Ghrelin and lipid metabolism: key partners in energy balance

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Abstract

Ghrelin, the endogenous ligand of the GH secretagogue receptor, has a pleiotropic role in the modulation of energy balance. Recent evidence has demonstrated that besides its orexigenic role, ghrelin regulates central and peripheral lipid metabolism through specific control of hypothalamic AMP-activated protein kinase (AMPK), a critical metabolic gauge regulating both cellular and whole-body energy homeostasis. In this review, we summarize the new milestones of ghrelin's actions on energy balance, with particular focus on its molecular interaction with hypothalamic AMPK and fatty acid metabolism. Understanding this new metabolic pathway can provide new therapeutic targets for the treatment of obesity and the metabolic syndrome.

Ghrelin: a stomach-derived peptide modulating energy balance

A large degree of attention has surrounded the prospect of ghrelin becoming a key factor in the fight against obesity. This 28 amino acid peptide hormone, with orexigenic properties produced in the stomach (Kojima *et al*. 1999, Tschop *et al*. 2000, Nakazato *et al*. 2001, Seoane *et al*. 2003, Nogueiras *et al*. 2004, López *et al*. 2008*a*), shows several characteristics that lead researchers to believe that its potential as antiobesity target is genuine (Foster-Schubert & Cummings 2006, Zorrilla *et al*. 2006). Investigations involving both humans and rodents have shown that ghrelin promotes feeding, weight gain, and adiposity (Tschop *et al*. 2000, Wren *et al*. 2001*a*). In fact, there is an inverse relationship between circulating ghrelin levels and body weight (Tschop *et al*. 2001*b*). Opposite, increased levels of ghrelin have been detected in patients diagnosed with Prader–Willi syndrome (Cummings *et al*. 2002). Moreover, research using ghrelin knockout (KO) mice (Wortley *et al*. 2005) or ghrelin receptor KO (GHS-R KO) mice (Zigman *et al*. 2005) has demonstrated that lack of ghrelin or ghrelin signaling protects against diet-induced obesity. Altogether, these data demonstrate that ghrelin is an important hormonal signal, promoting feeding and modulating circadian control of feeding patterns in humans and rodents (Cummings *et al*. 2001, Tschop *et al*. 2001*a*, Drazen *et al*. 2006).

The 'classical' mechanism mediating ghrelin orexigenic effect

The effect of ghrelin on feeding is mediated through the GH secretagogue receptor 1a (GHS-R 1a), as indicated by the lack of ghrelin's orexigenic effect in GHS-R KO mice (Sun *et al*. 2004). The relevance of this receptor is further supported by anatomical data showing that GHS-R 1a is highly expressed in hypothalamic cell populations that regulate feeding and body weight homeostasis, such as agouti-related peptide/neuropeptide Y (AGRP/NPY) neurons in the arcuate nucleus (ARC) and fatty acid synthase (FAS) neurons in the ventromedial nucleus (VMH; Bennett *et al*. 1997, Guan *et al*. 1997, Tannenbaum *et al*. 1998, Willesen *et al*. 1999, Mitchell *et al*. 2001, Nogueiras *et al*. 2004, Smith 2005, Zigman *et al*. 2006, López *et al*. 2008*a*, Lage *et al*. 2010). GHS-R 1a expression is not widespread, as it is absent in other regions, such as the lateral hypothalamic area (LHA; Guan *et al*. 1997), where a large number of orexigenic cell populations, such as orexins (OXs; Broberger *et al*. 1998, Elias *et al*. 1998, de Lecea *et al*. 1998, Peyron *et al*. 1998, Sakurai *et al*. 1998, Horvath *et al*. 1999, Seoane *et al*. 2003) and melanin-concentrating hormone (MCH)-producing neurons are located (Nahon *et al*. 1989, Bittencourt *et al*. 1992, Qu *et al*. 1996).

Along with this morphological evidence, central treatment with ghrelin massively increases the mRNA expression of the orexigenic neuropeptides AGRP and NPY in the ARC (Fig. 1), in fed or fasted rodents, an effect that is gender independent (Kamegai *et al*. 2001, Nakazato *et al*. 2001, Seoane *et al*. 2003, Lage *et al*. 2010). Ghrelin has also been reported to inhibit the firing of proopiomelanocortin (*Pomc*) neurons by increasing the frequency of spontaneous synaptic γ-aminobutyric acid release onto them (Cowley *et al*. 2003, Andrews *et al*. 2008), without affecting *Pomc* mRNA expression (Kamegai *et al*. 2000, 2001). Confirming that ghrelin's orexigenic effect is mediated by specific modulation of AGRP/NPY neurons in the ARC, no change was demonstrated in the mRNA levels of the other feeding-promoting neuropeptides, such as MCH and prepro-OX (Seoane *et al*. 2003). The physiological relevance of this molecular mechanism was firmly established using ghrelin in KO mice. Of interest, AGRP KO or NPY KO responded normally to ghrelin; however, the double AGRP/NPY KO failed to respond to ghrelin, indicating the existence of redundancy among these two neuropeptides as mediators of ghrelin's orexigenic action (Chen *et al*. 2004).
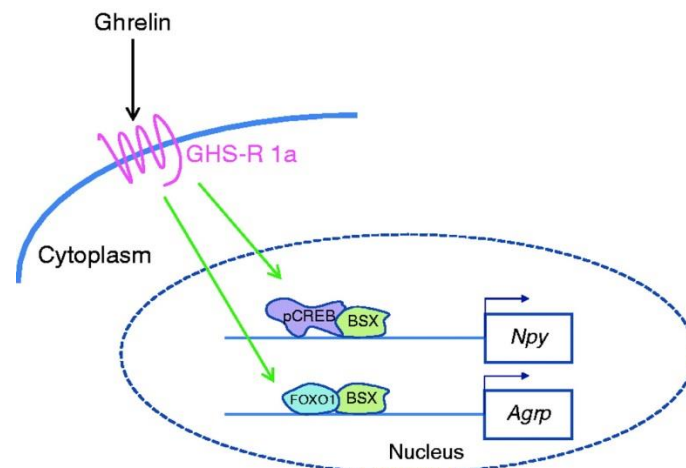

Figure 1. The 'classical' mechanism under ghrelin orexigenic effect. Ghrelin, acting on GH secretagogue receptor 1a (GHS-R 1a), stimulates the expression of hypothalamic homeobox domain transcription factor (BSX), forkhead box O1 (FOXO1), and the phosphorylated cAMP response-element-binding protein (pCREB). Subsequently, agouti-related peptide (*Agrp*) and neuropeptide Y (*Npy*) gene expressions are increased in the arcuate nucleus of the hypothalamus (ARC).

In addition to the control of the circadian patterns of feeding, the interaction between ghrelin and ARC neurons is also important in the metabolic adaptation to fasting, a situation characterized by elevated ghrelin-circulating levels (Tschop *et al*. 2000, Wren *et al*. 2001*a*, Nogueiras *et al*. 2004, López *et al*. 2008*a*). This is demonstrated by the fact that starvation-induced hunger and food intake are suppressed after central administration of anti-ghrelin antiserum (Nakazato *et al*. 2001). Indeed, it is worth mentioning that even in this state of hyperghrelinemia, the administration of this peptide to food-deprived rats led to a further increase in *Agrp* and *Npy* mRNA contents in the ARC, suggesting that this interaction is fundamental for hunger to occur (Seoane *et al*. 2003). It is also important that the ghrelin-induced fasting effect is also mediated by specific modulation of GHS-R. Thus, in the ARC of fasted rats (and also of obese Zucker rats, which lack leptin signaling), GHS-R expression is significantly increased in comparison to controls, an effect that can be blunted with central leptin replacement (Nogueiras *et al*. 2004). The physiological relevance of leptin action on GHS-R is unclear but it has been proposed that, considering that GHS-R is reported to be constitutively active (Holst *et al*. 2003), leptin downregulates GHS-R expression in the ARC in an attempt to decrease orexigenic signaling (Nogueiras *et al*. 2004). Consistently, ghrelin significantly increased GHS-R expression in the ARC of normal rats (Bennett *et al*. 1997, Nogueiras *et al*. 2004), demonstrating that both leptin and ghrelin have a dual and opposite role in modulating the GHS-R expression in the ARC.

