Leptin and estrogen signaling crosstalk in the brain modulates energy metabolism

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Abstract Leptin and estrogen are key hormones in regulating feeding, metabolic health, and body weight. In this review, we explore how the interaction between leptin and estrogen may modulate body weight through changes in metabolism and feeding behaviour. A significant proportion of arcuate neurons co-express receptors for leptin and estrogen, providing ample opportunity for signal crosstalk to occur in the brain. We conducted a narrative literature review and identified the major mechanisms through which leptin and estrogen interact, with a focus on signal transduction pathways. G-protein coupled receptor 30 (GPR30) is a good candidate for an inter-pathway connection because it interacts with estrogen receptors and affects the activation of signal transducer and activator of transcription (STAT3), an important downstream factor in both estrogen and leptin signaling pathways in the hypothalamus. Evidence suggests that estrogen and leptin receptors both utilize hypothalamic STAT3-activating pathways to modulate appetite and lipid storage, and that these pathways may depend on one another for adequate activation. While there are some physiological results to support this point of connection, the cellular and biochemical details remain unclear. Better understanding how leptin and estrogen interact will better inform the treatment of metabolic disorders, including type 2 diabetes, obesity, and post-menopausal weight gain.

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INTRODUCTION

Energy balance is key for homeostasis and is the net result of energy intake through feeding minus energy expenditure through exercise and metabolism. When energy intake chronically exceeds energy expenditure, obesity results, which can lead to comorbidities such as type 2 diabetes (T2D), atherosclerosis, and some cancers (Apovian, 2016). Due to the availability of high-calorie, inexpensive processed foods, obesity has become an epidemic worldwide, contributing to healthcare overburden and patient suffering (Di Cesare et al., 2019). Increasing exercise and decreasing caloric intake can ameliorate obesity but these outcomes have proven difficult to attain and understanding why this is so remains an important question in health research. This uncertainty is due, in part, to the complexity of energy-regulating circuits in the brain (van Swieten et al., 2014). Characterization of the neuroendocrine action of hormones such as leptin and estrogen may provide the insight needed to better understand and manage metabolic diseases, thus reducing the burden of obesity and its associated comorbidities on healthcare.

Leptin stimulates orexigenic and inhibits anorexigenic neurons, and therefore plays a critical role in reducing food intake, regulating body weight, and controlling energy homeostasis (Pelleymounter et al., 1995). Studies have reported that patients with rare homozygous leptin gene mutations have low blood leptin levels and increased obesity (Strobel et al., 1998). However, when patients with such leptin deficiencies are treated with leptin therapy, food intake is reduced and metabolic decompensation resolves, demonstrating that leptin plays an integral role in regulating body weight in humans (Farooqi et al., 1999). Paradoxically, leptin is replete in obesity, though its signaling pathways appear to be disrupted by negative feedback mechanisms such as SOCS3 (Ernst et al., 2009). Like leptin, estrogen contributes to decreasing food intake and increasing energy expenditure (Farooqi et al., 1999; Asarian & Geary, 2002). Studies have indicated that treating ovariectomized rats with estrogen therapy results in decreased body weight (Babaei et al., 2017). This illustrates the importance of estrogen in managing body weight and raises the possibility of signaling overlap with leptin.

This review aims to better characterize the relationship between estrogen and leptin at the level of STAT3 activation in hypothalamic neurons. To achieve this, we searched for peer-reviewed articles containing the keywords "estrogen," "leptin," "STAT3," and "hypothalamus" using Medline, PubMed, and Google Scholar. Though it is well-established that leptin and estrogen have overlapping metabolic roles in reducing food intake and body

weight, it is not yet clear if leptin and estrogen interact with each other's signaling pathways to regulate energy metabolism. Therefore, we will discuss the roles of estrogen and leptin in health and metabolic disease, their effect on the signal transducer STAT3, and how this molecule may link the pathways of these two important hormones.

