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Review

Hypothalamic ER stress: A bridge between leptin resistance and obesity

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ABSTRACT

The prevalence of obesity has increased worldwide at an alarming rate. However, non-invasive pharmacological treatments remain elusive. Leptin resistance is a general feature of obesity, thus strategies aimed at enhancing the sensitivity to this hormone may constitute an excellent therapeutical approach to counteract current obesity epidemics. Nevertheless, the etiology and neuronal basis of leptin resistance remains an enigma. A recent hypothesis gaining substantial experimental support is that hypothalamic endoplasmic reticulum (ER) stress plays a causal role in the development of leptin resistance and obesity. The objective of this review article is to provide an updated view on current evidence connecting hypothalamic ER stress with leptin resistance. We discuss the experimental findings supporting this hypothesis, as well as the potential causes and underlying mechanisms leading to this metabolic disorder. Understanding these mechanisms may provide key insights into the development of novel intervention approaches.

1. Introduction

Obesity has reached epidemic proportions worldwide causing major human and economic consequences [1]. The World Health Organization currently estimates that more than 1.9 billion adults are over-weight and more than 600 million obese, but effective and safe pharmacological treatments remain elusive [2].

A predominant attribute of obesity is leptin resistance, which is the inability of high circulating leptin levels to exert its anorexigenic actions. Thus, therapeutical strategies aimed at improving leptin sensitivity would constitute an excellent approach for the treatment of obesity. Unfortunately, and despite the strong connection between obesity and leptin resistance, its etiology is currently unknown.

Recently, the hypothesis that hypothalamic ER stress is causally linked with leptin resistance and obesity has progressively gained solid experimental support. The purpose of this article is to review and discuss current evidence related to this topic.

2. Hypothalamic control of energy balance: the melanocortin system

In mammals, the central nervous system (CNS) plays a critical role in energy homeostasis regulation through the modulation of complex and distributed neuronal networks. The hypothalamus, and in particular the arcuate nucleus (ARC), occupies a central position in the neural hierarchy implicated in the regulation of whole-body energy balance and metabolism. Extensive experimental evidence indicates that these biological processes are largely mediated by two specific subpopulations of ARC neurons: (a) neurons co-expressing orexigenic neuropeptides agouti-related protein (AgRP) and neuropeptide Y (NPY) and; (b) neighboring neurons co-expressing anorexigenic neuropeptides alpha-melanocyte stimulating hormone (α-MSH, a product of proopiomelanocortin (POMC) processing) and cocaine and amphetamine-related transcript (CART) [3]. These subsets of hypothalamic neurons (henceforth referred as AgRP and POMC, respectively), together with downstream target neurons expressing melanocortin receptors (MCR) 3 and 4, are the core of the melanocortin system. This is a critical collection of circuits that sense and integrate a wide variety of local (neurotransmitters and neuropeptides) and circulating (hormones and metabolites) signals to promote appropriate neuroendocrine, autonomic and behavioral responses to preserve systemic energy balance [3].

Genetic and pharmacological studies indicate that α-MSH and AgRP neuropeptides mediate, to large extent, the divergent physiological functions of these two subsets of neurons. While α-MSH is an endogenous MCR3 and 4 agonist, thereby generating an anorexigenic output by suppressing appetite and enhancing thermogenesis [4,5], AgRP is an antagonist of these receptors that counteracts the effects of α-MSH on food intake and body weight [6].

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Furthermore, the orexigenic outcome of AgRP neurons is also accomplished by the secretion of NPY, which binds to specific receptors, and by direct inhibitory GABAergic synapsis onto POMC neurons [7]. The most well-known factors regulating the activity of these circuits are peripheral hormones such as ghrelin, insulin and, specially, leptin [3].

3. Leptin biology, physiology and pathophysiology

The discovery of leptin in 1994 by Jeffrey Friedman laboratory [8] was an enormous breakthrough in metabolic research. Extensive experimental investigation over the last 20 years has undoubtedly positioned leptin as a major regulator of long-term energy balance. Leptin is a hormone produced by the adipose tissue in proportion to fat stores that conveys information about the energy status of the organism [9,10]. It mainly acts as an afferent signal to the brain, and in particular to the hypothalamus, where it coordinates energy homeostasis through the modulation of food intake and energy expenditure [11]. The expression of leptin, and its circulating plasma levels, is influenced by the organism nutritional state [12]. Disturbances in leptin signaling and synthesis have been extensively related with obesity. For example, defective leptin production leads to a dramatic obese phenotype both in mice (*ob/ob*) and humans [13,14]. A similar phenotype is found in mice (*db/db*) and with leptin receptor deficiency [15,16].

3.1. Leptin signaling

The leptin receptor (LepR) is expressed throught the CNS, but it is densely located in the hypothalamus [17]. Although there are six different isoforms of leptin receptors (LepRa-f) generated by alternative splicing, the long form (LepRb) is the responsible for the main effects of leptin in the hypothalamus [18]. Binding of leptin to LepRb results in the recruitment and activation of Janus kinase 2 (JAK2), which in turn phosphorylates different tyrosine residues of the LepRb thereby activating different signaling pathways and inducing distinct physiological functions [19,20]. Remarkably, the phosphorylation of Tyr_{1138} plays a prominent role in mediating the effects of leptin on energy homeostasis through the activation of the transcription factor Signal transducer and activator of transcription 3 (STAT3) [19,21]. The activation of STAT3 is associated with enhanced transcription of two proteins that serve as negative regulators of the leptin signaling: the feedback inhibitor suppressor of cytokine signaling 3 (SOCS3) and the phosphotyrosine phosphatase-1B (PTP1B) [22,23]. In addition to the JAK2-STAT3 pathway, leptin also modulates the activity of additional signaling mediators, that have also been implicated in energy balance control, such as the phosphoinositol-3-kinase (PI3K) and 5′-adenosine monophosphate-activated protein kinase (AMPK) cascade [18].

3.2. Leptin and the hypothalamic regulation of energy homeostasis

ARC POMC and AgRP neurons express LepRb, and thus they are direct targets of leptin action [7,24,25]. Generally speaking, the anorexigenic effects of leptin are primarily achieved through the activation of POMC neurons and the concomitant repression of AgRP neurons by different strategies. Leptin signaling cascade, through STAT3 transcriptional effects, promotes *Pomc* gene expression and its conversion into the bioactive anorexigenic neuropeptide α-MSH [26–28]. In addition to these transcriptional effects, leptin directly depolarizes POMC neurons thus promoting α-MSH release to target areas [7,29–31]. In contrast, leptin hinders *Npy* and *AgRP* gene transcription [32–34] while reduces the direct GABAergic tone onto POMC neurons thereby disinhibiting POMC neuronal activity [7]. In summary, leptin promotes an anorexigenic output through the coordination of direct and indirect effects on POMC and AgRP neurons.

3.3. Leptin resistance

The discovery of leptin created great hope on the potential of this hormone to become the miracle cure for human obesity. Encouraging studies showed that leptin administration was able to reduce hyperphagia and body weight in rodent models and humans with genetic leptin deficiency [35–37]. Furthermore, leptin treatment exerted potent dose–response anorectic effects in normal control mice [35,36]. However, subsequent experiments reported that most genetic and induced mouse models of obesity, as well as obese individuals, exhibited elevated circulating levels of leptin pointing to the existence of physiological resistance to the anorexigenic effects of the hormone [9,38]. Indeed, the results of leptin monotherapy in clinical trials were unsuccessful, with modest effects in the majority of human obese patients indicating relative leptin resistance with increasing adiposity [39].

Currently, the general hypotheses explaining leptin resistance are divided into three categories: (a) reduced leptin transport to the brain [40,41]; (b) impaired leptin signaling in target neurons; (c) defective signaling in downstream target cells and circuits [42,43]. The precise neuronal basis of leptin resistance remains elusive, although defects in different biological processes have been proposed to be the underlying cause including hypothalamic inflammation, defective autophagy and endoplasmic reticulum (ER) stress [43]. Despite all these processes are interconnected at multiple levels, in the following sections we will exclusively focus on the latter by summarizing current evidences linking hypothalamic ER stress with the development of leptin resistance and obesity. Excellent revisions on the role of hypothalamic inflammation upon energy balance can be found elsewhere [44,45].