BSX, forkhead box O1, and CREB are the key transcription factors in ghrelin receptor signaling pathway

Although the role of ghrelin as the main modulator of ARC-derived neuropeptides is well established, the molecular mechanisms mediating this action have just been identified. It has been recently reported that the hypothalamic homeobox domain transcription factor BSX is highly expressed in AGRP/NPY neurons in the ARC and regulates ghrelin's stimulatory effect on *Agrp* and *Npy* gene expression in male and female rodents (Sakkou *et al*. 2007, Nogueiras *et al*. 2008*a*, Lage *et al*. 2010). Although both genes share BSX as a common transcriptional factor, BSX needs to interact with another two transcription factors to activate *Agrp* and *Npy* mRNA expression: the forkhead box O1 (FOXO1) for *Agrp* gene and the phosphorylated cAMP response-element-binding protein (pCREB) for *Npy* gene respectively (Shimizu-Albergine *et al*. 2001, Kitamura *et al*. 2006, Nogueiras *et al*. 2008*a*, Lage *et al*. 2010). Our group has shown that BSX, FOXO1, and pCREB protein expression in the hypothalamus are stimulated after central ghrelin administration, also in a gender-independent manner (Fig. 1; Lage *et al*. 2010). Interestingly, the ghrelin–BSX–FOXO1–pCREP–AGRP/NPY pathway seems to exhibit a nucleus-specific pattern, since BSX expression in the dorsomedial nucleus of the hypothalamus (DMH) is unaffected by central ghrelin treatment (Nogueiras *et al*. 2008*a*).

The ghrelin-induced increase in FOXO1 protein levels is particularly interesting, since it affects both the nonphosphorylated (FOXO1) and the phosphorylated (pFOXO1) forms (Lage *et al*. 2010). Recent evidence has shown that hormonal signals, such as leptin and insulin, regulate *Agrp* and *Pomc* gene expression through modulation of the balance between FOXO1 (active form) and pFOXO1 (inactive form). Thus, leptin and insulin promote pFOXO1 and prevent its translocation to the nucleus (Kim *et al*. 2006). This results in increased expression of *Pomc* (Belgardt *et al*. 2008, Ernst *et al*. 2009, Plum *et al*. 2009) and decreased expression of *Agrp* (Kitamura *et al*. 2006). Quite opposite to leptin and insulin, we demonstrated that central ghrelin administration increased both hypothalamic FOXO1 and pFOXO1 to a similar extent. Consistent with previous literature, elevated levels of FOXO1 would be the mechanism leading to an increased transcription rate of the *Agrp* gene by ghrelin (Kitamura *et al*. 2006); however, the physiological relevance of increased pFOXO1 is unclear. Recent evidence has revealed that in pancreatic β-cells, ghrelin also stimulates both FOXO1 and pFOXO1 (Wang *et al*. 2010*a*). In this context, increased levels of pFOXO1 after ghrelin treatment elicit a protective effect against lipotoxicity, as shown by inhibition of endoplasmic reticulum (ER) stress and reduced levels of C/EBP homologous protein (CHOP-10; Wang *et al*. 2010*a*), a pro-apoptotic member of the C/EBP

family of transcription factors (Ron & Habener 1992, Oyadomari & Mori 2004), which is known to inhibit cell differentiation in response to metabolic stress (Tang & Lane 2000, Martínez de Morentin *et al*. 2010*b*, de Morentin & López 2010, Wang *et al*. 2010*b*). Similarly, ghrelin seems to protect against lipotoxicity-induced apoptosis and ER stress in an ischemic heart model (Zhang *et al*. 2009). Consistent with these observations, we demonstrated that ghrelin reduces the hypothalamic levels of CHOP-10 and the phosphorylated form of its upstream regulator phosphorylated eukaryotic translation initiation factor 2α (peIF2α; Lage *et al*. 2010). Taking into account that 1) hypothalamic ER stress has recently been suggested as a pathophysiological mechanism mediating leptin resistance and obesity (Hosoi *et al*. 2008, Zhang *et al*. 2008, Ozcan *et al*. 2009, Martínez de Morentin *et al*. 2010*b*, de Morentin & López 2010, Ropelle *et al*. 2010) and 2) central ghrelin administration increases hypothalamic reactive oxygen species (ROS; Andrews *et al*. 2008), which are well-recognized ER-stress inducers (Lee *et al*. 2007, Tagawa *et al*. 2008, Medina-Gomez *et al*. 2009, Santos *et al*. 2009, Martínez de Morentin *et al*. 2010*b*), we have recently proposed that ghrelin-induced elevation in hypothalamic pFOXO1 might be part of an allostatic response protecting against ER stress (Lage *et al*. 2010), a hypothesis that will involve further investigation.

Hypothalamic fatty acid metabolism: a housekeeping pathway modulating whole-body energy balance

Fatty acids are derived either from the diet or by *de novo* synthesis. The basic pathway for *de novo* fatty acid synthesis is summarized in Fig. 2 (Ruderman *et al*. 2003, Dowell *et al*. 2005, López *et al*. 2007, Lage *et al*. 2008, Martínez de Morentin *et al*. 2010*a*). Under lipogenic conditions, excess glucose in the cell is first converted to pyruvate via glycolysis in the cytoplasm. Pyruvate enters the mitochondria and is converted to acetyl-CoA and transported as citrate from the mitochondria into the cytoplasm. ATP citrate lyase then reconverts citrate to acetyl-CoA. Acetyl-CoA carboxylase (ACC) catalyzes the carboxylation of acetyl-CoA to malonyl-CoA in an ATP-dependent manner. Acetyl-CoA and malonyl-CoA are then used as the substrates for the production of palmitate by the seven enzymatic reactions catalyzed by FAS, at the expense of NADPH. The synthesis step of malonyl-CoA can be reversibly regulated as malonyl-CoA decarboxylase (MCD) converts malonyl-CoA back to acetyl-CoA. The resulting saturated fatty acid molecule produced by FAS can be subsequently desaturated to form unsaturated fatty acids, to contribute to the triglyceride pool, or be directed to specific biosynthetic pathways for the synthesis of phospholipids and derivatives for membrane and signaling functions (Kahn *et al*. 2005, López *et al*. 2007). Alternatively, fatty acids can also be further metabolized depending on requirements. During fatty acid oxidation, fatty acids are first activated in the outer mitochondrial membrane in a reaction catalyzed by long-chain fatty acyl-CoA synthetase. Next, they are translocated to the mitochondrial matrix, by the action of carnitine palmitoyltransferase 1 and 2 (CPT1 and CPT2), where oxidation takes place (Kahn *et al*. 2005, López *et al*. 2007). The complete oxidation of a palmitate molecule (C16:0) yields 129 molecules of ATP.
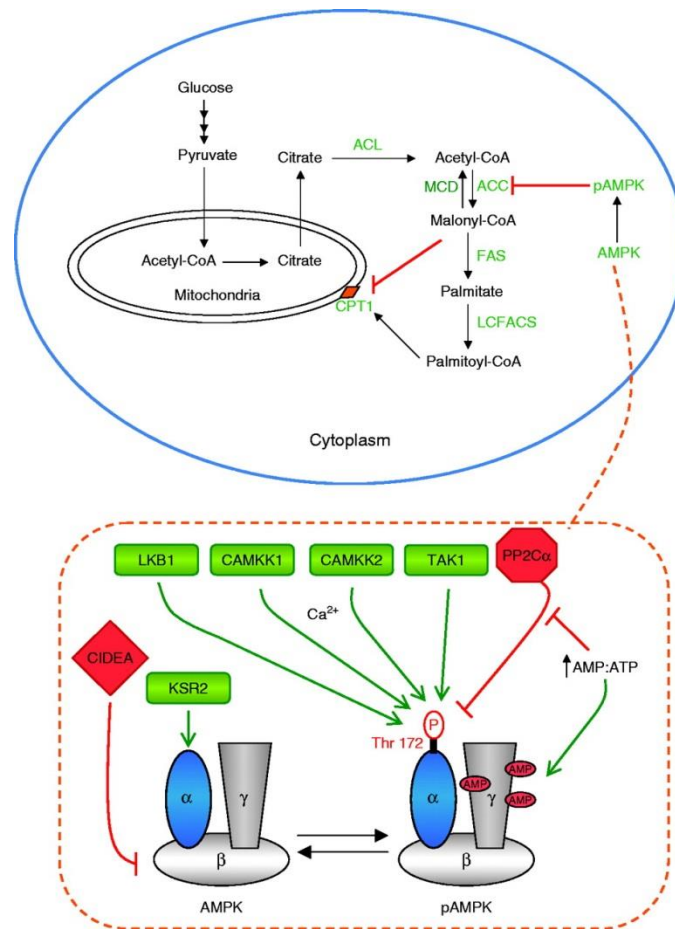
Figure 2. Fatty acid synthesis pathway. Excess glucose in the cell is first converted to pyruvate via glycolysis in the cytoplasm. Pyruvate enters the mitochondria and is converted to acetyl-CoA and transported as citrate from the mitochondria to the cytoplasm. ATP citrate lyase (ACL) then reconverts citrate to acetyl-CoA. Acetyl-CoA carboxylase (ACC) catalyzes the carboxylation of acetyl-CoA to malonyl-CoA. Both acetyl-CoA and malonyl-CoA are then used as the substrates for the production of palmitate catalyzed by fatty acid synthase (FAS). Malonyl-CoA decarboxylase (MCD) converts malonyl-CoA back to acetyl-CoA. Fatty acids are esterified in a reaction catalyzed by long-chain fatty acyl-CoA synthetase (LCFACS). Carnitine palmitoyltransferase 1 (CPT1) is the enzyme importing long-chain fatty acyl-CoA, such as palmitoyl-CoA, into the mitochondria for fatty acid oxidation; CPT1 activity is allosterically inhibited by malonyl-CoA. The resulting saturated fatty acid molecule produced by FAS can be further metabolized depending on the requirements, desaturated to form unsaturated fatty acids, derived to triglyceride molecules, or channeled to a range of phospholipids and derivatives for membrane and signaling functions. AMP-activated protein kinase (AMPK) is a serine/threonine protein kinase composed of a catalytic subunit (α1 or α2) and two regulatory subunits (β1 or β2 and γ1 or γ2 or γ3), which controls ACC, MCD, and FAS activities. Upstream regulation of AMPK is mediated by a complex mechanism involving several proteins such as LKB1 Ca^{2+}/calmodulin-dependent protein kinase kinase 1 or 2 (CAMKK1 or CAMKK2), transforming growth factor-β-activated kinase (TAK1), kinase suppressor of Ras (KSR2), protein phosphatase 2C α (PP2Cα), and cell-death-inducing-like-effector A (CIDEA), as well as cellular AMP concentration.

