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Neuroendocrine Regulation of Metabolism

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Abstract

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 are focused 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 to ensure the maintenance of any species because of the dependence of reproduction on energy stores. Adequate levels of energy reserves are necessary for proper functioning of the hypothalamus-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 overweight and obesity demonstrates the difficulty that people have maintaining proper energy balance. In 2014, 39% of adults age 18 or over were overweight (BMI>25kg/m²), while 11% of men and 15% of women were obese (BMI>32kg/m²) (1). That means an estimate of 1.9 billion overweight adults worldwide, of which 600 million are obese. The risks associated with overweight include cardiovascular diseases and type 2 diabetes mellitus, among 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 behavior and reproduction, circadian rhythms and emotional responses. The hypothalamus, comprised of 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, among others. Through changes in 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. It is also a key factor to ensure the maintenance of any species because of the dependence of reproduction on energy stores (3). Adequate levels of energy reserves are necessary for proper functioning of the hypothalamus-pituitary-gonadal axis. The neuroendocrine players that link energy balance with the reproductive system include hormones and neuropeptides that act on hypothalamic

gonadotropin releasing hormone neurons. As an example, leptin is one of the hormones recognized 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 will discuss valuable data presented in the 2015 edition of the International Workshop of Neuroendocrinology concerning the fundamental nature of the neuroendocrine regulation of hypothalamic neurons and the impact on energy balance and reproduction.

Main hypothalamic systems regulating metabolism

POMC and AgRP neurons 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. In order to fully understand the central regulation of energy balance, it is essential to understand the complete complement of neurotransmitters involved. Clearly, the peptide transmitters released from hypothalamic agouti-related peptide (AgRP) and proopiomelanocortin (POMC) neurons are important regulators of energy balance (4,5). However, it has become increasingly clear that these neurons also utilize 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 neurons.

Indications that AgRP and POMC neurons likely use a mix of peptide and amino acid transmitters date back several decades to immuno-electron microscopy studies showing both dense-core and small-clear vesicles in the axon terminals of these neurons (4,6). A role for amino acid transmitters in food intake and metabolism was suggested through pharmacologic studies carried out from the 1970s through the 1990s. However, experiments exploring the potential significance of GABA or glutamate release from neurons in energy balance circuits were not possible until advances were made in genetic manipulation. Using genetic approaches to delete the orexigenic peptides NPY, 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 neurons in adult mice blunted food intake and caused rapid wasting (9,10). This surprising difference between peptide deletion and neuronal ablation was eventually attributed to the loss of GABA co-release when the neurons were ablated as a whole (11), sparking 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 neurons in the stimulation of food intake and the downstream targets of AgRP neuron-derived GABA release are beginning to be revealed (11,12).

The release of GABA from AgRP neurons can be dynamically regulated. Fasting causes increased GABA release, whereas leptin decreases GABA release from AgRP neurons onto POMC neurons (13). While inhibition of POMC neurons may not be a necessary contributor to increased food intake upon robust AgRP neuron stimulation (14), the high degree of connectedness from AgRP to POMC neurons makes this a convenient synapse to examine changes in GABA release from AgRP neurons. It is tempting to infer that altered release at one target site likely reflects similar changes in other target regions, but this may not be the case. Neurons can release different sets of chemical transmitters from

distinct fibers (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 neurons affects GABA release in specific target sites to fully understand the dynamic regulation imposed by these neurons under select conditions.

