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REVIEW ARTICLE

# Neuroendocrine Regulation of Metabolism

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Given the current environment in most developed countries, it is a challenge to maintain a good balance between calories consumed and calories burned, although maintenance of metabolic balance is key to good health. Therefore, understanding how metabolic regulation is achieved and how the dysregulation of metabolism affects health is an area of intense research. Most studies focus on the hypothalamus, which is a brain area that acts as a key regulator of metabolism. Among the nuclei that comprise the hypothalamus, the arcuate nucleus is one of the major mediators in the regulation of food intake. The regulation of energy balance is also a key factor ensuring the maintenance of any species as a result of the dependence of reproduction on energy stores. Adequate levels of energy reserves are necessary for the proper functioning of the hypothalamic-pituitary-gonadal axis. This review discusses valuable data presented in the 2015 edition of the International Workshop of Neuroendocrinology concerning the fundamental nature of the hormonal regulation of the hypothalamus and the impact on energy balance and reproduction.

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## Introduction

Given the current environment in most developed countries, it is a challenge to maintain a good balance between calories consumed and calories burned, although maintenance of metabolic balance is key to good health. The worldwide increase in the incidence of being overweight and obese demonstrates the difficulty that people have maintaining a proper energy balance. In 2014, 39% of adults age $\geq$ 18 years were overweight (body mass index > 25 kg/m$^2$), whereas 11% of men and 15% of women were obese (body mass index > 32 kg/m$^2$) (1). This leads to an estimate of 1.9 billion overweight adults worldwide, of whom 600 million are obese. The risks associated with being overweight include cardiovascular disease and type 2 diabetes mellitus, amongst others. Therefore, understanding how metabolic regulation is achieved and how the dysregulation of metabolism affects health is of great importance.

The hypothalamus is a brain area that acts as a key regulator of metabolism and mediates numerous processes, including food intake, body temperature, sexual behaviour and reproduction, circadian rhythms and emotional responses. The hypothalamus, comprising many distinct neuronal nuclei, integrates neural, endocrine and metabolic signals. Among these nuclei, the importance of the arcuate nucleus (ARC) in the regulation of energy balance is well known (2). Its position is adjacent to the median eminence, allowing the ARC to sense circulating signals such as leptin, ghrelin and insulin, amongst others. Through changes in the activity of its neuronal populations and through many diverse and widespread outputs, the ARC is a major participant in the regulation of energy metabolism.

The regulation of energy balance is not only important for the survival of one individual, but also is a key factor ensuring the maintenance of any species as a result of the dependence of reproduction on energy stores (3). Adequate levels of energy reserves are necessary for the proper functioning of the hypothalamic-pituitary-gonadal axis. The neuroendocrine players that link energy balance with the reproductive system include hormones and neuropeptides that act on hypothalamic gonadotrophin-releasing hormone neurones. As an example, leptin is one of the hormones recognised as

a modulator of the reproductive axis, as well as a modulator of energy balance. Thus, the importance of energy metabolism and its regulation to guarantee reproduction is well established.

This review discusses valuable data presented in the 2015 edition of the International Workshop of Neuroendocrinology concerning the fundamental nature of the neuroendocrine regulation of hypothalamic neurones and the impact on energy balance and reproduction.

## Main hypothalamic systems regulating metabolism

### Pro-opiomelanocortin (POMC) and agouti-related peptide (AgRP) neurones in energy balance regulation: beyond the peptides

The central nervous system (CNS) plays a major role in maintaining a balance between the energy required for survival and the energy provided by feeding. To fully understand the central regulation of energy balance, it is essential to understand the complete compliment of neurotransmitters involved. Clearly, the peptide transmitters released from hypothalamic AgRP and POMC neurones are important regulators of energy balance (4,5). However, it has become increasingly clear that these neurones also utilise amino acid transmitters that may also impact energy balance regulation. Therefore, many recent studies have focused on the production, release and consequence of the amino acid transmitters GABA and glutamate from AgRP and POMC neurones.

Indications that AgRP and POMC neurones likely use a mix of peptide and amino acid transmitters date back several decades to immunoelectron microscopy studies showing both dense-core and small-clear vesicles in the axon terminals of these neurones (4,6). A role for amino acid transmitters in food intake and metabolism was suggested as a result of pharmacologic studies carried out from the 1970s up to the 1990s. However, experiments exploring the potential significance of GABA or glutamate release from neurones in energy balance circuits were not possible until advances were made in genetic manipulation. Using genetic approaches to delete the orexigenic peptides neuropeptide Y (NPY) or AgRP or both, it was found that energy balance could be largely maintained in the absence of these peptides (7,8), whereas ablation of the AgRP/NPY neurones in adult mice blunted food intake and caused rapid wasting (9,10). This unexpected difference between peptide deletion and neuronal ablation was eventually attributed to the loss of GABA co-release when the neurones were ablated as a whole (11), resulting in renewed interest in amino acid transmitters in energy balance circuits. Optogenetic and other approaches have been used to further show the importance of GABA release from AgRP neurones in the stimulation of food intake and the downstream targets of AgRP neurone-derived GABA release are beginning to be revealed (11,12).