The activities of ACC and MCD are regulated by phosphorylation by AMP-activated protein kinase (AMPK). AMPK is a serine/threonine protein kinase composed of a catalytic subunit (α1 or α2) and two regulatory subunits (β1 or β2 and γ1 or γ2 or γ3). AMPK is activated by phosphorylation on Thr172 of the α subunit, a process catalyzed by LKB1 or Ca^{2+}/calmodulin-dependent protein kinase kinase 1 or 2 (CAMKK1 or CAMKK2; Ruderman *et al*. 2003, Hawley *et al*. 2005, Kahn *et al*. 2005, Woods *et al*. 2005, Carling *et al*. 2008, Lage *et al*. 2008, Martínez de Morentin *et al*. 2010*a*). Transforming growth factor-β-activated kinase (TAK1; Xie *et al*. 2006) also activates AMPK, and kinase suppressor of Ras (KSR2) interacts with the α1 subunit of AMPK, modulating its activity (Costanzo-Garvey *et al*. 2009). The current data also point out that protein phosphatase 2Cα (PP2Cα) inactivates AMPK by dephosphorylation (Steinberg *et al*. 2006*a*, Martínez de Morentin *et al*. 2010*a*). AMPK is also allosterically activated by AMP, which also inhibits PP2C, increasing phosphorylation in Thr172. Finally, recent data have revealed that cell-death-inducing like-effector A (CIDEA) forms a complex with the β subunit of AMPK, which elicits an ubiquitination-mediated degradation of AMPK, reducing its activity (Fig. 2; Qi *et al*. 2008). Whatever the mechanism, activated (phosphorylated) AMPK is a counter-regulatory response to avoid ATP depletion in many tissues, leading to a switching off of ATP-consuming processes (such as fatty acid synthesis), while switching on catabolic processes that produce ATP (such as fatty acid β-oxidation) and restore the AMP:ATP ratio (Ruderman *et al*. 2003, Kahn *et al*. 2005, Carling *et al*. 2008, Lage *et al*. 2008, Martínez de Morentin *et al*. 2010*a*). In the particular case of the lipid metabolism, activated (phosphorylated) AMPK phosphorylates and inhibits ACC (pACC), while activating MCD (Ruderman *et al*. 2003, Dowell *et al*. 2005, López *et al*. 2007, Lage *et al*. 2008, Martínez de Morentin *et al*. 2010*a*). In addition, activated AMPK decreases FAS mRNA expression via a sterol regulatory element-binding protein-1 (SREBP-1)-dependent mechanism (Zhou *et al*. 2001, López *et al*. 2008*a,b*). Thus, the overall effect of AMPK activation reduces malonyl-CoA and the flux of substrates in the fatty acid biosynthetic pathway.

Evidence gleaned in the last decade has demonstrated that due to the fact that neurons and glial cells need lipid synthesis to sustain their metabolic homeostasis, enzymes of fatty acid metabolism are constitutively expressed in the brain (López *et al*. 2007). Importantly, AMPK, ACC, CPT1, FAS, and MCD mRNAs and proteins are highly expressed in several metabolically relevant hypothalamic nuclei, such as ARC, DMH, paraventricular (PVH), and VMH (Kim *et al*. 2002, Sorensen *et al*. 2002, Minokoshi *et al*. 2004, López *et al*. 2006, 2008*a,b*, 2010, Chakravarthy *et al*. 2007, Dai *et al*. 2007). In addition to this morphological evidence, pharmacologic and genetic results have demonstrated that altered levels and activities of these enzymes affect feeding through specific modulation of ARC-derived neuropeptides, namely the orexigenic AGRP and NPY, as well as the anorexigenic POMC and cocaine and amphetamine-regulated transcript (Loftus *et al*. 2000, Obici *et al*. 2003, Minokoshi *et al*. 2004, López *et al*. 2006, 2008*a*, Wolfgang *et al*. 2006, Chakravarthy *et al*. 2007). Since these impaired fatty acid metabolism altered the hypothalamic pool of either malonyl-CoA (Hu *et al*. 2003, López *et al*. 2006, 2008*a*, 2010, Chakravarthy *et al*. 2007, Lage *et al*. 2010) and/or long-chain fatty acids-CoA (LCFA-CoA; Obici *et al*. 2003, He *et al*. 2006, Pocai *et al*. 2006, López *et al*. 2010), these metabolites have been proposed as signals of nutrient abundance able to modulate feeding. Whether alternative hypothalamic lipid metabolites are involved in feeding control will require further investigation.

Hypothalamic AMPK: a crucial mediator of whole-body energy balance

Similarly to fatty acid metabolism enzymes, AMPK is expressed in several key hypothalamic nuclei, including ARC, LHA, PVH, and VMH (Minokoshi *et al*. 2004, López *et al*. 2008*a*, 2010). During physiological regulation of feeding, changes of hypothalamic AMPK are critical parts of the adaptive response. Fasting stimulates hypothalamic AMPK, while refeeding inhibits it (Minokoshi *et al*. 2004, Lage *et al*. 2008, López *et al*. 2008*a*, Vázquez *et al*. 2008, Martínez de Morentin *et al*. 2010*a*). Activation of AMPK in the hypothalamus (by using adenoviruses expressing constitutively active AMPK, AMPK-CA) elicits feeding and body weight gain (Minokoshi *et al*. 2004). On the other hand, inhibition of hypothalamic AMPK (by using

adenoviruses expressing dominant negative AMPK, AMPK-DN) promotes anorexia and weight loss (Minokoshi *et al*. 2004, López *et al*. 2008*a*, 2010). Importantly, as with the variations in the hypothalamic concentration of malonyl-CoA and LCFA-CoA, alterations in hypothalamic AMPK activity are associated with modifications of neuropeptide mRNA levels. Inhibition of hypothalamic AMPK with an AMPK-DN isoform decreases the mRNA expression of AGRP and NPY in the ARC; on the contrary, overexpression of an AMPK-CA isoform elevates the fasting-induced expression of AGRP and NPY in the ARC and also MCH in the LHA (Minokoshi *et al*. 2004).