4. Endoplasmic reticulum: an overview

4.1. The endoplasmic reticulum

The ER is a dynamic organelle that forms an interconnected network of convoluted membrane sacs that consists of two different domains: the smooth domain (SER) and the rough domain (RER; holder of ribosomes). The SER is important for fatty acid and phospholipid synthesis, carbohydrate metabolism, and regulation of Ca^{2+} homeostasis. In contrast, the RER is the region where the vast majority of secreted and transmembrane proteins are synthesized, folded and assembled into secondary and tertiary structures that confer a state of stability and maturation by specialized enzymes (chaperones) [46]. To ensure that these complex processes are adequately achieved, the cell has developed a collection of quality control mechanisms. Under normal circumstances, improperly folded proteins are delivered to the cytosol by the ER for proteosomal degradation (ERAD) [47]. Nevertheless, strong and prolonged cellular perturbations may alter ER homeostasis, leading to the accumulation of potentially toxic misfolded proteins and ER stress. To guarantee adequate ER performance under these conditions, this organelle activates a set of phylogenetically conserved, stress-responsive signaling pathways collectively termed the unfolded protein response (UPR) [48,49].

4.2. The unfolded protein response (UPR)

The UPR is mediated by three principal classes of ER-resident transmembrane protein sensors that are negatively regulated by the chaperone immunoglobulin heavy chain binding protein (BIP/GRP78). Under stress conditions, the increased burden of unfolded

proteins promotes the dissociation of BIP from these sensors and their subsequent activation in an attempt to alleviate the workload of the ER by expanding its abundance, temporarily reducing translation and increasing protein folding capacity [48,49]. These UPR branches operate in parallel and in a coordinated manner to restore ER homeostasis. The following is a summary of the basic signaling and specific functions of the different UPR branches.

4.2.1. PKR-like ER kinase (PERK)

PERK is a type I ER transmembrane protein. Upon ER stress, the two proteins dissociate leading to PERK dimerization, autophosphorylation and activation of the kinase domain [50]. Activated PERK inhibits α eukaryotic translation initiation factor 2 (eIF2α), thus reducing protein synthesis and load into the ER [51]. However, some genes containing an internal ribosome entry site (IRES) are selectively translated. One of them is the activating transcription factor 4 (ATF4) [52], which is implicated in the expression of genes related with UPR and cell survival.

4.2.2. Inositol-requiring protein-1 (IRE1)

IRE1 is a type I ER transmembrane protein. ER stress activates IRE1 RNAse domain by homodimerization and autophosphorilation, thereafter catalyzing the splicing of X-box binding protein 1 (*Xbp1*) mRNA generating a potent transcription factor called XBP1s. In the nucleus, XBP1s modulates the expression of UPR pathway genes involved in protein folding and ER biogenesis [53,54]. IRE1 also triggers the activation of other signaling events independent of XBP1, such as the degradation of a subset of mRNAs that probably assist in clearing the ER membrane [55].

4.2.3. Activating transcription factor 6 (ATF6)

ATF6 is a type II ER transmembrane protein belonging to the family of bZIP transcription factors. Under stress conditions, it translocates to the Golgi apparatus were it is processed by different proteases. The N-terminal active domain is released and migrates to the nucleus where regulates the expression of genes related with protein folding and ERAD [56,57].

4.2.4. UPR-mediated apoptosis

Prolonged activation of the UPR and lack of ER stress resolution results in cell death by the activation of autophagic programs or apoptosis. UPR-mediated apoptosis is complex but largely mediated by the mitochondrial canonical pathway, involving the activation of the pro-apoptotic BCL-2 family members BAX and BAK at the mitochondria, the release of cytochrome c and activation of downstream caspases.

PERK and eIF2α pathways induce the pro-apoptotic transcriptional factor CCAAT/-enhancer-binding protein homologous (CHOP), which is considered a master regulator of ER stress-induced apoptosis [58]. It promotes the activation of transcription factor growth arrest and DNA damage-inducible protein 34 (GADD34) and the endoplasmic reticulum oxidoreductase-1 (Ero1α) [59,60]. Furthermore, CHOP increases the level of pro-apoptotic BH3-only protein bim (BIM) and the p53 upregulated modulator of apoptosis (PUMA) while decreases the transcription of the pro-survival protein Bcl-2 [61].

The IRE1α branch also contributes to apoptosis by activating c-Jun N-terminal kinase (JNK) pathway and the apoptosis signal-regulating kinase 1 (ASK1), as well as by binding to the tumour necrosis factor receptor associated factor 2 (TRAF2). JNK pathway induces apoptosis through different mechanisms, including the activation of caspase-12, inhibiting Bcl-2 anti-apoptotic function or binding to BAX and BAK thus leading to the mitochondrial activation of apoptosis [62].

Apoptosis induction in the context of unmitigated ER stress may constitute a protective mechanism to the organism. However, the detailed molecular mechanisms and the cellular threshold switching from a homeostatic process to cell death remain incompletely understood.

4.3. The connection between ER and mitochondria

The ER establishes direct physical contacts with most membrane-bound organelles. These junctions are essential for structural and functional communication between organelles, and thus they necessary for adequate biological performance. The most studied contact sites are those established with the mitochondria, which form specialized ER domains termed mitochondrial-associated membranes (MAMs). These junctions allow bidirectional communication and trafficking of factors, including key signaling molecules such as lipids and Ca^{2+} [63]. Furthermore, the interaction between ER and mitochondria also plays a key role in the dynamic regulation (motility and shape) of these organelles. The accumulation of misfolded proteins in the ER causes a reinforcement of ER-mitochondria contacts and a Ca^{2+} efflux to mitochondria trough MAMs. During early states of ER stress, these changes promote mitochondrial respiration and ATP production that allow the ER to cope with the energy requirements associated with UPR activation [64]. However, sustained Ca^{2+} transmission during prolonged ER stress may lead to deleterious changes in pH and reactive oxygen species (ROS) production in the mitochondria. These alterations may cause modifications in mitochondrial membrane potential, changes in its permeability and a ATP depletion leading to apoptosis [65]. In summary, ER-mitochondria contacts are critical for a number of biological processes and organelle functions. Alterations in the establishment of these juctions may interfere with adequate ER homeostasis leading to ER stress.

5. ER stress and leptin resistance

Pioneering studies by Ozcan et al. demonstrated in 2004 a close relationship between ER stress and obesity [66]. Since then, a bulk of papers has extensively documented the relevance of adequate ER homeostasis in different peripheral tissues upon metabolic control [49].

In recent years, accumulating evidence indicate the existence of a causal link between hypothalamic ER stress and the development of leptin resistance. Both genetic and diet-induced obesity (DIO) models are associated with enhanced expression of ER stress markers in the hypothalamus [67–71] (Table 1). A direct involvement of hypothalamic ER stress in leptin resistance and obesity development comes from a number of in vitro and in vivo pharmacological and genetic studies. For example, culture cells and hypothalamic organotypic slice preparations treated with ER stress inducers (tunicamycin, thapsigargin, brefeldin A or dithiothreitol) markedly inhibited leptin-induced STAT3 phosphorylation [72,73] (Table 1). Furthermore, intracerebroventricular (ICV) administration of these ER stress inducers to control mice promoted hypothalamic ER stress and leptin resistance associated with increased food intake and body weight gain [68–70] (Table 1). Remarkably, different approaches aimed at alleviating ER stress in the hypothalamus were able to reverse these phenotypes. Treatment with the chemical chaperones 4-phenylbutyrate (4-PBA) or tauroursodeoxycholic acid (TUDCA) normalized the expression of ER stress markers and enhanced leptin sensitivity in both diet-induced and genetic models of obesity [67,68,70,72] (Table 1). Additionally, based on the hypothesis that increased UPR function would enhance leptin signaling, Ozcan and collaborators overexpressed XBP1s or ATF6 in MEFs. Overexpression of these critical UPR mediators increased the resistance of cells to the inhibitory effects of tunicamycin and prevented ER stress-mediated inhibition of leptin signaling [68].

Table 1
Modulation of hypothalamic ER stress and leptin pathways in rodent models.