Unlike AgRP neurons that appear to use only GABA as their amino acid transmitter, POMC neurons appear heterogeneous in their amino acid phenotype with ~50% of POMC neurons being GABAergic and ~10-40% glutamatergic (19,20). Interestingly, the amino acid transmitter phenotype of POMC neurons seems to be plastic throughout development with a large proportion of POMC neurons 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 neurons 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 since vGlut2 was constitutively deleted from POMC neurons, and potentially from a subset of other neurons that transcribe the POMC gene briefly during development (22). Therefore, further studies are needed to determine the role of glutamate release from POMC neurons in adulthood and to begin to explore the functional consequence of GABA release from POMC neurons. Additionally, determining if GABAergic and glutamatergic POMC neurons represent otherwise distinct subpopulations of POMC neurons 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 neurons 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 neurons. Although there is much more to learn regarding the roles of amino acid transmitters in energy balance, the recognition that POMC and AgRP neurons 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 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 centers 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 (GH) 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 behaviors, among 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 the GHSR1a, which is a G-protein coupled receptor highly expressed in brain centers associated with food intake (27). The main neuronal targets that mediate ghrelin's orexigenic action 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, lying adjacent to the ARC, and the area postrema, which is part of the DVC. Circumventricular organs are specialized 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 supposed that ghrelin is able to freely diffuse through the median eminence and reach GHSR1a-expressing neurons (31). Regarding to the DVC, ghrelin could directly activate GHSR1a-expressing neurons of the area postrema, which in turn regulate their targets in brainstem and hypothalamus (32).

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

The DVC is another important brain area that mediates ghrelin's orexigenic action. This brain region is made up by 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 administration of ghrelin directly on the DVC promotes food intake (37). Additionally, intra-cerebroventricular 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 shows 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 to regulate food intake but it is not sufficient to mediate ghrelin's orexigenic action.

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 ghrelin's orexigenic action are well established, the exact molecular mechanisms by which this hormone regulates feeding are not completely understood. Unraveling these mechanisms would be of extreme importance to consider them as potential therapeutic targets in the treatment of pathologies that affect food intake.

Oxysterols and LXRs.

Overweight and obesity are primarily linked to poor eating habits. Busy lifestyle induces an increase in the availability and consumption of fast foods. This type of food contains great amounts of animal fats, which contain a mixture of triglycerides, cholesterol and phospholipids. Cholesterol is necessary to guarantee the integrity and fluidity of 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 metabolize it and one important way is 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 liver X receptors (LXRs) acting as their endogenous ligands (41–43). The functional LXRs exist as two isoforms: LXR α and LXR β . The first is mainly expressed in the liver and to a lesser extent in the gut, adipose tissue, kidney, spleen and macrophages, whereas LXR β is expressed in almost all tissues (40). Upon exposure to excessive accumulation of intracellular cholesterol oxides, LXRs activate a program 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, LXRs activation promotes the direct conversion of excessive cholesterol to bile acids through the regulation of the limiting enzyme 7-alpha hydroxylase (CYP7a) (40).

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 increases 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 LXR β in particular plays an important role in pancreatic insulin secretion and LXR activators promote insulin secretion (47). LXR ligands have also showed 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 sensitizers (49,50).

While 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 LXR α and LXR β in the hypothalamus is sensitive to triglycerides and serum insulin levels. Animals with glucose intolerance show an upregulation of LXR β and a downregulation of LXR α in the hypothalamus. In addition, a correlation between this LXR expression and triglycerides or insulin levels was described indicating the importance of both subtypes in the risk of developing metabolic diseases (52). LXR β expression in the hypothalamus correlates negatively with the area under the curve in glucose tolerance tests in control animals, while a positive correlation is found in rats with abnormal glucose tolerance (52,53). The endogenous

receptor agonists can also contribute to 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 the cholesterol-24-hydroxylase (CYP46A1). This enzyme converts cholesterol from degraded neurons 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 this data is controversial (56,57).

The hypothalamus coordinates several complex homeostatic mechanisms and LXRs seem to be involved in some of them. The anatomical location of both receptor subtypes in the hypothalamus has been described using confocal microscopy (**Figure 1**). LXR α was found in the periventricular nuclei, medial preoptic area (mPOA) and in the VMH while LXR β was found in mPOA and the ARC (52). These nuclei contain neurons 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. Glucose concentrations higher than 5.5 mM decrease LXR β expression, while insulin treatment produces a similar effect only in the presence of 8.5 mM glucose. In both conditions, LXR α 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 LXR α , and cholic acid also promotes the expression of ABCA1. These results suggest that hypothalamic LXR β is mainly sensitive to carbohydrate changes (47) while LXR α responds to lipid changes (60).

-----FIGURE 1 SHOULD BE HERE-----

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 neurons 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 directed 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 in the introduction of this article, 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 centers controlling food intake (63).

