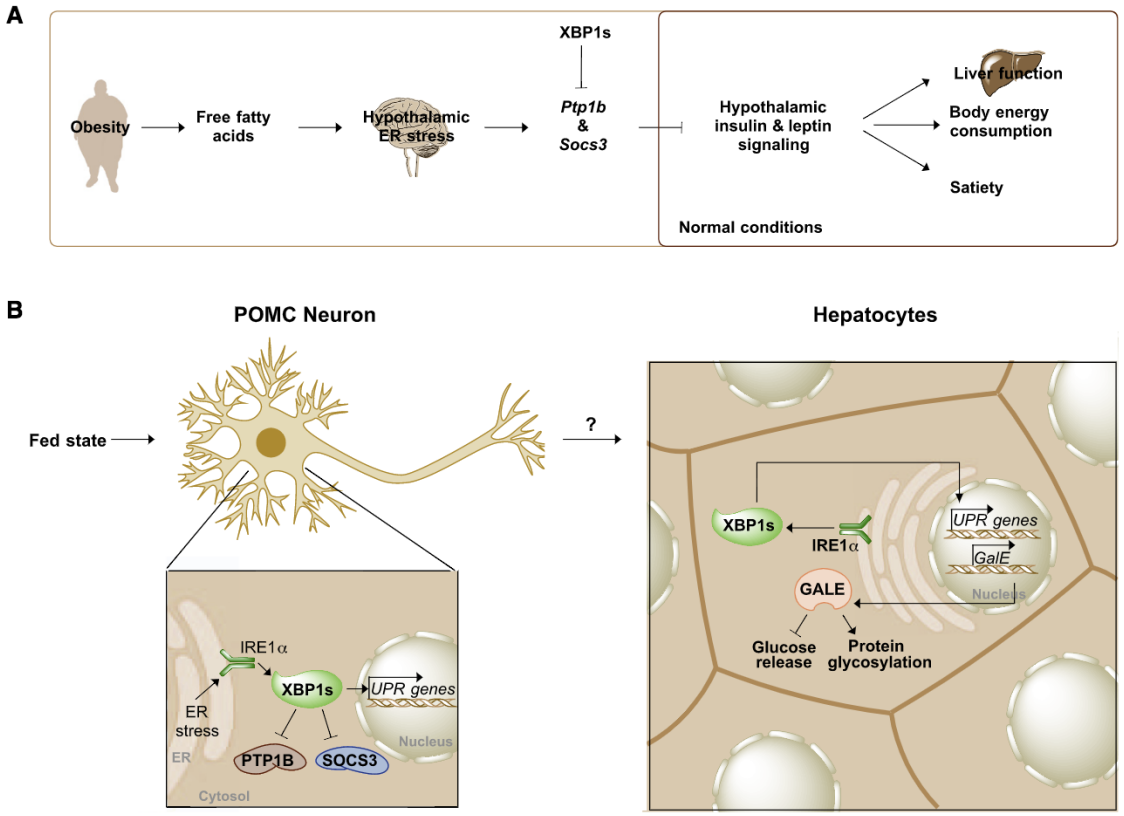


**Figure 1. A New Mechanism of Hypothalamic Control of Global Energy Balance through the UPR Transcription Factor XBP1**
(A) Insulin and leptin resistance in the hypothalamus is causally connected with ER stress triggered by circulating free fatty acids in models of obesity. ER stress induces *Ptp1b* and *Socs3*, two known blockers of leptin signaling. Expression of the active form of the UPR transcription factor XBP1s in POMC neurons has protective effects on these endocrine pathways, possibly enhancing the transmission of satiety signals to target organs and stimulating energy expenditure. (B) Apart from its cell-autonomous effect on leptin/insulin signaling (inset), XBP1s activation in POMC neurons propagates to the liver via a cell-nonautonomous mechanism, where XBP1 mRNA splicing by IRE1α activates canonical UPR target genes, such as *Bip*, *Edem1*, and *Erdj4*, and also the enzyme UDP-glucose-4-epimerase (GALE) in hepatocytes. These events may contribute to reduced hepatic glucose release and enhanced insulin sensitivity observed in *POMC*-specific inducible *Xbp1s* (*PIXs*) mice. Figure design by Dr. Gabriela Martínez.

without significant alterations of feeding behavior.