The release of GABA from AgRP neurones can be dynamically regulated. Fasting causes increased GABA release, whereas leptin decreases GABA release from AgRP neurones onto POMC neurones (13). Although inhibition of POMC neurones may not be a necessary contributor to increased food intake upon robust AgRP neurone stimulation (14), the high degree of connectedness from AgRP to POMC neurones makes this a convenient synapse for examining changes in GABA release from AgRP neurones. It is tempting to infer that altered release at one target site likely reflects similar changes in other target regions, although this may not be the case. Neurones can release different sets of chemical transmitters from distinct fibres (15,16) and terminals may preferentially express one transmitter or another from a given cell type (17,18). Therefore, it will be necessary to determine how changes in the activity of POMC or AgRP neurones affects GABA release in specific target sites to fully understand the dynamic regulation imposed by these neurones under select conditions.

Unlike AgRP neurones that appear to use only GABA as their amino acid transmitter, POMC neurones appear heterogeneous in their amino acid phenotype with approximately 50% of POMC neurones being GABAergic and approximately 10–40% being glutamatergic (19,20). Interestingly, the amino acid transmitter phenotype of POMC neurones appears to be plastic throughout development, with a large proportion of POMC neurones showing glutamatergic markers during the early postnatal period and tapering off into adulthood (21). The genetic deletion of the vesicular glutamate transporter vGlut2 to prevent glutamate release specifically from POMC neurones causes a modest sex-specific increase in body weight in male mice maintained on a high-fat diet (21). However, this phenotype may reflect a developmental effect because vGlut2 was constitutively deleted from POMC neurones and potentially from a subset of other neurones that transcribe the POMC gene briefly during development (22). Therefore, additional studies are needed to determine the role of glutamate release from POMC neurones in adulthood and to begin to explore the functional consequence of GABA release from POMC neurones. Additionally, determining whether GABAergic and glutamatergic POMC neurones represent otherwise distinct subpopulations of POMC neurones and whether the developmental reduction in glutamatergic phenotype is important in energy balance regulation will add to a more complete understanding and inform future studies that may manipulate or examine POMC neurones in a subtype-specific manner.

By identifying and understanding the actions of the array of transmitters involved in energy balance regulation, it will be possible to better identify potential points of dysfunction and perhaps therapeutic targets. The recent advances described above add essential information regarding the complex actions of POMC and AgRP neurones. Although there is much more to learn regarding the roles of amino acid transmitters in energy balance, the recognition that POMC and AgRP neurones use both peptide and amino acid transmitters to effect a variety of responses on distinct timescales in a state-dependent manner reflects a significant increase in the understanding of this system.

## Hormones regulating hypothalamic systems controlling metabolism

### Ghrelin and the regulation of feeding: an important player for energy homeostasis

To achieve the regulation of energy balance, CNS circuits must be able to interact with the endocrine system, which provides

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peripheral signals that indicate body energy status. Among them, ghrelin is the only mammalian peptide hormone known to increase food intake. Ghrelin acts primarily on CNS centres to affect not only homeostatic-driven food intake, but also to regulate hedonic-driven feeding.

Ghrelin is a 28-amino acid octanoylated peptide secreted by cells located within the gastrointestinal tract. It was first discovered as the endogenous ligand of the growth hormone secretagogue receptor 1a (GHSR1a), with the ability to stimulate the secretion of growth hormone from the anterior pituitary gland (23). Further studies in the area of ghrelin led to the discovery of a role for this hormone in the regulation of several processes, including food intake, glucose metabolism, gastrointestinal tract motility and stress- and anxiety-related behaviours, amongst others (24). Plasma ghrelin levels rise before meals and decrease after the ingestion of food (25). This pattern of variation promoted the idea of ghrelin as a 'meal initiation' signal, which held up for many years, although this simplistic view is now beginning to be displaced (26).

Ghrelin acts on the CNS by binding to GHSR1a, which is a G-protein coupled receptor highly expressed in brain centres associated with food intake (27). The main neuronal targets that mediate the orexigenic action of ghrelin are the ARC of the hypothalamus and the dorsal vagal complex (DVC) of the brainstem (28). The extent to which ghrelin is able to reach these sites of action is a matter of debate (29). These two brain areas display the important feature of having a circumventricular organ associated: the median eminence, which lies adjacent to the ARC, and the area postrema, which is part of the DVC. Circumventricular organs are specialised brain regions that lack the normal blood–brain barrier and present fenestrated capillaries that allow peripheral signals to reach their neuronal targets (30). In the case of the ARC, it is assumed that ghrelin is able to freely diffuse through the median eminence and reach GHSR1a-expressing neurones (31). Regarding the DVC, ghrelin could directly activate GHSR1a-expressing neurones of the area postrema, which in turn regulate their targets in the brainstem and hypothalamus (32).

The ARC contains two major neuronal populations with opposite effects on food intake: the orexigenic AgRP/NPY-expressing neurones and the anorexigenic POMC-expressing neurones. The importance of the ARC as a mediator of the orexigenic action of ghrelin emerges from the fact that the absence of AgRP/NPY neurones eliminates the ghrelin-triggered increase in food intake (33). Another piece of evidence supporting a key role of the ARC in mediating the orexigenic effects of ghrelin is that AgRP/NPY neurones express high levels of GHSR1a mRNA (34). The AgRP/NPY neurones send projections to other hypothalamic nuclei such as the paraventricular nucleus (PVN), the dorsomedial nucleus of the hypothalamus (DMH) and the lateral hypothalamic area (LHA), which are all involved in the control of feeding. These hypothalamic nuclei also express GHSR1a mRNA and show ghrelin binding when biotin- and fluorescent-labelled ghrelin binding assays are performed (31,35,36). Thus, the direct and/or indirect participation of these brain areas could be important for the regulation of food intake by ghrelin.

