REVIEW

Ghrelin and lipid metabolism: key partners in energy balance

Luis Varela^{1,2*}, María J Vázquez^{1,2*}, Fernando Córdido^{3,4}, Rubén Nogueiras^{1,2}, Antonio Vidal-Puig⁵, Carlos Diéguez^{1,2} and Miguel López^{1,2}

¹Department of Physiology, School of Medicine, University of Santiago de Compostela-Instituto de Investigación Sanitaria, Santiago de Compostela (A Coruña) 15782, Spain

²CIBER Fisiopatología de la Obesidad y Nutrición (CIBERObn), Santiago de Compostela 15706, Spain

³Department of Medicine, School of Health Science, University of A Coruña, A Coruña 15006, Spain

⁴Endocrine Department, Hospital A Coruña, A Coruña 15006, Spain

⁵Metabolic Research Laboratories, Institute of Metabolic Science, Addenbrooke's Hospital, University of Cambridge, Cambridge, CB2 0QQ, UK

(Correspondence should be addressed to M López at Department of Physiology, School of Medicine, University of Santiago de Compostela-Instituto de Investigación Sanitaria, CIBER Fisiopatología de la Obesidad y Nutrición (CIBERObn); Email: m.lopez@usc.es)

*(L Varela and M J Vázquez contributed equally to this work)

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.

Journal of Molecular Endocrinology (2011) **46**, R43–R63

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.* 2008a), 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.* 2001a). In fact, there is an inverse relationship between circulating ghrelin levels and body weight (Tschop *et al.* 2001b). 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.* 2001a, 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.* 2008a, 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).

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.* 2001a, Nogueiras *et al.* 2004, López *et al.* 2008a). 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.

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).

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.* 2008a, 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.* 2008a, 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–pCREB–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.* 2008a).

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.* 2010a). 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.* 2010a), 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.* 2010b, de Morentin & López 2010, Wang *et al.* 2010b). 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 2 α (pEIF2 α ; 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.* 2010b, 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.* 2010b), 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.* 2010a). 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.

The activities of ACC and MCD are regulated by phosphorylation by AMP-activated protein kinase (AMPK). AMPK is a serine/threonine protein kinase

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 ($\alpha 1$ or $\alpha 2$) and two regulatory subunits ($\beta 1$ or $\beta 2$ and $\gamma 1$ or $\gamma 2$ or $\gamma 3$), 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 (PP2C α), and cell-death-inducing-like-effector A (CIDEA), as well as cellular AMP concentration.

composed of a catalytic subunit ($\alpha 1$ or $\alpha 2$) and two regulatory subunits ($\beta 1$ or $\beta 2$ and $\gamma 1$ or $\gamma 2$ or $\gamma 3$). 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.* 2010a). Transforming growth factor- β -activated kinase (TAK1; Xie *et al.* 2006) also activates AMPK, and kinase suppressor of Ras (KSR2) interacts with the $\alpha 1$ subunit of AMPK, modulating its activity (Costanzo-Garvey *et al.* 2009). The current data also point out that protein phosphatase 2C α (PP2C α) inactivates AMPK by dephosphorylation (Steinberg *et al.* 2006a, Martínez de Morentin *et al.* 2010a). 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.* 2010a). 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.* 2010a). 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.* 2008a,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, 2008a,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, 2008a, 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, 2008a, 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.* 2008a, 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.* 2008a, Vázquez *et al.* 2008, Martínez de Morentin *et al.* 2010a). 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.* 2008a, 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

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

	Hypothalamic AMPK function	Agpr 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 <i>et al.</i> 2004, Ropelle <i>et al.</i> 2008a) ↓ (Cesquini <i>et al.</i> 2008) ↓ (Steinberg <i>et al.</i> 2006b)	– – ↔ (Ziotopoulou <i>et al.</i> 2000)	– ↓ (Stoppa <i>et al.</i> 2008) ↓ (Xu <i>et al.</i> 1998)	– – ↑ (Ambati <i>et al.</i> 2007)	– ↑ (Stoppa <i>et al.</i> 2008) ↑ (Ambati <i>et al.</i> 2007)	↓ (Kim <i>et al.</i> 2004, Ropelle <i>et al.</i> 2008a) ↓ (Cesquini <i>et al.</i> 2008, Stoppa <i>et al.</i> 2008) ↓ (Xu <i>et al.</i> 1998, Ziotopoulou <i>et al.</i> 2000, Steinberg <i>et al.</i> 2006b, Ambati <i>et al.</i> 2007)
Feeding	↓ (Minokoshi <i>et al.</i> 2004, López <i>et al.</i> 2008a)	↓ (Swart <i>et al.</i> 2002, Minokoshi <i>et al.</i> 2004, López <i>et al.</i> 2008a)	↓ (Sanacora <i>et al.</i> 1990, Kalra <i>et al.</i> 1991, Swart <i>et al.</i> 2002, Minokoshi <i>et al.</i> 2004, López <i>et al.</i> 2008a)	↑ (Kristensen <i>et al.</i> 1997, 1998, Minokoshi <i>et al.</i> 2004, López <i>et al.</i> 2008a)	↑ (Schwartz <i>et al.</i> 1997, Swart <i>et al.</i> 2002, Minokoshi <i>et al.</i> 1998, López <i>et al.</i> 2008a)	↓ (Sanacora <i>et al.</i> 1990, Kalra <i>et al.</i> 1991, Kristensen <i>et al.</i> 1998, Swart <i>et al.</i> 2002, Minokoshi <i>et al.</i> 2004, López <i>et al.</i> 2006, 2008a) ↓ (Scroochi <i>et al.</i> 1996, Seo <i>et al.</i> 2008) ↓ (Minokoshi <i>et al.</i> 2004, McCrimmon <i>et al.</i> 2004, 2006, Han <i>et al.</i> 2005, Wolfgang <i>et al.</i> 2007, Cha <i>et al.</i> 2008, Lane & Cha 2009)
GLP-1	↓ (Seo <i>et al.</i> 2008)	↓ (Seo <i>et al.</i> 2008)	↓ (Seo <i>et al.</i> 2008)	↑ (Seo <i>et al.</i> 2008)	↑ (Seo <i>et al.</i> 2008)	↓ (Ropelle <i>et al.</i> 2008b)
Glucose	↓ (McCrimmon <i>et al.</i> 2004, 2006, Minokoshi <i>et al.</i> 2004, Chang <i>et al.</i> 2005, Han <i>et al.</i> 2005, Wolfgang <i>et al.</i> 2007, Cha <i>et al.</i> 2008, Lane & Cha 2009) ↓ (Ropelle <i>et al.</i> 2008b)	↓ (Chang <i>et al.</i> 2005, Lee <i>et al.</i> 2005, Cha <i>et al.</i> 2008)	↓ (Chang <i>et al.</i> 2005, Cha <i>et al.</i> 2008)	↑ (Cha <i>et al.</i> 2008)	↑ (Cha <i>et al.</i> 2008)	↓ (Woods <i>et al.</i> 1979, Ikeda <i>et al.</i> 1986, Schwartz <i>et al.</i> 1992, Minokoshi <i>et al.</i> 2004, Namkoong <i>et al.</i> 2005) ↓ (Ropelle <i>et al.</i> 2008b)
High-protein diet/ aminoacids Insulin	↓ (Minokoshi <i>et al.</i> 2004, Namkoong <i>et al.</i> 2005) ↓ (Cha & Lane 2009) ↓ (Minokoshi <i>et al.</i> 2004, Gao <i>et al.</i> 2007)	↓ (Dunbar <i>et al.</i> 2005, Konner <i>et al.</i> 2007) ↔ (Fekete <i>et al.</i> 2006)	↓ (Schwartz <i>et al.</i> 1991, 1992, Sipols <i>et al.</i> 1995, Fekete <i>et al.</i> 2006)	↔ (Fekete <i>et al.</i> 2006)	↑ (Kim <i>et al.</i> 1999, Benoit <i>et al.</i> 2002, Fekete <i>et al.</i> 2006)	↓ (Woods <i>et al.</i> 1979, Ikeda <i>et al.</i> 1986, Schwartz <i>et al.</i> 1992, Minokoshi <i>et al.</i> 2004, Namkoong <i>et al.</i> 2005) ↓ (Cha & Lane 2009) ↓ (Schwartz <i>et al.</i> 1997, 1998, Fekete <i>et al.</i> 2006)
Lactate Leptin	↓ (Cha & Lane 2009) ↓ (Minokoshi <i>et al.</i> 2004, Gao <i>et al.</i> 2007)	↓ (Cha & Lane 2009) ↓ (Ahima & Flier 2000, Swart <i>et al.</i> 2002, Fekete <i>et al.</i> 2006)	↓ (Cha & Lane 2009) ↓ (Ahima & Flier 2000, Swart <i>et al.</i> 2002, Fekete <i>et al.</i> 2006)	↑ (Cha & Lane 2009) ↑ (Kristensen <i>et al.</i> 1998, Fekete <i>et al.</i> 2006)	↑ (Cha & Lane 2009) ↑ (Schwartz <i>et al.</i> 1997, Elias <i>et al.</i> 1999, Swart <i>et al.</i> 2002, Fekete <i>et al.</i> 2006)	↓ (Kim <i>et al.</i> 2004, Ropelle <i>et al.</i> 2008a) ↓ (Cesquini <i>et al.</i> 2008, Stoppa <i>et al.</i> 2008) ↓ (Xu <i>et al.</i> 1998, Ziotopoulou <i>et al.</i> 2000, Steinberg <i>et al.</i> 2006b, Ambati <i>et al.</i> 2007) ↓ (Sanacora <i>et al.</i> 1990, Kalra <i>et al.</i> 1991, Kristensen <i>et al.</i> 1998, Swart <i>et al.</i> 2002, Minokoshi <i>et al.</i> 2004, López <i>et al.</i> 2006, 2008a) ↓ (Scroochi <i>et al.</i> 1996, Seo <i>et al.</i> 2008) ↓ (Minokoshi <i>et al.</i> 2004, McCrimmon <i>et al.</i> 2004, 2006, Han <i>et al.</i> 2005, Wolfgang <i>et al.</i> 2007, Cha <i>et al.</i> 2008, Lane & Cha 2009) ↓ (Ropelle <i>et al.</i> 2008b)

(continued)