In a remarkable finding, ectopic XBP1s overexpression in POMC neurons induced the cell-nonautonomous activation of XBP1 mRNA splicing in the mouse liver, where it appears to mediate a postprandial transcriptional program. This XBP1s-dependent program resulted in increased levels of UDP-glucose-4-epimerase (GalE), which led to reduced endogenous glucose release, lower glycemia, and improved insulin sensitivity, in agreement with the phenotype of hepato-specific XBP1s overexpression in mice (Deng et al., 2013) (Figure 1B). Interestingly, an analogous cell-nonautonomous regulatory circuit for XBP1 was only recently described in the model organism *C. elegans*, where XBP1s expression in the nervous system engaged XBP1 mRNA splicing in the intestine in an IRE1-dependent manner, to increase lifespan and protect animals from ER stress (Taylor and Dillin, 2013).

The present study suggests for the first time that the basic concept of neuronal control of ER stress might be a conserved feature of the UPR in metazoans and opens many intriguing questions about the global integration of stress signals in the modulation of diverse physiological responses by the central nervous system (Taylor et al., 2014). A precise definition of this cell-nonautonomous circuit may have enormous therapeutic potential and can be outlined by answering: (1) What is the signal produced by XBP1s activity that propagates from neurons to peripheral tissues? (2) Does it rely on direct multisynaptic connection to the target tissue, or is it relayed through secondary downstream effectors? (3) How are the canonical ER-stress sensors engaged during the cell-nonautonomous activation of *Xbp1* splicing? (4) Is the activation of IRE1 in the hepatocyte stress independent? It is possible that signaling events may directly engage IRE1α activation through assembling distinct *UPRosome* regulatory complexes, as suggested in other systems (Hetz, 2012). In *C. elegans*, the direct release of small molecule neurotransmitters, rather than neuropeptides, is required for cell non-autonomous *xbp1* mRNA splicing in the intestine. In mammals, there are direct multisynaptic connections between ARC-POMC neurons and the liver as well as adipose tissue (Stanley et al., 2010), which could be targeted in future mechanistic studies.

The work of Williams et al. provides important implications to understand the central control of energy balance and identifies IRE1/XBP1 signaling in ARC-POMC neurons as a key module

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of global metabolic control, affecting adipose tissue physiology and glucose use by the liver through a novel UPR cell-nonautonomous mechanism. It remains to be shown whether long-term manipulation of this pathway influences other functional outputs of POMC neurons, but for the time being, the specificity and cell-type-restricted mode of action of XBP1s in the hypothalamus can be considered a promising therapeutic candidate to treat prevalent metabolic diseases.

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# *APOC3*, Coronary Disease, and Complexities of Mendelian Randomization

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**Two new studies report that triglyceride (TG)-lowering mutations in *APOC3* reduce coronary heart disease (CHD) (Crosby et al., 2014; Jørgensen et al., 2014). Here, we explore limitations of using Mendelian randomization to evaluate CHD risk, including potential confounding by the widespread use of statin therapy.**

The status of plasma TG levels as a risk factor for CHD has been debated for decades. In most studies, plasma TG levels are associated with CHD, but adjusting for confounding variables (e.g., smoking, insulin resistance, and diabetes) substantially attenuates the association (Di Angelantonio et al., 2009). Two recent studies take a genetic approach to untangle the Gordian knot between TG levels and CHD (Crosby et al., 2014; Jørgensen et al., 2014).

Instead of stratifying individuals based on plasma TG levels, the studies divide participants into two groups according to their *APOC3* genotypes. Plasma APOC3 and TG levels are highly correlated. Stratifying by *APOC3* genotype rather than plasma level of TG circumvents confounding by factors that affect both plasma TG levels and CHD, an approach referred to as Mendelian randomization (Katan, 1986).

One study, led by Sekar Kathiresan, identified four rare variants in *APOC3* that were associated with a 39% reduction in plasma TG levels (Crosby et al., 2014). The variants were then tested for association with CHD in 110,097 individuals from 15 different studies. Mutation carriers had a 40% reduction in CHD compared to noncarriers. The other study, led by Anne Tybjærg-Hansen, used a similar strategy (Jørgensen et al., 2014). They found three *APOC3* variants that were associated with a 44% reduction in plasma TG levels. In a cohort of 75,725 Danes, carriers of these variants had a 41% reduction in CHD. Taken together, these findings provide compelling evidence that reducing *APOC3* expression will reduce CHD risk. The question remains as to whether the reduced CHD risk in *APOC3* variant carriers is due to lower plasma TG levels or to other associated factors, such as lower plasma levels of LDL cholesterol (LDL-C), APOC3, or remnant lipoproteins, or to increased levels of HDL-C.

Reductions in LDL-C are consistently associated with reduced CHD. Figure 1A