The DVC is another important brain area that mediates the orexigenic action of ghrelin. This brain region is made up of three nuclei: the nucleus of the solitary tract, the area postrema and the dorsal motor nucleus of the vagus. The expression of GHSR1a mRNA has been described in all three components of the DVC (27), suggesting that ghrelin can directly act on them. Indeed, it has been shown that the administration of ghrelin directly on the DVC promotes food intake (37). Additionally, i.c.v. infusion of ghrelin activates c-Fos (a marker of neuronal activation) expression in the area postrema and the nucleus of the solitary tract (38). Nevertheless, one study showed that peripheral administration of ghrelin to mice that selectively express GHSR1a in the DVC does not result in an increase in food intake (39). This evidence suggests that the DVC is a target of ghrelin with respect to regulating food intake but it is not sufficient to mediate the orexigenic action of ghrelin.

Considering that ghrelin is the only peptide hormone known to increase food intake, its relevance in the regulation of energy homeostasis is highlighted. Although the brain targets for the orexigenic action of ghrelin are well established, the exact molecular mechanisms by which this hormone regulates feeding are not completely understood. Unravelling these mechanisms is of extreme importance with respect to considering them as potential therapeutic targets in the treatment of pathologies that affect food intake.

## Oxysterols and liver X receptors (LXRs)

Being overweight and obese are primarily linked to poor eating habits. A busy lifestyle induces an increase in the availability and consumption of fast foods. This type of food contains large amounts of animal fats, which contain a mixture of triglycerides, cholesterol and phospholipids. Cholesterol is necessary to guarantee the integrity and fluidity of the cell plasma membrane. It is produced by all animal cells and is also incorporated with the diet. At present, the quantity of cholesterol consumed represents an excess relative to the needs of human body. This implicates that the organism must be able to metabolise it and one important way is via the oxidation of cholesterol to oxysterols.

The oxysterols are involved in different mechanisms related to the removal of cholesterol from cells (40). These compounds are capable of binding to specific proteins called LXRs acting as their endogenous ligands (41–43). The functional LXRs exist as two isoforms: LXR$\alpha$ and LXR$\beta$. The first is mainly expressed in the liver and, to a lesser extent in the gut, adipose tissue, kidney, spleen and macrophages, whereas LXR$\beta$ is expressed in almost all tissues (40). Upon exposure to excessive accumulation of intracellular cholesterol oxides, LXRs activate a programme of gene expression for limiting the pathogenic accumulation of cholesterol (44). In the intestine, activation of LXRs decreases cholesterol absorption from the diet by promoting the expression of excretion transporters such as ABCA1, ABCG5 and ABCG8 (45). In macrophages, LXRs cause a rapid increase in the expression of genes involved in the formation of high-density lipoprotein and reverse cholesterol transport (46). In the liver, LXR activation promotes the direct conversion of excessive cholesterol to bile acids through the regulation of the limiting enzyme 7$\alpha$ hydroxylase (CYP7a) (40).


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In addition to the regulation of cholesterol homeostasis in multiple tissues, LXRs are also intimately involved in the control of hepatic lipid metabolism and in the physiological regulation of carbohydrate metabolism (47). Studies demonstrated that LXR agonists improve glucose tolerance in a mouse model of diet-induced insulin resistance. Treatment with synthetic LXR ligands alters the expression of genes in the liver and adipose tissue and causes a decrease in hepatic glucose production and an increase in glucose uptake by adipocytes. Furthermore, activation of LXRs indirectly suppresses the expression of hepatic gluconeogenesis enzymes (phosphoenolpyruvatecarboxykinase and glucose 6-phosphatase), whereas, in adipose tissue, LXRs regulate the expression of the insulin-sensitive glucose transporter GLUT4 (48). Further studies suggest that LXRβ in particular plays an important role in pancreatic insulin secretion and LXR activators promote insulin secretion (47). LXR ligands have also proven to be effective in other studies of insulin resistance and type 2 diabetes mellitus, in which spontaneously diabetic or age-developed glucose intolerant rodent strains (*db/db* mice, *ob/ob* mice, *fa/fa* Zucker rats) were used to highlight the potential of LXR agonists as insulin sensitisers (49,50).

Although the metabolic functions of the LXRs in peripheral organs have been widely investigated, little is known about the expression and functionality of LXRs in the brain. The activation of LXRs facilitates the excretion of cholesterol in the cerebellum and hippocampus (51). Recent studies show that the expression of LXRα and LXRβ in the hypothalamus is sensitive to triglycerides and serum insulin levels. Animals with glucose intolerance show an up-regulation of LXRβ and a down-regulation of LXRα in the hypothalamus. In addition, a correlation between this LXR expression and triglyceride or insulin levels was described, indicating the importance of both subtypes in the risk of developing metabolic diseases (52). LXRβ expression in the hypothalamus correlates negatively with the area under the curve in glucose tolerance tests in control animals, whereas a positive correlation is found in rats with abnormal glucose tolerance (52,53). The endogenous receptor agonists can also modulate LXR expression. The brain produces most of the 24(S)-hydroxycholesterol present in the body. This metabolite acts as an efficient LXR agonist (54) and is produced by cholesterol-24-hydroxylase (CYP46A1). This enzyme converts cholesterol from degraded neurones into 24(S)-hydroxycholesterol to allow the removal of cholesterol from the brain and is induced by oxidative stress (55). Glucose has also been described to induce the expression of LXR target genes at physiological concentrations, although the data are controversial (56,57).

