

Review Article

Regulation of Estrogen Receptor α Expression in the Hypothalamus by Sex Steroids: Implication in the Regulation of Energy Homeostasis

Xian Liu and Haifei Shi

Department of Biology, Miami University, 700 E. High Street, Oxford, OH 45056, USA

Correspondence should be addressed to Haifei Shi; shih@miamioh.edu

Received 19 May 2015; Revised 18 July 2015; Accepted 22 July 2015

Academic Editor: Mario Maggi

Copyright © 2015 X. Liu and H. Shi. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Sex differences exist in the complex regulation of energy homeostasis that utilizes central and peripheral systems. It is widely accepted that sex steroids, especially estrogens, are important physiological and pathological components in this sex-specific regulation. Estrogens exert their biological functions via estrogen receptors (ERs). ER α , a classic nuclear receptor, contributes to metabolic regulation and sexual behavior more than other ER subtypes. Physiological and molecular studies have identified multiple ER α -rich nuclei in the hypothalamus of the central nervous system (CNS) as sites of actions that mediate effects of estrogens. Much of our understanding of ER α regulation has been obtained using transgenic models such as ER α global or nuclei-specific knockout mice. A fundamental question concerning how ER α is regulated in wild-type animals, including humans, in response to alterations in steroid hormone levels, due to experimental manipulation (i.e., castration and hormone replacement) or physiological stages (i.e., puberty, pregnancy, and menopause), lacks consistent answers. This review discusses how different sex hormones affect ER α expression in the hypothalamus. This information will contribute to the knowledge of estrogen action in the CNS, further our understanding of discrepancies in correlation of altered sex hormone levels with metabolic disturbances when comparing both sexes, and improve health issues in postmenopausal women.

1. Introduction

Sex differences exist in the epidemic of obesity-related metabolic diseases. According to the recent National Center for Health Statistics [1], although there is no significant difference in obesity prevalence between men and women at any age, the incidence of obesity increases dramatically after women reach menopause. Additionally, the percentage of people who suffer metabolic diseases is particularly higher among men and postmenopausal women than in premenopausal women [1]. Further, obese premenopausal women have much less metabolic complications than obese men and postmenopausal women [1]. Among women, menopause is associated with a significant shift to an atherogenic lipid profile with elevated circulating level of triglyceride but lowered circulating level of high-density lipoprotein cholesterol [2, 3]. Consequently postmenopausal women are at an increased risk of developing visceral obesity, metabolic syndrome, and

cardiovascular diseases independent of age, due to loss of endogenous ovarian hormone production [4]. Various types of hormone replacement therapy aiming to increase estrogen levels in postmenopausal women have been shown to ameliorate visceral obesity and lower risks for metabolic disorders and cardiovascular diseases [5]. Interestingly, elevated levels of circulating estrogens are associated with obesity and metabolic dysfunctions in men [6–9]. Increased estrogen levels are also seen in male rabbits with increased body fat and metabolic syndrome due to high-fat diet [10]. The causal relationship between sex steroid and obesity in males and females has not been determined yet [11, 12]. Therefore, insights into how altered sex steroid levels affect metabolic regulation are vital.

It is well-established that obesity is frequently associated with irregular menstrual cycles, decreased fertility, and altered patterns of various reproductive and metabolic hormones in humans [13] as well as in rodents [14]. Sex steroids

play key roles in energy homeostasis and glucose regulation. Among sex steroids, including estrogens, androgens, and progestogens, deficit in levels of estrogens has been identified to be one of the major contributors to the metabolic syndrome and visceral lipid accumulation observed in postmenopausal women. In intact, young adult rodents [15] and young women [16] with regular estrous cycles, variation of feeding behavior follows natural fluctuation of estrogens such that, following the proestrus estradiol surge, daily food intake reaches its minimum during estrus, which is usually around 20% less than their maximum daily caloric intake during diestrus [17, 18]. In rodent models, ovariectomy (OVX), that is, removal of ovaries and thus majority of endogenous estrogens, is a typical experimental model for investigating metabolic disturbance due to estrogen deficiency. Increases in food intake, body weight, and body fat accumulation are usually observed following OVX, whereas exogenous, cyclic treatment of estradiol at physiological doses normalizes these changed variables to individuals with intact ovaries and normal estrous cycles [19–21]. Similar as in females, working estrogen signaling with normal range estrogen levels and functional estrogen receptors (ERs) is required for metabolic regulation in males. Male mice with deficient estrogen signaling due to either absence of aromatase, a key enzyme for converting androgen to estrogen, thus disrupting estrogen biosynthesis, or lack of receptors for estrogens develop similar phenotypes of increased adiposity, inflammation, and insulin resistance as individuals lack of estrogens [22–24]. Treatment of estrogen ameliorates hyperglycemia and insulin resistance in obese male mice [25]. In humans, men with congenital aromatase deficiency are insulin resistant, and estrogen replacement therapy has been successfully used to improve insulin sensitivity in these men [26]. Therefore, estrogens play critical roles in metabolic regulation in male and female animal models and humans. Understanding the metabolic regulation of estrogen's effects is of great interest to develop efficient therapy targeting on estrogen signaling to treat obesity and related metabolic disorders.

2. Estrogens Regulate Energy Balance in the Central Nervous System (CNS)

2.1. Hypothalamic Regulation of Energy Balance. The hypothalamus of the CNS is the integration center that controls homeostatic regulation of energy balance including energy intake and energy expenditure [27]. The major nuclei that contain neurotransmitter systems and circuits among neuronal and nonneuronal cells in the hypothalamus include the rostral part of medial preoptic hypothalamic area (MPOA) within the neural circuit for regulating thermogenesis [28] and the caudal medial basal hypothalamic region including the paraventricular nucleus of the hypothalamus (PVH) related to energy expenditure and HPA axis regulation, the arcuate nucleus (ARC), and the ventromedial nucleus of the hypothalamus (VMH) for controlling food intake (Figure 2). These nuclei are key brain areas in the neuroendocrine regulations of reproductive function and energy homeostasis. For instance, VMH-specific ablation of ERs abolishes sexual

behavior in females [29] and males [30] in genetic mouse models, indicating that VMH is a critical brain nucleus that regulates reproductive function. The VMH is also recognized as a “satiety center” that suppresses appetite when VMH cells are stimulated [31] and induces hyperphagia when VMH cells are damaged [32]. Sex differences exist in the central regulation of energy balance and body weight. Sex steroid hormones influence the regulation of body weight, body fat, energy balance, and metabolism [33], which is at least partially achieved via ERs expressed in key neuropeptide-expressing neurons in the ARC and VMH of the hypothalamus [34–39].

2.2. Estrogens Regulate Energy Balance in the Hypothalamus

2.2.1. Mechanisms of Estrogenic Action via ERs. Estrogenic activity is mediated through genomic along with nongenomic mechanisms. Like other sex steroids, estrogens exert their biological functions through binding to ERs, including classic nuclear ER α and ER β , membrane-localized nuclear ERs, and membrane-bound G protein-coupled ER (GPER, also known as GPR30) [40].

The classic nuclear receptors for sex steroid hormones, including estrogens, progestogens, and androgens, are activated by their respective steroid hormones and undergo conformational changes, dimerize with other hormone receptors, and recruit coactivator/corepressor molecules. The dimers then bind to their respective hormone response elements in the promoter of target genes and function as nuclear transcription factors, to alter transcription and expression of specific target hormone responsive genes and to mediate biological actions of their respective hormones [41, 42]. Such genomic activity between steroid hormones and their respective receptors typically occurs over a course of several hours to a day for the effects to be manifested, due to the time needed for transcription and translation of hormone responsive genes [43] (Figure 1). While the most abundant and potent female sex hormone for nuclear ER α is estradiol, ER α also binds to other natural forms of estrogens such as estrone and estriol with lower affinity than estradiol [44]. Additionally, many pharmacological, environmental, and food compounds are capable of binding ER α , either promoting or disturbing ER α genomic activity [45].