Besides the response to starvation, recent evidence has shed light on the central physiological role of hypothalamic AMPK in the regulation of energy homeostasis by integrating peripheral signals with hypothalamic networks. Of note, the effects of fasting and refeeding on hypothalamic AMPK are associated with the changes in circulating nutrients, hormones, and hypothalamic neuropeptides (Table 1 and Fig. 3). AMPK activation in several hypothalamic nuclei such as VMH, ARC, and PVH has a significant part in mediating counter-regulatory responses to acute hypoglycemia, such as attenuated hypoglycemia-induced increases in plasma corticosterone, glucagons, and epinephrine (Han *et al*. 2005, McCrimmon *et al*. 2006, Alquier *et al*. 2007, Cotero & Routh 2009, Fan *et al*. 2009), in hypoglycemia sensing (McCrimmon *et al*. 2004, Murphy *et al*. 2009), and glucose production (Yang *et al*. 2010). In concurrence with these findings, central administration of glucose suppresses AMPK activity in the hypothalamus, decreasing feeding (Minokoshi *et al*. 2004, Wolfgang *et al*. 2007, Cha *et al*. 2008, Lane & Cha 2009). Metabolites also modulate hypothalamic AMPK function. α-Lipoic acid, a cofactor of mitochondrial enzymes with antioxidant and anorectic properties (Kim *et al*. 2004, Ropelle *et al*. 2008*a*), citrate (Cesquini *et al*. 2008, Stoppa *et al*. 2008), and lactate (Cha & Lane 2009) elicit anorexigenic responses associated with inhibition of AMPK, activation of ACC, and subsequent increase in malonyl-CoA. Furthermore, anorectic hormones, such as leptin, insulin, glucagon-like peptide-1 (GLP-1), ciliary neurotrophic factor (CNTF), and melanocortin receptors agonists, including melanotan II (MTII), inhibit hypothalamic AMPK (Andersson *et al*. 2004, Minokoshi *et al*. 2004, Steinberg *et al*. 2006*b*, Gao *et al*. 2007, Wolfgang *et al*. 2007, Seo *et al*. 2008). In contrast, activation of hypothalamic AMPK is caused by orexigenic signals such as AGRP, adiponectin, cannabinoids, glucocorticoids, and ghrelin (see later; Andersson *et al*. 2004, Kola *et al*. 2005, Kubota *et al*. 2007, Andrews *et al*. 2008, López *et al*. 2008*a*, Shimizu *et al*. 2008, Lage *et al*. 2010, Sangiao-Alvarellos *et al*. 2010, Wen *et al*. 2010). Resistin (RSTN), despite its anorectic effect, activates hypothalamic AMPK (Vázquez *et al*. 2008). Finally, current evidence has demonstrated the hypothalamic AMPK mediates thyroid hormone actions on energy balance by controlling the brown adipose tissue (BAT) thermogenic program through activation of the sympathetic nervous system (SNS; López *et al*. 2010). Thus, in general, it can be stated that hypothalamic AMPK is a genuine element of the energy homeostasis system, as shown in the findings. Interestingly, in spite of the vast evidence linking this kinase to peripheral signals (Table 1), the molecular effects of these interactions have been mainly investigated only for ghrelin, which are summarized below.

Table 1. Regulation of hypothalamic AMP-activated protein kinase (AMPK), function of arcuate nucleus (ARC)-derived neuropeptides and feeding

	Hypothalamic AMPK function	*Agrp* mRNA levels in the ARC	*Npy* mRNA levels in the ARC	*Cart* mRNA levels in the ARC	*Pomc* mRNA levels in the ARC	Food intake
Anorexigenic signals						
α-Lipoic acid	↓ (Kim *et al*. 2004, Ropelle *et al*. 2008*a*)	–	–	–	–	↓ (Kim *et al*. 2004, Ropelle *et al*. 2008*a*)
Citrate	↓ (Cesquini *et al*. 2008, Stoppa *et al*. 2008)	–	↓ (Stoppa *et al*. 2008)	–	↑ (Stoppa *et al*. 2008)	↓ (Cesquini *et al*. 2008, Stoppa *et al*. 2008)
CNTF	↓ (Steinberg *et al*. 2006*b*)	↔ (Ziotopoulou *et al*. 2000)	↓ (Xu *et al*. 1998)	↑ (Ambati *et al*. 2007)	↑ (Ambati *et al*. 2007)	↓ (Xu *et al*. 1998, Ziotopoulou *et al*. 2000, Steinberg *et al*. 2006*b*, Ambati *et al*. 2007)
Feeding	↓ (Minokoshi *et al*. 2004, López *et al*. 2008*a*)	↓ (Swart *et al*. 2002, Minokoshi *et al*. 2004, López *et al*. 2008*a*)	↓ (Sanacora *et al*. 1990, Kalra *et al*. 1991, Swart *et al*. 2002, Minokoshi *et al*. 2004, López *et al*. 2008*a*)	↑ (Kristensen *et al*. 1998, Minokoshi *et al*. 2004, López *et al*. 2008*a*)	↑ (Schwartz *et al*. 1997, Swart *et al*. 2002, Minokoshi *et al*. 2004, López *et al*. 2008*a*)	↓ (Sanacora *et al*. 1990, Kalra *et al*. 1991, Kristensen *et al*. 1998, Swart *et al*. 2002, Minokoshi *et al*. 2004, López *et al*. 2006, 2008*a*)
GLP-1	↓ (Seo *et al*. 2008)	↓ (Seo *et al*. 2008)	↓ (Seo *et al*. 2008)	↑ (Seo *et al*. 2008)	↑ (Seo *et al*. 2008)	↓ (Scrocchi *et al*. 1996, Seo *et al*. 2008)
Glucose	↓ (McCrimmon *et al*. 2004, 2006, Minokoshi *et al*. 2004, Chang *et al*. 2005, Han *et al*. 2005, Wolfgang *et al*. 2007, Cha *et al*. 2008, Lane & Cha 2009)	↓ (Chang *et al*. 2005, Lee *et al*. 2005, Cha *et al*. 2008)	↓ (Chang *et al*. 2005, Cha *et al*. 2008)	↑ (Cha *et al*. 2008)	↑ (Cha *et al*. 2008)	↓ (Minokoshi *et al*. 2004, McCrimmon *et al*. 2004, 2006, Han *et al*. 2005, Wolfgang *et al*. 2007, Cha *et al*. 2008, Lane & Cha 2009)
High-protein diet/aminoacids	↓ (Ropelle *et al*. 2008*b*)	↓ (Morrison *et al*. 2007)	↓ (Ropelle *et al*. 2008*b*)	–	↑ (Ropelle *et al*. 2008*b*)	↓ (Ropelle *et al*. 2008*b*)
Insulin	↓ (Minokoshi *et al*. 2004, Namkoong *et al*. 2005)	↓ (Dunbar *et al*. 2005, Konner *et al*. 2007) ↔ (Fekete *et al*. 2006)	↓ (Schwartz *et al*. 1991, 1992, Sipols *et al*. 1995, Fekete *et al*. 2006)	↔ (Fekete *et al*. 2006)	↑ (Kim *et al*. 1999, Benoit *et al*. 2002, Fekete *et al*. 2006)	↓ (Woods *et al*. 1979, Ikeda *et al*. 1986, Schwartz *et al*. 1992, Minokoshi *et al*. 2004, Namkoong *et al*. 2005)
Lactate	↓ (Cha & Lane 2009)	↓ (Cha & Lane 2009)	↓ (Cha & Lane 2009)	↑ (Cha & Lane 2009)	↑ (Cha & Lane 2009)	↓ (Cha & Lane 2009)

Table 1. Regulation of hypothalamic AMP-activated protein kinase (AMPK), function of arcuate nucleus (ARC)-derived neuropeptides and feeding

