OVERVIEW

Central (mainly) actions of GPCRs in energy homeostasis/balance: view from the Chair

N Gallo-Payet

To maintain a constant body weight, energy intake must equal energy expenditure; otherwise, there is a risk of overweight and obesity. The hypothalamus is one of the primary brain regions where multiple nutrient-related signals from peripheral and central sources converge and become integrated to regulate both short- and long-term nutritional states. The aim of the afternoon session of the 15th Annual International Symposium of the Laval University Obesity Research Chair held in Quebec City on 9 November 2012 was to present the most recent insights into the complex molecular mechanisms regulating food intake. The aims were to emphasize on the interaction between central and peripheral actions of some of the key players acting not only at the hypothalamic level but also at the periphery. Presentations were focused on melanocortin-3 receptor (MC3R) and melanin-concentrating hormone (MCH) as anorexigenic and orexigenic components of the hypothalamus, on endocannabinoid receptors, initially as a central neuromodulatory signal, and on glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP) as peripheral signals. What becomes clear from these four presentations is that the regulation of food intake and energy homeostasis involves several overlapping pathways, and that we have only touched the tip of the iceberg. From the examples presented in this symposium, it could be expected that in the near future, in addition to a low-fat diet and exercise, a combination of appropriate peptides and small molecules is likely to become available to improve/facilitate the objectives of long-term maintenance of energy balance and body weight.

International Journal of Obesity Supplements (2014) 4, S21–S25; doi:10.1038/ijosup.2014.7

Keywords: energy balance; homeostasis; HFD

The alarming increase in the incidence of obesity and obesity-associated disorders (insulin resistance, type-2 diabetes and metabolic syndrome) has reached epidemic levels in modern society, not only in the United States but also now in Europe and even in Asian countries, afflicting not only adults but also children.^{1–4} Body weight and normal energy balance are regulated by a complex system that includes both peripheral and central factors. To maintain a constant weight, energy intake has to be equal to energy expenditure. When the energy balance becomes disrupted, this can eventually lead to sustained overweight and obesity. The hypothalamus is one of the primary brain regions where multiple nutrient-related signals from peripheral and central sources converge and become integrated to regulate both short- and long-term nutritional states. The aim of the afternoon session of the 15th Annual International Symposium of the Laval University Obesity Research Chair was to present the most recent insights by which some of the key players act not only at the hypothalamic level but also at the periphery. Presentations were focused on melanocortin-3 receptor (Mc3R; MC3R; Andrew Butler) and melanin-concentrating hormone (MCH; Jean-Louis Nahon) as anorexigenic and orexigenic components of the hypothalamus, on endocannabinoid (eCB) receptors, initially as a central neuromodulatory signal (Vincenzo Di Marzo), and on GLP-1 and gastric inhibitory polypeptide (GIP) as peripheral signals (Matthias Tschöp).

Hormonal and nutrient signals are processed in the hypothalamus and inform the brain as to the current and stored levels

of fuel available for the organism. In turn, hypothalamic neuronal circuits use this information to regulate energy intake, energy expenditure as well as peripheral lipid and glucose metabolism. In a simplistic overview of this sophisticated regulation, leptin and insulin are the first afferent signals to the hypothalamus that circulate in proportion to body fat mass. Leptin is indeed secreted exclusively by fat cells in proportion to body-fat content and is considered to function as an 'adiposity signal' that conveys information to the brain regarding the level of body energy stores. Input from both leptin and insulin is transduced into adaptive responses that reduce food intake, increase energy expenditure and promote normal glucose homeostasis by neurons in the hypothalamic arcuate nucleus (ARC). Within the ARC are two distinct populations of neurons that exert opposing effects on energy balance. Anabolic neuronal pathways contain molecules such as neuropeptide Y and agouti-related protein that stimulate food intake and weight gain, and are inhibited by insulin and leptin. Opposing these effects are the melanocortins, which are catabolic peptides that are encoded by the pro-opiomelanocortin (POMC) gene and reduce food intake and promote weight loss. These neurons are also targets for the action of the gastric hormone ghrelin, which stimulates appetite and is implicated in mealtime hunger. The ARC, therefore, has a key role in sensing, integrating and responding to peripheral signals that control food intake and body weight (for review, see refs 5–9). The neuropeptides produced in ARC neurons in response to adiposity signals are called 'first-order neurons'. They project to 'second-order neurons'

expressing their G protein-coupled receptors (GPCRs) in other parts of the hypothalamus (such as the lateral hypothalamic area, paraventricular nucleus and perifornical area).^{10,11}