The hypothalamus coordinates several complex homeostatic mechanisms and LXRs appear to be involved in some of them. The anatomical location of both receptor subtypes in the hypothalamus has been described using confocal microscopy (Fig. 1). LXRα was found in the periventricular nuclei, medial preoptic area (mPOA) and in the ventromedial nucleus of the hypothalamus (VMH), whereas LXRβ was found in mPOA and the ARC (52). These nuclei contain neurones reactive to nutrient-related signals that induce neurochemical responses to regulate energy homeostasis (58). On the other hand, recent results show that *in vitro* treatment with glucose or insulin may alter LXR expression in hypothalamic cells.

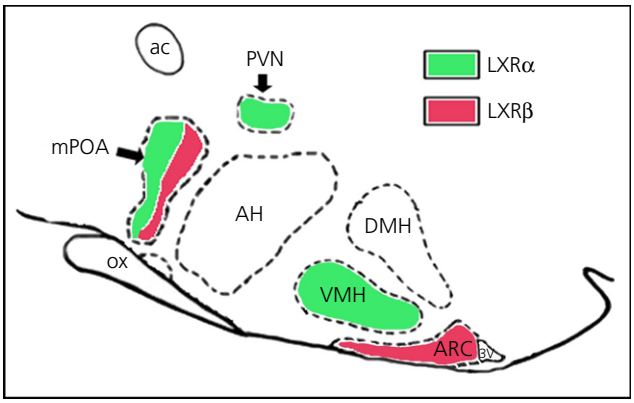


**Fig. 1.** Representative diagram of liver X receptor (LXR) expression in the hypothalamic nuclei of rats: LXR localisation in the hypothalamic nuclei was evaluated by immunocytochemistry using specific antibodies. An LXRα signal was observed in the paraventricular (PVN) and ventromedial (VMH) nuclei, whereas an LXRβ signal was found in the arcuate (ARC) nucleus; both LXR immunosignals were detected in the median preoptic area (mPOA) expressed in different cell types (52). DMH, dorsomedial nucleus of hypothalamus; AH, anterior hypothalamic area; ac, anterior commissure; ox, optic chiasm; 3V, third ventricle.

Glucose concentrations higher than 5.5 mM decrease LXRβ expression, whereas insulin treatment produces a similar effect only in the presence of 8.5 mM glucose. In both conditions, LXRα expression is unaffected (59). *In vitro* treatment with lipids also modifies the expression of this receptor. Incubation with cholic acid (4 h) and cholesterol increases the expression of LXRα, and cholic acid also promotes the expression of ABCA1. These results suggest that hypothalamic LXRβ is mainly sensitive to carbohydrate changes (47), whereas LXRα responds to lipid changes (60).

The data presented above indicate that oxysterols and LXRs are important players in the regulation of cholesterol metabolism in several organs. In addition, they mediate cholesterol removal from neurones in the CNS. There is also evidence that LXR expression in the hypothalamus is sensitive to nutrient levels, which suggests that they are involved in the regulation of energy balance. Thus, future studies that aim to understand the role that oxysterols and LXRs play in the regulation of energy metabolism would be of great interest.

## Food intake and metabolism: intertwined regulation by dopamine and prolactin

As mentioned at the beginning of this review, maintaining a proper energy balance is a key factor to guarantee reproduction. Metabolic adaptations to store energy during pregnancy in preparation for future demands is a biological allostatic hallmark in evolution. Females display a strong hyperphagia during pregnancy and lactation. The hormone prolactin may be a major factor mediating this hyperphagia (61,62), probably sustained by leptin-resistant hypothalamic centres controlling food intake (63).

Prolactin acts on peripheral tissues by activating a cytokine receptor (prolactin receptor; PRLR) of which there are long and short isoforms (64). Prolactin can reach the brain through an active

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re-uptake mechanism similar to the transport mechanisms described for leptin and insulin (65). In the brain, PRLR has been localised in the striatum, as well as in a number of hypothalamic nuclei associated with food intake and metabolism, including the ARC, VMH, PVN and the DMH (61). The presence of PRLR in brain areas associated with the regulation of energy balance and food intake, as well as in white and brown adipose tissue, liver and pancreas, raises the possibility that prolactin is involved in the regulation of energy balance acting at different levels (66) (Fig. 2).

Consistent with the hypothesis that prolactin has a significant role in the regulation of body weight, prolactin administration stimulates food intake (67,68), whereas PRLR-deficient mice exhibit lower body weight and a reduced fat mass (69). Nevertheles, female mice lacking dopamine D2 receptors (D2Rs), *Drd2*$^{-/-}$ mice, exhibit chronic hyperprolactinaemia and pituitary lactotroph hyperplasia (70,71) but similar body weight compared to wild-type females and only a minimal increase in food intake (72). However, *Drd2*$^{-/-}$ mice may not be an optimal model for studying the effects of chronic hyperprolactinaemia on energy balance given the fundamental importance of central D2Rs in food intake, reward mechanisms related to feeding behaviour (73,74) and growth hormone-releasing hormone-growth hormone regulation (75).