The importance of membrane steroid receptors is increasingly apparent. Besides nuclear-initiated genomic signaling via ER α and ER β localized inside nuclei, membrane-initiated nongenomic estrogenic signaling via membrane-bound ERs [46, 47] is also involved in the hypothalamic control of energy homeostasis [48, 49]. These membrane-localized ERs could be GPER or membrane isoforms of nuclear ERs. In order to differentiate which genes are translated to membrane-localized ERs, one could determine membrane ER expression in nuclear ER ablated genetic mouse model, or *vice versa*. Razandi et al. showed that no membrane ER could be detected in ER α and ER β double knockout mice [50], suggesting that membrane ERs and nuclear ERs are derived from the same genes. Kang et al. assessed transcription activity of nuclear ER α following knockdown or overexpression of membrane ER in breast cancer cells and

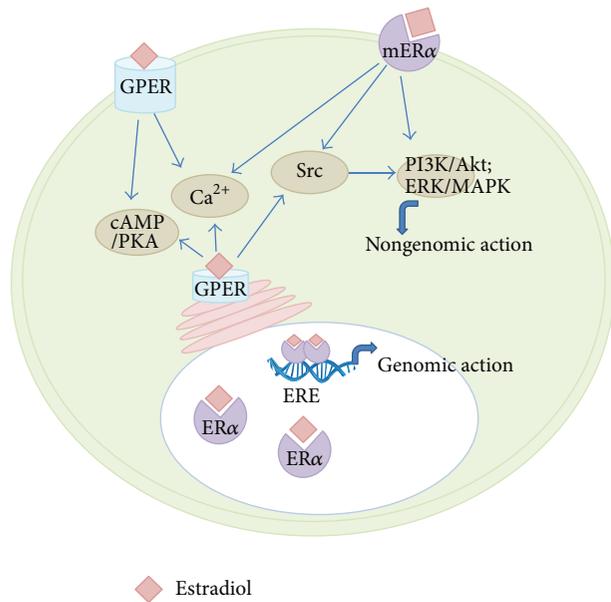


FIGURE 1: Schematic overview of estradiol-mediated genomic signaling pathway via nuclear ER α and rapid nongenomic signaling pathways via GPER and membrane isoform of ER α .

reported a positive correlation in expressions between nuclear and membrane ERs. Specifically, lowered expression of nuclear ER α was detected when membrane ER expression was decreased and enhanced nuclear ER α expression was detected when membrane ER was increased [51]. Thus, the membrane-localized ER, at least in breast cancer cells, is a related isoform of the classical nuclear ER α [51]. Similar findings have been reported in the hypothalamic neurons and astrocytes obtained from male and female rats, as both a full-length isoform and a truncated variant of the ER α have been detected using surface biotinylation and immunocytochemistry [52, 53]. Subsequent studies have shown that about 3–5% of classical nuclear ER α is distributed on membrane [54, 55]. These studies suggest that membrane-localized ER could be a variant of classical nuclear ER. A currently accepted view is that GPER and membrane-localized ER α are both presented in cell membrane. One possibility is that collaboration between membrane-localized ER α and GPER exists, and GPER could induce the expression of the membrane-localized ER α [51]. These studies have collectively demonstrated that a subpopulation of nuclear ER α is present at the plasma membrane in many cell types within the CNS, including the hypothalamus.

Nongenomic estrogenic signaling occurs more rapidly than the classical genomic estrogenic signaling, typically over a short course of seconds to minutes [56]. This nongenomic signaling starts at membrane, activates several kinase cascade pathways and triggers intracellular signaling, and subsequently induces multiple actions such as gene transcription and alteration in activity of neurons in the CNS. Extranuclear and membrane-associated isoforms of ERs can congregate with signaling molecules, such as G proteins and nonreceptor tyrosine kinases (Src) to facilitate interaction and

rapid intracellular signaling [49]. Binding of estrogens and nonnuclear ERs may induce several intracellular signaling kinase cascade pathways, including stimulation of adenylyl cyclase activity and cAMP-dependent protein kinase (PKA) pathway and cAMP-response element binding protein signaling cascade [57, 58], mobilization of intracellular Ca²⁺-dependent protein kinase C (PKC) pathway [59–61], activation of extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) pathway [62–65], and activation of receptor tyrosine kinase and phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway [66–68] (Figure 1). These abovementioned intracellular signaling events induce rapid actions that mediate a variety of estrogenic actions besides regulation of energy homeostasis and body fat, such as neuronal excitability, neuronal protection, reductions in inflammation, and neurite outgrowth [69].

In order to distinguish involvement of classical versus nonclassical ER α signaling in metabolic regulation of energy balance, a recent study investigated *in vivo* physiology using an ER α knockout mouse model with manipulation in nonclassical and classical estrogen signaling. Briefly, a mutant allele with disrupted estrogen response element (ERE) is introduced into the nuclear ER α knockout mouse model. Consequently, these ER α knockout mice express a mutant ER α with intact nonclassical signaling capacity but disrupted classical estrogen signaling capacity; thus ER α only signals via nongenomic pathway in this mouse model [70]. Interestingly, all of the metabolic dysfunctions presented in ER α knockout mice are normalized in these mice with nonclassical signaling but lack of genomic estrogen signaling [70], confirming that nonclassical estrogen signaling is a significant player that mediates the metabolic effects of estrogens. Therefore, estradiol and other ER α ligands at least act via membrane-localized ER α to regulate energy homeostasis.

2.2.2. Estrogenic Action on Energy Balance in the Hypothalamus via ERs. The hypothalamus is structurally and functionally sexually dimorphic [71] and is classically recognized as a steroid hormone-responsive area of the brain [72]. It is important that the brain remains sensitive to steroid hormones during postnatal development period even during adulthood, which is critical for several estrogen-dependent behaviors [73]. When acting in the hypothalamus, estrogens decrease food intake and increase energy expenditure [74], suppress lipogenesis [75, 76], and improve glucose metabolism [77]. Among all the ERs, ER α is considered to be the receptor primarily responsible for energy homeostatic modulation in the CNS and in the periphery [40, 49]. ER α -containing neuronal and nonneuronal cells are mostly located and much more abundant than other types of ERs in the ARC and the ventrolateral part of the VMH [78–81], the key brain nuclei regulating energy homeostasis. Recent studies have indicated that regulation of ER α expression in these nuclei by steroid hormones could provide direct mechanisms underlying how estrogens affect the activity of these brain nuclei to regulate energy balance, body fat distribution, and metabolism [34–39, 82].

Deletion of ER α , either whole-body knockout [23, 83] or hypothalamic nuclei-specific knockout [37, 84], increases