In the ARC, POMC produces melanocyte-stimulating hormone, which binds two GPCRs, namely the (Mc3r, MC3R and melanocortin-4 receptor (Mc4r, MC4R). Studies investigating the phenotypes of *Mc3r* and *Mc4r* gene in knockout (KO) mouse models indicate that the acute regulation of satiety, energy expenditure and glucose disposal by melanocortins is mediated by the Mc4r, and not Mc3r; *mc3r* KO mice are obese with increased fat mass and decreased lean body mass, but without hyperphagia, in contrast to *mc4r* KO mice^{12–14} (for review see refs 6,7,15–17). Although the Mc4r has been interrogated intensively for the treatment of obesity, the homeostatic functions of Mc3r have remained enigmatic. The regulation of energy homeostasis by Mc3r thus involves mechanisms that are not classically associated with obesity.^{7,14} In particular, although obesity in *Mc3r*^{−/−} mice is independent of hyperphagia, the group of Andrew Butler has observed hyperphagia restricted to the light-on periods.¹⁸ This aspect has been developed in the fourth presentation of this session.

The lateral hypothalamus (LHA) and perifornical area, as 'second-order neurons', produce the MCH, a highly orexigenic neuropeptide. KO mice for MCH eat less and lose weight, suggesting that MCH is required for animals to consume normal amounts of food. Hypothalamic MCH production is also increased under caloric restriction; hence, blocking MCH signalling would be an attractive target for obesity drug development 5. In the third presentation of the afternoon, Jean-Louis Nahon revealed that MCH has effects that go well beyond those directly linked with the regulation of food intake.

The eCB system consists of the endogenous agonists (in particular, anandamide and 2-arachidonoylglycerol, 2-AG), enzymes involved in the biosynthesis and degradation of the latter mediators and their GPCR, in particular the cannabinoid CB1 receptors (CB1-Rs). The eCB system is involved in stimulating both the hedonic and homeostatic aspects of food intake.¹⁹ The eCBs and their CB1-Rs are present not only in brain structures controlling energy intake but also in peripheral cells regulating energy homeostasis (for review, see refs 9,19–23). It is increasingly evident that eCBs affect food intake, energy expenditure and substrate metabolism by acting on peripheral sites. The presentation of Vincenzo Di Marzo ably summarized known and new pharmacological approaches that can be used for developing peripherally restricted CB1r antagonists.²⁰

Peripheral regulation includes adiposity signals (such as leptin), pancreatic signals (insulin), as well as appetite-stimulatory signal (such as ghrelin)²⁴ and satiety signals (such as GLP-1; for review, see refs 9,25–27). The proglucagon peptide is processed to produce the glucagon peptide, as well as GLP-1 and GLP-2. GLP-1 is a gut hormone that is released in response to food intake. It enhances glucose-induced stimulation of insulin synthesis and secretion, whereas GIP is known to potentiate glucose-stimulated insulin secretion.

The second session of the symposium was to present the most recent insights into the complex molecular mechanisms regulating food intake, focusing on the interaction between central and peripheral actions of the above-mentioned trophic factors; the perspectives of each of the presentations were to develop new efficient drugs for the treatment of obesity. The presentation of the first speaker, Vincenzo Di Marzo, was entitled 'New horizons in the targeting of cannabinoid receptors for the treatment of abdominal obesity and related dysmetabolism'. He first reviewed how the eCB system is involved in the central regulation of energy balance and how deregulation of the eCB system in the hypothalamus contributes to obesity. He then presented interesting new data demonstrating that the inhibition of eCB biosynthesis, as well as CB1-R antagonists that do not cross the