To unravel the role of elevated prolactin levels, with intact central D2Rs, lactotroph-specific D2R knockout (lacDrd2KO) female mice provide a unique model. In lacDrd2KO female mice, serum prolactin levels are chronically elevated and mice are subfertile, with altered oestrous cycles (76). Consistent with the presence of functional brain D2Rs, the haloperidol-induced catatonia test is normal and the growth hormone axis is preserved (77). In lacDrd2KO female mice, there is a marked increase in body weight, food intake and adiposity. In correlation with adiposity accretion, serum leptin is markedly elevated but hypothalamic anorexigenic peptides do not indicate leptin resistance. Hypothalamic POMC mRNA levels, as well as intermediate pituitary levels of α-melanocyte stimulating hormone (α-MSH), which are anorexigenic, are normal. Furthermore, mRNA levels of the orexigenic NPY, which are usually down-regulated by leptin, are increased.

Similarly, high prolactin levels in pregnancy or lactation induce a state of leptin resistance to meet the metabolic demands of the dams (78). Both suckling and prolactin increase NPY expression in the DMH, suggesting that prolactin might stimulate food intake by potentiating the effects of NPY input on the PVN (61).

On the other hand, in the global *Drd2*$^{-/-}$ knockout mouse, a loss of central D2Rs mediates a decrease in prepro-orexin (Ppo) mRNA levels and an increase in α-MSH levels and, to some extent, these two anorexigenic events may offset the effect of prolactin on food intake. Therefore, functional central D2R signalling in the lacDrd2KO mouse maintains POMC and Ppo mRNA levels and the central orexigenic effect of prolactin is fully demonstrated (Fig. 3).

These data indicate that central D2Rs, which are key elements in food intake homeostasis, interact with prolactin levels.

In lacDrd2KO female mice, heavier gonadal and retroperitoneal fat pads, larger adipocytes, and heavier livers were found. Increased adiposity correlated with higher serum triglycerides and non-esterified fatty acids, with no changes in cholesterol or adiponectin. In adipose tissue, prolactin has been shown to up-regulate the expression of its receptor, stimulate adipocyte differentiation and inhibit lipolysis (79,80). Adipose PRLR was not increased in the selective mutant, although lipolysis was decreased (76) and this may explain the increased adipocyte size found. Interestingly, the expression of a lipogenic enzyme, lipoprotein lipase, was also decreased in correlation with increased serum triglycerides. Livers were heavier in lacDrd2KO mutants. Abundant fat droplets were observed, as were a higher triglyceride content and PRLR mRNA levels. High fat content in the liver could not be attributed to changes in the

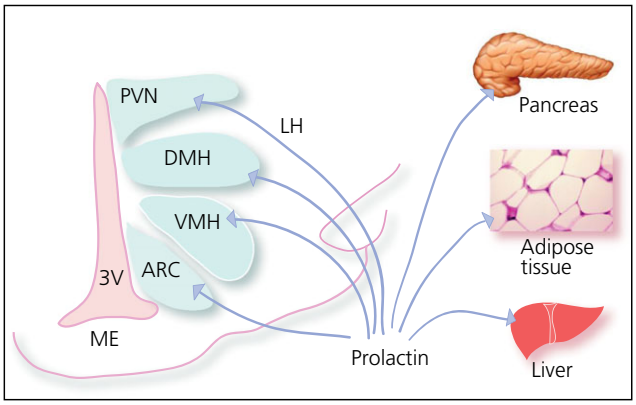


**Fig. 2.** Prolactin receptor (PRLR) in brain and tissues. In the rat brain, PRLR has been localised in a number of hypothalamic nuclei associated with food intake and metabolism, including the arcuate nucleus (ARC), ventromedial nucleus of the hypothalamus (VMH), paraventricular hypothalamic nucleus (PVN) and the dorsomedial nucleus of the hypothalamus (DMH). PRLR has also been described in the pancreas, adipose tissue and liver. ME, median eminence; LH, lateral hypothalamic area, 3V, third ventricle.

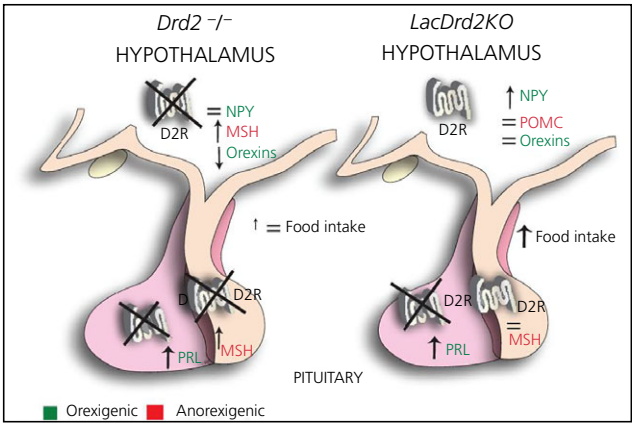


**Fig. 3.** Effect of global (*Drd2*$^{-/-}$) and lactotroph specific D2R knockout (LacDrd2KO) mouse models on anorexigenic and orexigenic factors in the pituitary and hypothalamus. In *Drd2*$^{-/-}$ females, two anorexigenic events [an increase in α-melanocyte stimulating hormone (MSH) and a decrease of orexin precursors] may offset the orexigenic action of prolactin (PRL), whereas, in lacDrd2KO mice, both *Pomc* and *Ppo* are not modified, and prolactin activates *Npy* expression, resulting in increased food intake. NPY, neuropeptide Y; POMC, pro-opiomelanocortin.