LITERATURE REVIEW

Leptin's role in energy metabolism

Leptin is a 167-amino acid hormone that is produced in adipose tissue and acts as a master regulator of metabolism (Zhang et al., 1994). Leptin maintains energy homeostasis by informing the body about its energy stores and subsequently mediating neurological and metabolic changes to modulate body weight and appetite accordingly (Park & Ahima, 2015). To perform these functions, leptin primarily targets hypothalamic neurons, which then signal to peripheral organs of metabolic import including the liver, skeletal muscles, and pancreas. However, leptin also functions outside of the central nervous system (CNS) to affect rates of glucose and lipid metabolism in adipose, liver, and the immune system (Pereira et al., 2021; Denroche et al., 2012; Gray et al., 2010). Importantly, this includes maintaining insulin sensitivity in adipose and skeletal muscle, which is likely a key component of leptin's glucoregulatory effects (Bates et al., 2005) and ability to regulate body weight.

The majority of leptin's weight-regulating effects are mediated by its action on neurons of the energy regulating centres in the brain. Once leptin travels through the bloodstream and crosses the blood brain barrier, it binds to leptin receptors (LepRs) present on hypothalamic neurons such as those in the arcuate nucleus (ARC). Leptin activates anorexigenic (appetite suppressing) neurons, which are responsible for the production of neuropeptides such as pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) (Cowley et al., 2001). Conversely, leptin inhibits orexigenic (appetite inducing) neurons, which are responsible for the production of agouti-related peptide (AgRP) and neuropeptide Y (NPY) (Cowley et al., 2001). When circulating leptin is deficient, AgRP and NPY are secreted by orexigenic neurons, while anorexigenic neurons decrease their expression of POMC and CART (Park & Ahima, 2015). Several metabolically important peptides, such as insulin and glucagon-like peptide-1, can alter leptin's ability to activate hypothalamic neurons and highlight the importance of hormonal interactivity in regulating energy homeostasis (Williams et al., 2006).

The concentration of circulating leptin is directly proportional to the amount of adipose tissue, suggesting that obese individuals produce more leptin as their bodies are less sensitive to the peptide hormone (Cowley et al., 2001). However, recent findings have suggested that restricted leptin entry to the CNS provides a novel method of leptin resistance (Duquenne et al., 2021). There are several other mechanisms, such as soluble leptin receptors in the blood, that decrease leptin availability and may dampen leptin's ability to reach the CNS (Tu et al., 2006). Any processes that significantly alter the body's perceived level of leptin invariably show metabolic disruption.

Leptin's homeostatic importance is illustrated by metabolic perturbations that are exhibited in conditions of leptin excess or deficiency. For example, obese subjects can have ten-fold higher than normal levels of leptin due to the linear relationship between adipose mass and serum leptin, which drives resistance to both leptin and insulin and further exacerbates weight gain (Zhao et al., 2019). Excess serum leptin in obesity may cause leptin resistance by increasing SOCS3, a negative regulator of the leptin signaling pathway (Ernst et al., 2009). Conversely, leptin deficiency seen in lipodystrophy syndromes induces insulin resistance and hypertriglyceridemia. Leptin replacement therapy in lipodystrophic patients restores metabolic functions such as glycemic regulation and fat metabolism (Oral et al., 2002; Chong et al., 2010). Leptin likely mediates this effect by restoring the adipose tissue, which is a key target for insulin action. In rodent models of type 1 diabetes, leptin corrects high plasma glucose levels by lowering hepatic glucose production and by increasing tissue uptake of glucose independently from insulin (Paz-Filho et al., 2012). These examples show the importance of serum leptin concentration to metabolic health.

Leptin is also a key regulator of appetite and leptin deficiency causes hyperphagia and obesity in humans as well as animal models. This can result from the inadequate production of leptin, as seen in the obese (ob/ob) mice, or from leptin resistance, in db/db mice, caused by leptin receptor gene mutation (Paz-Filho et al., 2012). Dyslipidemia is commonly the earliest risk factor of atherosclerotic cardiovascular disease in obesity and is due to abnormal cholesterol and lipid levels seen in hyperleptinemia (Tsai et al., 2017; Du et al., 2016). Ravussin et al. (1997) have indicated a correlation in humans between low concentrations of leptin in the plasma and abnormally high gains in body weight over short periods of time, which ties together the body weight regulating activity of leptin seen in animal models to human physiology.