blood–brain barrier, could provide a novel pharmacological approach to controlling obesity without the psychiatric side effects observed with global CB1r antagonists. In the hypothalamus and accumbens, the eCB system is activated after food deprivation and inhibited by refeeding.²⁸ Following temporary food restriction, CB1-R KO mice eat less than their wild-type (WT) littermates, and the CB1 antagonist SR141716A reduces food intake in WT mice but not in KO mice. By contrast, defective leptin signalling in obese Zucker rats is associated with elevated 2-AG levels in the hypothalamus compared with non-obese controls. Conversely, in *ob/ob* mice treated with leptin, hypothalamic 2-AG levels are similar to those measured in lean controls, whereas anandamide declines to undetectable levels. These findings indicate a negative regulation of eCBs by leptin, which appears to be specific for eCBs and the hypothalamus.²⁹ Cardinal and colleagues³⁰ more recently used an elegant model of specific deletion of the *CB1-R* gene in hypothalamic neurons. They used injection of a Cre recombinase-expressing recombinant adeno-associated virus into the hypothalamus of adult mice, leading to the generation of specific Hyp-CB1-KO mice. When the CB1r antagonist SR141716 (rimonabant) was administered to 24-h-fasted Hyp-CB1-WT and Hyp-CB1-KO mice, control and mutant mice comparably lost weight after the 24-h fast. However, although the appetite-suppressant effect of the drug was still present 24 h after refeeding in Hyp-CB1-WT mice, it was completely abolished in Hyp-CB1-KO littermates. Similarly, rimonabant reduced the 24-h refeeding-induced body-weight gain in Hyp-CB1-WT mice but not in mice carrying the hypothalamic deletion of the *CB1* gene.

Dr Di Marzo then presented new exciting results demonstrating that deficiency in leptin signalling in obesity 'rewires' orexinergic neurons of the LHA independently of eCB signalling. This 'rewiring' of orexinergic neurons is partly due to impaired leptin–mTOR signalling in neuropeptide Y neurons of the ARC; leptin signalling deficiency-induced 'rewiring' in obesity switches eCB–CB1 control of orexinergic neurons from inhibitory to disinhibitory. This switch in eCB control of orexinergic neurons results in increased orexinergic signalling in target brain areas, whereas activation of CB1 reduces leptin-induced generation of reactive oxygen species in hypothalamic neurons. An exciting segment then followed with the presentation of new results demonstrating that targeting the peripheral cannabinoid receptors for the treatment of abdominal obesity and related dysmetabolism could be an interesting new alternative to circumvent the adverse effects of central nervous system-mediated drugs. The first involved a novel irreversible inhibitor of 2-AG biosynthesis called O-7460. When administered to mice, O-7460 dose-dependently inhibits the intake of a high-fat diet (HFD) over a 14-h observation period, and, subsequently, slightly but significantly reduces body weight.³¹ The second pertained to a new generation of CB1-R antagonist, called O-2050. Using the conditioned place preference test, Higuchi *et al.*³² found that consumption of a HFD over either 3 or 7 days increased HFD preferences and transiently increased hypothalamic 2-AG levels. The administration of the cannabinoid 1 receptor antagonist O-2050 reduced preferences for HFDs after 3, 7 and 14 days of HFD consumption. The main conclusions of this presentation were as follows: (1) the eCB system and CB1-Rs control food intake at all levels investigated to date; (2) this control becomes dysfunctional under conditions leading to leptin resistance and obesity, such as those produced by HFD; (3) defective leptin signalling in the ARC produces a 'rewiring' of the LHA, which in turn changes the way eCBs and CB1 control orexinergic signalling; (4) eCBs control leptin signalling in the hypothalamus at the level of reactive oxygen species production; (5) inhibition of 2-AG biosynthesis may be useful to correct dysfunctional CB1 signalling in HFD-induced obesity.

The second presentation from Matthias Tschöp was dedicated to 'Novel Therapeutics for Diabetes & Obesity: Targeting the