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expression of lipogenic or lipolytic enzymes but, instead, to alteration of glucose homeostasis. In this respect, hyperprolactinaemic lacDrd2KO mice had glucose intolerance, and a blunted insulin response to glucose (76).

In conclusion, selective ablation of D2Rs from lactotrophs evokes persistent hyperprolactinaemia, which induces a state of leptin resistance and increases hypothalamic NPY levels, in correlation with a hyperphagic state. Increased food intake, together with prolactin acting at different organs, modifies energy metabolism. There is an increase in adiposity, higher serum non-esterified fatty acids and triglycerides. At the pancreatic level, the insulin response to glucose is impaired, which results in glucose intolerance, high serum glucose and hyperinsulinaemia. Altered glucose metabolism may be responsible for the increased lipid content in the liver. These results highlight the role of prolactin as a metabolic hormone acting on different organs to reinforce its role during pregnancy, which is to store energy for future demands.

## Impact of altered metabolism on reproductive function

### Metabolic control of reproduction: focus on leptin signalling

The processes involved in successful reproduction, including sexual maturation, production of gametes, pregnancy and lactation, are energetically demanding (81–83). As a consequence, conditions of low energy availability or high energy utilisation result in decreased activity of the reproductive axis. For example, gonadotrophin secretion and ovulation are compromised in females in negative energy balance as a result of inadequate feeding or excessive energy expenditure (84,85). The interaction between these complex systems (metabolism and reproduction) is orchestrated by hypothalamic neurones that sense changes in circulating levels of metabolic cues and adapt the system to the individual nutritional condition. Among these cues, leptin is essential for the regulation of the reproductive axis.

Humans and mice with loss-of-function mutations in leptin (LEP/Lep) or leptin receptor (LEPR/Lepr) genes are obese and infertile (86–88). Both have low circulating gonadotrophin levels, incomplete development of the reproductive tract and no pubertal maturation. Leptin is an adipocyte hormone secreted into the circulation in proportion to fat mass. It binds to cognate receptors expressed in many organs and tissues. The LepR is a class 1 cytokine receptor found in six isoforms (88–90). The LepR long form (LepRb) is the signalling isoform and contains a Box 3 motif associated with downstream phosphorylation of tyrosine residues. The best associated pathway described is the Janus kinase/signal transducer and activator of transcription (JAK/STAT) signalling pathway (91–94). Deletion of leptin-induced STAT3 signalling (Tyr1138, LRbS1138s/s mice) recapitulates the hyperphagic obesity and the well-described changes in the melanocortin system of the LepR deficient (*db*/*db*) mice (95). However, disruption of STAT3 signalling in this mutant line has little effect on glycaemic control and fertility (95). The *s*/*s* female mice show sexual maturation, development of the reproductive tract and ovarian signs of ovulation, suggesting that the effect of leptin on reproductive function is independent of the STAT3 signalling pathway (95).

Disruption of LepRb tyrosine residue 1107 (Tyr1107) blocks leptin-induced phosphorylation of STAT5. These mice develop mild obesity and show very small changes in oestrous cycle duration (96). However, conditional deletion of STAT5 in LepR cells causes no metabolic or reproductive deficits (97). Deletion of both STAT3 and STAT5 signalling produces mice with a phenotype similar to those with deletion of STAT3 alone (i.e. increased body weight and adiposity). On the other hand, mutation in Tyr985 residue of LepRb causes a lean phenotype potentially because of increased leptin sensitivity as a result of blockade of SOCS3 and phosphatases associated with feedback inhibition of leptin signalling (98). No reproductive phenotype was observed in Tyr985 mutant mice.

Leptin also recruits the phosphoinositide 3-kinase (PI3K) signalling pathways (92,99–101). In hypothalamic slices, the acute effects of leptin on cell activity and feeding require intact PI3K (99,100,102–105). However, the molecular mechanisms associated with these responses are not clear, although studies have suggested that phosphorylation of insulin receptor substrate-2 (IRS-2) is upstream of leptin-induced PI3K (106,107). IRS-2 is expressed in hypothalamic neurones and IRS-2 knockout mice show metabolic dysfunction and infertility (108). Females have low sex hormones and deficient reproductive tract development. However, mice with conditional deletion of IRS-2 in LepR cells are fertile, although whether pubertal development, cyclicity and hormone levels are normal has not been reported (109).

PI3K is found as multiple classes of enzymes. Leptin recruits the class 1a PI3K comprising heterodimers of one regulatory and one catalytic subunit. The regulatory subunits are collectively called p85s and the catalytic subunits are called p110s (110,111). The p110$\alpha$ and p110$\beta$ catalytic subunits are widely expressed, and global deletion of either one is incompatible with life (112–115). However, 50% loss-of-function of p110$\alpha$ activity decreases insulin and leptin responsiveness, causing hyperphagia, glucose intolerance and increased fat mass (116). Both catalytic subunits are expressed in LepR neurones of the hypothalamus (104,117). However, whether the lack of leptin-induced PI3K signalling results in metabolic or reproductive deficits has not been reported.