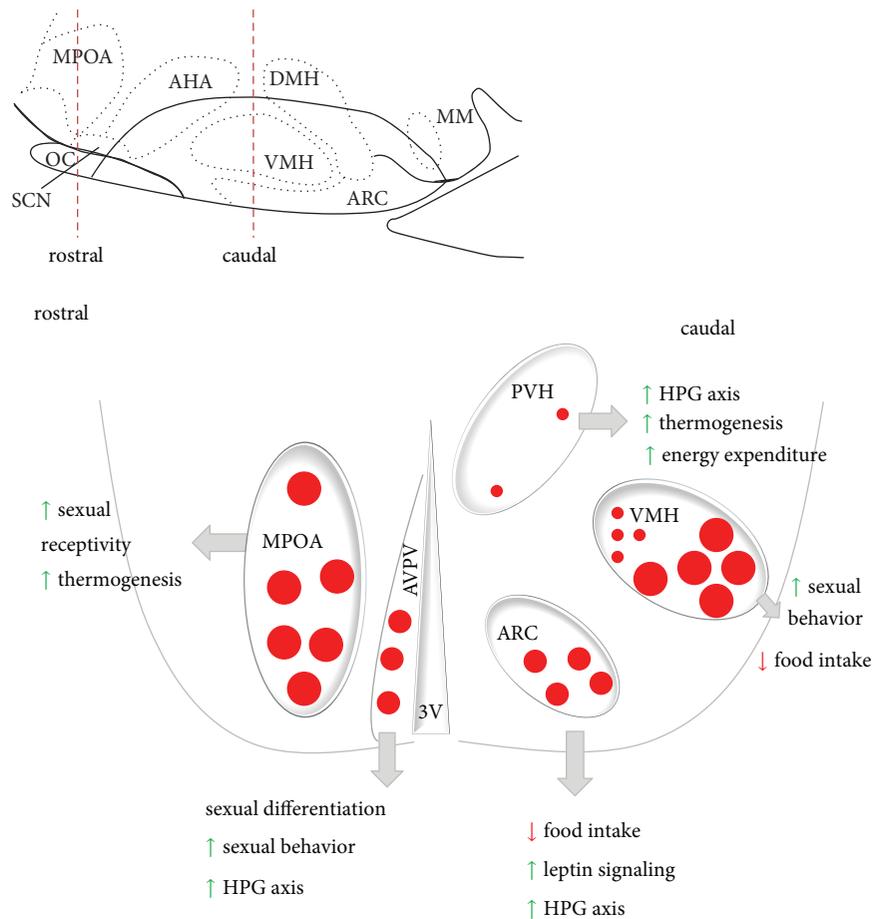


FIGURE 2: Distribution of ER α in the hypothalamus and their physiological effects. Schematic coronal section showing locations of major nuclei in the rostral (left) and caudal (right) hypothalamus containing ER α -expressing cells, the relative quantities of ER α in these nuclei (large red dots indicate abundant distribution and small red dots indicate scarce distribution), and their physiology effects in reproduction and energy homeostasis.

adiposity and causes the metabolic syndrome in male and female mice. It is noteworthy that suppression of ER α gene expression in the steroidogenic factor-1 (SF-1) neurons in the VMH or in the proopiomelanocortin (POMC) neurons in the ARC has a greater impact in females than males to increase food intake and body fat and to decrease energy expenditure and metabolism [37, 84]. These findings not only expand our understanding of the metabolic regulation of ER α in the hypothalamus but also support that ER α in the neurons in the VMH and ARC, at least SF-1 and POMC expressing neurons, plays important roles in regulating energy balance, especially in females.

2.2.3. ER α in Nonneuronal Cells in the Hypothalamus. There are more nonneuronal cells than neuronal cells within the CNS. Nonneuronal cells encompass diverse cell types, with microglia and astrocytes being the most abundant cell types. Regulation of energy homeostasis involving neuronal cells has been focused in majority studies previously, while microglia and astrocytes have been less studied. Two key points are pertinent to the topic reviewed here. First, microglia and astrocytes locate in the hypothalamic regions that respond

to steroid hormones including estrogens [85–87]. Microglia and astrocytes are well-documented for their contributions to estradiol-induced effects in neurogenesis and synaptogenesis, synaptic plasticity, and neural repair [88, 89] sexual differentiation of the brain [90, 91], modulation of estrogen-positive feedback in LH surge [92], and maintaining function of neural circuits among neighboring nonneuronal and neuronal cells during developmental stage and adulthood [91, 93]. Second, the significance of hypothalamic microglia and astrocytes in pathological metabolic processes associated with obesity, such as hypothalamic inflammation [94–98] and related insulin and leptin resistance [99–101], has become increasingly evident in rodent and human disease models. The ARC and the VMH of the hypothalamus are sexually dimorphic brain nuclei that control reproduction [102] and energy homeostasis [103], which contain ERs with ER α at a much higher concentration than ER β [104]. In fact, hypothalamic inflammation with activation of microglia and astrocytes in these brain nuclei caused by high-fat diet feeding and increased body fat have been demonstrated in recent studies [97, 100, 105]. Therefore, it is logical to presume the presence of ERs in hypothalamic microglia and astrocytes to contribute

to anti-inflammatory effects of estrogens and differential regulation of energy homeostasis between sexes [33, 106].

Whether ER α is expressed in microglia and astrocytes in the hypothalamus has been an ongoing debate. An early study observed ER α immunoreactivity in neuronal cells only but not in any nonneuronal glial cells including microglia and astrocytes [107]. Regarding ER expression in microglia, the above notion was questioned as ER α presence was demonstrated in primary microglial cell culture established from the forebrains of neonatal rats [108] and ER α was identified as the receptor modulating microglial activity [109]. A few recent studies, however, confirmed the absence of ER α protein and gene expression in primary mouse and human microglia [110–112], whereas ER β expression was abundant in microglia [112]. Regarding ER expression in astrocytes, some *in vitro* studies have reported that both ER α and ER β are expressed intracellularly and on plasma membrane of astrocytes from cultured CNS cells [86, 89, 113–116], although one study reported that ER β expression in astrocytes was uncertain [117]. Recently, ER α has been found in primary hypothalamic astrocytes cultured from male and female pups [53, 110, 118]. Using ER α knockout and ER β knockout mice, Mong and Blutstein have demonstrated that ER α but not ER β mediates the action of estradiol on astrocytes in the ARC [87], suggesting that, at least in the ARC, ER α is the receptor mediating the actions of estradiol in astrocyte morphology. To reconcile the literature, most studies on ER expression in microglia and astrocytes have been conducted using *in vitro* cultures and have demonstrated dominant ER α expression in astrocytes and dominant ER β expression in microglia. Future *in vivo* demonstration of ERs in hypothalamic microglia and astrocytes in neonatal and adult brains and assessment of their physiological function in metabolic regulation are needed to further our knowledge of estrogenic metabolic regulation in the CNS.

2.2.4. Regulation of ER α Expression in the Hypothalamus. ER α distribution in the hypothalamus is highly conserved across a number of species, including rat [81, 104, 119], mouse [79], hamster [120], guinea pig [121], ferret [122], opossum [123], musk shrew [124], bird [125, 126], cow [127], nonhuman primates at pubertal stage [128] and adulthood [129, 130], and adult human [131–133]. ER α is abundantly distributed in several key brain regions implicated in regulating energy homeostasis in both males and females, including the ventral lateral portion of the VMH and the ARC related to the control of food intake, the PVH related to energy expenditure and HPA axis regulation, the MPOA that influences thermogenesis, and the anteroventral periventricular nucleus (AVPV) regulating sexual behavior and HPG axis [78, 81, 104, 134] (Figure 2). In general, males express less amount of ER α than females in these key hypothalamic nuclei involved in metabolic regulation. The gene expression, protein levels, and distribution pattern of ER α in the hypothalamus can be modified by alterations in circulating steroid hormone levels. Hormone levels can be experimentally manipulated using methods of surgical removal of gonads and hormone replacement, or fluctuate due to natural variation across estrous cycles and during certain physiological stages such

as puberty, menopause and pregnancy. The degrees to which the reported differences, either between species or within one species, are indeed due to differences in sex or hormone status remains vague from the current literature.

The exact mechanism regulating ER α expression in the hypothalamus is largely uncertain but at least in part could be at the mRNA level, as seen in breast cancer cell lines with elevated ER α expression [135]. Recent studies have indicated that the genomic organization pattern of ER α gene (*Esr1*) is more complex than it was previously presumed, especially in humans [136]. Specifically, *Esr1* gene possesses promoters in its 5' end untranslated region, which could generate several mRNA splice variants to encode the same protein ER α [137, 138]. This multiple promoter system is believed to play a role in the tissue-specific and temporal regulation of ER α gene expression. For example, both human ER α promoters A and C are used in human breast adenocarcinoma cells MCF7 but only promoter A is used in human breast carcinoma cells ZR-75-1 [139]. Considering hypothalamus is a heterogeneous region in which nucleus groups have different origins and functions, it is highly possible that various nuclei utilize distinct promoters or promoter combinations of *Esr1* among one another at different stages of development. Usage of multiple promoters of *Esr1* changes an array of transcription activators and repressors and leads to variation of alternative splicing of mRNA transcripts while encoding the same full-length protein ER α . Furthermore, *Esr1* promoter region has been shown to undergo epigenetic modification, that is, DNA methylation, under normal and pathological conditions [140]. It is possible that, by changing the length and/or sequence of 5' end untranslated region, the stability of a specific transcript can be enhanced or compromised. The regulation of ER protein is also complicated, as ER mRNA is not always translated into functional ER protein, possibly due to brain region-specific posttranscriptional processing [141].