Estrogen's role in energy metabolism

Estrogen is another important energy regulating peptide that acts in the hypothalamus. It may have functional overlap in intracellular signaling with leptin, allowing the two hormones to integrate nutrient information and together mediate changes in behaviour and metabolism. Estrogen is a sex hormone derived from cholesterol and not only affects the gonadal organs but also impacts liver, heart, muscle, and bone tissue in both sexes (Cui et al., 2013). Estrogen is mainly produced by the ovaries, and less in testes, adipose tissue, and brain. Four types of estrogen exist: estrone (E1), estradiol (E2), estriol (E3), and estrane (E4), with estradiol being the most potent and common form. As a steroid hormone, estradiol can freely diffuse across the plasma membrane to interact with the estrogen receptors (ER) located in the cytosol.

The two main subtypes of estrogen receptors, ERα and ERβ, are coded by genes located on different chromosomes (6 and 14, respectively) but both consist of six domains with distinct functions. Starting from the N-terminus, the A/B domains contain activation function 1, which acts as a regulator for ER's transcriptional activity. The C domain is responsible for ER's binding to specific DNA sequences to regulate their expression. The D domain is a hinge region containing a nuclear localization sequence and is where post-translational modifications occur. At the E domain, or ligand-binding domain, estrogen binds and interacts with co-regulators. Lastly, the F domain at the C-terminus modulates the receptor's activity. When estradiol binds, ER is phosphorylated, dimerizes, and travels into the nucleus to bind estrogen response elements in DNA, facilitating assembly of the transcription complex (Figure 1) (Lee et al., 2012). Transcriptional modification by ER shifts metabolism towards increased energy expenditure, increased proliferation, and improved cell survival (Charpentier et al., 2000).

On a macroscopic level, there are estrogen-dependent systemic differences that exist between the sexes. There is a higher prevalence of obesity among females compared to males, though males have been shown to be more likely to develop obesity-associated comorbidities which may be a result of how estrogen differs in men and women (Ng et al., 2014; Meyer et al., 2006; Onat et al., 2016). It has been established that healthy, pre-menopausal females exhibit a defense against metabolic dysfunctions compared to men, primarily ascribed to the activity of estrogen (Sharma & Prossnitz, 2021). In a study conducted to identify the sexual dimorphism in inflammation in obese mice, it was found that although the females had more adipose tissue compared to the males overall, they were also more tolerant to glucose. This finding supports estrogen's ability to protect against metabolic defects, including insulin resistance, diet-induced obesity, and similar disorders (Nickelson et al., 2012; Varghese et al., 2017). However, it is important to note that for estrogen, murine models are limited in their generalizability to humans and as a result, mouse models should be interpreted with caution (Elsea & Lucas, 2002; Springer & Murphy, 2007; Rader, 2004).