Gut-Brain Axis'. The first part of the presentation was related to the consequences of HFD on the development of inflammation and related inflammatory responses in the brain, whereas the second part focused on novel therapeutics that combines two or more components. Rodent models of obesity induced by consuming HFD are characterized by inflammation both in peripheral tissues and in hypothalamic areas critical for energy homeostasis.^{4,33} However, unlike inflammation in peripheral tissues, which develops as a consequence of obesity, hypothalamic inflammatory signalling is evident in both rats and mice within 1–3 days of HFD onset, before substantial weight gain.³⁴ Furthermore, both reactive gliosis and markers of neuron injury are evident in the hypothalamic ARC of rats and mice within the first week of HFD feeding. Consistent with these data in rodents, the authors found increased gliosis in the mediobasal hypothalamus of obese humans, as assessed with magnetic resonance imaging. These findings collectively suggest that, in both humans and rodent models, obesity is associated with neuronal injury in a brain area crucial for body-weight control.³⁴ They also found a modification in the synaptic input organization of the melanocortin system with a significantly greater number of inhibitory inputs in the POMC neurons of vulnerable rats compared with resistant diet-induced obesity (DIO) rats. When exposed to a HFD, the POMC cells of vulnerable DIO animals lost synapses, whereas those of resistant rats recruited connections.³⁵ Thus, hypothalamic gliosis, inflammation and hypervascularization may represent a novel pathological mechanism for DIO and diabetes, which may be under the control of GPCRs. The next part of the presentation focused on the peripheral actions of GLP-1 alone or in combination. Over the past 7 years, Matthias Tschöp and collaborators have tested a large series of combination therapies based on multiple gastrointestinal- and adipocyte-derived signals. Balanced single-molecule peptide hormone-based GLP1–glucagon and GIP–GLP1 co-agonists exhibit superior body weight loss and glucose metabolism benefits in mouse models of obesity and diabetes, as compared with any established mono-agonists.³⁶ As co-infusion of a soluble and stable glucagon mono-agonist in parallel with GIP–GLP1 co-agonist treatment was found to provide additional benefits, a series of single-molecule GIP–GLP1–glucagon tri-agonists were generated and validated. These novel tri-agonists showed unprecedented metabolic and body-weight benefits in mouse and rat models of obesity and diabetes. More recently, they also reported the development of a GLP-1–oestrogen conjugate that has superior sex-independent efficacy over all of the individual hormones alone in correcting obesity, hyperglycaemia and dyslipidaemia in mice.³⁷ Selective activation of oestrogen receptors in GLP-1-targeted tissues enhances the metabolic benefits of GLP-1 agonism. Together, these studies underscore a novel and potentially important new direction for obesity and metabolic disease therapy. An advantage of the described approach is that a single-molecule combination therapy targeting multiple GPCRs offers promising novel opportunities for the treatment of metabolic diseases such as diabetes and obesity.

The third presentation from Jean-Louis Nahon was aimed at presenting neuronal and non-neuronal functions of the MCH receptors (MCH-Rs). The first MCH receptor (hereforth named MCH-R1) was identified almost simultaneously by several laboratories, using a reverse-pharmacology strategy. This receptor was initially called SLC-1 for its homology with the orphan somatostatin-like receptor 1. Transgenic mouse models and pharmacological studies have shown the importance of the MCH signalling pathway as a potential target for treating not only appetite disorders and obesity but also anxiety and psychiatric diseases. This receptor is expressed at high levels in many brain areas of rodents and primates and is also expressed in peripheral organs, albeit at a lower rate (for review, see ref. 38). After providing an extensive history on MCH and its receptors, as well as their respective localization in the brain and their phylogeny, Dr Nahon

presented new unexpected MCH actions on ependymocytes. Ependymocytes are the cells that delineate cerebral ventricles. These cells are covered by motile cilia, which allow the circulation of cerebrospinal fluid (CSF) containing factors that signal energy states and arousal. Defects in ventricular cilia result in hydrocephalus due to the accumulation of excess CSF in the ventricles. The MCH-R1 is among the 12 genes encoding proteins involved in vesicular transport to the primary cilium. In addition, in humans, there is a particular syndrome where the blood–brain barrier is altered. In this syndrome, there is obesity, retinal dystrophy, renal anomalies and cognitive deficits, suggesting a link between MCH and this syndrome. In fact, little is known regarding these cells, which are the guardians of appropriate filtration through the blood–brain barrier. As MCH antagonists that are able to cross the blood–brain barrier are not available, the group of Jean-Louis Nahon has used a strategy of knocked down expression of the MCRH1 receptor. In these MCH-R1-KO mice, they investigated ventricle volumes and verified whether or not a defect in MCH-R1 function contributes to hydrocephalus. From their studies, it could be concluded that (1) lack of blood–brain barrier proteins prevents MCH-R1 localization to the primary cilium in the mouse brain; (2) MCH-R1 is expressed by ependymal cells on the third ventricle; (3) MCH neuronal projections reach these ciliary cells and MCH can also be released into the CSF; (4) activation of MCH neurons in the LHA increases CSF in the third ventricle through a calcium-dependent mechanism; and (5) lack of MCH-R1 in mice results in hydrocephalus. Thus, a potential new role for MCH in maintaining CSF flow and homeostasis in the brain must be considered. MCH could accelerate CSF signalling molecule distribution under metabolic needs.