Table 1 Continued

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
MTII ↓ (Minokoshi <i>et al.</i> 2004)	↓ (Marsh <i>et al.</i> 1999, Hagan <i>et al.</i> 2000, Haskell-Luevano & Monck 2001, Haskell-Luevano <i>et al.</i> 2001, Trivedi <i>et al.</i> 2003)	↓ (Fan <i>et al.</i> 1997, Murphy <i>et al.</i> 1998, Raposinho <i>et al.</i> 2003)	–	↑ (Marsh <i>et al.</i> 1999, Hagan <i>et al.</i> 2000, Haskell-Luevano & Monck 2001, Haskell-Luevano <i>et al.</i> 2001, Trivedi <i>et al.</i> 2003)	↓ (Fan <i>et al.</i> 1997, Murphy <i>et al.</i> 1998, Marsh <i>et al.</i> 1999, Hagan <i>et al.</i> 2000, Haskell-Luevano & Monck 2001, Raposinho <i>et al.</i> 2003, Trivedi <i>et al.</i> 2003, Minokoshi <i>et al.</i> 2004, Nogueiras <i>et al.</i> 2007) ↓ (Tovar <i>et al.</i> 2005, Vázquez <i>et al.</i> 2008)
RSTN Orexigenic signals Adiponectin	↑ (Vázquez <i>et al.</i> 2008)	↓ (Vázquez <i>et al.</i> 2008)	↑ (Vázquez <i>et al.</i> 2008)	↔ (Vázquez <i>et al.</i> 2008)	↑ (Kubota <i>et al.</i> 2007)
AGRP	↑ (Kubota <i>et al.</i> 2007, Guillod-Maximin <i>et al.</i> 2009, Wen <i>et al.</i> 2010) ↑ (Minokoshi <i>et al.</i> 2004)	– ↓ (Korner <i>et al.</i> 2003)	↑ (Zheng <i>et al.</i> 2002) ↔ (Zheng <i>et al.</i> 2002, Korner <i>et al.</i> 2003)	↓ (Ollmann <i>et al.</i> 1997, Wilson <i>et al.</i> 1999, Marsh <i>et al.</i> 1999, Hagan <i>et al.</i> 2000, Haskell-Luevano & Monck 2001, Haskell-Luevano <i>et al.</i> 2001)	↑ (Ollmann <i>et al.</i> 1997, Marsh <i>et al.</i> 1999, Wilson <i>et al.</i> 1999, Hagan <i>et al.</i> 2000, Haskell-Luevano & Monck 2001, Haskell-Luevano <i>et al.</i> 2001, Zheng <i>et al.</i> 2002, Korner <i>et al.</i> 2003, Minokoshi <i>et al.</i> 2004, López <i>et al.</i> 2008a)
Cannabinoids	↑ (Kola <i>et al.</i> 2005, 2008)	↑ (Gamber <i>et al.</i> 2005)	↓ (Cota <i>et al.</i> 2003, Osei-Hyiaman <i>et al.</i> 2005)	↓ (Verty <i>et al.</i> 2004, Hentges <i>et al.</i> 2005, Nguyen & Wagner 2006)	↑ (Cota <i>et al.</i> 2003, Gamber <i>et al.</i> 2005, Kola <i>et al.</i> 2005, 2008, Osei-Hyiaman <i>et al.</i> 2005, Nogueiras <i>et al.</i> 2008b)
Fasting	↑ (Minokoshi <i>et al.</i> 2004, López <i>et al.</i> 2008a)	↑ (Swart <i>et al.</i> 2002, Minokoshi <i>et al.</i> 2004, López <i>et al.</i> 2008a)	↑ (Sanacora <i>et al.</i> 1990, Kalra <i>et al.</i> 1991, Swart <i>et al.</i> 2002, Minokoshi <i>et al.</i> 2004, López <i>et al.</i> 2008a)	↓ (Kristensen <i>et al.</i> 1998, Li <i>et al.</i> 2002, Minokoshi <i>et al.</i> 2004, López <i>et al.</i> 2008a)	↑ (Sanacora <i>et al.</i> 1990, Kalra <i>et al.</i> 1991, Schwartz <i>et al.</i> 1997, Kristensen <i>et al.</i> 1998, Swart <i>et al.</i> 2002, Minokoshi <i>et al.</i> 2004, López <i>et al.</i> 2006, 2008a)

(continued)

Table 1 Continued

	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 <i>et al.</i> 2004, Kola <i>et al.</i> 2005, 2008, Andrews <i>et al.</i> 2008, López <i>et al.</i> 2008a, Lage <i>et al.</i> 2010, Sangiao-Alvarellos <i>et al.</i> 2010)	↑ (Kamegai <i>et al.</i> 2000, 2001, Nakazato <i>et al.</i> 2001, Seoane <i>et al.</i> 2003, Andrews <i>et al.</i> 2008, López <i>et al.</i> 2008a, Lage <i>et al.</i> 2010, Sangiao-Alvarellos <i>et al.</i> 2010)	↑ (Nakazato <i>et al.</i> 2001, Andrews <i>et al.</i> 2008, López <i>et al.</i> 2008a, Lage <i>et al.</i> 2010, Sangiao-Alvarellos <i>et al.</i> 2010)	↔ (Wren <i>et al.</i> 2000, Hu <i>et al.</i> 2005)	↓ (Hu <i>et al.</i> 2005)	↑ (Tschop <i>et al.</i> 2000, Nakazato <i>et al.</i> 2001, Wren <i>et al.</i> 2001b, Seoane <i>et al.</i> 2003, Andersson <i>et al.</i> 2004, Hu <i>et al.</i> 2005, Kola <i>et al.</i> 2005, 2008, Andrews <i>et al.</i> 2008, López <i>et al.</i> 2008a, Lage <i>et al.</i> 2010, Sangiao-Alvarellos <i>et al.</i> 2010)
Glucocorticoids	↑ (Shimizu <i>et al.</i> 2008)	↑ (Savontaus <i>et al.</i> 2002, Coll <i>et al.</i> 2005, Shimizu <i>et al.</i> 2008)	↑ (Shimizu <i>et al.</i> 2008)	↑ (Savontaus <i>et al.</i> 2002, Vrang <i>et al.</i> 2003, Germano <i>et al.</i> 2007)	↓ (Arvaniti <i>et al.</i> 2001)	↑ (Arvaniti <i>et al.</i> 2001, Savontaus <i>et al.</i> 2002, Vrang <i>et al.</i> 2003, Coll <i>et al.</i> 2005, Germano <i>et al.</i> 2007, Shimizu <i>et al.</i> 2008)
Glucose deprivation	↑ (McCrimmon <i>et al.</i> 2004, 2006, Han <i>et al.</i> 2005, Alquier <i>et al.</i> 2007, Wolfgang <i>et al.</i> 2007, Cotero & Routh 2009, Fan <i>et al.</i> 2009)	↑ (Hidaka <i>et al.</i> 2001, Lee <i>et al.</i> 2005)	↑ (Hidaka <i>et al.</i> 2001, Sindelar <i>et al.</i> 2002, Tkacs & Levin 2004, Briski & Parihar 2008, Watanabe <i>et al.</i> 2008)	↓ (Nedungadi & Briski 2007)	↓ (Tkacs <i>et al.</i> 2000, Nedungadi & Briski 2007)	↑ (Dryden <i>et al.</i> 1998, Hidaka <i>et al.</i> 2001, Sindelar <i>et al.</i> 2002, 2004, McCrimmon <i>et al.</i> 2004, 2006, Han <i>et al.</i> 2005, Wolfgang <i>et al.</i> 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.

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.* 2008a), 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.* 2006b, 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.* 2008a, 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.

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.* 2008a,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.* 2008a,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.* 2008a,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.* 2008a). 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.* 2008a). 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, 2008b). 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.* 2008a,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, Willeesen *et al.* 1999, Mitchell *et al.* 2001, Nogueiras *et al.* 2004, Smith 2005, Zigman *et al.* 2006, López *et al.*

2008a) 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.* 2008a,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 (Tschoop *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.* 2008a,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 (Tschoop *et al.* 2000, Wren *et al.* 2000, Nakazato *et al.* 2001, Wren *et al.* 2001b), 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.* 2008a,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.* 2010b), 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.* 2008a, 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), ACC α , FAS, and stearyl-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

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.

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 from 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.* 2005b, Buettner *et al.* 2006, 2008), insulin (Obici *et al.* 2002a,b, Pocai *et al.* 2005a, 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).

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 ACC α , 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.* 2008a,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.* 2008a,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.* 2008a, 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

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007–2013) under grant agreements no. 245009 (R N, C D, and M L) and no. 018734 (A V P), Xunta de Galicia (F C: PS07/12; C D: PGIDIT06PXIB208063PR; M L: 10PXIB208164PR), Fondo Investigaciones Sanitarias (F C: PI051024 and PI070413; M L: PS09/01880), Ministerio de Educacion y Ciencia (R N: RyC-2008-02219; C D: BFU2008; M L: RyC-2007-00211), Medical Research Council (A V P) and Wellcome Trust (A V P). CIBER de Fisiopatología de la Obesidad y Nutrición is an initiative of ISCIII.