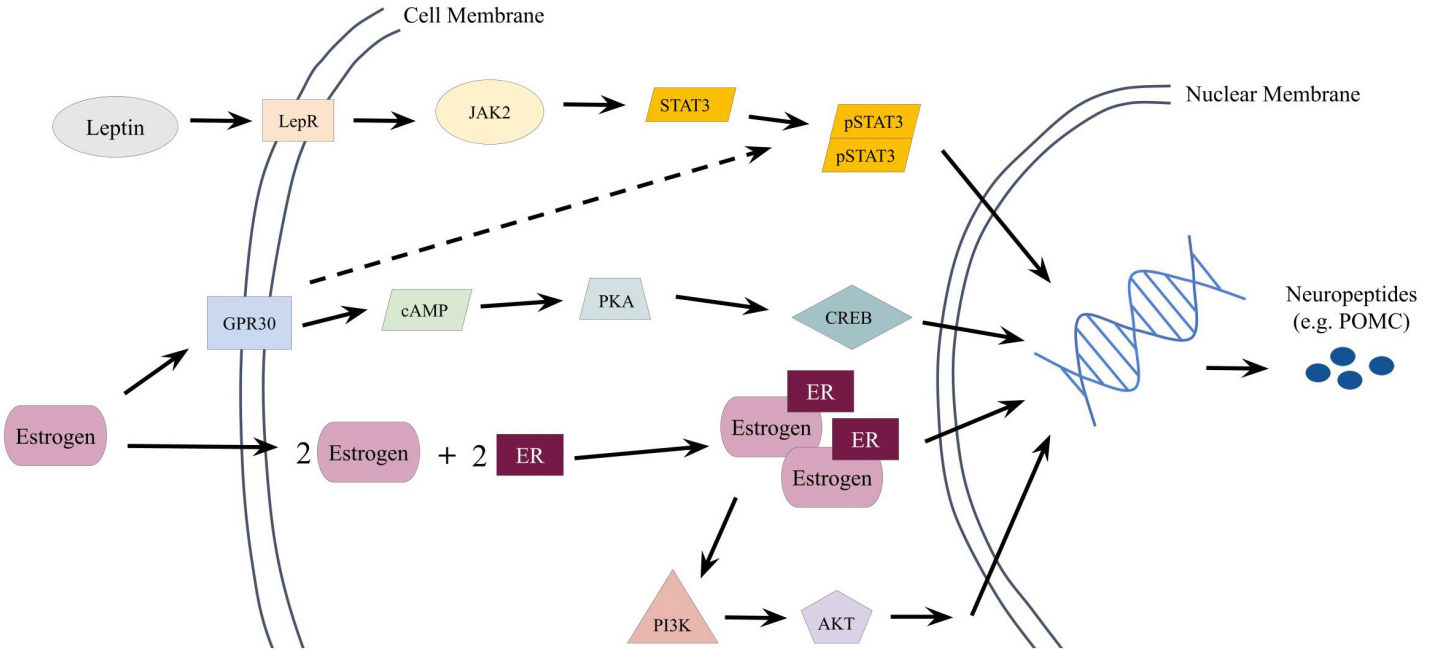

Figure 1 Signaling pathways of leptin and estrogen in hypothalamic neurons to indicate the proposed site of interaction between these hormones, indicated by a dashed arrow. All leptin and estrogen signaling pathways presented here are able to increase the mRNA expression of the orexigenic neuropeptide POMC, which can be translated into proteins such as alpha-melanocyte-stimulating hormone (not shown) to decrease appetite. Abbreviations: LepR, leptin receptor; JAK2, Janus kinase 2; STAT3, signal transducer and activator of transcription; p, phosphate group (some excluded for clarity); POMC, pro-opiomelanocortin; GPR, G protein-coupled receptor 30; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; CREB, cAMP response element-binding protein; ER, estrogen receptor; and PI3K, phosphoinositide 3-kinase.

A study conducted on 20 postmenopausal women attempting to identify the mechanisms related to T2D found that the people living with T2D have significantly higher estradiol concentrations compared to non-diabetics (Phillips et al., 2000). Moreover, elevated levels of estradiol have been implicated in being positively correlated with adiposity, further substantiating the relationship between estrogen, body weight, and T2D (Longcope et al., 1986).

A study by Varghese et al. (2017) proposed that as estrogen levels decline with age, menopause increases the proportion of testosterone in women, leading to increased visceral adiposity. Through the activity of testosterone, males store fat in visceral adipose tissue (VAT) preferentially to subcutaneous adipose, a pattern similarly seen amongst postmenopausal women (Bloor & Symonds, 2014). VAT is implicated in elevated inflammation as well as insulin resistance, and as VAT increases, the risk factor for metabolic disorders, such as obesity and T2D, increases as well (Smith et al., 2001; Meyer et al., 2011). Likewise, in a depressed estrogen state such as menopause, it has been identified that fat is redistributed in the body and the composition of VAT changes (Varghese et al., 2017; Pedersen et al., 2001). Heine (2000) showed that mice with knocked-out estrogen receptors had more white adipose tissue than control mice, as well as a significant reduction in energy expenditure. White adipose tissue functions as an energy storage depot by accumulating fatty acids and triglycerides after meals, and then releasing them when the body requires energy (Trayhurn & Beattie, 2001). Increased visceral adiposity, hyperinsulinemia, and overall increase in white adipose tissue may explain the weight gain experienced in a hypoestrogenic state.