In this review, we gather evidence with regard to how expression and distribution of ER α in the hypothalamus of the CNS are regulated by various sex hormones in both males and females from human and animal studies and discuss discrepancy in current literature. This information will contribute to understanding of estrogenic action in the CNS and improve health issues in postmenopausal women.

3. Regulation of ER α Expression by Sex Steroids

Sex steroid hormones profoundly affect metabolic regulation of many species. Although the distribution pattern of ER-containing cells in the hypothalamus is similar across species, the regulation of ER expression could be relatively dynamic depending on circulating sex hormone levels and reproductive status, indicating the plasticity in ER α regulation. In men, androgen levels rise during puberty and decrease gradually over the lifespan [142]. In contrast, from puberty through menopause, women continuously experience cycles of fluctuations of estrogens and progestogens in their lives, and all these sex hormones decrease suddenly following menopause [143, 144]. Additionally, the levels of progestogens rise rapidly following mating, and both estrogens and progestogens are at

sustained high levels during pregnancy. Towards the end of pregnancy, progesterone levels sharply decline whereas estrogen levels increase, which allows parturition and maternal behavior to be displayed [143, 144].

All circulating androgens in men and most of the estrogens and progesterones in premenopausal women are produced in the gonads, testes in males and ovaries in females. On the other hand, circulating estrogens and progesterones in men, androgens in premenopausal women, and all sex steroids in postmenopausal women are synthesized in tissues other than gonads, including the brain, adipose tissues, and adrenal glands. The sex steroids secreted by nongonad tissues may also act locally as intracrine or paracrine factors, distinct from endocrine hormones. Testosterone and 5 α -dihydrotestosterone, estradiol, and progesterone are the most potent respective androgens, estrogens, and progesterones that carry out physiological functions.

Regulation of ER α expression in the hypothalamus due to dynamic endocrine status and changes in circulating steroid levels could be region-specific and different among species, depending on dissimilar patterns of reproductive cycles (see below). It is noteworthy, however, that ER α expression could be regulated by organizational effects during developing stages rather than activational effects influenced by steroid hormones. This is demonstrated by a study using castrated male and female rats, both of which lack majority of endogenous sex hormones [145]. Lauber et al. reported that the castrated male rats had significantly lower amount of ER α mRNA levels, less than half, than OVX females in the VMH and the ARC [145]. Such finding suggests a sex difference in basal, constitutive ER α expression level in the rat hypothalamus since there is no impact of circulating sex hormones in either sex. Thus sex difference in ER α expression may not be merely due to sex hormones. Consequently, data on the sex steroid hormone regulation of sex steroid receptor expression must be interpreted with care.

Differential ER α expression would affect the sensitivity to steroid hormones and consequently affect their endocrine physiology and behavior, which contribute to the adaptation of neuroendocrine regulation of energy homeostasis in obesogenic environment. In this section, we focus on discussing steroidal regulation of hypothalamic ER α expression in the context of metabolic regulation of energy homeostasis.

3.1. Estradiol Regulates ER α Expression in the Hypothalamus. ER α expression in the hypothalamus changes with estrous cycle as a function of circulating levels of estradiol and progesterone [119, 145–149]. Various animal and human studies have demonstrated that ER α expression can be down- or upregulated by circulating levels of estrogens in a brain region-dependent manner across species [122, 150–152]. For example, estradiol increases ER α mRNA expression in the VMH, decreases ER α mRNA expression in the lateral septum, and has no effect on ER α mRNA expression in the dorsal hypothalamus in lizards [150]. In a separate study using male ferrets, ER α immunoreactivity is reduced in the MPOA, is increased in the medial VMH, and is unchanged in the lateral VMH and the ARC by estradiol treatment [122]. Therefore, regulation of ER α in the hypothalamus by estradiol, either at

expression level or protein level, is regulated in a brain nuclei-specific manner.

It is generally assumed that estradiol would downregulate its own receptor ER α , similar to what many other hormones do. In agreement with this, several groups have shown that removal of majority of endogenous estradiol by OVX increases the number of ER α mRNA-positive neurons in the VMH of the hypothalamus of female rats [146, 147]. Accordingly, OVX female or castrated male rats receiving either an acute [146, 153] or chronic [154] treatment of estradiol benzoate up to 1 mg [153] have decreased levels of ER α mRNA in the hypothalamus. Specifically, ER α mRNA levels decline in the VMH and ARC 18–24 hours after estradiol benzoate treatment [153, 154], and the numbers of ER α positive neurons reduce in the VMH and anterior hypothalamic area following a 2-week treatment of estradiol benzoate at pharmacologic dosages [154]. Thus, it seems that, at least in rats that were treated with pharmacologic level of estradiol, hypothalamic ER α mRNA and protein levels are inversely correlated with circulating estradiol concentration to maintain responsiveness to hormonal activation in ER-containing cells, which would ensure a balanced, homeostatic system to prevent excessive estrogenic action [145–147] (Table 1).

On the contrary, other groups have shown opposite results (Table 1). When either physiological or supraphysiological concentrations of estradiol are utilized to treat primary neurons isolated from the ventrolateral area of the VMH of young female rats, measurement of ER α using immunofluorescence reveals that estradiol increases the protein level of ER α in these primary neurons *in vitro* [155], suggesting a positive relationship between estradiol level and ER α expression. Findings from several *in vivo* studies have reached this same conclusion. When compared with ovarian intact, cycling females, males with lower endogenous estrogen levels than females express much less amount of ER α in the VMH [156, 157] or in the MPOA [152] seen in females. A recent study confirmed the importance of the dosage of estradiol treatment on ER α expression in the ARC [158]. OVX rats are injected with two different doses of estradiol, either at a pharmacologic dose of 50 μ g or at a physiological dose of 2 μ g. Not surprisingly, ER α expression in the ARC is significantly downregulated in the rats treated with the pharmacologic dose of estradiol but is not changed in the rats treated with the physiological dose of estradiol [158]. Devidze et al. [157] have demonstrated that the mRNA level of ER α within the VMH is increased by a two-day exposure to a physiological dose of estradiol that is much lower than the dosage of estradiol used in earlier studies. Consequently, ER α expression is greater in OVX rats with estradiol treatment than their control OVX rats receiving vehicle treatment. Indeed, the quantity of ER α in the VMH gradually rises in response to increasing concentrations of estradiol throughout the estrous cycle until it reaches its maximum during the proestrus [148]. Similarly, a human study that compared ER α immunoreactivity in young adult men and women has shown stronger ER α immunoreactivity in the VMH of female brains than male brains [131]. The physiological relevance of this ER α elevation by increased estradiol level is to reinforce and augment estrogenic action

TABLE 1: Regulation of ER α in the hypothalamus by treatment of steroid hormones in male and female rats.

Hormones	ER α expression	Sex	References
Estradiol	Increase	Female	Cultured VMH cells: [155] VMH: [157]
		Male	VMH: [157]
	Decrease	Female	ARC: [145–147, 153, 158] (high dose) VMH: [78, 145–147, 153] AVPV: [153] MPOA: [148]
		Male	ARC: [147, 153] VMH: [153]
		Female	VMH: [145] ARC: [145]
		Male	AVPV: [153] VMH: [158] (low dose)
Progesterone	Progesterone + estradiol decrease	Female	MPOA and medial basal hypothalamus: [175] Cultured VMH cells: [155]
	Progesterone alone increase	Female	Cultured VMH cells: [155]
Testosterone	Decrease	Female	VMH and ARC: [147]
		Male	MPOA: [184, 186] AVPV: [186] VMH and ARC: [147]

during active phase of reproductive cycle for regulating related metabolism and reproductive behavior and ultimately leads to the survival of individuals and species. This notion of promoting behavioral regulation by estrogenic action is indeed supported by an abovementioned chronic study when 5-day parenting experiment is paired with treatment of estradiol benzoate; there is an increase in the number of ER α positive cells in the VMH [154]. Such increased ER α expression provides a means for estrogens to carry out their physiological and behavioral effects critical for reproductive function and success.