The fourth and last presentation of the afternoon by Andrew Butler was related to 'the mysterious roles of MC3Rs in metabolic homeostasis and obesity: unravelling using mouse genetics'. Dr Butler presented the advantages of melanocortin-3-selective knocked down mice to better circumvent the effects mediated by the MC3R. On the basis of observations from studies examining daily rhythms in rats with various hypothalamic lesions, Andrew Butler hypothesizes that the melanocortin system acts through the Mc3r to link signals of energy status with the system expressing rhythms of food anticipatory behaviour.¹⁶ Clocks integrate cues from environmental and systemic signals of energy status to regulate diverse cellular and physiological functions. The integration of the circadian clock and energy metabolism is controlled by biological signals at multiple levels.^{39–41} The laboratory of Andrew Butler has focused on a role for MC3R in the expression of circadian rhythms in anticipatory behaviour.^{15,16,42} Restricted feeding (RF) paradigms with a periodicity of 24 h rapidly induce entrainment of rhythms anticipating food presentation that are independent of master clocks in the suprachiasmatic nucleus but do require other hypothalamic structures. Sutton *et al.*⁴³ reported that the melanocortin system is required for the expression of food-entrainable rhythms. In their study, food anticipatory activity was assessed in WT and Mc3r-deficient (Mc3r^{−/−}) C57BL/J mice by wheel running, spontaneous locomotory movement and measurement of wakefulness. WT mice housed in wheel cages subject to RF exhibited increased wheel activity during the 2-h preceding meal presentation, which corresponded with an increase in wakefulness around meal time and reduced wakefulness during the dark. WT mice also exhibited increased x- and z-movements centred on food initiation. The activity-based responses to RF were significantly impaired in mice lacking Mc3r. RF also failed to increase wakefulness in the 2-h before food presentation in Mc3r^{−/−} mice. Results thus indicate that Mc3r is required for the expression of anticipatory patterns of activity and wakefulness during periods of limited nutrient availability and for normal regulation of cortical clock function.⁴³ Furthermore, the ability to synchronize rhythms of increased vigilance and food-seeking behaviours that anticipate nutrient

consumption requires functional MC3R.^{15,16,18,43,44} The results from Begriche *et al.*^{15,16,45} suggest that MC3Rs modulate the expression of the 'anticipatory' response to nutrients. *Mc3r*^{-/-} mice subjected to daily RF regimes in which 60–70% of normal caloric intake is presented mid-light cycle or at 24-h intervals in constant dark exhibit attenuated 'food anticipatory activity'.^{15,43,45} In addition, although exhibiting weight loss during RF, *Mc3r*^{-/-} mice develop a metabolic phenotype suggesting partial insulin resistance.¹⁸ Collectively, these observations link MC3Rs to behavioural (that is, wakefulness, motivation) and metabolic adaptations when feeding occurs outside the normal photoperiod-driven circadian rhythm. *Mc3r*^{-/-} mice might thus be described as a circadian mutant that exhibits weakened responses to caloric cues and increased dependency on obesity and metabolic disease cues for the expression of circadian rhythm. Results shown thereby indicate the following: (1) central infusion of a melanocortin receptor antagonist disrupts normal regulation of clock genes in the forebrain; (2) *Mc3r*^{-/-} mice subjected to RF develop hyperinsulinaemia and glucose intolerance; and (3) Mc3r regulates hepatic glucose production while clock activity in liver is compromised in *Mc3r*^{-/-} mice.