Ultimately, it is evident that estrogen plays a critical role in body weight and energy homeostasis, as both an over- and under-production of the hormone result in metabolic disorders.

STAT3: the link between leptin and estrogen?

Whether estrogen and leptin cooperate and if this interaction affects body weight has not yet been definitively concluded. Research conducted by Clegg et al. (2006) found that rats with higher circulating estrogen levels had increased sensitivity to leptin and lower body weight. Furthermore, ovariectomy female rats injected with estradiol had lower body weight, highlighting estrogen's important role in regulating body weight. The authors found that, in the presence of estrogen, leptin sensitivity was high despite lower LepR isoform b (LepRb) protein expression in the hypothalamus. It was also suggested that estrogen potentially acts downstream of LepRb's signal cascade in ARC neurons, where ERα and LepRb are coexpressed (Clegg et al., 2006). Although Clegg, et al. (2006) were able to establish a correlation between estrogen and leptin, they were unable to confirm the pathway by which estrogen mediates body weight in the leptin signaling pathway.

The mechanism through which leptin regulates body weight has been heavily studied and a clear mechanism by which it functions has been determined. Once leptin is released from adipose tissue, it travels through the bloodstream and crosses the blood brain barrier and binds to leptin receptors present on hypothalamic neurons in hypothalamic nuclei including the ARC (Figure 1). LepRb is the most common isoform and is highly expressed on hypothalamic neurons. (Couce et al., 1997). Binding of leptin to LepRs activates Janus kinase 2 (JAK2) (Banks et al., 2000). Upon ligand binding, JAK2 phosphorylates three specific tyrosine residues located on LepRb, and when tyrosine 1388 is phosphorylated, STAT3 is transported to the LepRb-JAK2 complex (Gao & Horvath, 2008). JAK2 then phosphorylates STAT3. Following phosphorylation and dimerization, STAT3 travels to the nucleus where it binds to the promoter regions of genes to modulate their expression. In response to activation by leptin, STAT3 increases POMC and CART expression and decreases AgRP and NPY expression, thereby increasing food intake (Gao & Horvath, 2008; Ernst et al., 2009; Mesaros et al., 2008).

Estrogen acts through multiple signal pathways to mediate its metabolic effects and alter cell survival in neurons. It can act on cell surface receptors or pass through the cell membrane to bind ERs in the cytoplasm. Intracellular ERs bind estrogen in the cytosol, forming a complex, and can then utilize two separate pathways. In the best-characterized mechanism, the ER-estrogen complex binds to another ER-estrogen complex in a process called dimerization and together they translocate to the nucleus and act as a transcription factor (Figure 1). Alternatively, ER can act

through the phosphoinositide 3-kinase (PI3K) pathway to mediate changes in neuropeptides such as POMC (Malyala et al., 2008). G-protein coupled receptor 30 (GPR30) is a cell membrane-bound estrogen receptor that initiates the cyclic adenosine monophosphate/protein kinase A (cAMP/PKA) pathway and ultimately increases STAT3 activity, which may serve as the link between estrogen and leptin signaling (Kwon et al., 2014). The exact method of this interaction remains to be elucidated, as indicated by the dashed arrow in Figure 1.

DISCUSSION

Identifying the specific pathway where estrogen and leptin interact to regulate body weight is an important factor in developing therapeutics that target them. Gao et al. (2007) aimed to determine whether estrogen influences anorexigenic POMC hypothalamic neurons in experimental mouse models. They found that estradiol is dependent on the STAT3 pathway to alter body weight and that its mechanism of action on POMC tone may be to increase the number of excitatory synapses, parallelling leptin's effect on these neurons (Gao et al., 2007). With regards to body weight, it was seen that exogenous administration of estradiol significantly reduced body weight gain – a finding seen across both the ob/ob and db/db mouse lines as compared to wildtype controls (Gao et al., 2007). Estradiol caused no reduction in body weight in STAT3-knockout mice when compared to controls, supporting the dependence on the STAT3 pathway for estradiol's ability to alter body weight (Gao et al., 2007). Furthermore, estradiol mediates the phosphorylation of STAT3 in a similar manner to JAK2 (Ma et al., 2020).