Prolactin acts on peripheral tissues by activating a cytokine receptor (PRLR) of which there are long and short isoforms (64). Prolactin can reach the brain through an active reuptake mechanism similar to the transport mechanisms described for leptin and insulin (65). In the brain, PRLR has been localized 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) **(Figure 2)**.

-----FIGURE 2 SHOULD BE HERE-----

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

To unravel the role of elevated prolactin levels, with intact central D2Rs, lactotrope specific D2R knockout (lacDrd2KO) female mice provide a unique model. In lacDrd2KO female mice serum prolactin levels are chronically elevated, mice are subfertile and have altered estrous cycles (76). Consistent with the presence of functional brain D2Rs, haloperidol-induced catatonia test is normal and the GH 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 downregulated 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, loss of central D2Rs mediates a decrease in prepro-orexin (Ppo) mRNA levels and an increase in α -MSH levels, and these two anorexigenic events may offset to some extent the effect of prolactin on food intake. Therefore, functional central D2R signaling in the lacDrd2KO mouse maintains POMC and Ppo mRNA levels and the central orexigenic effect of prolactin is fully evidenced **(Figure 3)**.

-----FIGURE 3 SHOULD BE HERE-----

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 upregulate the expression of its receptor, stimulate adipocyte differentiation and inhibit lipolysis (79,80). Adipose PRLR was not increased in the selective mutant, but 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 well as higher triglyceride content and PRLR

mRNA levels. High fat content in the liver could not be attributed to changes in the expression of lipogenic or lipolytic enzymes, but to alteration of glucose homeostasis. In this respect, hyperprolactinemic lacDrd2KO mice had glucose intolerance, and a blunted insulin response to glucose (76).

In conclusion, selective ablation of D2Rs from lactotropes evokes persistent hyperprolactinemia 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, insulin response to glucose is impaired, which results in glucose intolerance, high serum glucose and hyperinsulinemia. Altered glucose metabolism may be causal of 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 signaling.

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 utilization result in decreased activity of the reproductive axis. For example, gonadotropin secretion and ovulation are compromised in females in negative energy balance caused by inadequate feeding or excessive energy expenditure (84,85). The interaction between these complex systems (metabolism and reproduction) is orchestrated by hypothalamic neurons 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 gonadotropin levels, incomplete development of the reproductive tract and no pubertal maturation. Leptin is an adipocyte hormone secreted into 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 signaling isoform and contains a Box 3 motif associated with downstream phosphorylation of tyrosine residues. The best associated pathway described is the JAK/STAT signaling pathway (91–94). Deletion of leptin-induced STAT3 signaling (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 signaling in this mutant line has little effect on glycemic control and fertility (95). The s/s female mice show sexual maturation, development of the reproductive tract and ovarian signs of ovulation suggesting that leptin's effect on reproductive function is independent from STAT3 signaling 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 estrous cycle duration (96). However, conditional deletion of STAT5 in LepR cells causes no metabolic or reproductive deficits (97). Deletion of both STAT3 and STAT5 signaling 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 due to increased leptin sensitivity due to blockade of SOCS3 and phosphatases associated with feedback inhibition of leptin signaling (98). No reproductive phenotype was observed in Tyr985 mutant mice.

Leptin also recruits the phosphoinositide 3-kinase (PI3K) signaling 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, but 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 neurons 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, but whether pubertal development, cyclicity and hormone levels are normal have not been reported (109).

PI3K is found as multiple classes of enzymes. Leptin recruits the class 1a PI3K comprised of heterodimers of one regulatory and one catalytic subunit. The regulatory subunits are collectively called p85s, and the catalytic subunits, p110s (110,111). The p110 α and p110 β catalytic subunits are widely expressed, and global deletion of either one is incompatible with life (112–115). However, 50% loss-of-function of p110 α activity decreases insulin and leptin responsiveness causing hyperphagia, glucose intolerance and increased fat mass (116). Both catalytic subunits are expressed in LepR neurons of the hypothalamus (104,117). However, whether the lack of leptin-induced PI3K signaling 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 permissive factor for pubertal maturation and as a signal of energy sufficiency for successful reproduction is still unsettled.