	Hypothalamic AMPK function	*Agrp* mRNA levels in the ARC	*Npy* mRNA levels in the ARC	*Cart* mRNA levels in the ARC	*Pomc* mRNA levels in the ARC	Food intake
Leptin	↓ (Minokoshi *et al*. 2004, Gao *et al*. 2007)	↓ (Ahima & Flier 2000, Swart *et al*. 2002, Fekete *et al*. 2006)	↓ (Ahima & Flier 2000, Swart *et al*. 2002, Fekete *et al*. 2006)	↑ (Kristensen *et al*. 1998, Fekete *et al*. 2006)	↑ (Schwartz *et al*. 1997, Elias *et al*. 1999, Swart *et al*. 2002, Fekete *et al*. 2006)	↓ (Halaas *et al*. 1995, Stephens *et al*. 1995, Ahima *et al*. 1996, Schwartz *et al*. 1997, Hahn *et al*. 1998, Kristensen *et al*. 1998, Elias *et al*. 1999, Ahima 2000, Swart *et al*. 2002, Minokoshi *et al*. 2004, Gao *et al*. 2007, López *et al*. 2008*a*)
MTII	↓ (Minokoshi *et al*. 2004)	↓ (Marsh *et al*. 1999, Hagan *et al*. 2000, Haskell-Luevano & Monck 2001, Haskell-Luevano *et al*. 2001, Trivedi *et al*. 2003)	↓ (Fan *et al*. 1997, Murphy *et al*. 1998, Raposinho *et al*. 2003)	–	↑ (Marsh *et al*. 1999, Hagan *et al*. 2000, Haskell-Luevano & Monck 2001, Haskell-Luevano *et al*. 2001, Trivedi *et al*. 2003)	↓ (Fan *et al*. 1997, Murphy *et al*. 1998, Marsh *et al*. 1999, Hagan *et al*. 2000, Haskell-Luevano & Monck 2001, Raposinho *et al*. 2003, Trivedi *et al*. 2003, Minokoshi *et al*. 2004, Nogueiras *et al*. 2007)
RSTN	↑ (Vázquez *et al*. 2008)	↓ (Vázquez *et al*. 2008)	↓ (Vázquez *et al*. 2008)	↑ (Vázquez *et al*. 2008)	↔ (Vázquez *et al*. 2008)	↓ (Tovar *et al*. 2005, Vázquez *et al*. 2008)
Orexigenic signals						
Adiponectin	↑ (Kubota *et al*. 2007, Guillod-Maximin *et al*. 2009, Wen *et al*. 2010)	–	↑ (Kubota *et al*. 2007)	–	↓ (Kubota *et al*. 2007)	↑ (Kubota *et al*. 2007)
AGRP	↑ (Minokoshi *et al*. 2004)	↓ (Korner *et al*. 2003)	↔ (Zheng *et al*. 2002)	↑ (Zheng *et al*. 2002, Korner *et al*. 2003)	↓ (Ollmann *et al*. 1997, Wilson *et al*. 1999, Marsh *et al*. 1999, Hagan *et al*. 2000, Haskell-Luevano & Monck 2001, Haskell-Luevano *et al*. 2001)	↑ (Ollmann *et al*. 1997, Marsh *et al*. 1999, Wilson *et al*. 1999, Hagan *et al*. 2000, Haskell-Luevano & Monck 2001, Haskell-Luevano *et al*. 2001, Zheng *et al*. 2002, Korner *et al*. 2003, Minokoshi *et al*. 2004, López *et al*. 2008*a*)
Cannabinoids	↑ (Kola *et al*. 2005, 2008)	↑ (Gamber *et al*. 2005)	↑ (Gamber *et al*. 2005)	↓ (Cota *et al*. 2003, Osei-Hyiaman *et al*. 2005)	↓ (Verty *et al*. 2004, Hentges *et al*. 2005, Nguyen & Wagner 2006)	↑ (Cota *et al*. 2003, Gamber *et al*. 2005, Kola *et al*. 2005, 2008, Osei-Hyiaman *et al*. 2005, Nogueiras *et al*. 2008*b*)
Fasting	↑ (Minokoshi *et al*. 2004, López *et al*. 2008*a*)	↑ (Swart *et al*. 2002, Minokoshi *et al*. 2004, López *et al*. 2008*a*)	↑ (Sanacora *et al*. 1990, Kalra *et al*. 1991, Swart *et al*. 2002, Minokoshi *et al*. 2004, López *et al*. 2008*a*)	↓ (Kristensen *et al*. 1998, Li *et al*. 2002, Minokoshi *et al*. 2004, López *et al*. 2008*a*)	↓ (Schwartz *et al*. 1997, Swart *et al*. 2002, Minokoshi *et al*. 2004, López *et al*. 2008*a*)	↑ (Sanacora *et al*. 1990, Kalra *et al*. 1991, Schwartz *et al*. 1997, Kristensen *et al*. 1998, Swart *et al*. 2002, Minokoshi *et al*. 2004, López *et al*. 2006, 2008*a*)

Table 1. Regulation of hypothalamic AMP-activated protein kinase (AMPK), function of arcuate nucleus (ARC)-derived neuropeptides and feeding

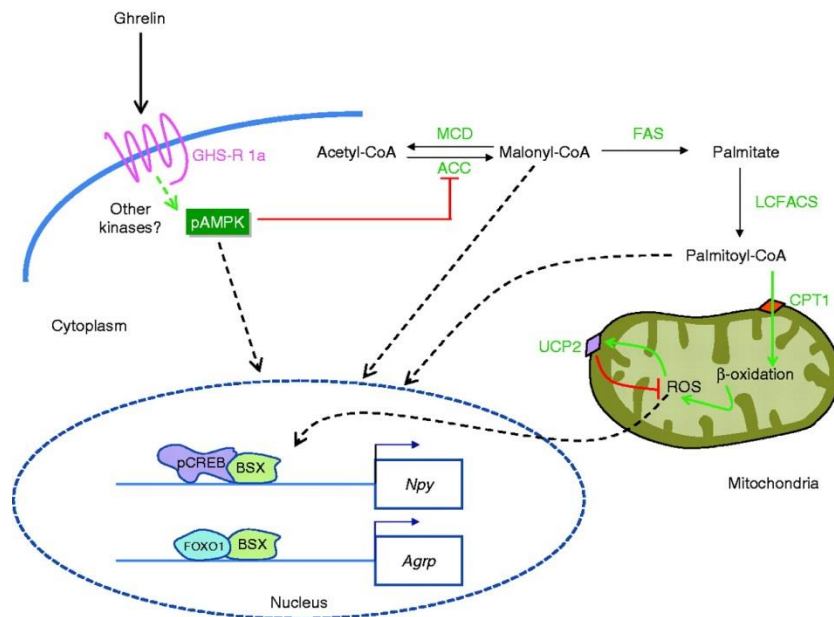|	Hypothalamic AMPK function	*Agrp* mRNA levels in the ARC	*Npy* mRNA levels in the ARC	*Cart* mRNA levels in the ARC	*Pomc* mRNA levels in the ARC	Food intake
Ghrelin	↑ (Andersson *et al.* 2004, Kola *et al.* 2005, 2008, Andrews *et al.* 2008, López *et al.* 2008*a*, Lage *et al.* 2010, Sangiao-Alvarellos *et al.* 2010)	↑ (Kamegai *et al.* 2000, 2001, Nakazato *et al.* 2001, Seoane *et al.* 2003, Andrews *et al.* 2008, López *et al.* 2008*a*, Lage *et al.* 2010, Sangiao-Alvarellos *et al.* 2010)	↑ (Nakazato *et al.* 2001, Andrews *et al.* 2008, López *et al.* 2008*a*, Lage *et al.* 2010, Sangiao-Alvarellos *et al.* 2010)	↔ (Wren *et al.* 2000, Hu *et al.* 2005)	↓ (Hu *et al.* 2005)	↑ (Tschop *et al.* 2000, Nakazato *et al.* 2001, Wren *et al.* 2001*b*, Seoane *et al.* 2003, Andersson *et al.* 2004, Hu *et al.* 2005, Kola *et al.* 2005, 2008, Andrews *et al.* 2008, López *et al.* 2008*a*, Lage *et al.* 2010, Sangiao-Alvarellos *et al.* 2010)
Glucocorticoids	↑ (Shimizu *et al.* 2008)	↑ (Savontaus *et al.* 2002, Coll *et al.* 2005, Shimizu *et al.* 2008)	↑ (Shimizu *et al.* 2008)	↑ (Savontaus *et al.* 2002, Vrang *et al.* 2003, Germano *et al.* 2007)	↓ (Arvaniti *et al.* 2001)	↑ (Arvaniti *et al.* 2001, Savontaus *et al.* 2002, Vrang *et al.* 2003, Coll *et al.* 2005, Germano *et al.* 2007, Shimizu *et al.* 2008)
Glucose deprivation	↑ (McCrimmon *et al.* 2004, 2006, Han *et al.* 2005, Alquier *et al.* 2007, Wolfgang *et al.* 2007, Cotero & Routh 2009, Fan *et al.* 2009)	↑ (Hidaka *et al.* 2001, Lee *et al.* 2005)	↑ (Hidaka *et al.* 2001, Sindelar *et al.* 2002, 2004, Tkacs & Levin 2004, Briski & Parihar 2008, Watanabe *et al.* 2008)	↓ (Nedungadi & Briski 2007)	↓ (Tkacs *et al.* 2000, Nedungadi & Briski 2007)	↑ (Dryden *et al.* 1998, Hidaka *et al.* 2001, Sindelar *et al.* 2002, 2004, McCrimmon *et al.* 2004, 2006, Han *et al.* 2005, Wolfgang *et al.* 2007)