The regulation of ER α mRNA by estradiol also could differ among species. For example, estradiol treatment decreases ER α mRNA in the VMH in female rats [146, 147] but increases ER α mRNA in the VMH in female lizards [150, 159]. The species difference between rats and lizards could be due to their distinct reproductive physiology [160]. Briefly, the duration of follicular phase is brief in rats; thus estradiol surge during proestrus is immediately followed by behavioral estrus and onset of sexual receptivity with declining estradiol level. Therefore, in female rodents, increasing ER α expression in response to falling estradiol level is necessary for maintaining neural sensitivity after ovulation when individuals are sexually receptive. In contrast, the duration of follicular phase in lizards is much longer and extends to overlap with behavioral estrus. As a result, the onset of sexual receptivity occurs when estradiol level is still high in lizards, as well as many other vertebrates including rabbits, dogs, cats, bears, and primates. Therefore, lizards and many other mammal species need enhanced neural sensitivity during these latter stages of follicular phase when circulating estradiol level is high and individuals are sexually receptive, attributed to the

increase in ER α expression in response to initial estradiol surge during early stages of follicular growth in these species.

Differences in the regulation of ER α expression have also been reported between the sexes, but such observed sex difference is not consistent among different studies using similar *in situ* hybridization histochemistry method. Lauber et al. reported that estradiol reduced ER α gene expression in the VMH and the ARC of OVX female laboratory rats, but estradiol failed to regulate ER α expression in castrated male rats [145]. In contrast, Simerly and Young reported comparable reduction of ER α gene expression in the VMH and the ARC between castrated male rats and OVX and estrous female rats [147].

To summarize, the abovementioned studies strongly suggest that the mRNA and protein levels of ER α within the key brain nuclei are positively correlated with circulating estradiol levels within physiological ranges, whereas they are negatively correlated with estradiol levels if estradiol levels reach pharmacologic levels. The reason for such discrepancy is not clear when considering the massive degree of complexity of living animals. We speculate that several factors may be involved. First, the response of neurons in these hypothalamic nuclei might be multiphasic, which is regulated by a negative feedback mechanism. Since there is no feedback control leading to downregulation when estradiol directly acts on isolated neurons *in vitro*, it is likely that ER α in these isolated VMH neurons neither autoregulate its own expression nor are controlled by any feedback mechanism, as seen in some other cell lines including cancer cells [161], osteoblasts [162], and macrophages [163]. The second possibility for such discrepancy involves the dosages and methods adopted to introduce estradiol among different studies. Using

supraphysiological or pharmacologic dose of any hormone is likely to induce adverse impact or hyperstimulation to neurons, whose effect is of low physiological significance [164]. Besides using different concentrations of estradiol, different methods have been used to replace estradiol. Chronic, prolonged estradiol capsule treatment potentially desensitizes neurons, which may disable feedback mechanisms and result in uncontrolled adaptation. Third, the effects of estradiol might be concealed by another major ovarian hormone, progesterone, which is also being removed by OVX. When considering progesterone, it is not surprising that we observe different results when adult mature females are compared with young females that do not cycle regularly [157] or *in vitro* isolated neurons [155]. Furthermore, it is worth noticing that a number of factors that may be implicated, including aging [165, 166] and chronic exposure to environmental factors such as bisphenol-A [167, 168], are capable of altering endogenous ER α and E2 level as well. Thus the discrepancies among different studies may reflect the differences in ages, composition of diet, or dietary origins.

3.2. Progesterone Regulates ER α Expression in the Hypothalamus. Progesterone is another sex steroid that is mainly synthesized and secreted in the ovaries and the adrenal gland. During vertebrate ovarian cycles in primates and rodents, growing follicles secrete increasing level of estrogens during latter stage of diestrus which peaks at proestrus and declines following ovulation, while progesterone level increases following the luteinizing hormone (LH) surge, ovulation, and formation of the corpus luteum during diestrus and proestrus [169]. The elevation in plasma progesterone during diestrus is eliminated following adrenalectomy, confirming the adrenal origin of progesterone [169].

Progesterone is an important modulator of estrogenic action in the CNS and it affects LH release and ovulation in a biphasic manner [170]. The role of progesterone on estrogenic function is complicated, as it can be either inhibitory or stimulatory depending on the timing and sequence of injections [171]. For example, in OVX rats, estradiol injection alone can induce daily surges of LH [172]. These daily LH surges are blocked by progesterone treatment [173]. Thus, one of the functions of the increased progesterone secretion in the proestrus is to abolish LH surge [173]. On the contrary, treatment with progesterone following estrogen priming, which mimics the estrogen peak that precedes progesterone elevation in cycling females, activates sexual behavior [171]. Therefore, sequential secretion of estrogens and progestogens during ovarian cycles determines timing of ovulation and leads to the onset of a series of sexual behavior [171].

The theory of progesterone regulation on ER α expression is supported by the fact that both progesterone receptor (PR) and ER α are coexpressed in certain nuclei in the CNS [174]. Blaustein and Brown [175] have reported that when estradiol-treated OVX female rats are injected with progesterone *in vivo*, ER α binding is significantly reduced in the anterior pituitary, the MPOA, and the medial basal hypothalamus where the VMH and the ARC are located. Such progesterone's effect on ER α expression is not evenly displayed among the brain areas with coexpression of PR and ER α .

Additionally, when progesterone and estradiol are treated together to primary disassociated VMH cells *in vitro*, physiological concentrations of progesterone counteract estradiol-induced elevation of ER α expression, whereas progesterone treatment alone upregulates ER α expression [155] (Table 1). One possible explanation for the dual effects of progesterone is interinhibition of transcriptional activities, due to activated PRs localizing to the progesterone response element (PRE) in the promoter region of ERE. Thus, simultaneous activation of both receptors may lead to protein-protein interaction that eventually blocks the activation site for estrogens, causing counteraction between progesterone and estradiol. Furthermore, under certain conditions when PR ligands do not activate transcription, PR isoforms potently suppress transcriptional activity of ER in cultured primary uterine cells from rats [176]. It is also possible that binding of PR at PRE may not be required for upregulation of ER α expression by progesterone. PR agonist can increase expression of reporter gene in the cells artificially expressing PR or ERs and transfected with vectors containing ERE but not PRE in their promoters [177]. It is mentioned earlier in this review that ER α expression increases after OVX in many species, which could be at least partially due to the removal of progesterone by OVX and thus release inhibition of progesterone on ER α expression [176]. Alternatively, decrease in progesterone level following OVX might also attenuate progesterone inhibition on other factors. For example, decrease in progesterone concentration increases the amount of neurotransmitter norepinephrine in the CNS, which has been shown to augment number of ERs in the hypothalamus [178] as well as lordosis behavior [179] in guinea pigs. The aforementioned studies collectively indicate that when treated alone, progesterone upregulates ER α expression, while when cotreated with estrogens, progesterone downregulates ER α expression.

Progesterone has these similar effects on the regulation of ER α expression during gestation. In rodents, progesterone level rapidly increases following mating and reaches plateaus during gestation days 15–20 and then it sharply declines, whereas estradiol level increases towards the end of pregnancy after day 20, a shift allowing parturition and maternal behavior to be displayed. Levels of progesterone and estradiol change naturally but dramatically during gestation and thus can be used as a naturally occurring model to study impact of progesterone and estradiol on the expression of ER α . During gestation, ER α expression in the ARC remains constant, except for an increase in ER α expression on days 12 and 19 of gestation when progesterone level reaches its plateaus before rise of estrogens level. Consequently, ER α expression in the ARC on days 12 and 19 of gestation is greater compared with ER α expression of diestrus rats and rats at postpartum lactation when their estrogens levels are climbing but progesterone levels are declining [180]. Similarly, Mann and Babb [181] have reported that ER α mRNA levels are constant between days 15 and 21 of gestation when progesterone level reaches the peak.

To summarize, ER α expression increases when progesterone level is high and estrogen level is low, as seen in animals treated with progesterone alone or during gestation; ER α expression decreases in the presence of progesterone when

animals are cotreated with estrogens or during postpartum phase when estrogen level sharply rises.