Rodent model	Tissue	ER stress Phenotype	Treatments						Reference
			ER stress relievers		ER stress inducers		ER stress reliever/inducer	Other treatment	
			4-PBA	TUDCA	Tm	Tg	+ LEPTIN		
Lean mice (C57Bl/6)	Hyp. Protein levels				↑pPERK				[67]
DIO mice (chronic HFD)		↑pPERK		↓pPERK, FI , BW					
Lean mice (C57Bl/6)	Hyp. protein levels and gene exp. of Xpb1s					↑ FI, BW, pIRE1,CHOP, pSTAT3	**Tg + L** ↓ FI, BW, pSTAT3		[69]
DIO mice (8-10 w HFD)		↑ pIRE1, BIP,CHOP, Xbp1s					**4-PBA + L** ↓ FI, BW		
XNKO (13 w HFD)	Hyp. Protein levels	↑pPERK , FI, BW ↓pSTAT3							[68]
Ob/Ob mice	Hyp. protein levels and gene exp. of Xpb1s and CHOP	↑pPERK, Xbp1s, CHOP ↓ pSTAT3	↓pPERK		↓pPERK		**4-PBA + L** ↑pSTAT3 ↓ FI, BW **TUDCA + L** ↓ FI, BW		
DIO mice (25 w HFD)			↓pPERK				**4-PBA + L** ↑pSTAT3 ↓ FI, BW		
Lean rats (Sprague-Dawley)	ARC Protein levels				↑ pPERK, peIF2α	↑ SOCS3, PTP1B			[70]
DIO rat (12 w HFD)		↑ pPERK, peIF2α		↓peIF2α, FI, BW		↑ SOCS3, PTP1B			
Mfn2KO mice	Hyp. gene exp.	**6 w age** ↑Xbp1s, ATF4 **12 w age** ↑CHOP, Xbp1s, ATF4	↓Xbp1s, CHOP, ATF4, FI, BW, leptin						[71]
DIO mice (12 w HFD)	ARC gene exp.							**Overexpression of Mfn2** ↓ BIP, Xbp1s, CHOP, ATF4, ATF6	
PIXs mice	ARC or POMC neurons gene exp. and pSTAT3 protein levels in ARC	**ARC** ↑ Xbp1s **POMC neu.** ↑ BIP, Xbp1s, ATF6, PTP1B, SOCS3					**Tg or Tm** ↑ pEIF2α (only Tm), SOCS3, PTP1B ↓pSTAT3		[73]
Lean rats (Sprague-Dawley)								**Ceramide icv** ↑ GRP78, pIRE, pPERK, peIF2α, ATF6, CHOP, BW	
	Hyp. protein levels							**Overexpression of GRP78 VMH** ↑ GRP78 ↓ peIF2α, pIRE1, ATF6, CHOP, BW	[82]
OZR		↑ Ceramide pIRE, pPERK, peIF2α						**Overexpression of GRP78 VMH** ↑ GRP78, pSTAT3 ↓ pPERK, peIF2α, CHOP	
Lean rats (Wistar)	Hyp. protein levels				↑ pPERK ↓pSTAT3			**Tg+ IL-6 or exercise** ↓pPERK ↑pSTAT3	[83]
DIO rats (3 m HFD)		**No exercise** ↑pPERK, CHOP ↓ pSTAT3						**IL-6 or exercise** ↑ pSTAT3 ↓ pPERK, CHOP	
DIO mice (3 w HFD)	Hyp. gene exp.							**Exercise** ↑Xbp1, ATF6, eIF2α, GRP78	[84]

↑, Increased; ↓, decreased; ARC, arcuate nucleus; ATF4, activating transcription factor 4; ATF6, activating transcription factor 6; BIP/GRP78, binding immunoglobulin protein; BW, body weight; CHOP, CCAAT-enhancer-binding protein homologous protein; DIO, diet induced obesity; eIF2α, the alpha subunit of eukaryotic initiation factor 2; ER, endoplasmic reticulum; exp, expression; FI, food intake; HFD, high fat diet; Hyp, hypothalamus; ICV, intracerebroventricular; IL-6, interleukina-6; IRE1, serine/threonine-protein kinase/endoribonuclease; L, leptin; m, month; p, phosphate; PERK, protein kinase RNA-like endoplasmic reticulum kinase; PTP1B, tyrosine-protein phosphatase non-receptor type 1; SOCS3, suppressor of cytokine signaling 3; STAT3, signal transducer and activator of transcription 3; Tg, thapsigargin; Tm, tunicamycin; TUDCA, tauroursodeoxycholic acid; VMH, ventromedial hypothalamus; w, week; Xbp1s, spliced form of X-box binding protein 1; 4-PBA: 4-phenylbutyric acid.

To further confirm the link between ER stress and leptin signaling, these authors generated a mouse model with reduced ER folding capacity in the brain by deleting XBP1 in neurons (XNKO). Under high-fat diet (HFD) conditions these mice displayed an obesogenic phenotype, associated with hyperphagia and reduced oxygen consumption. As predicted, hypothalamic expression levels of phosphorylated PERK were markedly upregulated and leptin-induced STAT3 phosphorylation was significantly blunted in the XNKO mice [68] (Table 1).

Collectively, these reports demonstrate that the hypothalamic ER folding capacity links leptin resistance with obesity and that modulation of this process is able to enhance leptin sensitivity. A critical aspect is to unveil the causes and downstream molecular mechanisms involved, which are summarized in the following section.

5.1. Causes leading to hypothalamic ER stress-induced leptin resistance

5.1.1. Hypothalamic lipotoxicity

High-fat rich diets elicit ER stress, leptin resistance and obesity. But how HFD feeding can directly perturb hypothalamic neuronal function? A considerable body of evidence has demonstrated that hypothalamic neurons are able to sense circulating fatty acids, and that endogenous lipid metabolism in this CNS region is a key mechanism regulating whole-body energy balance [74]. Obese rodents exposed to a HFD exhibit elevated concentrations of free fatty acids in the hypothalamus, which leads to an accumulation of palmitoyl-CoA and other harmful species [75,76]. This ectopic accumulation of lipids in the hypothalamus, generically termed lipotoxicity, is conceptually similar to the process described in peripheral tissues in obesity states. A number of reports have linked hypothalamic lipotoxicity with ER stress as a possible explanation for the onset of obesity. Studies in hypothalamic cell lines have demonstrated that palmitate, a lipotoxic metabolite in excess, triggers ER stress and apoptosis [77–80]. Interestingly, mild palmitate stress decreases protein abundance and function of the α-MSH receptor MCR4 [80]. To specifically test the role of ER stress in this process, these authors co-treated cells with palmitate and 4-PBA. Administration of this chemical chaperone was able to correct the propensity of endogenous MC4R to misfold, restoring its protein levels and the correct response to α-MSH [80]. These results connect lipotoxicity with ER stress and downstream melanocortin system.

In in vivo studies, it has also been described that ICV injection of saturated fatty acids, in particular arachidonic acid (C20:0), induces ER stress in the hypothalamus of rats [81]. More recently, elegant studies from Contreras and collaborators have identified ceramides as another lipotoxic metabolite able to elicit hypothalamic liptoxicity and ER stress [82]. Central ceramide delivery enhances hypothalamic expression of ER stress markers and causes overweight due to decreased sympathetic tone to BAT and reduced thermogenesis. Genetic over-expression of BIP/GRP78, a chaperone that facilitates the proper protein folding acting upstream the UPR pathways, specifically in the ventromedial nucleus of the hypothalamus (VMH) reversed ceramide-induced ER stress and metabolic alterations. Opposite biological effects were observed in experiments in which BIP/GRP78 was inactivated in the VMH using dominant negative adenovirus. Interestingly, obese Zucker rats exhibited increased levels of ceramides and increased UPR response in the VMH. Overexpression of BIP/GRP78 in the VMH of obese Zucker rats was able to ameliorate their metabolic phenotype and enhance leptin (pSTAT3) signaling in this hypothalamic region. Overall, these data identified ceramide accumulation in the VMH as a novel lipotoxic process that eventually leads to ER stress and obesity [82] (Table 1).

As lipotoxicity in the hypothalamus exerts harmful effects and metabolic abnormalities, it is reasonable to predict that preventing hypothalamic lipid accumulation and formation of toxic lipid species would overcome these alterations. Two different strategies have experimentally tested this hypothesis. On one hand, increasing fatty acid oxidation (FAOx) through pharmacologic approaches in vitro [77,79], and on the other hand extending the demand and utilization of these lipids with physical exercise in vivo [83,84]. Mayer et al. reported enhanced apoptosis in an embryonic mouse hypothalamic cell line treated with palmitate that was prevented with the co-treatment with the AMPK activator 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR) [77]. Similarly, two different FAOx inducers (C75 and FSG67) were also tested in primary hypothalamic neurons. Increased fatty acid catabolism limited palmitate availability and prevented the production of other toxic lipids, thus generating favorable changes in ER stress and inflammation [79]. Enhanced FAOx augments ROS production due to mitochondrial activity. In the hypothalamus, ROS act as important signaling molecules and thus their levels are tightly balanced [85]. However, persistent ROS cause protein, lipid and DNA damage in addition to ER stress. C75 or FSG67 treatment did not compromise mitochondrial health or ROS levels, as mitochondrial membrane potential, mitochondrial function and ROS levels were unaltered [79].