Kwon et al. (2014) found there may be two distinct pathways through which estrogen initiates the STAT3 signaling pathway and modulates body weight. Along with the known pathway of ERα-mediated STAT3 activation, GPR30 may be involved. As a member of the G-protein family, GPR30 has 7 transmembrane domains with an extracellular ligand binding site. Previous research has demonstrated that estradiol is a ligand for GPR30 (Revankar et al., 2005). Interestingly, Kwon and colleagues (2014) also found that when a GPR30-specific antagonist was added, the STAT3 signaling pathway was inhibited. Estrogen was found to bind GPR30 and thereby initiate pSTAT3 formation, resulting in the activation of the anorectic pathway in the ARC, leading to weight loss.

More recently, it has also been proposed that no STAT3-dependent interaction exists between ERα and LepRb with respect to estradiol's ability to modulate body weight via the hypothalamus (Kim et al., 2016). Administration of estradiol did not increase the leptin-dependent phosphorylation of STAT3, which indicates a lack of potentiation of the hypothalamic leptin signaling pathway by estradiol but may not necessarily suggest a lack of connection between ERα and LepRb signaling pathways in the modulation of metabolism. This study indicated that the knockout of either STAT3 or ERα from LepRb-expressing cells did not significantly alter estradiol's ability to reduce body weight, suggesting that STAT3 of the LepRb pathway is not necessarily required for body weight homeostasis. This finding seems to contradict the putative position that the interaction between LepRb and estradiol is crucial for the STAT3-mediated regulation of body weight.

There are several important caveats related to the results in the Kim et al. (2016) study. Immunohistochemistry was used to determine the overlap in the number of LepRb- and ERα-coexpressing cells, estimated to be less than 15%. Counting of coexpressing cells was performed by hand and perhaps not blinded to experimental groupings, which may have introduced bias. Furthermore, in the representative images, there are coexpressing cells that were not indicated and appear to have been missed in the analysis. Lastly, knocking down ERα in LepRb-expressing neurons causes significant changes in feeding, suggesting that ERα plays a role in the intracellular pathways related to feeding behaviour, which are also acted upon by leptin. Taken together, these data seem to indicate a fairly large and metabolically important population of cells that coexpress ERα and LepRb and run counter to the conclusions presented in the study.

Kim and colleagues (2016) failed to report the circulating estrogen levels in their estrogen device implant studies, and therefore the dose of estrogen released from these uncontrolled devices may be above physiological levels, which would preclude the interpretation of physiological phenomena. Furthermore, Cre recombinase-mediated excision of genes can be unpredictable, and Kim et al. (2016) did not report the efficacy of the STAT3 knockdown in LepRb neurons, which is an essential step to ensure that STAT3 expression was adequately reduced. Interestingly, estrogen treatment significantly decreased pSTAT3 in leptin neurons, presenting another piece of evidence that suggests these pathways do, in fact, interact with one another.

CONCLUSION

The understanding of neuroendocrine regulation of body weight by key hormones such as estrogen and leptin is paramount to combating the obesity epidemic. Currently, the ERα-mediated and GPR30-mediated STAT3 pathway both provide compelling evidence to support the assertion that estrogen pathways interact with leptin pathways to regulate body weight. The Kim et al. (2016) study discussed here contradicts this idea but suffers from experimental caveats and conflicting data. It is also possible that leptin and estrogen may interact indirectly through the modulation of many other factors known to affect STAT3. Therefore, we believe this finding is interesting, but not sufficient to contradict the larger body of literature supporting the existence of a relationship between estrogen and leptin in hypothalamic signaling. As such, the currently available evidence supports the claim that the STAT3 pathway is central to body weight modulation, mediated by the relationship between estradiol and leptin. We believe that both ERα and GPR30 are capable of initiating STAT3-activating pathways, though it is not clear whether one pathway predominates over the other or whether these pathways depend on one another for adequate STAT3 activation. We conclude that by identifying the site of interaction between leptin and estrogen signaling, future research will further our understanding of this site's role in metabolic disorders such as obesity and T2D.

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Conflicts of interest
The authors declare no conflicts of interest.

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