3.3. Androgens Regulate ER α Expression in the Hypothalamus. Androgens influence gene transcription through activation of androgen receptor (AR), a type of nuclear receptor that shares similar genomic signaling mechanism as ER and PR. Briefly, upon ligand binding, AR dimerizes and binds with specific DNA motifs androgen response element (ARE) in its target genes [41]. In males, testosterone's function mediated by AR has antiobesity features. Testosterone deprivation in men contributes to the development of metabolic syndrome. Male mice lacking AR develop insulin resistance, leptin resistance, and late onset obesity primarily due to decrease in locomotor activity, a component of energy expenditure [182, 183]. Although male sexual behavior is mediated by androgens, male reproductive behavior is also influenced by estradiol transformed by testosterone aromatization in the brain.

Castration and thus reduced androgen levels have been reported to increase ER α mRNA in the MPOA [119, 151, 184] and in the VMH [147] of male rats and male mandarin voles [185], suggesting that ER α expression in the CNS is inhibited by androgens. Findings by Wu and Gore support this notion and demonstrate that, in the MPOA, ER α cell density is substantially higher in vehicle-treated compared with testosterone-treated castrated rats of both young and old ages [186]. Additionally, in the AVPV, testosterone decreases ER α cell density but to a lesser extent [186]. Furthermore, androgens also affect ER α expression in females. Testosterone treatment attenuates ER α mRNA expression in the VMH and ARC [147] and in the MPOA [151] in both males and females. Thus ER α expression in the hypothalamus is greater in females than in males [145], increased by castration in males, and inhibited by androgen treatment in both males and females, indicating that ER expression in the hypothalamus is downregulated by circulating androgens in both sexes (Table 1).

Similarly both men and women could suffer from various forms of metabolic dysfunction and abnormalities due to androgen imbalance, including hypoandrogenism in aging men [187, 188] and hyperandrogenism in women with polycystic ovary syndrome (PCOS) [187, 189]. Lowered circulating levels of androgens in men are associated with insulin resistance and obesity, while testosterone treatment in hypogonadal men improves insulin sensitivity and reduces body fat content [187, 188, 190]. The functional role of androgens in female energy metabolism is not well-characterized and has not been directly tested yet, but it is suggested from human studies with women suffering PCOS or animal studies using a PCOS animal model induced by injection of letrozole, a third-generation aromatase inhibitor. Androgen excess in women, one of the key diagnostic characteristics of PCOS [191], is associated with insulin resistance and obesity [187, 189]. Briefly, women with PCOS [192–194] or female rats of PCOS model [195] have abnormally low levels of estrogens but high levels of androgens due to exaggerated androgen synthesis from the ovaries and adrenal glands. Additionally, PCOS women develop central visceral obesity, due to the fact that androgens promote abdominal fat deposition. In a

letrozole-induced PCOS animal model, letrozole treatment decreases estradiol secretion and ER α expression in the ovaries of female rats [195] and decreases estradiol content and ER α expression in the hippocampus of male mice [196]. It is not clear whether or not ER α expression in the hypothalamus changes in females with high androgen levels.

There are two possible mechanisms through which androgen treatment may regulate ER α expression: through its aromatization to estradiol or through binding to ARs and inducing genomic changes in AR and ER α coexpressing neurons. The former possibility has been tested by DonCarlos et al., as aromatizable androgen testosterone but not nonaromatizable androgen dihydrotestosterone downregulates the levels of ER mRNA in the POA [151], confirming that estrogen derived from aromatization of testosterone suppresses ER expression in the hypothalamus.

4. Conclusion

Sex differences exist in the prevalence of obesity and related metabolic diseases [1]. Sex steroids, especially estrogens, contribute critically in the regulation of energy homeostasis and play protective roles in the development of metabolic complications. Menopause, characterized by a sudden reduction in female sex hormones, leads to visceral lipid deposition and increased risk in metabolic disorders [197]. Discrepancies exist however in the clinical correlation of altered sex hormone levels with metabolic disturbances when comparing the two sexes. Some studies have shown that hormone replacement therapy in postmenopausal women has positive effects on treating visceral obesity and related metabolic diseases [198–200], while other studies have failed to confirm the positive effects [201–203] and even questioned beneficial effects and safety of hormone replacement therapy [204]. Understanding estrogenic actions is required when developing safe and effective hormone replacement therapy.

Recent basic, translational, and clinic studies have greatly advanced our understanding of the mechanism underlying estrogenic effects. Estrogens utilize both genomic and nongenomic signaling mechanisms for estrogenic effects in the central and peripheral systems, primarily via ER α [40]. ER α is abundantly located in the MPOA, the VMH, and the ARC of the hypothalamus [78, 81, 104]. It is well-accepted that all major sex steroid hormones regulate ER α expression in the CNS. In this review, three understudied areas are identified that await further investigation.

First, inconsistency exists in the literature regarding the regulation of estrogen receptors by manipulation of sex hormones. The discrepancy exists in the literature due to different model systems, dosages of hormones, and experimental paradigms being used for testing. It is necessary to gain a more thorough understanding of estrogenic action in order to improve hormone therapeutics in humans. The consensus is that, within the physiological ranges, estrogens or progestogens alone increase ER α expression in the hypothalamus (Table 1), whereas androgens or progestogens in combination with estrogens inhibit ER α expression in the hypothalamus. Such effects would disappear or even become opposite if the paradigm and/or dosage are changed. Thus,

dosages and combination of hormones are critical components for safe and effective hormone replacement therapies. Second, the importance of membrane steroid receptors is evident. The relationship between genomic and nongenomic estrogenic actions via respective nuclear and membrane-bound estrogen receptors, especially in *in vivo* physiology and biological functions, is uncertain, due to the fact that limited research has been done. The time courses of genomic and nongenomic estrogenic effects are dramatically different [56]. Understanding the coordination between nuclear and membrane estrogen receptors and their respective intracellular signaling pathways would contribute to developing more effective hormone therapies in future. Third, the actions of ERs in hypothalamic nonneuronal cells, such as microglia and astrocytes, are basically unknown, although it is clear that these cells play critical roles in regulating reproductive functions and metabolic processes and they are action targets for estrogens. Limited literature suggests that different ER subtypes play distinct roles in microglia and astrocytes, with ER α being dominant in astrocytes and ER β being dominant in microglia. Future *in vivo* studies are needed to assess their physiological function in regulating reproduction and metabolism.

Abbreviations

AR:	Androgen receptor
ARE:	Androgen response element
ARC:	Arcuate nucleus
AVPV:	Anteroventral periventricular nucleus
CNS:	Central nervous system
ER:	Estrogen receptor
ERE:	Estrogen response element
GPOR:	G protein-coupled estrogen receptor
LH:	Luteinizing hormone
MPOA:	Medial preoptic area
OVX:	Ovariectomy
PCOS:	Polycystic ovary syndrome
PR:	Progesterone receptor
PRE:	Progesterone response element
PVH:	Paraventricular nucleus of hypothalamus
VMH:	Ventromedial nucleus of hypothalamus.

Conflict of Interests

The authors declare that there is no conflict of interests regarding publication of this paper.

Acknowledgments

The authors acknowledge the funding from National Institutes of Health R15 DK090823 and Madalene and George Shetler Diabetes Research Award from Miami University to Haifei Shi. They also thank the Department of Biology at Miami University for providing Graduate Assistantship to Xian Liu.