These findings suggest that situations of elevated demand of energy and FAOx, such as during intense exercise, may constitute an alternative way to improve ER stress and metabolism. It has been described that during exercise interleukin 6 (IL-6) is released from skeletal muscle, and recent data indicate that IL-6 anti-inflammatory effects are mediated through IL-10 or IL-1 [86]. In line with these observations, it has been reported that IL-6 and IL-10 expression are elevated in the hypothalamus during exercise [83]. Interestingly, in DIO models, exercise and IL-6 administration exerted an inhibitory role upon hypothalamic ER stress and inflammation in the hypothalamus. Exercise was unable to mitigate ER stress when IL-6 signaling was blocked in these animals. Furthermore, thapsigargin-induced hypothalamic ER stress was reduced by exercise or IL-6 [83] (Table 1). However, controversy exist in that point, as another report has shown that 3 weeks of exercise actually increased hypothalamic ER stress in both lean and obese mice [84] (Table 1). These discrepancies may be the consequence of different experimental strategies. Further studies are required to establish the precise role of exercise in hypothalamic ER stress.

5.2. Downstream mechanisms underlying hypothalamic ER stress-induced leptin resistance

5.2.1. The role of POMC neurons and altered neuropeptide processing

The anorexigenic effects of leptin are critically mediated by the expression and release of α-MSH. This bioactive neuropeptide is generated through the sequential cleavage of the POMC precursor by different convertases [87]. As the ER is the site of protein folding and assembling of secretory proteins, a plausible hypothesis is that ER stress may interfere with proper synthesis and processing of POMC thus preventing the release of α-MSH in response to leptin. Hypothalamic POMC neurons are direct targets of leptin, and evidence indicate that these neurons may to some extent mediate the development of leptin resistance [88–90].

Recently, we and others have investigated the link between leptin resistance and ER stress in POMC neurons [70,71,73] (Table 1). Rodent DIO is associated with unchanged POMC transcript levels with increased POMC protein content but reduced α-MSH in the hypothalamus [70,91]. These disparate observations suggest accumulation of unfolded POMC and the existence of compensatory processing mechanisms in the setting of hypothalamic ER-stress.

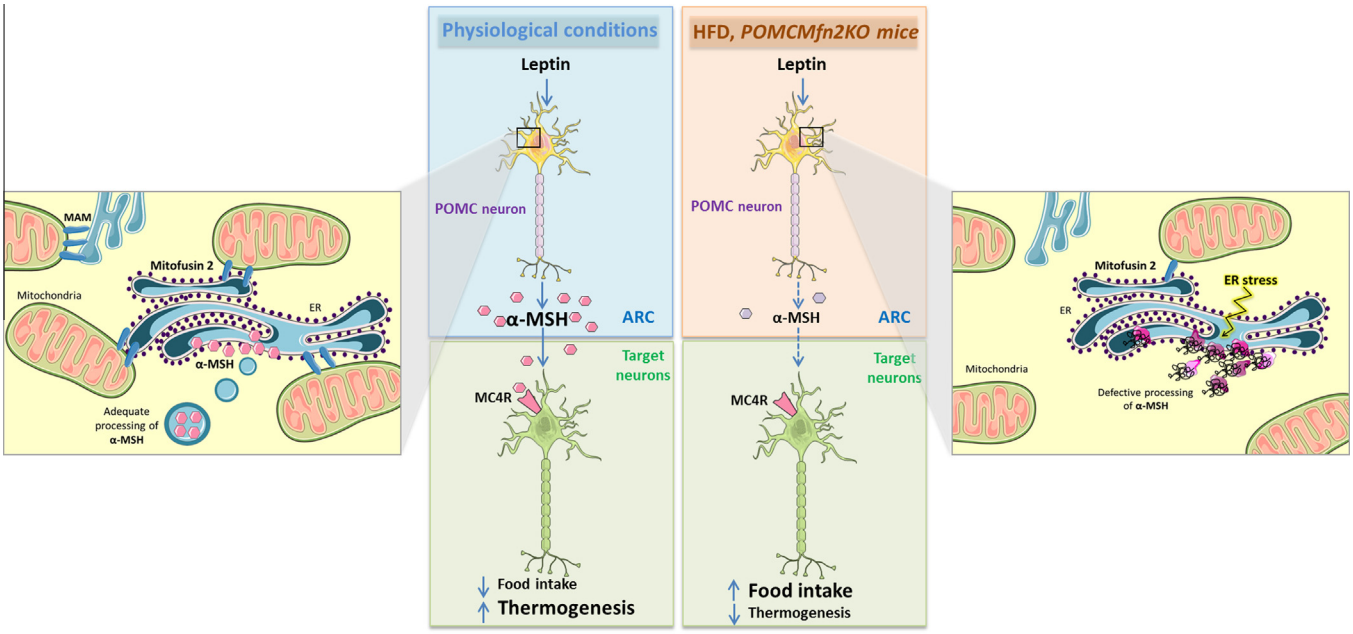

Fig. 1. Overview of leptin effects and POMC processing in the hypothalamus under physiological and obesogenic conditions. In physiological conditions the action of leptin enhances the synthesis of α-MSH in the POMC neurons of the ARC. This neuropeptide is released in target areas where interacts with MC4R promoting decreased food intake and increased thermogenesis. The accurate interaction between mitochondria and ER (MAMs) results in proper α-MSH folding and subsequent correct effect of leptin. HFD feeding or obese *POMCMfn2KO* mice exhibit decreased Mfn2 expression, triggering ER stress, defective α-MSH folding and leptin resistance. ARC, arcuate nucleus; ER, endoplasmic reticulum; HFD, high fat diet; MC4R, melanocortin receptor 4; Mfn2, mitofusin 2; α-MSH, α-melanocyte-stimulating hormone.

Consistent with these results, expression of pro-convertase 2 (PC2), which catalyzes the conversion of adrenocorticotropin (ACTH) into α-MSH, was reduced in the hypothalami from obese rodents. Further confirming that ER stress was the cause of POMC defective processing, pharmacological induction of ER stress in the hypothalamus reduced ACTH/α-MSH ratio and PC2 expression as in the case of DIO. Central TUDCA treatment to obese rats was able to normalize POMC processing and phenotype [70].

Electron microscopy studies showed that DIO is associated with reduced number of ER-mitochondria contacts specifically in POMC neurons [71]. This finding was related to diminished expression of hypothalamic Mitofusin 2 (Mfn2), a dynamin-like GTPase protein which is fundamental for the establishment of ER-mitochondria contacts [92]. In addition to this critical function, Mfn2 has also been reported to mediate cellular responses to ER stress through the interaction and modulation of PERK activity [93]. Overexpression of Mfn2 in the ARC of DIO mice was able to attenuate the obese phenotype and enhanced ER stress characteristic of obesity (Table 1). Collectively, these results suggest that alterations in Mfn2-mediated ER-mitochondria contacts may underlie the development of leptin resistance and obesity. Interestingly, a recent study reports that the number of ER-mitochondria junctions in POMC neurons is increased in a mouse model with enhanced leptin sensitivity [94]. Thus, this parameter may be a potential readout of leptin sensitivity.

To further confirm the importance of Mfn2 in POMC neurons, we generated a POMC-specific Mfn2 knockout mouse (*POMCMfn2KO*). *POMCMfn2KO* mice displayed early development of leptin resistance and a dramatic obese phenotype [71]. Similar to DIO mice, *POMCMfn2KO* mice exhibited a decrease in the mitochondria-ER contacts in POMC neurons and increased ER stress in the hypothalamus. Furthermore, the hypothalamic POMC/α-MSH ratio was altered indicating defective POMC processing. Central administration of chemical chaperones to *POMCMfn2KO* mice was able to normalize the obese phenotype and related metabolic parameters, including POMC processing and α-MSH levels [71] (Table 1). In summary, Mfn2 deletion in POMC neurons elicits ER stress, thus altering α-MSH processing and leading to leptin resistance and obesity (Fig. 1). These results argue for a critical role of Mfn2 in ER homeostasis maintenance and ER stress-induced leptin resistance.

Constitutive expression of a dominant Xbp1s form in POMC neurons leads to a lean phenotype, characterized by increased energy expenditure and leptin sensitivity, further supporting a fundamental role for POMC neurons in the systemic deleterious metabolic effects of hypothalamic ER stress [73]. Remarkably, ER stress inducers failed to blunt leptin-induced depolarization of POMC neurons and also failed to induce leptin resistance in mice lacking Xbp1s in POMC neurons. Expression studies also showed the requirement of PTP1B and SOCS3 in ER stress-induced acute leptin resistance of POMC neurons [73] (Table 1).