References

- Ahima RS 2000 Leptin and the neuroendocrinology of fasting. *Frontiers of Hormone Research* **26** 42–56. (doi:10.1159/000061014)
- Ahima RS & Flier JS 2000 Leptin. *Annual Review of Physiology* **62** 413–437. (doi:10.1146/annurev.physiol.62.1.413)
- Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E & Flier JS 1996 Role of leptin in the neuroendocrine response to fasting. *Nature* **382** 250–252. (doi:10.1038/382250a0)
- Alquier T, Kawashima J, Tsuji Y & Kahn BB 2007 Role of hypothalamic adenosine 5'-monophosphate-activated protein kinase in the impaired counterregulatory response induced by repetitive neuroglucopenia. *Endocrinology* **148** 1367–1375. (doi:10.1210/en.2006-1039)
- Ambati S, Duan J, Duff E, Choi YH, Hartzell DL, Della-Fera MA & Baile CA 2007 Gene expression in arcuate nucleus-median eminence of rats treated with leptin or ciliary neurotrophic factor. *Biofactors* **31** 133–144. (doi:10.1002/biof.5520310204)
- Anderson KA, Ribar TJ, Lin F, Noeldner PK, Green MF, Muehlbauer MJ, Witters LA, Kemp BE & Means AR 2008 Hypothalamic CaMKK2 contributes to the regulation of energy balance. *Cell Metabolism* **7** 377–388. (doi:10.1016/j.cmet.2008.02.011)
- Andersson U, Filipsson K, Abbott CR, Woods A, Smith K, Bloom SR, Carling D & Small CJ 2004 AMP-activated protein kinase plays a role in the control of food intake. *Journal of Biological Chemistry* **279** 12005–12008. (doi:10.1074/jbc.C300557200)
- Andrews ZB, Liu ZW, Wallingford N, Erion DM, Borok E, Friedman JM, Tschop MH, Shanabrough M, Cline G, Shulman GI *et al.* 2008 UCP2 mediates ghrelin's action on NPY/AgRP neurons by lowering free radicals. *Nature* **454** 846–851. (doi:10.1038/nature07181)
- Andrews ZB, Erion DM, Beiler R, Choi CS, Shulman GI & Horvath TL 2010 Uncoupling protein-2 decreases the lipogenic actions of ghrelin. *Endocrinology* **151** 2078–2086. (doi:10.1210/en.2009-0850)
- Arvaniti K, Huang Q & Richard D 2001 Effects of leptin and corticosterone on the expression of corticotropin-releasing hormone, agouti-related protein, and proopiomelanocortin in the brain of ob/ob mouse. *Neuroendocrinology* **73** 227–236. (doi:10.1159/000054639)
- Belgardt BF, Husch A, Rother E, Ernst MB, Wunderlich FT, Hampel B, Klockner T, Alessi D, Kloppenburg P & Bruning JC 2008 PDK1 deficiency in POMC-expressing cells reveals FOXO1-dependent and -independent pathways in control of energy homeostasis and stress response. *Cell Metabolism* **7** 291–301. (doi:10.1016/j.cmet.2008.01.006)
- Bennett PA, Thomas GB, Howard AD, Feighner SD, Van Der Ploeg LH, Smith RG & Robinson IC 1997 Hypothalamic growth hormone secretagogue-receptor (GHS-R) expression is regulated by growth hormone in the rat. *Endocrinology* **138** 4552–4557. (doi:10.1210/en.138.11.4552)
- Benoit SC, Air EL, Coolen LM, Strauss R, Jackman A, Clegg DJ, Seeley RJ & Woods SC 2002 The catabolic action of insulin in the brain is mediated by melanocortins. *Journal of Neuroscience* **22** 9048–9052.
- Bittencourt JC, Presse F, Arias C, Peto C, Vaughan J, Nahon JL, Vale W & Sawchenko PE 1992 The melanin-concentrating hormone system of the rat brain: an immuno- and hybridization histochemical characterization. *Journal of Comparative Neurology* **319** 218–245. (doi:10.1002/cne.903190204)
- Briski KP & Parihar M 2008 Effects of orchidectomy on adaptation of arcuate neuropeptide Y, proopiomelanocortin, and cocaine- and amphetamine-related transcript gene profiles to recurring insulin-induced hypoglycemia in the male rat. *Neuropeptides* **42** 585–591. (doi:10.1016/j.npep.2008.09.001)
- Broberger C, de Lecea L, Sutcliffe JG & Hokfelt T 1998 Hypocretin/orexin- and melanin-concentrating hormone-expressing cells form distinct populations in the rodent lateral hypothalamus: relationship to the neuropeptide Y and agouti gene-related protein systems. *Journal of Comparative Neurology* **402** 460–474. (doi:10.1002/(SICI)1096-9861(19981228)402:4<460::AID-CNE3>3.0.CO;2-S)
- Buettner C, Pocai A, Muse ED, Eigen AM, Myers MG Jr & Rossetti L 2006 Critical role of STAT3 in leptin's metabolic actions. *Cell Metabolism* **4** 49–60. (doi:10.1016/j.cmet.2006.04.014)
- Buettner C, Muse ED, Cheng A, Chen L, Scherer T, Pocai A, Su K, Cheng B, Li X, Harvey-White J *et al.* 2008 Leptin controls adipose tissue lipogenesis via central, STAT3-independent mechanisms. *Nature Medicine* **14** 667–675. (doi:10.1038/nm1775)
- Carling D, Sanders MJ & Woods A 2008 The regulation of AMP-activated protein kinase by upstream kinases. *International Journal of Obesity* **32** (Supplement 4) S55–S59. (doi:10.1038/ijo.2008.124)
- Cesquini M, Stoppa GR, Prada PO, Torsoni AS, Romanatto T, Souza A, Saad MJ, Velloso LA & Torsoni MA 2008 Citrate diminishes hypothalamic acetyl-CoA carboxylase phosphorylation and modulates satiety signals and hepatic mechanisms involved in glucose homeostasis in rats. *Life Sciences* **82** 1262–1271. (doi:10.1016/j.lfs.2008.04.015)

- Cha SH & Lane MD 2009 Central lactate metabolism suppresses food intake via the hypothalamic AMP kinase/malonyl-CoA signaling pathway. *Biochemical and Biophysical Research Communications* **386** 212–216. (doi:10.1016/j.bbrc.2009.06.017)
- Cha SH, Wolfgang M, Tokutake Y, Chohnan S & Lane MD 2008 Differential effects of central fructose and glucose on hypothalamic malonyl-CoA and food intake. *PNAS* **105** 16871–16875. (doi:10.1073/pnas.0809255105)
- Chakravarthy MV, Zhu Y, López M, Yin L, Wozniak DW, Coleman T, Hu Z, Wolfgang M, Vidal-Puig A, Lane MD *et al.* 2007 Brain fatty acid synthase activates PPAR- α to maintain energy homeostasis. *Journal of Clinical Investigation* **117** 2539–2552. (doi:10.1172/JCI31183)
- Chang GQ, Karatayev O, Daydova Z, Wortley K & Leibowitz SF 2005 Glucose injection reduces neuropeptide Y and agouti-related protein expression in the arcuate nucleus: a possible physiological role in eating behavior. *Brain Research. Molecular Brain Research* **135** 69–80. (doi:10.1016/j.molbrainres.2004.12.017)
- Chen HY, Trumbauer ME, Chen AS, Weingarth DT, Adams JR, Frazier EG, Shen Z, Marsh DJ, Feighner SD, Guan XM *et al.* 2004 Orexigenic action of peripheral ghrelin is mediated by neuropeptide Y (NPY) and agouti-related protein (AgRP). *Endocrinology* **145** 2607–2612. (doi:10.1210/en.2003-1596)
- Coll AP, Challis BG, López M, Piper S, Yeo GS & O'Rahilly S 2005 Proopiomelanocortin-deficient mice are hypersensitive to the adverse metabolic effects of glucocorticoids. *Diabetes* **54** 2269–2276. (doi:10.2337/diabetes.54.8.2269)
- Costanzo-Garvey DL, Pfluger PT, Dougherty MK, Stock JL, Boehm M, Chaika O, Fernandez MR, Fisher K, Kortum RL, Hong EG *et al.* 2009 KSR2 is an essential regulator of AMP kinase, energy expenditure, and insulin sensitivity. *Cell Metabolism* **10** 366–378. (doi:10.1016/j.cmet.2009.09.010)
- Cota D, Marsicano G, Tschop M, Grubler Y, Flachskamm C, Schubert M, Auer D, Yassouridis A, Thone-Reineke C, Ortman S *et al.* 2003 The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. *Journal of Clinical Investigation* **112** 423–431. (doi:10.1172/JCI200317725)
- Cotero VE & Routh VH 2009 Insulin blunts the response of glucose-excited neurons in the ventrolateral-ventromedial hypothalamic nucleus to decreased glucose. *American Journal of Physiology. Endocrinology and Metabolism* **296** E1101–E1109. (doi:10.1152/ajpendo.90932.2008)
- Cowley MA, Smith RG, Diano S, Tschop M, Pronchuk N, Grove KL, Strassburger CJ, Biddlingmaier M, Esterman M, Heiman ML *et al.* 2003 The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. *Neuron* **37** 649–661. (doi:10.1016/S0896-6273(03)00063-1)
- Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE & Weigle DS 2001 A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* **50** 1714–1719. (doi:10.2337/diabetes.50.8.1714)
- Cummings DE, Clement K, Purnell JQ, Vaisse C, Foster KE, Frayo RS, Schwartz MW, Basdevant A & Weigle DS 2002 Elevated plasma ghrelin levels in Prader–Willi syndrome. *Nature Medicine* **8** 643–644. (doi:10.1038/nm0702-643)
- Dai Y, Wolfgang MJ, Cha SH & Lane MD 2007 Localization and effect of ectopic expression of CPT1c in CNS feeding centers. *Biochemical and Biophysical Research Communications* **359** 469–474. (doi:10.1016/j.bbrc.2007.05.161)
- Dowell P, Hu Z & Lane MD 2005 Monitoring energy balance: metabolites of fatty acid synthesis as hypothalamic sensors. *Annual Review of Biochemistry* **74** 515–534. (doi:10.1146/annurev.biochem.73.011303.074027)
- Drazen DL, Vahl TP, D'Alessio DA, Seeley RJ & Woods SC 2006 Effects of a fixed meal pattern on ghrelin secretion: evidence for a learned response independent of nutrient status. *Endocrinology* **147** 23–30. (doi:10.1210/en.2005-0973)
- Dryden S, Pickavance L, Henderson L & Williams G 1998 Hyperphagia induced by hypoglycemia in rats is independent of leptin and hypothalamic neuropeptide Y (NPY). *Peptides* **19** 1549–1555. (doi:10.1016/S0196-9781(98)00106-5)
- Dunbar J, Lapanowski K, Barnes M & Rafols J 2005 Hypothalamic agouti-related protein immunoreactivity in food-restricted, obese, and insulin-treated animals: evidence for glia cell localization. *Experimental Neurology* **191** 184–192. (doi:10.1016/j.expneurol.2004.09.002)
- Egecioglu E, Bjursell M, Ljungberg A, Dickson SL, Kopchick JJ, Bergstrom G, Svensson L, Oscarsson J, Tornell J & Bohllooly Y 2006 Growth hormone receptor deficiency results in blunted ghrelin feeding response, obesity, and hypolipidemia in mice. *American Journal of Physiology. Endocrinology and Metabolism* **290** E317–E325. (doi:10.1152/ajpendo.00181.2005)
- Elias CF, Saper CB, Maratos-Flier E, Tritos NA, Lee C, Kelly J, Tatro JB, Hoffman GE, Ollmann MM, Barsh GS *et al.* 1998 Chemically defined projections linking the mediobasal hypothalamus and the lateral hypothalamic area. *Journal of Comparative Neurology* **402** 442–459. (doi:10.1002/(SICI)1096-9861(19981228)402:4<442::AID-CNE2>3.0.CO;2-R)
- Elias CF, Aschkenasi C, Lee C, Kelly J, Ahima RS, Bjorbaek C, Flier JS, Saper CB & Elmquist JK 1999 Leptin differentially regulates NPY and POMC neurons projecting to the lateral hypothalamic area. *Neuron* **23** 775–786. (doi:10.1016/S0896-6273(01)80035-0)
- Ernst MB, Wunderlich CM, Hess S, Paehler M, Mesaros A, Korolov SB, Kleinriders A, Husch A, Munzberg H, Hampel B *et al.* 2009 Enhanced Stat3 activation in POMC neurons provokes negative feedback inhibition of leptin and insulin signaling in obesity. *Journal of Neuroscience* **29** 11582–11593. (doi:10.1523/JNEUROSCI.5712-08.2009)
- Fan W, Boston BA, Kesterson RA, Hruby VJ & Cone RD 1997 Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. *Nature* **385** 165–168. (doi:10.1038/385165a0)
- Fan X, Ding Y, Brown S, Zhou L, Shaw M, Vella MC, Cheng H, McNay EC, Sherwin RS & McCrimmon RJ 2009 Hypothalamic AMP-activated protein kinase activation with AICAR amplifies counter-regulatory responses to hypoglycemia in a rodent model of type 1 diabetes. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* **296** R1702–R1708. (doi:10.1152/ajpregu.90600.2008)
- Fekete C, Singru PS, Sanchez E, Sarkar S, Christoffolete MA, Riberio RS, Rand WM, Emerson CH, Bianco AC & Lechan RM 2006 Differential effects of central leptin, insulin, or glucose administration during fasting on the hypothalamic–pituitary–thyroid axis and feeding-related neurons in the arcuate nucleus. *Endocrinology* **147** 520–529. (doi:10.1210/en.2005-0956)
- Foster-Schubert KE & Cummings DE 2006 Emerging therapeutic strategies for obesity. *Endocrine Reviews* **27** 779–793. (doi:10.1210/er.2006-0041)
- Gamber KM, Macarthur H & Westfall TC 2005 Cannabinoids augment the release of neuropeptide Y in the rat hypothalamus. *Neuropharmacology* **49** 646–652. (doi:10.1016/j.neuropharm.2005.04.017)
- Gao S, Kinzig KP, Aja S, Scott KA, Keung W, Kelly S, Strynadka K, Chohnan S, Smith WW, Tamashiro KL *et al.* 2007 Leptin activates hypothalamic acetyl-CoA carboxylase to inhibit food intake. *PNAS* **104** 17358–17363. (doi:10.1073/pnas.0708385104)
- Garcia A, Alvarez CV, Smith RG & Dieguez C 2001 Regulation of pit-1 expression by ghrelin and ghrelin-6 through the GH secretagogue receptor. *Molecular Endocrinology* **15** 1484–1495. (doi:10.1210/me.15.9.1484)
- Germano CM, de Castro M, Rorato R, Laguna MT, Antunes-Rodrigues J, Elias CF & Elias LL 2007 Time course effects of adrenalectomy and food intake on cocaine- and amphetamine-regulated transcript expression in the hypothalamus. *Brain Research* **1166** 55–64. (doi:10.1016/j.brainres.2007.05.077)