References

- [1] C. L. Ogden, M. D. Carroll, B. K. Kit, and K. M. Flegal, "Prevalence of obesity in the United States, 2009-2010," *NCHS Data Brief*, vol. 82, pp. 1-8, 2012.
- [2] P. Anagnostis, J. C. Stevenson, D. Crook, D. G. Johnston, and I. F. Godsland, "Effects of menopause, gender and age on lipids and high-density lipoprotein cholesterol subfractions," *Maturitas*, vol. 81, no. 1, pp. 62-68, 2015.
- [3] G. Kolovou, D. Damaskos, K. Anagnostopoulou, and D. V. Cokkinos, "Apolipoprotein E gene polymorphism and gender," *Annals of Clinical & Laboratory Science*, vol. 39, no. 2, pp. 120-133, 2009.
- [4] E. S. Ford, "Prevalence of the metabolic syndrome defined by the international diabetes federation among adults in the U.S.," *Diabetes Care*, vol. 28, no. 11, pp. 2745-2749, 2005.
- [5] H. Yüksel, A. R. Odabaşı, S. Demircan, K. Köseoğlu, K. Kizilkaya, and E. Onur, "Effects of postmenopausal hormone replacement therapy on body fat composition," *Gynecological Endocrinology*, vol. 23, no. 2, pp. 99-104, 2007.
- [6] G. Schneider, M. A. Kirschner, R. Berkowitz, and N. H. Ertel, "Increased estrogen production in obese men," *Journal of Clinical Endocrinology and Metabolism*, vol. 48, no. 4, pp. 633-638, 1979.
- [7] M. A. Gates, R. A. Mekary, G. R. Chiu, E. L. Ding, G. A. Wittert, and A. B. Araujo, "Sex steroid hormone levels and body composition in men," *Journal of Clinical Endocrinology and Metabolism*, vol. 98, no. 6, pp. 2442-2450, 2013.
- [8] M. Muller, I. den Tonkelaar, J. H. H. Thijssen, D. E. Grobbee, and Y. T. van der Schouw, "Endogenous sex hormones in men aged 40-80 years," *European Journal of Endocrinology*, vol. 149, no. 6, pp. 583-589, 2003.
- [9] A. Vermeulen, J. M. Kaufman, S. Goemaere, and I. van Pottelberg, "Estradiol in elderly men," *Aging Male*, vol. 5, no. 2, pp. 98-102, 2002.
- [10] A. Morelli, E. Sarchielli, P. Comeglio et al., "Metabolic syndrome induces inflammation and impairs gonadotropin-releasing hormone neurons in the preoptic area of the hypothalamus in rabbits," *Molecular and Cellular Endocrinology*, vol. 382, no. 1, pp. 107-119, 2014.
- [11] C. Couillard, J. Gagnon, J. Bergeron et al., "Contribution of body fatness and adipose tissue distribution to the age variation in plasma steroid hormone concentrations in men: the HERITAGE family study," *The Journal of Clinical Endocrinology & Metabolism*, vol. 85, no. 3, pp. 1026-1031, 2000.
- [12] T. A. C. M. van Geel, P. P. Geusen, B. Winkens, J.-P. J. E. Sels, and G.-J. Dinant, "Measures of bioavailable serum testosterone and estradiol and their relationships with muscle mass, muscle strength and bone mineral density in postmenopausal women: a cross-sectional study," *European Journal of Endocrinology*, vol. 160, no. 4, pp. 681-687, 2009.
- [13] E. S. Jungheim, J. L. Travieso, K. R. Carson, and K. H. Moley, "Obesity and reproductive function," *Obstetrics and Gynecology Clinics of North America*, vol. 39, no. 4, pp. 479-493, 2012.
- [14] P. Bermejo-Alvarez, C. S. Rosenfeld, and R. M. Roberts, "Effect of maternal obesity on estrous cyclicity, embryo development and blastocyst gene expression in a mouse model," *Human Reproduction*, vol. 27, no. 12, pp. 3513-3522, 2012.
- [15] N. Geary, "Endocrine controls of eating: CCK, leptin, and ghrelin," *Physiology and Behavior*, vol. 81, no. 5, pp. 719-733, 2004.

- [16] C. L. Pelkman, M. Chow, R. A. Heinbach, and B. J. Rolls, "Short-term effects of a progestational contraceptive drug on food intake, resting energy expenditure, and body weight in young women," *The American Journal of Clinical Nutrition*, vol. 73, no. 1, pp. 19–26, 2001.
- [17] L. Asarian and N. Geary, "Sex differences in the physiology of eating," *The American Journal of Physiology—Regulatory Integrative and Comparative Physiology*, vol. 305, no. 11, pp. R1215–R1267, 2013.
- [18] L. Asarian and N. Geary, "Modulation of appetite by gonadal steroid hormones," *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 361, no. 1471, pp. 1251–1263, 2006.
- [19] L. Asarian and N. Geary, "Cyclic estradiol treatment normalizes body weight and restores physiological patterns of spontaneous feeding and sexual receptivity in ovariectomized rats," *Hormones and Behavior*, vol. 42, no. 4, pp. 461–471, 2002.
- [20] J. Hong, R. E. Stubbins, R. R. Smith, A. E. Harvey, and N. P. Nunez, "Differential susceptibility to obesity between male, female and ovariectomized female mice," *Nutrition Journal*, vol. 8, no. 1, article 11, 2009.
- [21] X. Liu, Z. Zhu, M. Kalyani, J. M. Janik, and H. Shi, "Effects of energy status and diet on Bdnf expression in the ventromedial hypothalamus of male and female rats," *Physiology and Behavior*, vol. 130, pp. 99–107, 2014.
- [22] M. E. E. Jones, A. W. Thorburn, K. L. Britt et al., "Aromatase-deficient (ArKO) mice have a phenotype of increased adiposity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 23, pp. 12735–12740, 2000.
- [23] P. A. Heine, J. A. Taylor, G. A. Iwamoto, D. B. Lubahn, and P. S. Cooke, "Increased adipose tissue in male and female estrogen receptor- α knockout mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 23, pp. 12729–12734, 2000.
- [24] V. Ribas, M. T. A. Nguyen, D. C. Henstridge et al., "Impaired oxidative metabolism and inflammation are associated with insulin resistance in ER α -deficient mice," *American Journal of Physiology—Endocrinology and Metabolism*, vol. 298, no. 2, pp. E304–E319, 2010.
- [25] R. S. Dakin, B. R. Walker, J. R. Seckl, P. W. F. Hadoke, and A. J. Drake, "Estrogens protect male mice from obesity complications and influence glucocorticoid metabolism," *International Journal of Obesity*, 2015.
- [26] V. Rochira, B. Madoe, L. Zirilli, G. Caffagni, L. Maffei, and C. Carani, "Oestradiol replacement treatment and glucose homeostasis in two men with congenital aromatase deficiency: evidence for a role of oestradiol and sex steroids imbalance on insulin sensitivity in men," *Diabetic Medicine*, vol. 24, no. 12, pp. 1491–1495, 2007.
- [27] T. G. Ramamoorthy, G. Begum, E. Harno, and A. White, "Developmental programming of hypothalamic neuronal circuits: impact on energy balance control," *Frontiers in Neuroscience*, vol. 9, 2015.
- [28] S. F. Morrison, C. J. Madden, and D. Tupone, "Central neural regulation of brown adipose tissue thermogenesis and energy expenditure," *Cell Metabolism*, vol. 19, no. 5, pp. 741–756, 2014.
- [29] S. Musatov, W. Chen, D. W. Pfaff, M. G. Kaplitt, and S. Ogawa, "RNAi-mediated silencing of estrogen receptor α in the ventromedial nucleus of hypothalamus abolishes female sexual behaviors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 27, pp. 10456–10460, 2006.
- [30] K. Sano, M. C. Tsuda, S. Musatov, T. Sakamoto, and S. Ogawa, "Differential effects of site-specific knockdown of estrogen receptor α in the medial amygdala, medial pre-optic area, and ventromedial nucleus of the hypothalamus on sexual and aggressive behavior of male mice," *European Journal of Neuroscience*, vol. 37, no. 8, pp. 1308–1319, 2013.
- [31] M. Saito, Y. Minokoshi, and T. Shimazu, "Accelerated norepinephrine turnover in peripheral tissues after ventromedial hypothalamic stimulation in rats," *Brain Research*, vol. 481, no. 2, pp. 298–303, 1989.
- [32] A. W. Hetherington and S. W. Ranson, "Hypothalamic lesions and adiposity in the rat," *The Anatomical Record*, vol. 78, no. 2, pp. 149–172, 1940.
- [33] H. Shi, R. J. Seeley, and D. J. Clegg, "Sexual differences in the control of energy homeostasis," *Frontiers in Neuroendocrinology*, vol. 30, no. 3, pp. 396–404, 2009.
- [34] D. J. Clegg, C. A. Riedy, K. A. B. Smith, S. C. Benoit, and S. C. Woods, "Differential sensitivity to central leptin and insulin in male and female rats," *Diabetes*, vol. 52, no. 3, pp. 682–687, 2003.
- [35] D. J. Clegg, L. M. Brown, S. C. Woods, and S. C. Benoit, "Gonadal hormones determine sensitivity to central leptin and insulin," *Diabetes*, vol. 55, no. 4, pp. 978–987, 2006.
- [36] D. J. Clegg, L. M. Brown, J. M. Zigman et al., "Estradiol-dependent decrease in the orexigenic potency of ghrelin in female rats," *Diabetes*, vol. 56, no. 4, pp. 1051–1058, 2007.
- [37] Y. Xu, T. P. Nedungadi, L. Zhu et al., "Distinct hypothalamic neurons mediate estrogenic effects on energy homeostasis and reproduction," *Cell Metabolism*, vol. 14, no. 4, pp. 453–465, 2011.
- [38] L. Shen, D. Q.-H. Wang, C.-M. Lo et al., "Estradiol increases the anorectic effect of central apolipoprotein A-IV," *Endocrinology*, vol. 151, no. 7, pp. 3163–3168, 2010.
- [39] Z. Zhu, X. Liu, S. P. D. Senthil Kumar, J. Zhang, and H. Shi, "Central expression and anorectic effect of brain-derived neurotrophic factor are regulated by circulating estradiol levels," *Hormones and Behavior*, vol. 63, no. 3, pp. 533–542, 2013.
- [40] H. Shi, S. P. D. Senthil Kumar, and X. Liu, "G protein-coupled estrogen receptor in energy homeostasis and obesity pathogenesis," in *Progress in Molecular Biology and Translational Science*, Y.-X. Tao, Ed., vol. 114, pp. 193–250, Academic Press, 2013.
- [41] M. Beato and J. Klug, "Steroid hormone receptors: an update," *Human Reproduction Update*, vol. 6, no. 3, pp. 225–236, 2000.
- [42] N. J. McKenna and B. W. O'Malley, "Combinatorial control of gene expression by nuclear receptors and coregulators," *Cell*, vol. 108, no. 4, pp. 465–474, 2002.
- [43] R. O'Lone, M. C. Frith, E. K. Karlsson, and U. Hansen, "Genomic targets of nuclear estrogen receptors," *Molecular Endocrinology*, vol. 18, no. 8, pp. 1859–1875, 2004.
- [44] S. Pipil, V. Kumar, V. S. Rawat, L. Sharma, and N. Sehgal, "In silico and in vivo analysis of binding affinity of estrogens with estrogen receptor alpha in Channa punctatus (Bloch)," *Fish Physiology and Biochemistry*, vol. 41, no. 1, pp. 31–40, 2014.
- [45] D. P. McDonnell and S. E. Wardell, "The molecular mechanisms underlying the pharmacological actions of ER modulators: implications for new drug discovery in breast cancer," *Current Opinion in Pharmacology*, vol. 10, no. 6, pp. 620–628, 2010.
- [46] S. R. Hammes and E. R. Levin, "Extranuclear steroid receptors: nature and actions," *Endocrine Reviews*, vol. 28, no. 7, pp. 726–741, 2007.
- [47] P. E. Micevych and M. J. Kelly, "Membrane estrogen receptor regulation of hypothalamic function," *Neuroendocrinology*, vol. 96, no. 2, pp. 103–110, 2012.