POMC neurons have also been directly related with apoptosis triggered by HFD feeding in the context of obesity and leptin resistance. Velloso and colleagues reported that DIO alters the expression of 57% of genes associated with neuronal apoptosis, pointing to a clear effect of dietary fats in inducing hypothalamic neuronal cell death. Interestingly, HFD administration reduced the number of POMC, but not NPY neurons, leading to an imbalance in energy homeostasis [95]. Similarly, another study also reported that HFD administration was associated with a reduction in the number of ARC POMC neurons and enhanced presence of autophagosomes [96]. The reason why POMC neurons appear to be particularly sensitive to the deleterious effects of fat-rich diets is currently unknown, but it could be related with excessive ROS production or reduced buffering capacity in these neurons.

5.2.2. Leptin signaling molecules

An obvious molecular candidate to contribute to ER stress-induced leptin resistance is LepR. However, folding and translocation of LepR in culture cells were unaffected after pharmacological ER stress induction, thereby excluding LepR processing as the main molecular mediator of this process [68]. Additional potential candidates are SOCS3 and PTP1B, which are negative regulators of leptin signal transduction. Both molecules have been reported to be

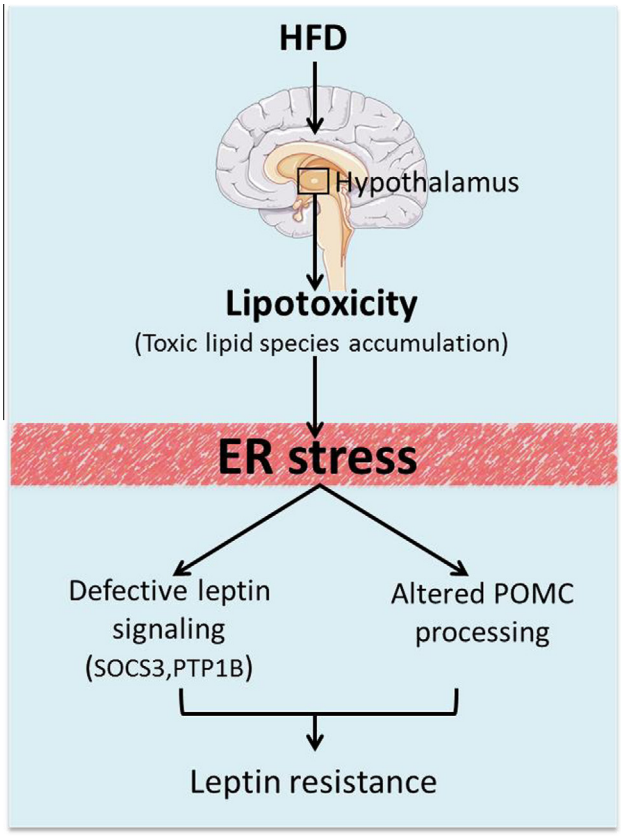

Fig. 2. Schematic summary representation of the development of leptin resistance in the hypothalamus due to HFD and the generation of ER stress.

highly expressed in the hypothalamus of rodents under obese conditions [21,70,91,97,98]. Furthermore, ICV administration of ER stress inducers increased the expression of SOCS3 and PTP1B in the ARC [70] (Table 1). Similarly, culture cells treated with a non-competitive allosteric inhibitor of PTP1B, and also with a specific PTP1B siRNA, showed reversion of ER stress-induced leptin resistance [72]. In contrast, no differences were observed when SOCS3 was tested suggesting that PTP1B mediates this effect. In line with these results, it has been recently described that sleep fragmentation in mice promote hyperphagic behaviors and reduced leptin signaling in the hypothalamus. These effects are mediated by enhanced ER stress, associated with increased PTP1B activity with no changes in the SOCS3 pathway [99]. Nevertheless, the involvement of PTP1B is controversial as other studies have identified SOCS3 as the major determinant involved in leptin resistance mediated by hypothalamic ER stress [67].

Fig. 2 summarizes the relationship between hypothalamic lipotoxicity, ER stress and downstream molecular defects leading to leptin resistance.

6. Alleviating ER stress as a potential treatment for leptin resistance and obesity

Leptin resistance is a hallmark of obesity. Given the strong link between ER stress and obesity development, strategies aimed at reducing aberrant ER stress activation in hypothalamic neurons may constitute a promising approach to improve leptin sensitivity and treat obesity. In this regard, chemical chaperones are therapeutical candidates to re-sensitize neurons to leptin in the context of overnutrition and overweight. In general terms, these molecules promote protein folding and reduce protein aggregation, thus attenuating ER stress. TUDCA and 4-PBA, which have been reported to mitigate ER stress and enhance leptin sensitivity in a variety of in vitro and in vivo models and cell types, are approved by the FDA for other clinical applications. In fact, clinical trials have already demonstrated the efficacy of these compounds in improving glucose homeostasis and insulin signaling in human obese subjects [100,101].

Recent studies have further tested the ability of other clinical compounds in the resolution of ER stress. Fluvoxamine, a selective serotonin reuptake inhibitor primarily used for a number of anxiety and depressive disorders, attenuates ER stress-induced leptin resistance in neuronal cell lines and mice [102]. Flurbiprofen is a phenylalkanoic acid derivative of non-steroidal anti-inflammatory drugs which is prescribed to treat arthritis. This drug exhibited chaperone properties, reducing protein aggregation and improving ER stress-induced leptin resistance in neuronal cell lines. Moreover, flurbiprofen administration showed weight-reducing effects in mice during and after HFD administration suggesting preventive and therapeutic effects [103,104]. Finally, caffeine has also shown to exert chaperone effects and to ameliorate leptin resistance induced by ER stress in neuronal culture cells [105].

The beneficial effects of drugs with chaperone function in cells, rodent models and humans upon leptin sensitivity suggest that this class of compounds may constitute a promising therapeutical approach to counteract the growing obesity epidemics. Nevertheless, detailed studies on the metabolic outcomes, fundamental mechanisms and potential side effects are needed. Another important current limitation is the route of administration of these drugs. As ER stress has been reported to be abnormally upregulated in a range of tissues in metabolic disorders, enteral and parenteral options may be suitable approaches that have been proved to be useful [100,101]. However, targeting the hypothalamus or specific subsets of neurons will require substantial research to design targeted pharmacological strategies to achieve such specificity.

7. New concepts, hypothesis and future directions

The presented evidence posits hypothalamic ER stress as a relevant pathogenic mechanism underlying leptin resistance and obesity. Recent progress has raised novel concepts and key questions around this topic. In this section we summarize some of these ideas and outstanding enigmas that require further investigation.

7.1. Nutritional causes of hypothalamic ER stress

Lipid overload, especially saturated fatty acids, trigger ER stress in the hypothalamus. A straightforward explanation is that this lipid excess causes alterations in the ER membrane composition and biophysical properties that can be sensed by UPR transducers such as IRE1 and PERK [106,107]. The toxic effects of lipids may be minimized through their accumulation in lipid droplets, which are originated from the ER, thereby attenuating UPR activation and ER stress. Thus a conceivable hypothesis, that requires experimental support, is that hypothalamic ER stress occurs when the amount of toxic lipids surpasses the storage capacity of lipid droplets. The contribution of specific lipid species and other nutrients to ER-stress related leptin resistance, and the potential of beneficial dietary nutrients (such as unsaturated fatty acids) to counteract it remains to be investigated.

7.2. Hypothalamic ER stress anatomy

The hypothalamus is made up of a number of nuclei, which contain different populations of neurons exerting distinct biological functions. In this regard, it is unquestionable that understanding

the contribution of specific regions and cells to ER stress-induced leptin resistance will provide valuable insights in the field. ARC POMC neurons are direct targets of leptin, and we and other have shown that these neurons are especially vulnerable to ER stress. However, other cell types and brain areas implicated in energy balance control also respond to leptin. For example, specific populations of neurons in other hypothalamic areas, the reward system (ventral tegmental area, nucleus accumbens) or the brainstem do express LepR. Thus it is reasonable to believe that these regions are also implicated in ER stress-driven leptin resistance. Furthermore, recent research has shown that non-neuronal cells (astrocytes, microglia, tanycytes) also mediate leptin signaling and play regulatory roles upon energy homeostasis [108–112]. The potential role of these cell types in leptin resistance development remains to be elucidated.