- Gonzalez CR, Vazquez MJ, Lopez M & Dieguez C 2008 Influence of chronic undernutrition and leptin on GOAT mRNA levels in rat stomach mucosa. *Journal of Molecular Endocrinology* **41** 415–421. (doi:10.1677/JME-08-0102)
- Guan XM, Yu H, Palyha OC, McKee KK, Feighner SD, Sirinathsinghji DJ, Smith RG, Van Der Ploeg LH & Howard AD 1997 Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. *Brain Research. Molecular Brain Research* **48** 23–29. (doi:10.1016/S0169-328X(97)00071-5)
- Guillod-Maximin E, Roy AF, Vacher CM, Aubourg A, Bailleux V, Lorsignol A, Penicaud L, Parquet M & Taouis M 2009 Adiponectin receptors are expressed in hypothalamus and colocalized with proopiomelanocortin and neuropeptide Y in rodent arcuate neurons. *Journal of Endocrinology* **200** 93–105. (doi:10.1677/JOE-08-0348)
- Gutierrez JA, Solenberg PJ, Perkins DR, Willency JA, Knierman MD, Jin Z, Witcher DR, Luo S, Onyia JE & Hale JE 2008 Ghrelin octanoylation mediated by an orphan lipid transferase. *PNAS* **105** 6320–6325. (doi:10.1073/pnas.0800708105)
- Hagan MM, Rushing PA, Pritchard LM, Schwartz MW, Strack AM, Van Der Ploeg LH, Woods SC & Seeley RJ 2000 Long-term orexigenic effects of AgRP-(83–132) involve mechanisms other than melanocortin receptor blockade. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* **279** R47–R52.
- Hahn TM, Breininger JF, Baskin DG & Schwartz MW 1998 Coexpression of AgRP and NPY in fasting-activated hypothalamic neurons. *Nature Neuroscience* **1** 271–272. (doi:10.1038/1082)
- Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK & Friedman JM 1995 Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* **269** 543–546. (doi:10.1126/science.7624777)
- Han SM, Namkoong C, Jang PG, Park IS, Hong SW, Katakami H, Chun S, Kim SW, Park JY, Lee KU *et al.* 2005 Hypothalamic AMP-activated protein kinase mediates counter-regulatory responses to hypoglycaemia in rats. *Diabetologia* **48** 2170–2178. (doi:10.1007/s00125-005-1913-1)
- Haskell-Luevano C & Monck EK 2001 Agouti-related protein functions as an inverse agonist at a constitutively active brain melanocortin-4 receptor. *Regulatory Peptides* **99** 1–7. (doi:10.1016/S0167-0115(01)00234-8)
- Haskell-Luevano C, Cone RD, Monck EK & Wan YP 2001 Structure activity studies of the melanocortin-4 receptor by *in vitro* mutagenesis: identification of agouti-related protein (AGRP), melanocortin agonist and synthetic peptide antagonist interaction determinants. *Biochemistry* **40** 6164–6179. (doi:10.1021/bi010025q)
- Hawley SA, Pan DA, Mustard KJ, Ross L, Bain J, Edelman AM, Frenguelli BG & Hardie DG 2005 Calmodulin-dependent protein kinase kinase- β is an alternative upstream kinase for AMP-activated protein kinase. *Cell Metabolism* **2** 9–19. (doi:10.1016/j.cmet.2005.05.009)
- He W, Lam TK, Obici S & Rossetti L 2006 Molecular disruption of hypothalamic nutrient sensing induces obesity. *Nature Neuroscience* **9** 227–233. (doi:10.1038/nm1626)
- Hentges ST, Low MJ & Williams JT 2005 Differential regulation of synaptic inputs by constitutively released endocannabinoids and exogenous cannabinoids. *Journal of Neuroscience* **25** 9746–9751. (doi:10.1523/JNEUROSCI.2769-05.2005)
- Hidaka S, Yoshimatsu H, Kondou S, Oka K, Tsuruta Y, Sakino H, Itateyama E, Noguchi H, Himeno K, Okamoto K *et al.* 2001 Hypoleptinemia, but not hypoinsulinemia, induces hyperphagia in streptozotocin-induced diabetic rats. *Journal of Neurochemistry* **77** 993–1000. (doi:10.1046/j.1471-4159.2001.00317.x)
- Holst B, Cygankiewicz A, Halkjar JT, Ankersen M & Schwartz TW 2003 High constitutive signaling of the ghrelin receptor-identification of a potent inverse agonist. *Molecular Endocrinology* **17** 2201–2210. (doi:10.1210/me.2003-0069)
- Horvath TL, Diano S & van den Pol AN 1999 Synaptic interaction between hypocretin (orexin) and neuropeptide Y cells in the rodent and primate hypothalamus: a novel circuit implicated in metabolic and endocrine regulations. *Journal of Neuroscience* **19** 1072–1087.
- Hosoi T, Sasaki M, Miyahara T, Hashimoto C, Matsuo S, Yoshii M & Ozawa K 2008 Endoplasmic reticulum stress induces leptin resistance. *Molecular Pharmacology* **74** 1610–1619. (doi:10.1124/mol.108.050070)
- Hu Z, Cha SH, Chohann S & Lane MD 2003 Hypothalamic malonyl-CoA as a mediator of feeding behavior. *PNAS* **100** 12624–12629. (doi:10.1073/pnas.1834402100)
- Hu Z, Cha SH, van Haasteren G, Wang J & Lane MD 2005 Effect of centrally administered C75, a fatty acid synthase inhibitor, on ghrelin secretion and its downstream effects. *PNAS* **102** 3972–3977. (doi:10.1073/pnas.0500619102)
- Ikeda H, West DB, Pustek JJ, Figlewicz DP, Greenwood MR, Porte D Jr & Woods SC 1986 Intraventricular insulin reduces food intake and body weight of lean but not obese Zucker rats. *Appetite* **7** 381–386.
- Joseph JW, Koshkin V, Zhang CY, Wang J, Lowell BB, Chan CB & Wheeler MB 2002 Uncoupling protein 2 knockout mice have enhanced insulin secretory capacity after a high-fat diet. *Diabetes* **51** 3211–3219.
- Kahn BB, Alquier T, Carling D & Hardie DG 2005 AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. *Cell Metabolism* **1** 15–25. (doi:10.1016/j.cmet.2004.12.003)
- Kalra SP, Dube MG, Sahu A, Phelps CP & Kalra PS 1991 Neuropeptide Y secretion increases in the paraventricular nucleus in association with increased appetite for food. *PNAS* **88** 10931–10935. (doi:10.1073/pnas.88.23.10931)
- Kamegai J, Tamura H, Shimizu T, Ishii S, Sugihara H & Wakabayashi I 2000 Central effect of ghrelin, an endogenous growth hormone secretagogue, on hypothalamic peptide gene expression. *Endocrinology* **141** 4797–4800. (doi:10.1210/en.141.12.4797)
- Kamegai J, Tamura H, Shimizu T, Ishii S, Sugihara H & Wakabayashi I 2001 Chronic central infusion of ghrelin increases hypothalamic neuropeptide Y and agouti-related protein mRNA levels and body weight in rats. *Diabetes* **50** 2438–2443. (doi:10.2337/diabetes.50.11.2438)
- Kim EM, Grace MK, Welch CC, Billington CJ & Levine AS 1999 STZ-induced diabetes decreases and insulin normalizes POMC mRNA in arcuate nucleus and pituitary in rats. *American Journal of Physiology* **276** R1320–R1326.
- Kim EK, Miller I, Landree LE, Borisov-Rudin FF, Brown P, Tihan T, Townsend CA, Witters LA, Moran TH, Kuhajda FP *et al.* 2002 Expression of FAS within hypothalamic neurons: a model for decreased food intake after C75 treatment. *American Journal of Physiology. Endocrinology and Metabolism* **283** E867–E879.
- Kim MS, Park JY, Namkoong C, Jang PG, Ryu JW, Song HS, Yun JY, Namgoong IS, Ha J, Park IS *et al.* 2004 Anti-obesity effects of α -lipoic acid mediated by suppression of hypothalamic AMP-activated protein kinase. *Nature Medicine* **10** 727–733. (doi:10.1038/nm1061)
- Kim MS, Pak YK, Jang PG, Namkoong C, Choi YS, Won JC, Kim KS, Kim SW, Kim HS, Park JY *et al.* 2006 Role of hypothalamic Foxo1 in the regulation of food intake and energy homeostasis. *Nature Neuroscience* **9** 901–906. (doi:10.1038/nm1731)
- Kirchner H, Gutierrez JA, Solenberg PJ, Pfluger PT, Czyzyk TA, Willency JA, Schurmann A, Joost HG, Jandacek RJ, Hale JE *et al.* 2009 GOAT links dietary lipids with the endocrine control of energy balance. *Nature Medicine* **15** 741–745. (doi:10.1038/nm.1997)
- Kitamura T, Feng Y, Ido KY, Chua SC, Xu AW, Barsh GS, Rossetti L & Accili D 2006 Forkhead protein FoxO1 mediates AgRP-dependent effects of leptin on food intake. *Nature Medicine* **12** 534–540. (doi:10.1038/nm1392)
- Koch L, Wunderlich FT, Seibler J, Konner AC, Hampel B, Irlenbusch S, Brabant G, Kahn CR, Schwenk F & Bruning JC 2008 Central insulin action regulates peripheral glucose and fat metabolism in mice. *Journal of Clinical Investigation* **118** 2132–2147. (doi:10.1172/JCI1073)

- Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H & Kangawa K 1999 Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* **402** 656–660. (doi:10.1038/45230)
- Kola B, Hubina E, Tucci SA, Kirkham TC, Garcia EA, Mitchell SE, Williams LM, Hawley SA, Hardie DG, Grossman AB *et al.* 2005 Cannabinoids and ghrelin have both central and peripheral metabolic and cardiac effects via AMP-activated protein kinase. *Journal of Biological Chemistry* **280** 25196–25201. (doi:10.1074/jbc.C500175200)
- Kola B, Farkas I, Christ-Crain M, Wittmann G, Lolli F, Amin F, Harvey-White J, Liposits Z, Kunos G, Grossman AB *et al.* 2008 The orexigenic effect of ghrelin is mediated through central activation of the endogenous cannabinoid system. *PLoS ONE* **3** e1797. (doi:10.1371/journal.pone.0001797)
- Konner AC, Janoschek R, Plum L, Jordan SD, Rother E, Ma X, Xu C, Enriori P, Hampel B, Barsh GS *et al.* 2007 Insulin action in AgRP-expressing neurons is required for suppression of hepatic glucose production. *Cell Metabolism* **5** 438–449. (doi:10.1016/j.cmet.2007.05.004)
- Korner J, Wissig S, Kim A, Conwell IM & Wardlaw SL 2003 Effects of agouti-related protein on metabolism and hypothalamic neuropeptide gene expression. *Journal of Neuroendocrinology* **15** 1116–1121. (doi:10.1111/j.1365-2826.2003.01113.x)
- Kristensen P, Judge ME, Thim L, Ribel U, Christjansen KN, Wulff BS, Clausen JT, Jensen PB, Madsen OD, Vrang N *et al.* 1998 Hypothalamic CART is a new anorectic peptide regulated by leptin. *Nature* **393** 72–76. (doi:10.1038/29993)
- Kubota N, Yano W, Kubota T, Yamauchi T, Itoh S, Kumagai H, Kozono H, Takamoto I, Okamoto S, Shiuchi T *et al.* 2007 Adiponectin stimulates AMP-activated protein kinase in the hypothalamus and increases food intake. *Cell Metabolism* **6** 55–68. (doi:10.1016/j.cmet.2007.06.003)
- Lage R, Diéguez C, Vidal-Puig A & López M 2008 AMPK: a metabolic gauge regulating whole-body energy homeostasis. *Trends in Molecular Medicine* **14** 539–549. (doi:10.1016/j.molmed.2008.09.007)
- Lage R, Vázquez MJ, Varela L, Saha AK, Vidal-Puig A, Nogueiras R, Diéguez C & López M 2010 Ghrelin effects on neuropeptides in the rat hypothalamus depend on fatty acid metabolism actions on BSX but not on gender. *FASEB Journal* **24** 2670–2679. (doi:10.1096/fj.09-150672)
- Lane MD & Cha SH 2009 Effect of glucose and fructose on food intake via malonyl-CoA signaling in the brain. *Biochemical and Biophysical Research Communications* **382** 1–5. (doi:10.1016/j.bbrc.2009.02.145)
- de Lecea L, Kilduff TS, Peyron C, Gao X, Foye PE, Danielson PE, Fukuhara C, Battenberg EL, Gautvik VT, Bartlett FS *et al.* 1998 The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *PNAS* **95** 322–327. (doi:10.1073/pnas.95.1.322)
- Lee K, Li B, Xi X, Suh Y & Martin RJ 2005 Role of neuronal energy status in the regulation of adenosine 5'-monophosphate-activated protein kinase, orexigenic neuropeptides expression, and feeding behavior. *Endocrinology* **146** 3–10. (doi:10.1210/en.2004-0968)
- Lee GH, Kim HK, Chae SW, Kim DS, Ha KC, Cuddy M, Kress C, Reed JC, Kim HR & Chae HJ 2007 Bax inhibitor-1 regulates endoplasmic reticulum stress-associated reactive oxygen species and heme oxygenase-1 expression. *Journal of Biological Chemistry* **282** 21618–21628. (doi:10.1074/jbc.M700053200)
- van der Lely AJ 2009 Ghrelin and new metabolic frontiers. *Hormone Research* **71** (Supplement 1) 129–133. (doi:10.1159/000178055)
- van der Lely AJ, Tschöp M, Heiman ML & Ghigo E 2004 Biological, physiological, pathophysiological, and pharmacological aspects of ghrelin. *Endocrine Reviews* **25** 426–457. (doi:10.1210/er.2002-0029)
- Li HY, Hwang HW & Hu YH 2002 Functional characterizations of cocaine- and amphetamine-regulated transcript mRNA expression in rat hypothalamus. *Neuroscience Letters* **323** 203–206. (doi:10.1016/S0304-3940(02)00151-9)
- Loftus TM, Jaworsky DE, Frehywot GL, Townsend CA, Ronnett GV, Lane MD & Kuhajda FP 2000 Reduced food intake and body weight in mice treated with fatty acid synthase inhibitors. *Science* **288** 2379–2381. (doi:10.1126/science.288.5475.2379)
- López M, Lelliott CJ, Tovar S, Kimber W, Gallego R, Virtue S, Blount M, Vázquez MJ, Finan N, Powles T *et al.* 2006 Tamoxifen-induced anorexia is associated with fatty acid synthase inhibition in the ventromedial nucleus of the hypothalamus and accumulation of malonyl-CoA. *Diabetes* **55** 1327–1336. (doi:10.2337/db05-1356)
- López M, Lelliott CJ & Vidal-Puig A 2007 Hypothalamic fatty acid metabolism: a housekeeping pathway that regulates food intake. *BioEssays* **29** 248–261. (doi:10.1002/bies.20539)
- López M, Lage R, Saha AK, Pérez-Tilve D, Vázquez MJ, Varela L, Sangiao-Alvarellos S, Tovar S, Raghu K, Rodríguez-Cuenca S *et al.* 2008a Hypothalamic fatty acid metabolism mediates the orexigenic action of ghrelin. *Cell Metabolism* **7** 389–399. (doi:10.1016/j.cmet.2008.03.006)
- López M, Saha AK, Diéguez C & Vidal-Puig A 2008b The AMPK–malonyl-CoA–CPT1 axis in the control of hypothalamic neuronal function – reply. *Cell Metabolism* **8** 176. (doi:10.1016/j.cmet.2008.08.003)
- López M, Varela L, Vázquez MJ, Rodríguez-Cuenca S, González CR, Velagapudi VR, Morgan DA, Schoenmakers E, Agassandian K, Lage R *et al.* 2010 Hypothalamic AMPK and fatty acid metabolism mediate thyroid regulation of energy balance. *Nature Medicine* **16** 1001–1008. (doi:10.1038/nm.2207)
- Maccario M, Grotoli S, Procopio M, Oleandri SE, Rossetto R, Gauna C, Arvat E & Ghigo E 2000 The GH/IGF-I axis in obesity: influence of neuro-endocrine and metabolic factors. *International Journal of Obesity and Related Metabolic Disorders* **24** (Supplement 2) S96–S99.
- Marsh DJ, Hollopeter G, Huszar D, Laufer R, Yagaloff KA, Fisher SL, Burn P & Palmiter RD 1999 Response of melanocortin-4 receptor-deficient mice to anorectic and orexigenic peptides. *Nature Genetics* **21** 119–122. (doi:10.1038/5070)
- Martínez de Morentin PB, González CR & López M 2010a AMP-activated protein kinase: 'a cup of tea' against cholesterol-induced neurotoxicity. *Journal of Pathology* **222** 329–334. (doi:10.1002/path.2778)
- Martínez de Morentin PB, Varela L, Ferno J, Nogueiras R, Diéguez C & López M 2010b Hypothalamic lipotoxicity and the metabolic syndrome. *Biochimica et Biophysica Acta* **1801** 350–361. (doi:10.1016/j.bbalip.2009.09.016)
- Masuda Y, Tanaka T, Inomata N, Ohnuma N, Tanaka S, Itoh Z, Hosoda H, Kojima M & Kangawa K 2000 Ghrelin stimulates gastric acid secretion and motility in rats. *Biochemical and Biophysical Research Communications* **276** 905–908. (doi:10.1006/bbrc.2000.3568)
- McCrimmon RJ, Fan X, Ding Y, Zhu W, Jacob RJ & Sherwin RS 2004 Potential role for AMP-activated protein kinase in hypoglycemia sensing in the ventromedial hypothalamus. *Diabetes* **53** 1953–1958. (doi:10.2337/diabetes.53.8.1953)
- McCrimmon RJ, Fan X, Cheng H, McNay E, Chan O, Shaw M, Ding Y, Zhu W & Sherwin RS 2006 Activation of AMP-activated protein kinase within the ventromedial hypothalamus amplifies counter-regulatory hormone responses in rats with defective counter-regulation. *Diabetes* **55** 1755–1760. (doi:10.2337/db05-1359)
- Medina-Gomez G, Yetukuri L, Velagapudi V, Campbell M, Blount M, Jimenez-Linan M, Ros M, Oresic M & Vidal-Puig A 2009 Adaptation and failure of pancreatic β cells in murine models with different degrees of metabolic syndrome. *Disease Models and Mechanisms* **2** 582–592. (doi:10.1242/dmm.003251)
- Minokoshi Y, Alquier T, Furukawa N, Kim YB, Lee A, Xue B, Mu J, Foufelle F, Ferre P, Birnbaum MJ *et al.* 2004 AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. *Nature* **428** 569–574. (doi:10.1038/nature02440)
- Mitchell V, Bouret S, Beauvillain JC, Schilling A, Perret M, Kordon C & Epelbaum J 2001 Comparative distribution of mRNA encoding the growth hormone secretagogue-receptor (GHS-R) in *Microcebus*