Figure 3. Central ghrelin actions on hypothalamic lipid metabolism and AMPK. Ghrelin, acting on GH secretagogue receptor 1a (GHS-R 1a), regulates hypothalamic AMP-activated protein kinase (AMPK), phosphorylating (pAMPK) and activating it, which in turn phosphorylates and inactivates acetyl-CoA carboxylase (ACC), decreasing the cytoplasmatic pool of malonyl-CoA. The net result of this action is an increase in carnitine palmitoyltransferase 1 (CPT1) activity and then fatty acid oxidation, which promotes the generation of reactive oxygen species (ROS), which are buffered by uncoupling protein 2 (UCP2). This mechanism is critical for ghrelin-induced electric activation of agouti-related peptide/neuropeptide Y (AGRP/NPY) neurons, ghrelin-induced upregulation of *Agrp* and *Npy* gene expression in the ARC, and ghrelin-induced feeding. The molecular events connecting the CPT1–ROS–UCP2 axis with the transcription factors (BSX, pCREB and FOXO1) involved in the gene expression of *Agrp* and *Npy* remain unclear. Alternatively, a direct interaction of AMPK, malonyl-CoA, palmitoyl-CoA (or other lipid species) with that signaling pathway may be possible. Dotted lines represent indirect or unknown interactions.

Central ghrelin actions on hypothalamic lipid metabolism and AMPK

Although the 'classical' pathway involving AGRP/NPY neurons, and more recently BSX–FOXO1–pCREB, has revealed some of the mechanisms underlying the orexigenic effect of ghrelin, it was apparent that some of the key factors implicated in the transduction pathway of the activated GHS-R1a were still missing. Recent data indicate that ghrelin modulates hypothalamic AMPK (Andersson *et al*. 2004, Kola *et al*. 2005, 2008, Andrews *et al*. 2008, López *et al*. 2008*a*,*b*, Lage *et al*. 2010, Sangiao-Alvarellos *et al*. 2010). By using a combination of pharmacological, physiological, and genetic approaches, several groups, including ours, have demonstrated that the physiological orexigenic response to ghrelin involves specific AMPK-induced inhibition of fatty acid biosynthesis, which results in decreased hypothalamic levels of malonyl-CoA and increased CPT1 activity (Andrews *et al*. 2008, López *et al*. 2008*a*,*b*, Lage *et al*. 2010, Sangiao-Alvarellos *et al*. 2010). In this context, ghrelin-induced activation of hypothalamic fatty acid oxidation leads to robust changes in hypothalamic mitochondrial respiration and production of ROS, which are buffered by uncoupling protein 2 (UCP2; Andrews *et al*. 2008). This mechanism is critical for ghrelin-induced electric activation of AGRP/NPY neurons, ghrelin-triggered synaptic plasticity of POMC neurons and ghrelin-dependent gene transcription events in those cells (Fig. 3).

Thus, ghrelin-induced upregulation of *Agrp*, *Npy*, *Ucp2*, *Cpt1*, and nuclear respiratory factor 1 (*Nrf1*) gene expression is blunted in UCP2 KO mice (Andrews *et al*. 2008). Finally, recent elegant data from Korbonits and colleagues have also demonstrated that hypothalamic cannabinoids cross talk with the ghrelin–AMPK signaling. Food intake is induced through the cannabinoid receptor type 1 (CB1; Pagotto *et al*. 2006) and stimulation of AMPK (Kola *et al*. 2005) by both exogenous and endogenous cannabinoids. The occurrence of an intact functional CB1 receptor is needed for ghrelin's effect on AMPK activity and food intake, as shown in CB1 KO animals, as well as using a CB1 antagonist (Kola *et al*. 2008). These data imply that an intact endocannabinoid–CB1 pathway is essential for the AMPK-mediated ghrelin's orexigenic effect (Kola *et al*. 2008).

Although these results provide an interesting mechanism, linking ghrelin-induced changes in fatty acid metabolism and neuropeptide expression, one particular caveat remained unresolved: how alterations in mitochondrial function could lead to nuclear transcriptional events. Our group has recently demonstrated that BSX, FOXO1, and pCREB connect ghrelin-promoted activation of hypothalamic fatty acid β-oxidation in the mitochondria with nuclear *Agrp* and *Npy* gene expression in the ARC (Lage *et al*. 2010). Furthermore, we demonstrate that this association is a physiological gender-independent mechanism modulating feeding. In fact, pharmacological blockage of hypothalamic fatty acid β-oxidation, by using the specific CPT1 inhibitor etomoxir, blocks the ghrelin-induced effect on BSX and subsequently on *Agrp* and *Npy* mRNA expression in the ARC of both male and female rats (Lage *et al*. 2010). Overall, these results indicate that the BSX–FOXO1–pCREB signaling pathway plays a key role in integrating hypothalamic ghrelin actions. Further experiments, using BSX KO mice (Sakkou *et al*. 2007), are essential to confirm the existence of alternative pathways to BSX linking ghrelin fatty acid metabolism and neuropeptide expression. Actually, taking into account that BSX KO mice exhibit lower fasting-induced response in *Agrp* and *Npy* mRNA levels (Sakkou *et al*. 2007), the involvement of alternative additional transcription factors cannot be excluded.

In addition to its effects on the AMPK–malonyl-CoA–CPT1 axis, ghrelin decreases FAS expression in a region-specific manner and this effect is AMPK dependent (Fig. 3; López *et al*. 2008*a,b*). This particular effect is interesting because of its physiological significance. The transient response to ghrelin is displayed in the fact that malonyl-CoA returns to normal levels only 6 h after the initial decrease (López *et al*. 2008*a*). Although the process decreasing malonyl-CoA levels may be attributed to the termination of ghrelin stimulation of AMPK (and the subsequent ghrelin-induced inactivation of ACC by AMPK), recent data also suggest that a direct effect of ghrelin decreasing FAS expression and activity in the VMH may also contribute to this process (López *et al*. 2008*a*). Thus, it has been proposed that this effect prevents the decrease in hypothalamic malonyl-CoA in this nucleus (secondary to fasting-induced inactivation of ACC by AMPK) from reaching deleteriously low levels in the context of food deprivation. This action would increase the level of β-oxidation and in turn could compromise neuronal viability by thwarting lipid biosynthesis and by allowing potentially harmful neuronal fatty acid oxidation during fasting, a condition of low-energy surplus. Supporting this concept, the reduction in FAS levels is not further decreased by prolonged fasting over a period of 48 h (López *et al*. 2006, 2008*b*). This suggests a tightly controlled FAS threshold in hypothalamic neurons, which is mediated by a mechanism involving the transcriptional regulation of SREBP-1 by AMPK (López *et al*. 2008*a,b*). In addition, the decrease in FAS after fasting or ghrelin administration is circumscribed to the VMH, where the GHS-R is highly expressed (Bennett *et al*. 1997, Guan *et al*. 1997, Tannenbaum *et al*. 1998, Willesen *et al*. 1999, Mitchell *et al*. 2001, Nogueiras *et al*. 2004, Smith 2005, Zigman *et al*. 2006, López *et al*. 2008*a*) is appealing. The VMH integrates peripheral signals alongside other hypothalamic nuclei

and the brainstem, connected through specific neuronal projections (Tong *et al*. 2007). Thus, these data indicate that FAS in the VMH may play a role as a sensor of the nutritional state, besides its lipogenic effect (López *et al*. 2008*a,b*).