In summary, the role of leptin in reproductive control is well established. However, the molecular pathways associated with leptin action as a permissive factor for pubertal maturation and as a signal of energy sufficiency for successful reproduction are still unsettled.

## Sexual dimorphism in the control of metabolism

### Sex steroids regulate metabolism

The differences in metabolic function between males and females highlight the role of sex steroids in the regulation of energy balance and body composition. In this regard, androgens and oestrogens are primary regulators of metabolism in both sexes. The

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action of sex steroids is focused on the hypothalamic nuclei that regulate food intake and energy balance, although they also regulate metabolism in peripheral tissue (muscle, liver and adipose tissue) (Fig. 4). Oestrogens exert their effects through binding to the nuclear oestrogen receptors (ER) isoforms α (ERα) and β (ERβ), or the membrane G protein-coupled oestrogen receptor (GPER30), dictating the activation of genomic or nongenomic pathways, respectively. At the same time, androgens bind to androgen receptors (AR) located in the nucleus or the cytoplasm of cells, with both exerting their actions in the nucleus.

## Androgens modulate metabolism in females and males

Multiple animal models and clinical studies have demonstrated that androgens play important roles in the control of metabolic function in both sexes. In males, androgens stimulate lean mass growth and inhibit fat accumulation (114). Therefore, it is not surprising that testosterone deficiency induces obesity, accumulation of visceral adipose tissue (VAT) and increases the risk of developing insulin resistance and diabetes mellitus (115). In females, androgen excess provokes a similar condition to androgen deficiency in males, including abdominal obesity, a pro-inflammatory profile and insulin resistance (116). The mechanisms associated with androgen deficiency-induced insulin-resistance probably include modifications in the muscle transcriptome, mainly a reduction in the expression of the transcription factor peroxisome proliferator-activated receptor-γ coactivator α (PGC1α), which plays important roles in the stimulation of mitochondrial biogenesis and skeletal muscle oxidative fibres

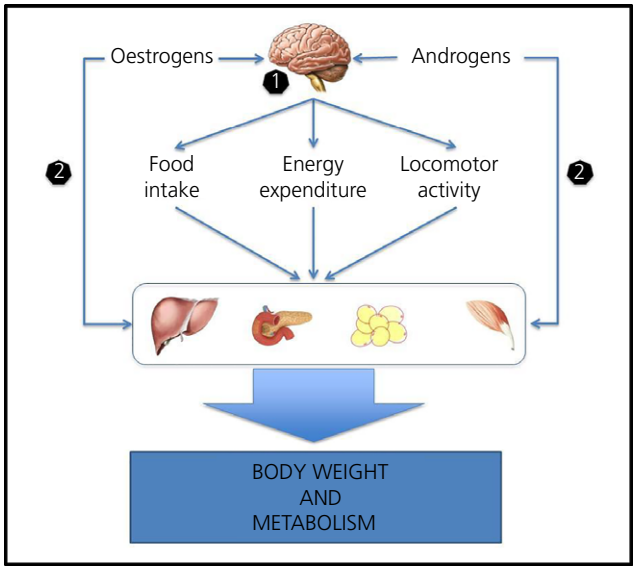


**Fig. 4.** Oestrogens and androgens regulate metabolic function. (1) Androgens and oestrogens act on the hypothalamus, mainly in the arcuate nucleus (ARC) and ventromedial nucleus of the hypothalamus (VMH), regulating food intake, energy expenditure and locomotor activity. This action impacts on the metabolic function of liver, pancreas, adipose tissue and muscle, leading to the regulation of body weight and whole-body metabolic function. (2) Moreover, androgens and oestrogens can directly modulate the function of these tissues.

(117). Moreover, testosterone and dihydrotestosterone (DHT) can modulate adipogenesis in subcutaneous adipose tissue (SAT) and VAT in both sexes. However, androgens can limit the number of mature adipose cells in females. By contrast, testosterone in males induces the proliferation of visceral pre-adipocytes (118).

It is difficult to isolate the role of androgens from the role of oestrogens with respect to metabolic function because androstenedione and testosterone are converted to oestrone and oestradiol, respectively, by the P450 aromatase. DHT, which cannot be metabolised to oestrogen, can be reduced by the aldo-ketoreductase family 1C to androstenediol, which has oestrogen-like activity via ERβ (119). However, AR knockout (ARKO) male mice develop late-onset obesity with an increase in both SAT and VAT. ARKO female mice lack these changes but present a reduction in energy expenditure (120), which demonstrates that androgens are directly involved in the control of body weight and metabolism.

Interestingly, ARs are more abundantly expressed in the brains of males than females, mainly in the VMH, ARC, anteroventral periventricular nucleus, mPOA and bed nucleus of the stria terminalis. In this regard, it has been observed that hypothalamic ARs are associated with the activation of STAT3 leptin-induced signalling in ARC neurones (121). In addition, *in vitro* studies demonstrate that ARs are necessary to maintain hypothalamic insulin sensitivity, which is mediated by the inhibition of nuclear factor-kappa B (122). In female mice, androgen-induced increase of visceral fat mass appears to be mediated by a decrease in hypothalamic POMC expression and POMC neuronal innervation to the DMH, resulting in the failure of leptin to activate brown adipose tissue thermogenesis and energy expenditure (123). These antecedents indicate that the hypothalamic ARs contribute to the suppression of food intake and the control of whole-body metabolism in both males and females.