Sexual dimorphism in the control of metabolism

Sex steroids regulate metabolism.

The differences in metabolic function between males and females point out the role of sex steroids in the regulation of energy balance and body composition. In this regard, androgens and estrogens are primary regulators of metabolism in both sexes. The action of sex steroids is focused on the hypothalamic nuclei that regulate food intake and energy balance, but they also regulate metabolism in peripheral tissue (muscle, liver and adipose tissue; **Figure 4**). Estrogens exert their effects through binding to the nuclear estrogen receptors (ER) isoforms α (ER α) and β (ER β), or the membrane G protein-coupled estrogen receptor (GPER30), dictating the activation of genomic or non-genomic pathways, respectively. Meanwhile, androgens bind to androgen receptors (AR) located in the nucleus or the cytoplasm of cells, both exerting their actions in the nucleus.

-----FIGURE 4 SHOULD BE HERE-----

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-gamma coactivator alpha (PGC1 α), which plays important roles in the stimulation of mitochondrial biogenesis and skeletal muscle oxidative fibers (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. In 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 estrogens on metabolic function because androstenedione and testosterone are converted to estrone and estradiol, respectively, by the P450 aromatase. DHT, which cannot be metabolized to estrogen, can be reduced by the aldo-ketoreductase family 1C to androstenediol, which has estrogen-like activity through ER β (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 (90), 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 signaling in ARC neurons (121). In addition, *in vitro* studies demonstrate that ARs are necessary to maintain hypothalamic insulin sensitivity, which is mediated by the inhibition of NF- κ B (122). In female mice, androgens-induced increase of visceral fat mass seems 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.

Estrogens modulate metabolism in females and males.

The metabolic role of estrogens 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 estrogen levels and an increase in adiposity, larger adipose cells and insulin resistance (125).

Animal models show that estrogens improve insulin sensitivity, body composition and 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 ER α probably more relevant than ER β signaling (127). The deletion of ER α induces insulin resistance, dyslipidemia, β -pancreatic cell dysfunction and impaired glucose tolerance. In the same way, the selective activation of ER α or ER β by specific pharmacological agonists, propylpyrazoletriol and diarylpropionitrile respectively, has demonstrated similar effects (128). It seems that ER β could have anti-obesogenic actions during high-fat diet challenge in mice, which is associated to the inhibition of PPAR γ -induced adipogenesis, as it has been demonstrated in ER β 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 ER α KO mice but not affected in ER β KO mice (131).

In hypothalamus, ER α expression is markedly higher than ER β in VMH, ARC, PVN, POA and LHA. Of interest, the hypothalamic expression of estrogen and androgen receptors is dependent on sex and age, indicating the importance of metabolism on reproductive function (132). Brain deletion of ER α 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 estradiol into the PVN, ARC and VMH are the most effective in reducing food intake, body weight and increasing locomotor activity, especially in females (134). In this regard, the specific loss of ER α in ARC POMC neurons increases food intake but does not directly affect energy expenditure (133), whereas the deletion of ER α in VMH neurons 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 estrogen membrane receptor GPER30 increases body weight (136). In turn, the activation of the GPER30 alone is able to trigger the STAT3 signaling pathway. Interestingly, ER α KO mice exhibit altered leptin-induced STAT3 activation (137). Overall, these antecedents suggest a crosstalk between nuclear and/or membrane estrogen receptors and leptin-induced STAT3 signaling 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 estrogens 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 presented recent studies indicating that hypothalamic AgRP and POMC neurons utilize not only peptide transmitters to exert their roles but also amino acid transmitters (GABA and glutamate), and this utilization 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 article, the role of ghrelin, prolactin and oxysterols as participants in the regulation of metabolism was discussed. Although these three metabolites 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, but recent studies 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 (it is secreted in proportion to fat mass) with the reproductive axis acting on hypothalamic gonadotropin neurons. This article presented 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 introduced valuable data showing the intertwined regulation of energy metabolism by estrogens and androgens.

Altogether, the whole concert of hormones and metabolites that regulate energy metabolism act on interrelated pathways forming a complex network. Proper functioning of this network finally determines the capacity of an organism to survive and to breed. This review 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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