Overall, these data show that hypothalamic fatty acid metabolism plays an integrative role in the short-term orexigenic response to ghrelin. However, the current data have challenged the long-term relevance of this mechanism, and thus its contribution to the increase in body mass and adiposity promoted by chronic central ghrelin administration (Tschop *et al*. 2000, Theander-Carrillo *et al*. 2006, Sangiao-Alvarellos *et al*. 2009). The current data show that chronic central ghrelin administration does not elicit large changes in either AMPK or ACC activities in normal rats, but expressly inhibits FAS expression in the VMH and hypothalamic CPT1 activity (Sangiao-Alvarellos *et al*. 2010). Bearing in mind that chronic ghrelin treatment exerted a profound orexigenic action and parallel changes in AGRP and NPY expression in the ARC (Sangiao-Alvarellos *et al*. 2010), we are able to speculate that 1) in long-term altered nutritional conditions, AMPK-induced changes in hypothalamic fatty acid metabolism cannot play a key role in feeding control; in accordance with this hypothesis it has been suggested that hypothalamic fatty acid metabolism could be a regulatory mechanism engineered to maintain energy homeostasis in starvation (Andrews *et al*. 2008, López *et al*. 2008*a,b*) and 2) contrary to short-term ghrelin action, chronic ghrelin inhibits hypothalamic CPT1 activity, which shows that in the long-term setting ghrelin could block hypothalamic β-oxidation, following the results obtained in the liver after long-term ghrelin treatment (Theander-Carrillo *et al*. 2006). The physiological significance of this action is uncertain, but it has been hypothesized that it could be a compensatory mechanism to the large orexigenic signal promoted by central hyperghrelinemia (Sangiao-Alvarellos *et al*. 2010). This concept originates from earlier data showing that pharmacological inhibition and/or genetic ablation of hypothalamic CPT1 (Obici *et al*. 2003, Pocai *et al*. 2006, Wolfgang *et al*. 2006, Wolfgang & Lane 2008) or overexpression of MCD (He *et al*. 2006) induces the cytoplasmic accumulation of fatty acyl-CoA concentration and decreases food intake and body weight.

GH-dependent effects of ghrelin on hypothalamic lipid metabolism

In addition to its role in the modulation of energy homeostasis, ghrelin also controls the secretion of GH, by acting as a powerful GHS in rodents and humans (Kojima *et al*. 1999, Masuda *et al*. 2000, Peino *et al*. 2000, Takaya *et al*. 2000). There are several studies highlighting the importance of GH signaling on the effect of ghrelin on metabolism. In spite of data demonstrating that GH-deficient rats show weight gain and adiposity induced by ghrelin (Tschop *et al*. 2000, Wren *et al*. 2000, Nakazato *et al*. 2001, Wren *et al*. 2001*b*), i.c.v. ghrelin treatment did not increase food intake in GH receptor gene-deficient mice (Egecioglu *et al*. 2006) and ghrelin failed to increase the expression of GHS-R in the ARC of dwarf rats (Nogueiras *et al*. 2004). Examination of ghrelin's effect on the fatty acid metabolism pathway in the hypothalamus of GH-deficient dwarf rats showed increased pAMPK levels after chronic ghrelin administration, without changes in CPT1 activity (which is decreased in dwarf rats) and no change in FAS mRNA and protein expression (Sangiao-Alvarellos *et al*. 2010). These data indicate that the actions of ghrelin on hypothalamic fatty acid metabolism are independent of GH and, in contrast to normal rats, chronic ghrelin-induced hyperphagia in dwarf rats could be mediated by specific modulation of the lipogenic pathway. It is possible that this effect is due to elevated hypothalamic GHS-R expression in GH deficiency (Nogueiras *et al*. 2004), but the molecular foundation of the effect is yet to be determined. In this context, it would be critical to assess whether that action is mediated by the canonical BSX–FOXO1–pCREP pathway. Equally intriguing are the results showing that ghrelin-mediated decrease in FAS expression is

blunted in the VMH of dwarf rats. As stated earlier, to avert a harmful drop in the levels of malonyl-CoA in the hypothalamus, data have shown that a physiological adaptive mechanism causes ghrelin- and fasting-induced decreases in FAS levels in the VMH (Andrews *et al*. 2008, López *et al*. 2008*a,b*). Since this regulatory mechanism is lost in dwarf rats, it is possible that an accumulation of toxic lipid species and lipotoxicity-associated phenomena, such as ER stress (Hosoi *et al*. 2008, Zhang *et al*. 2008, Ozcan *et al*. 2009, Won *et al*. 2009, Martínez de Morentin *et al*. 2010*b*), might be caused by altered fatty acid fluxes in the hypothalamic neurons, which may be induced from GH-deficiency, thus hindering hypothalamic metabolism in these animals. Further work will be necessary to address these issues. As seen in this evidence, the hypothalamus of dwarf rats displayed decreases in both *de novo* lipogenesis and β-oxidation after chronic ghrelin treatment (Sangiao-Alvarellos *et al*. 2010). These results are quite opposite to the observed data in normal rats, where ghrelin decreases both *de novo* lipogenesis but stimulates β-oxidation in the hypothalamus (Andersson *et al*. 2004, Kola *et al*. 2005, 2008, Andrews *et al*. 2008, López *et al*. 2008*a*, Sangiao-Alvarellos *et al*. 2010) and suggest that ghrelin actions on hypothalamic fatty acid metabolism are GH independent. Considering the essential role of GH on peripheral lipid metabolism (Maccario *et al*. 2000, van der Lely *et al*. 2004, van der Lely 2009), to determine the physiological relevance of these data is difficult. Following this theme, current findings indicate that GH dependency is not shown in the central ghrelin effects on either adipose or hepatic liver metabolism (Sangiao-Alvarellos *et al*. 2009).

Central ghrelin actions on hypothalamic CAMKK2

The ghrelin receptor, a Gq-coupled receptor, increases intracellular Ca^{2+} via phospholipase C and protein kinase C induction (Garcia *et al*. 2001, van der Lely *et al*. 2004). Since rises in intracellular Ca^{2+} lead to activation of CAMKK2, an upstream AMPK kinase (Hawley *et al*. 2005, Woods *et al*. 2005), which is abundantly expressed in the ARC (Anderson *et al*. 2008), it has recently been proposed that this interaction could be important for feeding control. Supporting this hypothesis, the CAMKK2 KO mice (CAMKK2 KO) showed reduced expression of AGRP and NPY in the ARC (Anderson *et al*. 2008). Consistent with this, hypothalamic AMPK activity is also decreased in the CAMKK2 KO mice, which also show resistance to the ghrelin orexigenic effect (Anderson *et al*. 2008). In keeping with these observations, STO-609, a selective CAMKK2 inhibitor, inhibited NPY expression and reduced food intake and body weight in wild-type animals. Moreover, the loss of CAMKK2 protects mice from high-fat diet (HFD)-induced obesity, insulin resistance, and glucose intolerance (Anderson *et al*. 2008).

Central ghrelin actions on peripheral lipid metabolism

In addition to its role as the main modulator of hypothalamic lipid metabolism, recent data have also highlighted the role of ghrelin as a main modulator of peripheral lipid metabolism (Nogueiras *et al*. 2010). Central administration of ghrelin directly elicits adiposity by the stimulation of the lipogenic program in the white adipose tissue (WAT) in a food intake-independent manner (Theander-Carrillo *et al*. 2006, Nogueiras *et al*. 2007, Sangiao-Alvarellos *et al*. 2009, Andrews *et al*. 2010). In particular, central ghrelin administration stimulates AGRP/NPY neurons, which promotes the blockade of the melanocortin receptors 3 and 4 (MC3R and MC4R) and regulation of peripheral lipid metabolism through the SNS (Theander-Carrillo *et al*. 2006, Nogueiras *et al*. 2007). As a result of these events, mRNA expression of various fat storage-promoting enzymes such as lipoprotein lipase (LPL), ACCα, FAS, and stearoyl-CoA desaturase-1 (SCD1) is induced in WAT; on the other hand, the rate-limiting step in fat oxidation,