## Oestrogens modulate metabolism in females and males

The metabolic role of oestrogens is better understood in female physiology. It is clear that the decline of ovarian function induces important changes in body composition, increasing the accumulation of total body fat, abdominal obesity and reducing energy expenditure (124). Interestingly, similar findings have been observed in female rats exposed to an inhibitor of the P450 aromatase. These animals present elevated androgens but reduced oestrogen levels and an increase in adiposity, larger adipose cells and insulin resistance (125).

Animal models show that oestrogens improve insulin sensitivity, body composition and the lipid profile in both sexes (126). Receptor-specific KO models have demonstrated that both receptors are involved in metabolic function. However, they can have different and sometimes antagonistic actions, with ERα probably being more relevant than ERβ signalling (127). The deletion of ERα induces insulin resistance, dyslipidaemia, β-pancreatic cell dysfunction and impaired glucose tolerance. In the same way, the selective activation of ERα or ERβ by specific pharmacological agonists, propylpyrazoletriol and diarylpropionitrile, respectively, has demonstrated similar effects (128). It appears that ERβ could have anti-


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obesogenic actions during high-fat diet challenge in mice, which is associated with the inhibition of PPARγ-induced adipogenesis, as demonstrated in ERβ KO mice (129,130). In this regard, it has been observed that the total and plasma membrane fraction of GLUT4 is strongly reduced in skeletal muscle of ERα KO mice but not affected in ERβ KO mice (131).

In the hypothalamus, ERα expression is markedly higher than ERβ in the VMH, ARC, PVN, POA and LHA. Of interest, the hypothalamic expression of oestrogen and androgen receptors is dependent on sex and age, indicating the importance of metabolism on reproductive function (132). Brain deletion of ERα induces hyperphagia and decreased energy expenditure and locomotor activity, leading to fat accumulation in visceral depots (133). Although these functions are determined by different hypothalamic areas, the direct injection of oestradiol into the PVN, ARC and VMH is the most effective method of reducing food intake and body weight and increasing locomotor activity, especially in females (134). In this regard, the specific loss of ERα in ARC POMC neurones increases food intake but does not directly affect energy expenditure (133), whereas the deletion of ERα in VMH neurones decreases energy expenditure but does not affect food intake (133,135). In addition to the metabolic effects exerted by their nuclear receptors, it has been observed that the deletion of oestrogen membrane receptor GPER30 increases body weight (136). In turn, the activation of the GPER30 alone is able to trigger the STAT3 signalling pathway. Interestingly, ERα KO mice exhibit altered leptin-induced STAT3 activation (137). Overall, these antecedents suggest a cross-talk between nuclear and/or membrane oestrogen receptors and leptin-induced STAT3 signalling in the control of food intake and energy expenditure.

In summary, it is clear that sex steroids are central regulators of metabolic function. Probably, androgens and oestrogens act coordinately at different organs such as brain, skeletal muscle, adipose tissue and liver. The different profile in sex steroids between females and males results in sex-dependent patterns of body composition, insulin sensitivity and energy expenditure.

## Concluding remarks

The importance of the regulation of energy metabolism is highlighted by the fact that survival and reproduction strongly depend on energy levels. It is clear that the CNS regulation of energy homeostasis is principally mediated by the hypothalamus, which contains neuronal populations with the ability to sense nutrient-related signals and affect food intake. This review highlights recent studies indicating that hypothalamic AgRP and POMC neurones utilise not only peptide transmitters to exert their roles, but also amino acid transmitters (GABA and glutamate) and this utilisation can be dynamically regulated depending on body energy status. The regulatory role of the hypothalamus is influenced by the action of peripheral hormones and metabolites produced by different organs, such as adipose tissue, gonads and gastrointestinal tract. In this review, the role of ghrelin, prolactin and oxysterols as participants in the regulation of metabolism has been discussed. Although these three molecules affect energy homeostasis and have their neuronal targets mainly located in the hypothalamus, their role becomes relevant in different states. Ghrelin is important when negative energy balance is present, stimulating food intake and preparing the body for the ingestion of food. Prolactin has a prominent role when females face pregnancy and lactation, promoting food intake with the final objective of storing energy for the future demands of the offspring. Oxysterols and their receptors are involved in the excretion of cholesterol when excessive cholesterol is present in cells, although recent studies have suggested that they are also implicated in the hypothalamic regulation of carbohydrates and lipid metabolism.

The hypothalamus is also important in the regulation of reproductive function, which is also influenced by body energy status. Leptin acts as a link between energy status (i.e. it is secreted in proportion to fat mass) with the reproductive axis acting on hypothalamic gonadotrophin neurones. This review has highlighted recent work that aimed to establish the molecular mechanism by which leptin modulates sexual maturation and fertility. It is also known that sex steroids are determinants of reproductive function and the present review has introduced valuable data showing the intertwined regulation of energy metabolism by oestrogens and androgens.

Altogether, the whole concert of hormones and metabolites that regulate energy metabolism act on interrelated pathways forming a complex network. The proper functioning of this network finally determines the capacity of an organism to survive and to breed. This review has discussed some of the advances made in areas that are part of this complex network, in the framework of an environment that predisposes humans to energy balance disorders. The efforts made in these research areas contribute to the overall objective of understanding the elaborate regulation of energy metabolism.

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