7.3. Defective neuropeptide processing

Evidence indicates that leptin resistance, in the context of hypothalamic ER stress, is largely mediated by defective POMC processing. However, how ER stress alters the processing of other neuropeptides is unknown. PC2 expression is reduced by ER stress and is primarily responsible for the reduced α-MSH levels observed in DIO [70]. However, it is important to note that defective PC2 activity could also potentially alter the biosynthesis of other key neuropeptides such as NPY and AgRP. In line with this, it is conceivable that ER stress should affect most of the cellular proteins processed by this organelle, but protein physicochemical properties may be a key factor in determining their proneness to suffer folding defects. Interestingly, the hypothalamic content of CART, which is colocalized in POMC neurons in this particular region [113], is not altered under ER stress and obesity conditions (unpublished results). The reason why ER stress alters POMC but not CART processing is currently unclear.

7.4. Peripheral consequences of hypothalamic ER stress

The hypothalamus, through multisynaptic relays, regulates peripheral tissues through the autonomic nervous system. Emerging data indicates that hypothalamic ER stress may further contribute to obesity and the metabolic deterioration through effects in brown adipose tissue or the liver [82,114,115]. The molecular mechanisms underlying these observations will provide valuable information on the regulation of fundamental biological processes such as thermogenesis and hepatic glucose production.

7.5. Hypothalamic ER stress in humans

The UPR is highly conserved, from yeast to humans. In fact, a number of human diseases have been associated with alterations in protein folding and ER stress, including metabolic disorders such as diabetes and obesity [60,116]. However, current evidence implicates ER stress in peripheral tissues, such as the adipose tissue, liver or pancreas, in the development of metabolic conditions. Thus, a key question to address is the significance of hypothalamic ER stress in human obesity. Future research will undoubtedly unveil this enigma, and will provide valuable insights into the potential use of drugs targeting ER stress to treat human obesity.

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References

[1] Finucane, M.M. et al. (2011) National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9.1 million participants. Lancet 377, 557–567.
[2] Dietrich, M.O. and Horvath, T.L. (2012) Limitations in anti-obesity drug development: the critical role of hunger-promoting neurons. Nat. Rev. Drug Discov. 11, 675–691.
[3] Schneeberger, M., Gomis, R. and Claret, M. (2014) Hypothalamic and brainstem neuronal circuits controlling homeostatic energy balance. J. Endocrinol. 220, T25–T46.
[4] Poggioli, R., Vergoni, A.V. and Bertolini, A. (1986) ACTH-(1-24) and alpha-MSH antagonize feeding behavior stimulated by kappa opiate agonists. Peptides 7, 843–848.
[5] Wirth, M.M., Olszewski, P.K., Yu, C., Levine, A.S. and Giraudo, S.Q. (2001) Paraventricular hypothalamic alpha-melanocyte-stimulating hormone and MTII reduce feeding without causing aversive effects. Peptides 22, 129–134.
[6] Ollmann, M.M., Wilson, B.D., Yang, Y.K., Kerns, J.A., Chen, Y., Gantz, I. and Barsh, G.S. (1997) Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein. Science 278, 135–138.
[7] Cowley, M.A., Smart, J.L., Rubinstein, M., Cerdan, M.G., Diano, S., Horvath, T.L., Cone, R.D. and Low, M.J. (2001) Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. Nature 411, 480–484.
[8] Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L. and Friedman, J.M. (1994) Positional cloning of the mouse obese gene and its human homologue. Nature 372, 425–432.
[9] Maffei, M. et al. (1995) Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat. Med. 1, 1155–1161.
[10] Frederich, R.C., Hamann, A., Anderson, S., Lollmann, B., Lowell, B.B. and Flier, J.S. (1995) Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. Nat. Med. 1, 1311–1314.
[11] Park, H.K. and Ahima, R.S. (2015) Physiology of leptin: energy homeostasis, neuroendocrine function and metabolism. Metabolism 64, 24–34.
[12] Saladin, R., De Vos, P., Guerre-Millo, M., Leturque, A., Girard, J., Staels, B. and Auwerx, J. (1995) Transient increase in obese gene expression after food intake or insulin administration. Nature 377, 527–529.
[13] Montague, C.T. et al. (1997) Congenital leptin deficiency is associated with severe early-onset obesity in humans. Nature 387, 903–908.
[14] Farooqi, I.S. et al. (1999) Effects of recombinant leptin therapy in a child with congenital leptin deficiency. N. Engl. J. Med. 341, 879–884.
[15] Tartaglia, L.A. et al. (1995) Identification and expression cloning of a leptin receptor, OB-R. Cell 83, 1263–1271.
[16] Chua Jr., S.C., Chung, W.K., Wu-Peng, X.S., Zhang, Y., Liu, S.M., Tartaglia, L. and Leibel, R.L. (1996) Phenotypes of mouse diabetes and rat fatty due to mutations in the OB (leptin) receptor. Science 271, 994–996.
[17] Elmquist, J.K., Bjorbaek, C., Ahima, R.S., Flier, J.S. and Saper, C.B. (1998) Distributions of leptin receptor mRNA isoforms in the rat brain. J. Compd. Neurol. 395, 535–547.
[18] Munzberg, H. and Morrison, C.D. (2015) Structure, production and signaling of leptin. Metabolism 64, 13–23.
[19] Banks, A.S., Davis, S.M., Bates, S.H. and Myers Jr., M.G. (2000) Activation of downstream signals by the long form of the leptin receptor. J. Biol. Chem. 275, 14563–14572.
[20] Kloek, C., Haq, A.K., Dunn, S.L., Lavery, H.J., Banks, A.S. and Myers Jr., M.G. (2002) Regulation of Jak kinases by intracellular leptin receptor sequences. J. Biol. Chem. 277, 41547–41555.
[21] Munzberg, H., Flier, J.S. and Bjorbaek, C. (2004) Region-specific leptin resistance within the hypothalamus of diet-induced obese mice. Endocrinology 145, 4880–4889.
[22] Bjorbaek, C., El-Haschimi, K., Frantz, J.D. and Flier, J.S. (1999) The role of SOCS-3 in leptin signaling and leptin resistance. J. Biol. Chem. 274, 30059–30065.
[23] Zabolotny, J.M. et al. (2002) PTP1B regulates leptin signal transduction in vivo. Dev. Cell 2, 489–495.
[24] Cheung, C.C., Clifton, D.K. and Steiner, R.A. (1997) Proopiomelanocortin neurons are direct targets for leptin in the hypothalamus. Endocrinology 138, 4489–4492.
[25] Elias, C.F. et al. (1999) Leptin differentially regulates NPY and POMC neurons projecting to the lateral hypothalamic area. Neuron 23, 775–786.
[26] Schwartz, M.W., Seeley, R.J., Woods, S.C., Weigle, D.S., Campfield, L.A., Burn, P. and Baskin, D.G. (1997) Leptin increases hypothalamic pro-opiomelanocortin mRNA expression in the rostral arcuate nucleus. Diabetes 46, 2119–2123.
[27] Thornton, J.E., Cheung, C.C., Clifton, D.K. and Steiner, R.A. (1997) Regulation of hypothalamic proopiomelanocortin mRNA by leptin in ob/ob mice. Endocrinology 138, 5063–5066.
[28] Mizuno, T.M., Kleopoulos, S.P., Bergen, H.T., Roberts, J.L., Priest, C.A. and Mobbs, C.V. (1998) Hypothalamic pro-opiomelanocortin mRNA is reduced by fasting and [corrected] in ob/ob and db/db mice, but is stimulated by leptin. Diabetes 47, 294–297.