CPT1, was decreased (Fig. 4; Theander-Carrillo *et al*. 2006, Sangiao-Alvarellos *et al*. 2009, Andrews *et al*. 2010). Opposite expression patterns seen throughout i.c.v. ghrelin treatment were discovered in ghrelin KO mice. This discovery is consistent with this pharmacological data, recognizing the fact that the endogenous ghrelin system is a major factor in the physiological regulation of WAT metabolism (Nogueiras *et al*. 2010). Specifically, *Lpl* and *Scd1* mRNA expression was decreased in ghrelin KO mice compared with wild-type controls (Theander-Carrillo *et al*. 2006). Taken as a whole, this evidence indicates that central ghrelin action is of physiological relevance in the control of adipocyte metabolism and indicated that ghrelin could elicit the processes in the central nervous system in readiness for the ingestion of food (Nogueiras *et al*. 2010). Interestingly, central ghrelin reduced UCP1 and UCP3 in BAT (Theander-Carrillo *et al*. 2006), suggesting that, besides increased lipogenesis, decreased thermogenesis and energy expenditure might contribute to adiposity. In this sense, a recent and elegant report form Horvath *et al*. demonstrated that the feeding-independent lipogenic actions of ghrelin are enhanced in mice lacking UCP2 (UCP2 KO; Andrews *et al*. 2010). The molecular mechanisms under this effect are not fully understood, but UCP2 KO mice display high expression of lipogenic enzymes, such as FAS, SCD1, and LPL, and decreased expression of CPT1a in WAT (Andrews *et al*. 2010). These data correlate with the higher susceptibility to gain weight in an HFD observed in this model (Joseph *et al*. 2002), as well as with that well-established UCP2-mediated increase in fatty acid oxidation under fasting (Andrews *et al*. 2008, Pecqueur *et al*. 2008, Sheets *et al*. 2008). These data identify UCP2 as a common factor modulating energy homeostasis at hypothalamic (Andrews *et al*. 2008) and peripheral level (Andrews *et al*. 2010). Moreover, they suggest that ghrelin-induced UCP2 expression in WAT may be a protective mechanism to prevent excessive weight gain and obesity. Additional work will be required to address whether the AMPK–malonyl-CoA–CPT1–UCP2 axis is specific to the actions of ghrelin on feeding and peripheral lipogenesis or a common mechanism for hormonal signals modulating both processes, such as leptin (Pocai *et al*. 2005*b*, Buettner *et al*. 2006, 2008), insulin (Obici *et al*. 2002*a*,*b*, Pocai *et al*. 2005*a*, Plum *et al*. 2006, Koch *et al*. 2008), melanocortins (Nogueiras *et al*. 2007), RSTN (Vázquez *et al*. 2008), and GLP-1 (Nogueiras *et al*. 2009).
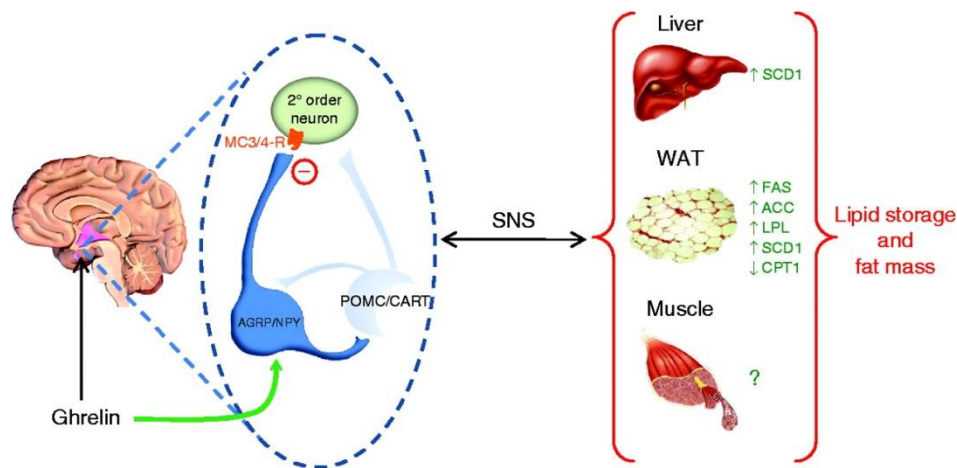

Figure 4. Central ghrelin actions on peripheral lipid metabolism. Ghrelin stimulates agouti-related peptide/neuropeptide Y (AGRP/NPY) neurons. This action leads to a blockade of the melanocortin receptor 3 and 4 (MC3R and MC4R) by AGRP and subsequent modulation of peripheral lipid metabolism through the sympathetic nervous system (SNS). Blockade of MC3R and MC4R increases lipid storage and adiposity.

GH-dependent effects of ghrelin on peripheral lipid metabolism

Considering the role of ghrelin as a GHS (Kojima *et al*. 1999, Masuda *et al*. 2000, Peino *et al*. 2000, Takaya *et al*. 2000), as well as the important actions of GH on peripheral lipid metabolism (Maccario *et al*. 2000, van der Lely *et al*. 2004, van der Lely 2009), we have recently examined the effects of chronic central ghrelin administration on peripheral lipid metabolism in dwarf rats. Our results indicate that central chronic ghrelin administration regulates adipose lipid metabolism, mainly in a GH-independent manner, as a result of increased mRNA, protein expression, and activity levels of ACCα, FAS, and SCD1, which increased in dwarf rats when compared with normal wild-type rats (Sangiao-Alvarellos *et al*. 2009). Conversely, central ghrelin regulates hepatic *de novo* lipogenesis in a GH-independent manner but fatty acid oxidation in a GH-dependent manner, because CPT1 was inhibited only in normal rats (Sangiao-Alvarellos *et al*. 2009). Furthermore, and in contrast to the hypothalamus (Andrews *et al*. 2008, López *et al*. 2008*a,b*), we showed that in peripheral tissues the increased ghrelin levels during food deprivation do not mediate the effects of fasting. In these tissues, starvation downregulates the expression of lipogenic enzymes, and activates (in the liver) or downregulates (in WAT) CPT1, which are the opposite effects to those observed after the ghrelin treatment (Sangiao-Alvarellos *et al*. 2009).

Concluding remarks

The positives of ensuring accurate regulation of homeostatic systems are complexity and redundancy. Lipid metabolism is a central element, which modulates energy balance downstream the ghrelin signaling pathway. Not only do recent anatomical and pharmacological data demonstrate this, but it is also backed up by current genetic and physiological evidence as well. At the hypothalamic level, the short-term (but not the long-term) orexigenic effect of ghrelin is mediated by AMPK-driven changes in hypothalamic lipid metabolism (ACC, FAS, and CPT1) and UCP2 that subsequently influence neuropeptide gene expression through the transcription factors BSX, FOXO1, and pCREB (Andersson *et al*. 2004, Kola *et al*. 2005, 2008, Andrews *et al*. 2008, López *et al*. 2008*a,b*, Lage *et al*. 2010, Sangiao-Alvarellos *et al*. 2010). Similarly, central ghrelin also acts by modulating the adipose lipogenic pathway through the SNS in a GH-independent but UCP2-dependent manner (Theander-Carrillo *et al*. 2006, Sangiao-Alvarellos *et al*. 2009, Andrews *et al*. 2010).

On the basis of these data, it is tempting to speculate that ghrelin favors energy stores in order to minimize the negative effects on periods of food scarcity (Nogueiras *et al*. 2010). During fasting, increased ghrelin levels stimulate appetite and facilitates anabolic processes when food becomes available by triggering biological responses that modulate the efficiency of energy storage, i.e. increasing lipogenesis and inducing UCP2 in WAT, which shifts the organism from a negative energy balance state to a neutral energy balance state, avoiding overweight and obesity (Tsubone *et al*. 2005, Andrews *et al*. 2008, 2010, López *et al*. 2008*a*, Sangiao-Alvarellos *et al*. 2009, Nogueiras *et al*. 2010). However, this mechanism, which was primarily designed as a response to fasting, under conditions of HFD (Wortley *et al*. 2005, Zigman *et al*. 2005) or GH deficiency (Sangiao-Alvarellos *et al*. 2009), seems to increase excessively positive energy balance and fat mass, which ultimately may lead to harmful pro-obesogenic and diabetic states.

Alternatively, a new and crucial link between ghrelin and lipids has been recently revealed by studying ghrelin *O*-acyl transferase (GOAT), the enzyme responsible for ghrelin acylation (Gonzalez *et al*. 2008, Gutierrez *et al*. 2008, Yang *et al*. 2008). Tschop and colleagues have elegantly demonstrated that GOAT is regulated by nutrient

availability and depends on specific dietary lipids, such as medium-chain fatty acids, which act as acylation substrates (Kirchner *et al*. 2009). This evidence links ingested lipids to ghrelin action and suggests that, to obtain optimal nutrient partitioning, the availability of high caloric food is signaled to the hypothalamus through readily absorbable medium-chain fatty acids, originating from the GOAT–ghrelin system working as a nutrient sensor.

Altogether, these data identify the ghrelin–lipid metabolism interaction as a key homeostatic process modulating energy balance. Further work will be necessary to investigate the therapeutic implication of the ghrelin–lipid metabolism partnership for the treatment of obesity and metabolic syndrome.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

Funding

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