To investigate the roles of central MC3Rs, the authors inserted a 'lox-stop-lox' (LoxTB) 5' of the translation initiation codon of the mouse *Mc3r* gene and reactivated transcription using neuron-specific Cre transgenic mice (*Mc3r*(TB/TB) mice). As predicted, these mice displayed reduced lean mass, increased fat mass and accelerated DIO, suggesting that actions of MC3Rs on energy homeostasis involve both central and peripheral sites of action, which, however, remains to be investigated. Indeed, the ventromedial hypothalamus MC3R signalling improves metabolic homeostasis but does not significantly have an impact on the expression of behaviours anticipating nutrient availability.⁴⁴ These results suggest that MC3R regulates input into systems responsible for the expression of a circadian rhythm in the absence of light. Future studies should identify specific neuronal pathways involved in the regulation of food entrainment and the regulation of glucose homeostasis by MC3R.¹⁵

CONCLUSION AND FUTURE DIRECTIONS

The incidence of obesity, type-2 diabetes and associated cardiovascular diseases has grown to pandemic proportions. Accumulating evidence suggests that the communication pathways linking the brain, gut and adipose tissue could be promising intervention points for metabolic disorders.⁴⁶ What does become clear from these four presentations is that the regulation of food intake and energy homeostasis involves several overlapping pathways, and we have only touched the tip of the iceberg, as a great number of endogenous substances are increasingly involved in the overall regulation of feeding and feeding behaviour. For example, the development of compounds with limited brain penetrance could be considered for peripherally located CB1-R. Among these, those described in this afternoon's session appear to be quite promising. Inadequate responses of an individual to environmental challenges such as unbalanced diet or lack of physical exercise has been recognized to increase the risk of obesity-induced cardiometabolic diseases. In addition, recent evidence suggests that this may involve alterations in the settings of the circadian clock system and of the hypothalamic–pituitary–adrenal axis, which regulates stress responses. These two systems are now known to interact in producing an integrated response to environmental challenges.⁴⁷ The results presented by Andrew Butler raise the possibility that MC3Rs are a potential target for treating disorders of metabolism. As diet and exercise have significant effects on energy homeostasis, the use of solely therapeutic drugs to treat obesity is certainly not sufficient. Several convincing studies have already established that the most effective treatment is provided by a combination of diet, exercise

and therapeutic drugs. Thus, taken together, as reviewed by many, the best strategy to accomplish long-term changes in body weight appears to be the use of potential anti-obesity agents in combination with a low-fat diet and sufficient exercise.^{1–4,9,27,48} From the examples presented in this symposium, a multitude of other possible combinations of peptides and small molecules could certainly be offered in the near future.

CONFLICT OF INTEREST

The author declares no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by grants from the Canadian Institute of Health Research to Nicole Gallo-Payet (MOP27912). NGP is a past recipient of a Canada Research Chair in Endocrinology of the Adrenal Gland.