[29] Claret, M. et al. (2007) AMPK is essential for energy homeostasis regulation and glucose sensing by POMC and AgRP neurons. J. Clin. Invest. 117, 2325–2336.
[30] Hill, J.W. et al. (2008) Acute effects of leptin require PI3K signaling in hypothalamic proopiomelanocortin neurons in mice. J. Clin. Invest. 118, 1796–1805.
[31] Al-Qassab, H. et al. (2009) Dominant role of the p110beta isoform of PI3K over p110alpha in energy homeostasis regulation by POMC and AgRP neurons. Cell Metab. 10, 343–354.
[32] Stephens, T.W. et al. (1995) The role of neuropeptide Y in the antiobesity action of the obese gene product. Nature 377, 530–532.
[33] Schwartz, M.W. et al. (1996) Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in ob/ob mice. Diabetes 45, 531–535.
[34] Mizuno, T.M. and Mobbs, C.V. (1999) Hypothalamic agouti-related protein messenger ribonucleic acid is inhibited by leptin and stimulated by fasting. Endocrinology 140, 814–817.
[35] Halaas, J.L. et al. (1995) Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269, 543–546.
[36] Halaas, J.L., Boozer, C., Blair-West, J., Fidahusein, N., Denton, D.A. and Friedman, J.M. (1997) Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. Proc. Natl. Acad. Sci. U.S.A. 94, 8878–8883.
[37] Farooqi, I.S. et al. (2002) Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. J. Clin. Invest. 110, 1093–1103.
[38] Considine, R.V. et al. (1996) Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N. Engl. J. Med. 334, 292–295.
[39] Heymsfield, S.B. et al. (1999) Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. JAMA 282, 1568–1575.
[40] Caro, J.F. et al. (1996) Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. Lancet 348, 159–161.
[41] Gao, A.W., Canto, C. and Houtkooper, R.H. (2014) Mitochondrial response to nutrient availability and its role in metabolic disease. EMBO Mol. Med..
[42] Coppari, R. and Bjorbaek, C. (2012) Leptin revisited: its mechanism of action and potential for treating diabetes. Nat. Rev. Drug Discov. 11, 692–708.
[43] Jung, C.H. and Kim, M.S. (2013) Molecular mechanisms of central leptin resistance in obesity. Arch. Pharm. Res. 36, 201–207.
[44] de Git, K.C. and Adan, R.A. (2015) Leptin resistance in diet-induced obesity: the role of hypothalamic inflammation. Obes. Rev. 16, 207–224.
[45] Purkayastha, S. and Cai, D. (2013) Neuroinflammatory basis of metabolic syndrome. Mol. Metab. 2, 356–363.
[46] Braakman, I. and Hebert, D.N. (2013) Protein folding in the endoplasmic reticulum. Cold Spring Harb. Perspect. Biol. 5, a013201.
[47] Ruggiano, A., Foresti, O. and Carvalho, P. (2014) Quality control: ER-associated degradation: protein quality control and beyond. J. Cell Biol. 204, 869–879.
[48] Walter, P. and Ron, D. (2011) The unfolded protein response: from stress pathway to homeostatic regulation. Science 334, 1081–1086.
[49] Lee, J. and Ozcan, U. (2014) Unfolded protein response signaling and metabolic diseases. J. Biol. Chem. 289, 1203–1211.
[50] Ma, Y., Brewer, J.W., Diehl, J.A. and Hendershot, L.M. (2002) Two distinct stress signaling pathways converge upon the CHOP promoter during the mammalian unfolded protein response. J. Mol. Biol. 318, 1351–1365.
[51] Harding, H.P., Zhang, Y., Bertolotti, A., Zeng, H. and Ron, D. (2000) Perk is essential for translational regulation and cell survival during the unfolded protein response. Mol. Cell 5, 897–904.
[52] Lu, P.D., Harding, H.P. and Ron, D. (2004) Translation reinitiation at alternative open reading frames regulates gene expression in an integrated stress response. J. Cell Biol. 167, 27–33.
[53] Calfon, M., Zeng, H., Urano, F., Till, J.H., Hubbard, S.R., Harding, H.P., Clark, S.G. and Ron, D. (2002) IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. Nature 415, 92–96.
[54] Lee, K. et al. (2002) IRE1-mediated unconventional mRNA splicing and S2P-mediated ATF6 cleavage merge to regulate XBP1 in signaling the unfolded protein response. Genes Dev. 16, 452–466.
[55] Hollien, J. and Weissman, J.S. (2006) Decay of endoplasmic reticulum-localized mRNAs during the unfolded protein response. Science 313, 104–107.
[56] Yamamoto, K., Sato, T., Matsui, T., Sato, M., Okada, T., Yoshida, H., Harada, A. and Mori, K. (2007) Transcriptional induction of mammalian ER quality control proteins is mediated by single or combined action of ATF6alpha and XBP1. Dev. Cell 13, 365–376.
[57] Adachi, Y., Yamamoto, K., Okada, T., Yoshida, H., Harada, A. and Mori, K. (2008) ATF6 is a transcription factor specializing in the regulation of quality control proteins in the endoplasmic reticulum. Cell Struct. Funct. 33, 75–89.
[58] Rutkowski, D.T. et al. (2006) Adaptation to ER stress is mediated by differential stabilities of pro-survival and pro-apoptotic mRNAs and proteins. PLoS Biol. 4, e374.
[59] Campos, G. et al. (2014) The transcription factor CHOP, a central component of the transcriptional regulatory network induced upon CCl4 intoxication in mouse liver, is not a critical mediator of hepatotoxicity. Arch. Toxicol. 88, 1267–1280.
[60] Gregor, M.F., Yang, L., Fabbrini, E., Mohammed, B.S., Eagon, J.C., Hotamisligil, G.S. and Klein, S. (2009) Endoplasmic reticulum stress is reduced in tissues of obese subjects after weight loss. Diabetes 58, 693–700.
[61] Ghosh, A.P., Klocke, B.J., Ballestas, M.E. and Roth, K.A. (2012) CHOP potentially co-operates with FOXO3a in neuronal cells to regulate PUMA and BIM expression in response to ER stress. PLoS ONE 7.
[62] Kim, B.J., Ryu, S.W. and Song, B.J. (2006) JNK- and p38 kinase-mediated phosphorylation of Bax leads to its activation and mitochondrial translocation and to apoptosis of human hepatoma HepG2 cells. J. Biol. Chem. 281, 21256–21265.
[63] Rowland, A.A. and Voeltz, G.K. (2012) Endoplasmic reticulum–mitochondria contacts: function of the junction. Nat. Rev. Mol. Cell Biol. 13, 607–625.
[64] Bravo, R. et al. (2012) Endoplasmic reticulum: ER stress regulates mitochondrial bioenergetics. Int. J. Biochem. Cell Biol. 44, 16–20.
[65] Raturi, A. and Simmen, T. (2013) Where the endoplasmic reticulum and the mitochondrion tie the knot: the mitochondria-associated membrane (MAM). Biochim. Biophys. Acta 1833, 213–224.
[66] Ozcan, U. et al. (2004) Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science 306, 457–461.
[67] Zhang, X., Zhang, G., Zhang, H., Karin, M., Bai, H. and Cai, D. (2008) Hypothalamic IKKbeta/NF-kappaB and ER stress link overnutrition to energy imbalance and obesity. Cell 135, 61–73.
[68] Ozcan, L., Ergin, A.S., Lu, A., Chung, J., Sarkar, S., Nie, D., Myers Jr., M.G. and Ozcan, U. (2009) Endoplasmic reticulum stress plays a central role in development of leptin resistance. Cell Metab. 9, 35–51.
[69] Won, J.C., Jang, P.G., Namkoong, C., Koh, E.H., Kim, S.K., Park, J.Y., Lee, K.U. and Kim, M.S. (2009) Central administration of an endoplasmic reticulum stress inducer inhibits the anorexigenic effects of leptin and insulin. Obesity (Silver Spring) 17, 1861–1865.
[70] Cakir, I., Cyr, N.E., Perello, M., Litvinov, B.P., Romero, A., Stuart, R.C. and Nillni, E.A. (2013) Obesity induces hypothalamic endoplasmic reticulum stress and impairs proopiomelanocortin (POMC) post-translational processing. J. Biol. Chem. 288, 17675–17688.
[71] Schneeberger, M. et al. (2013) Mitofusin 2 in POMC neurons connects ER stress with leptin resistance and energy imbalance. Cell 155, 172–187.
[72] Hosoi, T., Sasaki, M., Miyahara, T., Hashimoto, C., Matsuo, S., Yoshii, M. and Ozawa, K. (2008) Endoplasmic reticulum stress induces leptin resistance. Mol. Pharmacol. 74, 1610–1619.
[73] Williams, K.W. et al. (2014) Xbp1s in Pomc neurons connects ER stress with energy balance and glucose homeostasis. Cell Metab. 20, 471–482.
[74] Martinez de Morentin, P.B., Varela, L., Ferno, J., Nogueiras, R., Dieguez, C. and Lopez, M. (2010) Hypothalamic lipotoxicity and the metabolic syndrome. Biochim. Biophys. Acta 1801, 350–361.
[75] Benoit, S.C. et al. (2009) Palmitic acid mediates hypothalamic insulin resistance by altering PKC-theta subcellular localization in rodents. J. Clin. Invest. 119, 2577–2589.
[76] Posey, K.A. et al. (2009) Hypothalamic proinflammatory lipid accumulation, inflammation, and insulin resistance in rats fed a high-fat diet. Am. J. Physiol. Endocrinol. Metab. 296, E1003–E1012.
[77] Mayer, C.M. and Belsham, D.D. (2010) Palmitate attenuates insulin signaling and induces endoplasmic reticulum stress and apoptosis in hypothalamic neurons: rescue of resistance and apoptosis through adenosine 5′ monophosphate-activated protein kinase activation. Endocrinology 151, 576–585.
[78] Choi, S.J., Kim, F., Schwartz, M.W. and Wisse, B.E. (2010) Cultured hypothalamic neurons are resistant to inflammation and insulin resistance induced by saturated fatty acids. Am. J. Physiol. Endocrinol. Metab. 298, E1122–E1130.
[79] McFadden, J.W., Aja, S., Li, Q., Bandaru, V.V., Kim, E.K., Haughey, N.J., Kuhajda, F.P. and Ronnett, G.V. (2014) Increasing fatty acid oxidation remodels the hypothalamic neurometabolome to mitigate stress and inflammation. PLoS ONE 9, e115642.
[80] Cragle, F.K. and Baldini, G. (2014) Mild lipid stress induces profound loss of MC4R protein abundance and function. Mol. Endocrinol. 28, 357–367.
[81] Milanski, M. et al. (2009) Saturated fatty acids produce an inflammatory response predominantly through the activation of TLR4 signaling in hypothalamus: implications for the pathogenesis of obesity. J. Neurosci. 29, 359–370.
[82] Contreras, C. et al. (2014) Central ceramide-induced hypothalamic lipotoxicity and ER stress regulate energy balance. Cell Rep. 9, 366–377.
[83] Ropelle, E.R. et al. (2010) IL-6 and IL-10 anti-inflammatory activity links exercise to hypothalamic insulin and leptin sensitivity through IKKbeta and ER stress inhibition. PLoS Biol. 8.
[84] Kim, Y., Park, M., Boghossian, S. and York, D.A. (2010) Three weeks voluntary running wheel exercise increases endoplasmic reticulum stress in the brain of mice. Brain Res. 1317, 13–23.
[85] Drougard, A., Fournel, A., Valet, P. and Knauf, C. (2015) Impact of hypothalamic reactive oxygen species in the regulation of energy metabolism and food intake. Front. Neurosci. 9, 56.
[86] Petersen, A.M. and Pedersen, B.K. (2005) The anti-inflammatory effect of exercise. J. Appl. Physiol. 98 (1985), 1154–1162.
[87] Wardlaw, S.L. (2011) Hypothalamic proopiomelanocortin processing and the regulation of energy balance. Eur. J. Pharmacol. 660, 213–219.
[88] Bjorbaek, C. (2009) Central leptin receptor action and resistance in obesity. J. Investig. Med. 57, 789–794.