- murinus* (Primate, lemurian) and rat forebrain and pituitary. *Journal of Comparative Neurology* **429** 469–489. (doi:10.1002/1096-9861(20010115)429:3<469::AID-CNE8>3.0.CO;2#)
- de Morentin PB & López M 2010 “Mens sana in corpore sano”: exercise and hypothalamic ER stress. *PLoS Biology* **8** e1000464. (doi:10.1371/journal.pbio.1000464)
- Morrison CD, Xi X, White CL, Ye J & Martin RJ 2007 Amino acids inhibit AgRP gene expression via an mTOR-dependent mechanism. *American Journal of Physiology, Endocrinology and Metabolism* **293** E165–E171. (doi:10.1152/ajpendo.00675.2006)
- Murphy B, Nunes CN, Ronan JJ, Harper CM, Beall MJ, Hanaway M, Fairhurst AM, Van Der Ploeg LH, MacIntyre DE & Mellin TN 1998 Melanocortin mediated inhibition of feeding behavior in rats. *Neuropeptides* **32** 491–497. (doi:10.1016/S0143-4179(98)90077-4)
- Murphy BA, Fakira KA, Song Z, Beuve A & Routh VH 2009 AMP-activated protein kinase and nitric oxide regulate the glucose sensitivity of ventromedial hypothalamic glucose-inhibited neurons. *American Journal of Physiology, Cell Physiology* **297** C750–C758. (doi:10.1152/ajpcell.00127.2009)
- Nahon JL, Presse F, Bittencourt JC, Sawchenko PE & Vale W 1989 The rat melanin-concentrating hormone messenger ribonucleic acid encodes multiple putative neuropeptides coexpressed in the dorsolateral hypothalamus. *Endocrinology* **125** 2056–2065. (doi:10.1210/endo-125-4-2056)
- Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K & Matsukura S 2001 A role for ghrelin in the central regulation of feeding. *Nature* **409** 194–198. (doi:10.1038/35051587)
- Namkoong C, Kim MS, Jang PG, Han SM, Park HS, Koh EH, Lee WJ, Kim JY, Park IS, Park JY *et al.* 2005 Enhanced hypothalamic AMP-activated protein kinase activity contributes to hyperphagia in diabetic rats. *Diabetes* **54** 63–68. (doi:10.2337/diabetes.54.1.63)
- Nedungadi TP & Briski KP 2007 Effects of estradiol on acute and recurrent insulin-induced hypoglycemia-associated patterns of arcuate neuropeptide Y, proopiomelanocortin, and cocaine- and amphetamine-related transcript gene expression in the ovariectomized rat. *Neuroendocrinology* **86** 270–276. (doi:10.1159/000109678)
- Nguyen QH & Wagner EJ 2006 Estrogen differentially modulates the cannabinoid-induced presynaptic inhibition of amino acid neurotransmission in proopiomelanocortin neurons of the arcuate nucleus. *Neuroendocrinology* **84** 123–137. (doi:10.1159/000096996)
- Nogueiras R, Tovar S, Mitchell SE, Rayner DV, Archer ZA, Dieguez C & Williams LM 2004 Regulation of growth hormone secretagogue receptor gene expression in the arcuate nuclei of the rat by leptin and ghrelin. *Diabetes* **53** 2552–2558. (doi:10.2337/diabetes.53.10.2552)
- Nogueiras R, Wiedmer P, Perez-Tilve D, Veyrat-Durebex C, Keogh JM, Sutton GM, Pfluger PT, Castaneda TR, Neschen S, Hofmann SM *et al.* 2007 The central melanocortin system directly controls peripheral lipid metabolism. *Journal of Clinical Investigation* **117** 3475–3488. (doi:10.1172/JCI31743)
- Nogueiras R, López M, Lage R, Pérez-Tilve D, Pfluger P, Mendieta-Zeron H, Sakkou M, Wiedmer P, Benoit S, Datta R *et al.* 2008a Bsx, a novel hypothalamic factor linking feeding with locomotor activity, is regulated by energy availability. *Endocrinology* **149** 3009–3015. (doi:10.1210/en.2007-1684)
- Nogueiras R, Veyrat-Durebex C, Suchanek PM, Klein M, Tschöp J, Caldwell C, Woods SC, Wittmann G, Watanabe M, Liposits Z *et al.* 2008b Peripheral, but not central, CB1 antagonism provides food intake-independent metabolic benefits in diet-induced obese rats. *Diabetes* **57** 2977–2991. (doi:10.2337/db08-0161)
- Nogueiras R, Pérez-Tilve D, Veyrat-Durebex C, Morgan DA, Varela L, Haynes WG, Patterson TJ, Disse E, Pfluger P, López M *et al.* 2009 Direct control of peripheral lipid deposition by CNS GLP-1 receptor signaling is mediated by the sympathetic nervous system and blunted in diet induced obesity. *Journal of Neuroscience* **29** 5916–5925. (doi:10.1523/JNEUROSCI.5977-08.2009)
- Nogueiras R, López M & Diéguez C 2010 Regulation of lipid metabolism by energy availability: a role for the central nervous system. *Obesity Reviews* **11** 185–201. (doi:10.1111/j.1467-789X.2009.00669.x)
- Obici S, Feng Z, Karkanias G, Baskin DG & Rossetti L 2002a Decreasing hypothalamic insulin receptors causes hyperphagia and insulin resistance in rats. *Nature Neuroscience* **5** 566–572. (doi:10.1038/nn0602-861)
- Obici S, Zhang BB, Karkanias G & Rossetti L 2002b Hypothalamic insulin signaling is required for inhibition of glucose production. *Nature Medicine* **8** 1376–1382. (doi:10.1038/nm798)
- Obici S, Feng Z, Arduini A, Conti R & Rossetti L 2003 Inhibition of hypothalamic carnitine palmitoyltransferase-1 decreases food intake and glucose production. *Nature Medicine* **9** 756–761. (doi:10.1038/nm873)
- Ollmann MM, Wilson BD, Yang YK, Kerns JA, Chen Y, Gantz I & Barsh GS 1997 Antagonism of central melanocortin receptors *in vitro* and *in vivo* by agouti-related protein. *Science* **278** 135–138. (doi:10.1126/science.278.5335.135)
- Osei-Hyiaman D, Depetrillo M, Harvey-White J, Bannon AW, Cravatt BF, Kuhar MJ, Mackie K, Palkovits M & Kunos G 2005 Cocaine- and amphetamine-related transcript is involved in the orexigenic effect of endogenous anandamide. *Neuroendocrinology* **81** 273–282. (doi:10.1159/000087925)
- Oyadomari S & Mori M 2004 Roles of CHOP/GADD153 in endoplasmic reticulum stress. *Cell Death and Differentiation* **11** 381–389. (doi:10.1038/sj.cdd.4401373)
- Ozcan L, Ergin AS, Lu A, Chung J, Sarkar S, Nie D, Myers MG Jr & Ozcan U 2009 Endoplasmic reticulum stress plays a central role in development of leptin resistance. *Cell Metabolism* **9** 35–51. (doi:10.1016/j.cmet.2008.12.004)
- Pagotto U, Marsicano G, Cota D, Lutz B & Pasquali R 2006 The emerging role of the endocannabinoid system in endocrine regulation and energy balance. *Endocrine Reviews* **27** 73–100. (doi:10.1210/er.2005-0009)
- Pecqueur C, Bui T, Gelly C, Hauchard J, Barbot C, Bouillaud F, Ricquier D, Miroux B & Thompson CB 2008 Uncoupling protein-2 controls proliferation by promoting fatty acid oxidation and limiting glycolysis-derived pyruvate utilization. *FASEB Journal* **22** 9–18. (doi:10.1096/fj.07-8945com)
- Peino R, Baldelli R, Rodriguez-Garcia J, Rodriguez-Segade S, Kojima M, Kangawa K, Arvat E, Ghigo E, Dieguez C & Casanueva FF 2000 Ghrelin-induced growth hormone secretion in humans. *European Journal of Endocrinology* **143** R11–R14. (doi:10.1530/eje.0.143R011)
- Peyron C, Tighe DK, van den Pol AN, de Lecea L, Heller HC, Sutcliffe JG & Kilduff TS 1998 Neurons containing hypocretin (orexin) project to multiple neuronal systems. *Journal of Neuroscience* **18** 9996–10015.
- Plum L, Belgardt BF & Bruning JC 2006 Central insulin action in energy and glucose homeostasis. *Journal of Clinical Investigation* **116** 1761–1766. (doi:10.1172/JCI29063)
- Plum L, Lin HV, Dutia R, Tanaka J, Aizawa KS, Matsumoto M, Kim AJ, Cawley NX, Paik JH, Loh YP *et al.* 2009 The obesity susceptibility gene Cpe links FoxO1 signaling in hypothalamic pro-opiomelanocortin neurons with regulation of food intake. *Nature Medicine* **15** 1195–1201. (doi:10.1038/nm.2026)
- Pocai A, Lam TK, Gutierrez-Juarez R, Obici S, Schwartz GJ, Bryan J, Aguilar-Bryan L & Rossetti L 2005a Hypothalamic K(ATP) channels control hepatic glucose production. *Nature* **434** 1026–1031. (doi:10.1038/nature03439)
- Pocai A, Morgan K, Buettner C, Gutierrez-Juarez R, Obici S & Rossetti L 2005b Central leptin acutely reverses diet-induced hepatic insulin resistance. *Diabetes* **54** 3182–3189. (doi:10.2337/diabetes.54.11.3182)
- Pocai A, Lam TK, Obici S, Gutierrez-Juarez R, Muse ED, Arduini A & Rossetti L 2006 Restoration of hypothalamic lipid sensing