REFERENCES

- Cote M, Matias I, Lemieux I, Petrosino S, Almeras N, Despres JP *et al.* Circulating endocannabinoid levels, abdominal adiposity and related cardiometabolic risk factors in obese men. *Int J Obes (Lond)* 2007; **31**: 692–699.
- Despres JP, Lemieux I. Abdominal obesity and metabolic syndrome. *Nature* 2006; **444**: 881–887.
- Lim S, Despres JP, Koh KK. Prevention of atherosclerosis in overweight/obese patients. In need of novel multi-targeted approaches. *Circ J* 2011; **75**: 1019–1027.
- Despres JP. Abdominal obesity and cardiovascular disease: is inflammation the missing link? *Can J Cardiol* 2012; **28**: 642–652.
- Schwartz MW, Gelling RW. Rats lighten up with MCH antagonist. *Nat Med* 2002; **8**: 779–781.
- Cone RD. Anatomy and regulation of the central melanocortin system. *Nat Neurosci* 2005; **8**: 571–578.
- Cone RD. Studies on the physiological functions of the melanocortin system. *Endocr Rev* 2006; **27**: 736–749.
- Garfield AS, Lam DD, Marston OJ, Przydzial MJ, Heisler LK. Role of central melanocortin pathways in energy homeostasis. *Trends Endocrinol Metab* 2009; **20**: 203–215.
- Klok MD, Jakobsdottir S, Drent ML. The role of leptin and ghrelin in the regulation of food intake and body weight in humans: a review. *Obes Rev* 2007; **8**: 21–34.
- Barsh GS, Schwartz MW. Genetic approaches to studying energy balance: perception and integration. *Nat Rev Genet* 2002; **3**: 589–600.
- Schwartz MW, Woods SC, Porte Jr D, Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature* 2000; **404**: 661–671.
- Farooqi IS, Drop S, Clements A, Keogh JM, Biernacka J, Lowenbein S *et al.* Heterozygosity for a POMC-null mutation and increased obesity risk in humans. *Diabetes* 2006; **55**: 2549–2553.
- Yaswen L, Diehl N, Brennan MB, Hochgeschwender U. Obesity in the mouse model of pro-opiomelanocortin deficiency responds to peripheral melanocortin. *Nat Med* 1999; **5**: 1066–1070.
- Butler AA. The melanocortin system and energy balance. *Peptides* 2006; **27**: 281–290.
- Begriche K, Sutton GM, Butler AA. Homeostatic and non-homeostatic functions of melanocortin-3 receptors in the control of energy balance and metabolism. *Physiol Behav* 2011; **104**: 546–554.
- Begriche K, Sutton GM, Fang J, Butler AA. The role of melanocortin neuronal pathways in circadian biology: a new homeostatic output involving melanocortin-3 receptors? *Obes Rev* 2009; **10**(Suppl 2): 14–24.
- Butler AA, Kesterson RA, Khong K, Cullen MJ, Pelleymounter MA, Dekoning J *et al.* A unique metabolic syndrome causes obesity in the melanocortin-3 receptor-deficient mouse. *Endocrinology* 2000; **141**: 3518–3521.
- Sutton GM, Begriche K, Kumar KG, Gimble JM, Perez-Tilve D, Nogueiras R *et al.* Central nervous system melanocortin-3 receptors are required for synchronizing metabolism during entrainment to restricted feeding during the light cycle. *FASEB J* 2010; **24**: 862–872.
- Di Marzo V. The endocannabinoid system in obesity and type 2 diabetes. *Diabetologia* 2008; **51**: 1356–1367.
- Quarta C, Mazza R, Obici S, Pasquali R, Pagotto U. Energy balance regulation by endocannabinoids at central and peripheral levels. *Trends Mol Med* 2011; **17**: 518–526.
- Kunos G, Tam J. The case for peripheral CB(1) receptor blockade in the treatment of visceral obesity and its cardiometabolic complications. *Br J Pharmacol* 2011; **163**: 1423–1431.
- Di Marzo V. Endocannabinoids: an appetite for fat. *Proc Natl Acad Sci USA* 2011; **108**: 12567–12568.