[89] Diano, S. et al. (2011) Peroxisome proliferation-associated control of reactive oxygen species sets melanocortin tone and feeding in diet-induced obesity. Nat. Med. 17, 1121–1127.
[90] Gamber, K.M., Huo, L., Ha, S., Hairston, J.E., Greeley, S. and Bjorbaek, C. (2012) Over-expression of leptin receptors in hypothalamic POMC neurons increases susceptibility to diet-induced obesity. PLoS ONE 7, e30485.
[91] Enriori, P.J. et al. (2007) Diet-induced obesity causes severe but reversible leptin resistance in arcuate melanocortin neurons. Cell Metab. 5, 181–194.
[92] de Brito, O.M. and Scorrano, L. (2008) Mitofusin 2 tethers endoplasmic reticulum to mitochondria. Nature 456, 605–610.
[93] Munoz, J.P. et al. (2013) Mfn2 modulates the UPR and mitochondrial function via repression of PERK. EMBO J. 32, 2348–2361.
[94] Long, L., Toda, C., Jeong, J.K., Horvath, T.L. and Diano, S. (2014) PPARgamma ablation sensitizes proopiomelanocortin neurons to leptin during high-fat feeding. J. Clin. Invest. 124, 4017–4027.
[95] Moraes, J.C. et al. (2009) High-fat diet induces apoptosis of hypothalamic neurons. PLoS ONE 4, e5045.
[96] Thaler, J.P. et al. (2012) Obesity is associated with hypothalamic injury in rodents and humans. J. Clin. Invest. 122, 153–162.
[97] White, C.L., Whittington, A., Barnes, M.J., Wang, Z., Bray, G.A. and Morrison, C.D. (2009) HF diets increase hypothalamic PTP1B and induce leptin resistance through both leptin-dependent and -independent mechanisms. Am. J. Physiol. Endocrinol. Metab. 296, E291–E299.
[98] Zabolotny, J.M., Kim, Y.B., Welsh, L.A., Kershaw, E.E., Neel, B.G. and Kahn, B.B. (2008) Protein-tyrosine phosphatase 1B expression is induced by inflammation in vivo. J. Biol. Chem. 283, 14230–14241.
[99] Hakim, F., Wang, Y., Carreras, A., Hirotsu, C., Zhang, J., Peris, E. and Gozal, D. (2015) Chronic sleep fragmentation during the sleep period induces hypothalamic endoplasmic reticulum stress and PTP1b-mediated leptin resistance in male mice. Sleep 38, 31–40.
[100] Kars, M. et al. (2010) Tauroursodeoxycholic acid may improve liver and muscle but not adipose tissue insulin sensitivity in obese men and women. Diabetes 59, 1899–1905.
[101] Xiao, C., Giacca, A. and Lewis, G.F. (2011) Sodium phenylbutyrate, a drug with known capacity to reduce endoplasmic reticulum stress, partially alleviates lipid-induced insulin resistance and beta-cell dysfunction in humans. Diabetes 60, 918–924.
[102] Hosoi, T., Miyahara, T., Kayano, T., Yokoyama, S. and Ozawa, K. (2012) Fluvoxamine attenuated endoplasmic reticulum stress-induced leptin resistance. Front. Endocrinol. (Lausanne) 3, 12.
[103] Hosoi, T. et al. (2014) Flurbiprofen ameliorated obesity by attenuating leptin resistance induced by endoplasmic reticulum stress. EMBO Mol. Med. 6, 335–346.
[104] Hosoi, T., Baba, S. and Ozawa, K. (2014) Therapeutic potential of flurbiprofen against obesity in mice. Biochem. Biophys. Res. Commun. 449, 132–134.
[105] Hosoi, T., Toyoda, K., Nakatsu, K. and Ozawa, K. (2014) Caffeine attenuated ER stress-induced leptin resistance in neurons. Neurosci. Lett. 569, 23–26.
[106] Promlek, T., Ishiwata-Kimata, Y., Shido, M., Sakuramoto, M., Kohno, K. and Kimata, Y. (2011) Membrane aberrancy and unfolded proteins activate the endoplasmic reticulum stress sensor Ire1 in different ways. Mol. Biol. Cell 22, 3520–3532.
[107] Volmer, R., van der Ploeg, K. and Ron, D. (2013) Membrane lipid saturation activates endoplasmic reticulum unfolded protein response transducers through their transmembrane domains. Proc. Natl. Acad. Sci. U.S.A. 110, 4628–4633.
[108] Garcia-Caceres, C., Fuente-Martin, E., Argente, J. and Chowen, J.A. (2012) Emerging role of glial cells in the control of body weight. Mol. Metab. 1, 37–46.
[109] Chowen, J.A., Argente, J. and Horvath, T.L. (2013) Uncovering novel roles of nonneuronal cells in body weight homeostasis and obesity. Endocrinology 154, 3001–3007.
[110] Kim, J.G. et al. (2014) Leptin signaling in astrocytes regulates hypothalamic neuronal circuits and feeding. Nat. Neurosci. 17, 908–910.
[111] Jayaram, B. et al. (2013) Astrocytic leptin-receptor knockout mice show partial rescue of leptin resistance in diet-induced obesity. J. Appl. Physiol. 114, 734–741.
[112] Balland, E. et al. (2014) Hypothalamic tanycytes are an ERK-gated conduit for leptin into the brain. Cell Metab. 19, 293–301.
[113] Elias, C.F. et al. (1998) Leptin activates hypothalamic CART neurons projecting to the spinal cord. Neuron 21, 1375–1385.
[114] Purkayastha, S., Zhang, H., Zhang, G., Ahmed, Z., Wang, Y. and Cai, D. (2011) Neural dysregulation of peripheral insulin action and blood pressure by brain endoplasmic reticulum stress. Proc. Natl. Acad. Sci. U.S.A. 108, 2939–2944.
[115] Zhang, Q., Yu, J., Liu, B., Lv, Z., Xia, T., Xiao, F., Chen, S. and Guo, F. (2013) Central activating transcription factor 4 (ATF4) regulates hepatic insulin resistance in mice via S6K1 signaling and the vagus nerve. Diabetes 62, 2230–2239.
[116] Boden, G., Duan, X., Homko, C., Molina, E.J., Song, W., Perez, O., Cheung, P. and Merali, S. (2008) Increase in endoplasmic reticulum stress-related proteins and genes in adipose tissue of obese, insulin-resistant individuals. Diabetes 57, 2438–2444.