- normalizes energy and glucose homeostasis in overfed rats. *Journal of Clinical Investigation* **116** 1081–1091. (doi:10.1172/JCI26640)
- Qi J, Gong J, Zhao T, Zhao J, Lam P, Ye J, Li JZ, Wu J, Zhou HM & Li P 2008 Downregulation of AMP-activated protein kinase by Cidea-mediated ubiquitination and degradation in brown adipose tissue. *EMBO Journal* **27** 1537–1548. (doi:10.1038/emboj.2008.92)
- Qu D, Ludwig DS, Gammeltoft S, Piper M, Pellemounter MA, Cullen MJ, Mathes WF, Przypek R, Kanarek R & Maratos-Flier E 1996 A role for melanin-concentrating hormone in the central regulation of feeding behaviour. *Nature* **380** 243–247. (doi:10.1038/380243a0)
- Raposo PD, White RB & Aubert ML 2003 The melanocortin agonist melanotan-II reduces the orexigenic and adipogenic effects of neuropeptide Y (NPY) but does not affect the NPY-driven suppressive effects on the gonadotropic and somatotrophic axes in the male rat. *Journal of Neuroendocrinology* **15** 173–181. (doi:10.1046/j.1365-2826.2003.00962.x)
- Ron D & Habener JF 1992 CHOP, a novel developmentally regulated nuclear protein that dimerizes with transcription factors C/EBP and LAP and functions as a dominant-negative inhibitor of gene transcription. *Genes and Development* **6** 439–453. (doi:10.1101/gad.6.3.439)
- Ropelle ER, Fernandes MF, Flores MB, Ueno M, Rocco S, Marin R, Cintra DE, Velloso LA, Franchini KG, Saad MJ *et al.* 2008a Central exercise action increases the AMPK and mTOR response to leptin. *PLoS ONE* **3** e3856. (doi:10.1371/journal.pone.0003856)
- Ropelle ER, Pauli JR, Fernandes MF, Rocco SA, Marin RM, Morari J, Souza KK, Dias MM, Gomes-Marcondes MC, Gontijo JA *et al.* 2008b A central role for neuronal AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) in high-protein diet-induced weight loss. *Diabetes* **57** 594–605. (doi:10.2337/db07-0573)
- Ropelle ER, Flores MB, Cintra DE, Rocha GZ, Pauli JR, Morari J, de Souza CT, Moraes JC, Prada PO, Guadagnini D *et al.* 2010 IL-6 and IL-10 anti-inflammatory activity links exercise to hypothalamic insulin and leptin sensitivity through IKK β and ER stress inhibition. *PLoS Biology* **8** e1000465. (doi:10.1371/journal.pbio.1000465)
- Ruderman NB, Saha AK & Kraegen EW 2003 Minireview: malonyl CoA, AMP-activated protein kinase, and adiposity. *Endocrinology* **144** 5166–5171. (doi:10.1210/en.2003-0849)
- Sakkou M, Wiedmer P, Anlag K, Hamm A, Seuntjens E, Ettwiller L, Tschoep MH & Treier M 2007 A role for brain-specific homeobox factor bxs in the control of hyperphagia and locomotory behavior. *Cell Metabolism* **5** 450–463. (doi:10.1016/j.cmet.2007.05.007)
- Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S *et al.* 1998 Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* **92** 573–585. (doi:10.1016/S0092-8674(00)80949-6)
- Sanacora G, Kershaw M, Finkelstein JA & White JD 1990 Increased hypothalamic content of preproneuropeptide Y messenger ribonucleic acid in genetically obese Zucker rats and its regulation by food deprivation. *Endocrinology* **127** 730–737. (doi:10.1210/endo-127-2-730)
- Sangiao-Alvarellos S, Vázquez MJ, Varela L, Nogueiras R, Saha AK, Cordido F, López M & Diéguez C 2009 Central ghrelin regulates peripheral lipid metabolism in a growth hormone-independent fashion. *Endocrinology* **150** 4562–4574. (doi:10.1210/en.2009-0482)
- Sangiao-Alvarellos S, Varela L, Vázquez MJ, Boit KD, Saha AK, Cordido F, Diéguez C & López M 2010 Influence of ghrelin and GH deficiency on AMPK and hypothalamic lipid metabolism. *Journal of Neuroendocrinology* **22** 543–556. (doi:10.1111/j.1365-2826.2010.01994.x)
- Santos CX, Tanaka LY, Wosniak J & Laurindo FR 2009 Mechanisms and implications of reactive oxygen species generation during the unfolded protein response: roles of endoplasmic reticulum oxidoreductases, mitochondrial electron transport, and NADPH oxidase. *Antioxidants & Redox Signaling* **11** 2409–2427. (doi:10.1089/ars.2009.2625)
- Savontaus E, Conwell IM & Wardlaw SL 2002 Effects of adrenalectomy on AGRP, POMC, NPY and CART gene expression in the basal hypothalamus of fed and fasted rats. *Brain Research* **958** 130–138. (doi:10.1016/S0006-8993(02)03674-0)
- Schwartz MW, Marks JL, Sipols AJ, Baskin DG, Woods SC, Kahn SE & Porte D Jr 1991 Central insulin administration reduces neuropeptide Y mRNA expression in the arcuate nucleus of food-deprived lean (Fa/Fa) but not obese (fa/fa) Zucker rats. *Endocrinology* **128** 2645–2647. (doi:10.1210/endo-128-5-2645)
- Schwartz MW, Sipols AJ, Marks JL, Sanacora G, White JD, Scheurink A, Kahn SE, Baskin DG, Woods SC, Flegler DP *et al.* 1992 Inhibition of hypothalamic neuropeptide Y gene expression by insulin. *Endocrinology* **130** 3608–3616. (doi:10.1210/en.130.6.3608)
- Schwartz MW, Seeley RJ, Woods SC, Weigle DS, Campfield LA, Burn P & Baskin DG 1997 Leptin increases hypothalamic pro-opiomelanocortin mRNA expression in the rostral arcuate nucleus. *Diabetes* **46** 2119–2123. (doi:10.2337/diabetes.46.12.2119)
- Scrocchi LA, Brown TJ, McCluskey N, Brubaker PL, Auerbach AB, Joyner AL & Drucker DJ 1996 Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene. *Nature Medicine* **2** 1254–1258. (doi:10.1038/nm1196-1254)
- Seo S, Ju S, Chung H, Lee D & Park S 2008 Acute effects of glucagon-like peptide-1 on hypothalamic neuropeptide and AMP activated kinase expression in fasted rats. *Endocrine Journal* **55** 867–874. (doi:10.1507/endocrj.K08E-091)
- Seoane LM, López M, Tovar S, Casanueva F, Señaris R & Diéguez C 2003 Agouti-related peptide, neuropeptide Y, and somatostatin-producing neurons are targets for ghrelin actions in the rat hypothalamus. *Endocrinology* **144** 544–551. (doi:10.1210/en.2002-220795)
- Sheets AR, Fulop P, Derdak Z, Kassai A, Sabo E, Mark NM, Paragh G, Wands JR & Baffy G 2008 Uncoupling protein-2 modulates the lipid metabolic response to fasting in mice. *American Journal of Physiology. Gastrointestinal and Liver Physiology* **294** G1017–G1024. (doi:10.1152/ajpgi.00016.2008)
- Shimizu H, Arima H, Watanabe M, Goto M, Banno R, Sato I, Ozaki N, Nagasaki H & Oiso Y 2008 Glucocorticoids increase neuropeptide Y and agouti-related peptide gene expression via AMP-activated protein kinase signaling in the arcuate nucleus of rats. *Endocrinology* **149** 4544–4553. (doi:10.1210/en.2008-0229)
- Shimizu-Albergine M, Ippolito DL & Beavo JA 2001 Downregulation of fasting-induced cAMP response element-mediated gene induction by leptin in neuropeptide Y neurons of the arcuate nucleus. *Journal of Neuroscience* **21** 1238–1246.
- Sindelar DK, Mystkowski P, Marsh DJ, Palmiter RD & Schwartz MW 2002 Attenuation of diabetic hyperphagia in neuropeptide Y-deficient mice. *Diabetes* **51** 778–783. (doi:10.2337/diabetes.51.3.778)
- Sindelar DK, Ste ML, Miura GI, Palmiter RD, McMinn JE, Morton GJ & Schwartz MW 2004 Neuropeptide Y is required for hyperphagic feeding in response to neuroglucopenia. *Endocrinology* **145** 3363–3368. (doi:10.1210/en.2003-1727)
- Sipols AJ, Baskin DG & Schwartz MW 1995 Effect of intracerebroventricular insulin infusion on diabetic hyperphagia and hypothalamic neuropeptide gene expression. *Diabetes* **44** 147–151. (doi:10.2337/diabetes.44.2.147)
- Smith RG 2005 Development of growth hormone secretagogues. *Endocrine Reviews* **26** 346–360. (doi:10.1210/er.2004-0019)
- Sorensen A, Travers MT, Vernon RG, Price NT & Barber MC 2002 Localization of messenger RNAs encoding enzymes associated with malonyl-CoA metabolism in mouse brain. *Brain Research. Gene Expression Patterns* **1** 167–173. (doi:10.1016/S1567-133X(02)00013-3)