- 23 Pertwee RG. Emerging strategies for exploiting cannabinoid receptor agonists as medicines. *Br J Pharmacol* 2009; **156**: 397–411.
- 24 Tschoop M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. *Nature* 2000; **407**: 908–913.
- 25 Schwartz MW, Morton GJ. Obesity: keeping hunger at bay. *Nature* 2002; **418**: 595–597.
- 26 Castaneda TR, Tong J, Datta R, Culler M, Tschoop MH. Ghrelin in the regulation of body weight and metabolism. *Front Neuroendocrinol* 2010; **31**: 44–60.
- 27 Rodgers RJ, Tschoop MH, Wilding JP. Anti-obesity drugs: past, present and future. *Dis Model Mech* 2012; **5**: 621–626.
- 28 Soria-Gomez E, Marquez-Diosdado MI, Montes-Rodriguez CJ, Estrada-Gonzalez V, Prospero-Garcia O. Oleamide administered into the nucleus accumbens shell regulates feeding behaviour via CB1 and 5-HT2C receptors. *Int J Neuropsychopharmacol* 2010; **13**: 1247–1254.
- 29 Di Marzo V, Goparaju SK, Wang L, Liu J, Batkai S, Jarai Z *et al.* Leptin-regulated endocannabinoids are involved in maintaining food intake. *Nature* 2001; **410**: 822–825.
- 30 Cardinal P, Bellocchio L, Clark S, Cannich A, Klugmann M, Lutz B *et al.* Hypothalamic CB1 cannabinoid receptors regulate energy balance in mice. *Endocrinology* 2012; **153**: 4136–4143.
- 31 Bisogno T, Mahadevan A, Coccarello R, Chang JW, Allara M, Chen Y *et al.* A novel fluorophosphonate inhibitor of the biosynthesis of the endocannabinoid 2-arachidonoylglycerol with potential anti-obesity effects. *Br J Pharmacol* 2013; **169**: 784–793.
- 32 Higuchi S, Irie K, Yamaguchi R, Katsuki M, Araki M, Ohji M *et al.* Hypothalamic 2-arachidonoylglycerol regulates multistage process of high-fat diet preferences. *PLoS One* 2012; **7**: e38609##
- 33 Yi CX, Tschoop MH, Woods SC, Hofmann SM. High-fat-diet exposure induces IgG accumulation in hypothalamic microglia. *Dis Model Mech* 2012; **5**: 686–690.
- 34 Thaler JP, Yi CX, Schur EA, Guyenet SJ, Hwang BH, Dietrich MO *et al.* Obesity is associated with hypothalamic injury in rodents and humans. *J Clin Invest* 2012; **122**: 153–162.
- 35 Horvath TL, Diano S. The floating blueprint of hypothalamic feeding circuits. *Nat Rev Neurosci* 2004; **5**: 662–667.
- 36 Day JW, Ottaway N, Patterson JT, Gelfanov V, Smiley D, Gidda J *et al.* A new glucagon and GLP-1 co-agonist eliminates obesity in rodents. *Nat Chem Biol* 2009; **5**: 749–757.
- 37 Finan B, Yang B, Ottaway N, Stemmer K, Muller TD, Yi CX *et al.* Targeted estrogen delivery reverses the metabolic syndrome. *Nat Med* 2012; **18**: 1847–1856.
- 38 Nahon JL. The melanocortins and melanin-concentrating hormone in the central regulation of feeding behavior and energy homeostasis. *C R Biol* 2006; **329**: 623–638; discussion 653–625##
- 39 Peek CB, Ramsey KM, Marcheva B, Bass J. Nutrient sensing and the circadian clock. *Trends Endocrinol Metab* 2012; **23**: 312–318.
- 40 Saper CB, Scammell TE, Lu J. Hypothalamic regulation of sleep and circadian rhythms. *Nature* 2005; **437**: 1257–1263.
- 41 Scheer FA, Hilton MF, Mantzoros CS, Shea SA. Adverse metabolic and cardiovascular consequences of circadian misalignment. *Proc Natl Acad Sci USA* 2009; **106**: 4453–4458.
- 42 Butler AA, Kozak LP. A recurring problem with the analysis of energy expenditure in genetic models expressing lean and obese phenotypes. *Diabetes* 2010; **59**: 323–329.
- 43 Sutton GM, Perez-Tilve D, Nogueiras R, Fang J, Kim JK, Cone RD *et al.* The melanocortin-3 receptor is required for entrainment to meal intake. *J Neurosci* 2008; **28**: 12946–12955.
- 44 Begriche K, Levasseur PR, Zhang J, Rossi J, Skorupa D, Solt LA *et al.* Genetic dissection of the functions of the melanocortin-3 receptor, a seven-transmembrane G-protein-coupled receptor, suggests roles for central and peripheral receptors in energy homeostasis. *J Biol Chem* 2011; **286**: 40771–40781.
- 45 Begriche K, Marston OJ, Rossi J, Burke LK, McDonald P, Heisler LK *et al.* Melanocortin-3 receptors are involved in adaptation to restricted feeding. *Genes Brain Behav* 2012; **11**: 291–302.
- 46 Yi CX, Tschoop MH. Brain-gut-adipose-tissue communication pathways at a glance. *Dis Model Mech* 2012; **5**: 583–587.
- 47 Cagampang FR, Poore KR, Hanson MA. Developmental origins of the metabolic syndrome: body clocks and stress responses. *Brain Behav Immun* 2011; **25**: 214–220.
- 48 Heyman E, Gamelin FX, Aucouturier J, Di Marzo V. The role of the endocannabinoid system in skeletal muscle and metabolic adaptations to exercise: potential implications for the treatment of obesity. *Obes Rev* 2012; **13**: 1110–1124.