- Steinberg GR, Michell BJ, van Denderen BJ, Watt MJ, Carey AL, Fam BC, Andrikopoulos S, Proietto J, Gorgun CZ, Carling D *et al.* 2006a Tumor necrosis factor α -induced skeletal muscle insulin resistance involves suppression of AMP-kinase signaling. *Cell Metabolism* **4** 465–474. (doi:10.1016/j.cmet.2006.11.005)
- Steinberg GR, Watt MJ, Fam BC, Proietto J, Andrikopoulos S, Allen AM, Febbraio MA & Kemp BE 2006b Ciliary neurotrophic factor suppresses hypothalamic AMP-kinase signaling in leptin-resistant obese mice. *Endocrinology* **147** 3906–3914. (doi:10.1210/en.2005-1587)
- Stephens TW, Basinski M, Bristow PK, Bue-Valleskey JM, Burgett SG, Craft L, Hale J, Hoffmann J, Hsiung HM, Kraicunas A *et al.* 1995 The role of neuropeptide Y in the antiobesity action of the obese gene product. *Nature* **377** 530–532. (doi:10.1038/377530a0)
- Stoppa GR, Cesquini M, Roman EA, Prada PO, Torsoni AS, Romanatto T, Saad MJ, Velloso LA & Torsoni MA 2008 Intracerebroventricular injection of citrate inhibits hypothalamic AMPK and modulates feeding behavior and peripheral insulin signaling. *Journal of Endocrinology* **198** 157–168. (doi:10.1677/JOE-07-0428)
- Sun Y, Wang P, Zheng H & Smith RG 2004 Ghrelin stimulation of growth hormone release and appetite is mediated through the growth hormone secretagogue receptor. *PNAS* **101** 4679–4684. (doi:10.1073/pnas.0305930101)
- Swart L, Jahng JW, Overton JM & Houpt TA 2002 Hypothalamic NPY, AGRP, and POMC mRNA responses to leptin and refeeding in mice. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* **283** R1020–R1026. (doi:10.1152/ajpregu.00501.2001)
- Tagawa Y, Hiramatsu N, Kasai A, Hayakawa K, Okamura M, Yao J & Kitamura M 2008 Induction of apoptosis by cigarette smoke via ROS-dependent endoplasmic reticulum stress and CCAAT/enhancer-binding protein-homologous protein (CHOP). *Free Radical Biology and Medicine* **45** 50–59. (doi:10.1016/j.free-radbiomed.2008.03.003)
- Takaya K, Ariyasu H, Kanamoto N, Iwakura H, Yoshimoto A, Harada M, Mori K, Komatsu Y, Usui T, Shimatsu A *et al.* 2000 Ghrelin strongly stimulates growth hormone release in humans. *Journal of Clinical Endocrinology and Metabolism* **85** 4908–4911. (doi:10.1210/jc.85.12.4908)
- Tang QQ & Lane MD 2000 Role of C/EBP homologous protein (CHOP-10) in the programmed activation of CCAAT/enhancer-binding protein- β during adipogenesis. *PNAS* **97** 12446–12450. (doi:10.1073/pnas.220425997)
- Tannenbaum GS, Lapointe M, Beaudet A & Howard AD 1998 Expression of growth hormone secretagogue-receptors by growth hormone-releasing hormone neurons in the mediobasal hypothalamus. *Endocrinology* **139** 4420–4423. (doi:10.1210/en.139.10.4420)
- Theander-Carrillo C, Wiedmer P, Cettour-Rose P, Nogueiras R, Perez-Tilve D, Pfluger P, Castaneda TR, Muzzin P, Schurmann A, Szanto I *et al.* 2006 Ghrelin action in the brain controls adipocyte metabolism. *Journal of Clinical Investigation* **116** 1983–1993. (doi:10.1172/JCI25811)
- Tkacs NC & Levin BE 2004 Obesity-prone rats have preexisting defects in their counterregulatory response to insulin-induced hypoglycemia. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* **287** R1110–R1115.
- Tkacs NC, Dunn-Meynell AA & Levin BE 2000 Presumed apoptosis and reduced arcuate nucleus neuropeptide Y and pro-opiomelanocortin mRNA in non-coma hypoglycemia. *Diabetes* **49** 820–826. (doi:10.2337/diabetes.49.5.820)
- Tong Q, Ye C, McCrimmon RJ, Dhillon H, Choi B, Kramer MD, Yu J, Yang Z, Christiansen LM, Lee CE *et al.* 2007 Synaptic glutamate release by ventromedial hypothalamic neurons is part of the neurocircuitry that prevents hypoglycemia. *Cell Metabolism* **5** 383–393. (doi:10.1016/j.cmet.2007.04.001)
- Tovar S, Nogueiras R, Tung LY, Castaneda TR, Vazquez MJ, Morris A, Williams LM, Dickson SL & Dieguez C 2005 Central administration of resistin promotes short-term satiety in rats. *European Journal of Endocrinology* **153** R1–R5. (doi:10.1530/eje.1.01999)
- Trivedi P, Jiang M, Tamvakopoulos CC, Shen X, Yu H, Mock S, Fenyk-Melody J, Van Der Ploeg LH & Guan XM 2003 Exploring the site of anorectic action of peripherally administered synthetic melanocortin peptide MT-II in rats. *Brain Research* **977** 221–230. (doi:10.1016/S0006-8993(03)02683-0)
- Tschöp M, Smiley DL & Heiman ML 2000 Ghrelin induces adiposity in rodents. *Nature* **407** 908–913. (doi:10.1038/35038090)
- Tschöp M, Wawarta R, Riepl RL, Friedrich S, Bidlingmaier M, Landgraf R & Folwaczny C 2001a Post-prandial decrease of circulating human ghrelin levels. *Journal of Endocrinological Investigation* **24** RC19–RC21.
- Tschöp M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E & Heiman ML 2001b Circulating ghrelin levels are decreased in human obesity. *Diabetes* **50** 707–709. (doi:10.2337/diabetes.50.4.707)
- Tsubone T, Masaki T, Katsuragi I, Tanaka K, Kakuma T & Yoshimatsu H 2005 Ghrelin regulates adiposity in white adipose tissue and UCP1 mRNA expression in brown adipose tissue in mice. *Regulatory Peptides* **130** 97–103. (doi:10.1016/j.regpep.2005.04.004)
- Vázquez MJ, González CR, Varela L, Lage R, Tovar S, Sangiao-Alvarellos S, Williams LM, Vidal-Puig A, Nogueiras R, López M *et al.* 2008 Central resistin regulates hypothalamic and peripheral lipid metabolism in a nutritional-dependent fashion. *Endocrinology* **149** 4534–4543. (doi:10.1210/en.2007-1708)
- Verty AN, McFarlane JR, McGregor IS & Mallet PE 2004 Evidence for an interaction between CB1 cannabinoid and melanocortin MCR-4 receptors in regulating food intake. *Endocrinology* **145** 3224–3231. (doi:10.1210/en.2004-0059)
- Vrang N, Larsen PJ, Tang-Christensen M, Larsen LK & Kristensen P 2003 Hypothalamic cocaine-amphetamine regulated transcript (CART) is regulated by glucocorticoids. *Brain Research* **965** 45–50. (doi:10.1016/S0006-8993(02)04064-7)
- Wang W, Liu Y, Chen Y, Cao C, Xiang Y, Zhang D, Han L, Zhao H & Liu G 2010a Inhibition of Foxo1 mediates protective effects of ghrelin against lipotoxicity in MIN6 pancreatic β -cells. *Peptides* **31** 307–314. (doi:10.1016/j.peptides.2009.11.011)
- Wang W, Zhang D, Zhao H, Chen Y, Liu Y, Cao C, Han L & Liu G 2010b Ghrelin inhibits cell apoptosis induced by lipotoxicity in pancreatic β -cell line. *Regulatory Peptides* **161** 43–50. (doi:10.1016/j.regpep.2009.12.017)
- Watanabe M, Arima H, Fukushima K, Goto M, Shimizu H, Hayashi M, Banno R, Sato I, Ozaki N, Nagasaki H *et al.* 2008 Direct and indirect modulation of neuropeptide Y gene expression in response to hypoglycemia in rat arcuate nucleus. *FEBS Letters* **582** 3632–3638. (doi:10.1016/j.febslet.2008.09.045)
- Wen JP, Liu CE, Hu YT, Chen G & Lin LX 2010 Globular adiponectin regulates energy homeostasis through AMP-activated protein kinase-acetyl-CoA carboxylase (AMPK/ACC) pathway in the hypothalamus. *Molecular and Cellular Biochemistry* **344** 109–115. (doi:10.1007/s11010-010-0534-2)
- Willemsen MG, Kristensen P & Romer J 1999 Co-localization of growth hormone secretagogue receptor and NPY mRNA in the arcuate nucleus of the rat. *Neuroendocrinology* **70** 306–316. (doi:10.1159/000054491)
- Wilson BD, Bagnol D, Kaelin CB, Ollmann MM, Gantz I, Watson SJ & Barsh GS 1999 Physiological and anatomical circuitry between agouti-related protein and leptin signaling. *Endocrinology* **140** 2387–2397. (doi:10.1210/en.140.5.2387)
- Wolfgang MJ & Lane MD 2008 Hypothalamic malonyl-coenzyme A and the control of energy balance. *Molecular Endocrinology* **22** 2012–2020. (doi:10.1210/me.2007-0538)
- Wolfgang MJ, Kurama T, Dai Y, Suwa A, Asaumi M, Matsumoto S, Cha SH, Shimokawa T & Lane MD 2006 The brain-specific carnitine palmitoyltransferase-1c regulates energy homeostasis. *PNAS* **103** 7282–7287. (doi:10.1073/pnas.0602205103)

- Wolfgang MJ, Cha SH, Sidhaye A, Chohnan S, Cline G, Shulman GI & Lane MD 2007 Regulation of hypothalamic malonyl-CoA by central glucose and leptin. *PNAS* **104** 19285–19290. (doi:10.1073/pnas.0709778104)
- Won JC, Jang PG, Namkoong C, Koh EH, Kim SK, Park JY, Lee KU & Kim MS 2009 Central administration of an endoplasmic reticulum stress inducer inhibits the anorexigenic effects of leptin and insulin. *Obesity* **17** 1861–1865. (doi:10.1038/oby.2009.194)
- Woods SC, Lotter EC, McKay LD & Porte D Jr 1979 Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. *Nature* **282** 503–505. (doi:10.1038/282503a0)
- Woods A, Dickerson K, Heath R, Hong SP, Momcilovic M, Johnstone SR, Carlson M & Carling D 2005 Ca(2+)/calmodulin-dependent protein kinase kinase- β acts upstream of AMP-activated protein kinase in mammalian cells. *Cell Metabolism* **2** 21–33. (doi:10.1016/j.cmet.2005.06.005)
- Wortley KE, Del Rincon JP, Murray JD, Garcia K, Iida K, Thorner MO & Sleeman MW 2005 Absence of ghrelin protects against early-onset obesity. *Journal of Clinical Investigation* **115** 3573–3578. (doi:10.1172/JCI26003)
- Wren AM, Small CJ, Ward HL, Murphy KG, Dakin CL, Taheri S, Kennedy AR, Roberts GH, Morgan DG, Ghatei MA *et al.* 2000 The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinology* **141** 4325–4328. (doi:10.1210/en.141.11.4325)
- Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, Dhillon WS, Ghatei MA & Bloom SR 2001a Ghrelin enhances appetite and increases food intake in humans. *Journal of Clinical Endocrinology and Metabolism* **86** 5992. (doi:10.1210/jc.86.12.5992)
- Wren AM, Small CJ, Abbott CR, Dhillon WS, Seal I, Cohen MA, Batterham RL, Taheri S, Stanley SA, Ghatei MA *et al.* 2001b Ghrelin causes hyperphagia and obesity in rats. *Diabetes* **50** 2540–2547. (doi:10.2337/diabetes.50.11.2540)
- Xie M, Zhang D, Dyck JR, Li Y, Zhang H, Morishima M, Mann DL, Taffet GE, Baldini A, Khoury DS *et al.* 2006 A pivotal role for endogenous TGF- β -activated kinase-1 in the LKB1/AMP-activated protein kinase energy-sensor pathway. *PNAS* **103** 17378–17383. (doi:10.1073/pnas.0604708103)
- Xu B, Dube MG, Kalra PS, Farmerie WG, Kaibara A, Moldawer LL, Martin D & Kalra SP 1998 Anorectic effects of the cytokine, ciliary neurotrophic factor, are mediated by hypothalamic neuropeptide Y: comparison with leptin. *Endocrinology* **139** 466–473. (doi:10.1210/en.139.2.466)
- Yang J, Brown MS, Liang G, Grishin NV & Goldstein JL 2008 Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone. *Cell* **132** 387–396. (doi:10.1016/j.cell.2008.01.017)
- Yang CS, Lam CK, Chari M, Cheung GW, Kokorovic A, Gao S, Leclerc I, Rutter GA & Lam TK 2010 Hypothalamic AMP-activated protein kinase regulates glucose production. *Diabetes* **59** 2435–2443. (doi:10.2337/db10-0221)
- Zhang X, Zhang G, Zhang H, Karin M, Bai H & Cai D 2008 Hypothalamic IKK β /NF- κ B and ER stress link overnutrition to energy imbalance and obesity. *Cell* **135** 61–73. (doi:10.1016/j.cell.2008.07.043)
- Zhang GG, Teng X, Liu Y, Cai Y, Zhou YB, Duan XH, Song JQ, Shi Y, Tang CS, Yin XH *et al.* 2009 Inhibition of endoplasmic reticulum stress by ghrelin protects against ischemia/reperfusion injury in rat heart. *Peptides* **30** 1109–1116. (doi:10.1016/j.peptides.2009.03.024)
- Zheng H, Corkern MM, Crousillac SM, Patterson LM, Phifer CB & Berthoud HR 2002 Neurochemical phenotype of hypothalamic neurons showing Fos expression 23 h after intracranial AgRP. *American Journal of Physiology, Regulatory, Integrative and Comparative Physiology* **282** R1773–R1781.
- Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Wu M, Ventre J, Doebber T, Fujii N *et al.* 2001 Role of AMP-activated protein kinase in mechanism of metformin action. *Journal of Clinical Investigation* **108** 1167–1174.
- Zigman JM, Nakano Y, Coppari R, Balthasar N, Marcus JN, Lee CE, Jones JE, Deysher AE, Waxman AR, White RD *et al.* 2005 Mice lacking ghrelin receptors resist the development of diet-induced obesity. *Journal of Clinical Investigation* **115** 3564–3572. (doi:10.1172/JCI26002)
- Zigman JM, Jones JE, Lee CE, Saper CB & Elmquist JK 2006 Expression of ghrelin receptor mRNA in the rat and the mouse brain. *Journal of Comparative Neurology* **494** 528–548. (doi:10.1002/cne.20823)
- Ziotopoulou M, Erani DM, Hileman SM, Bjorbaek C & Mantzoros CS 2000 Unlike leptin, ciliary neurotrophic factor does not reverse the starvation-induced changes of serum corticosterone and hypothalamic neuropeptide levels but induces expression of hypothalamic inhibitors of leptin signaling. *Diabetes* **49** 1890–1896. (doi:10.2337/diabetes.49.11.1890)
- Zorrilla EP, Iwasaki S, Moss JA, Chang J, Otsuji J, Inoue K, Meijler MM & Janda KD 2006 Vaccination against weight gain. *PNAS* **103** 13226–13231. (doi:10.1073/pnas.0605376103)

Received in final form 6 December 2010

Accepted 17 December 2010

Made available online as an Accepted Preprint 17 December 2010