- [48] T. A. Roepke, "Oestrogen modulates hypothalamic control of energy homeostasis through multiple mechanisms," *Journal of Neuroendocrinology*, vol. 21, no. 2, pp. 141–150, 2009.
- [49] A. Frank, L. M. Brown, and D. J. Clegg, "The role of hypothalamic estrogen receptors in metabolic regulation," *Frontiers in Neuroendocrinology*, vol. 35, no. 4, pp. 550–557, 2014.
- [50] M. Razandi, A. Pedram, I. Merchenthaler, G. L. Greene, and E. R. Levin, "Plasma membrane estrogen receptors exist and functions as dimers," *Molecular Endocrinology*, vol. 18, no. 12, pp. 2854–2865, 2004.
- [51] L. Kang, X. Zhang, Y. Xie et al., "Involvement of estrogen receptor variant ER- α 36, not GPR30, in nongenomic estrogen signaling," *Molecular Endocrinology*, vol. 24, no. 4, pp. 709–721, 2010.
- [52] R. Dominguez and P. Micevych, "Estradiol rapidly regulates membrane estrogen receptor α levels in hypothalamic neurons," *The Journal of Neuroscience*, vol. 30, no. 38, pp. 12589–12596, 2010.
- [53] G. Bondar, J. Kuo, N. Hamid, and P. Micevych, "Estradiol-induced estrogen receptor- α trafficking," *The Journal of Neuroscience*, vol. 29, no. 48, pp. 15323–15330, 2009.
- [54] J. Rainville, K. Pollard, and N. Vasudevan, "Membrane-initiated non-genomic signaling by estrogens in the hypothalamus: cross-talk with glucocorticoids with implications for behavior," *Frontiers in Endocrinology*, vol. 6, article 18, 2015.
- [55] E. R. Levin, "Membrane oestrogen receptor α signalling to cell functions," *Journal of Physiology*, vol. 587, no. 21, pp. 5019–5023, 2009.
- [56] T. A. Roepke, A. Malyala, M. A. Bosch, M. J. Kelly, and O. K. Rønnekleiv, "Estrogen regulation of genes important for K⁺ channel signaling in the arcuate nucleus," *Endocrinology*, vol. 148, no. 10, pp. 4937–4951, 2007.
- [57] C. M. Szego and J. S. Davis, "Adenosine 3',5'-monophosphate in rat uterus: acute elevation by estrogen," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 58, no. 4, pp. 1711–1718, 1967.
- [58] Y. Zhou, J. J. Watters, and D. M. Dorsa, "Estrogen rapidly induces the phosphorylation of the cAMP response element binding protein in rat brain," *Endocrinology*, vol. 137, no. 5, pp. 2163–2166, 1996.
- [59] M.-T. Sutter-Dub, "Rapid non-genomic and genomic responses to progestogens, estrogens, and glucocorticoids in the endocrine pancreatic B cell, the adipocyte and other cell types," *Steroids*, vol. 67, no. 2, pp. 77–93, 2002.
- [60] A. C. B. Cato, A. Nestl, and S. Mink, "Rapid actions of steroid receptors in cellular signaling pathways," *Science Signaling*, vol. 2002, no. 138, article re9, 2002.
- [61] J. Kuo, N. Hamid, G. Bondar, P. Dewing, J. Clarkson, and P. Micevych, "Sex differences in hypothalamic astrocyte response to estradiol stimulation," *Biology of Sex Differences*, vol. 1, no. 1, article 7, 2010.
- [62] J. J. Watters, J. S. Campbell, M. J. Cunningham, E. G. Krebs, and D. M. Dorsa, "Rapid membrane effects of steroids in neuroblastoma cells: effects of estrogen on mitogen activated protein kinase signalling cascade and c-fos immediate early gene transcription," *Endocrinology*, vol. 138, no. 9, pp. 4030–4033, 1997.
- [63] R. Bi, M. R. Foy, R.-M. Vouimba, R. F. Thompson, and M. Baudry, "Cyclic changes in estradiol regulate synaptic plasticity through the MAP kinase pathway," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 23, pp. 13391–13395, 2001.
- [64] S.-H. Yang, A. D. Sharrocks, and A. J. Whitmarsh, "Transcriptional regulation by the MAP kinase signaling cascades," *Gene*, vol. 320, no. 1-2, pp. 3–21, 2003.
- [65] A. Malyala, M. J. Kelly, and O. K. Rønnekleiv, "Estrogen modulation of hypothalamic neurons: activation of multiple signaling pathways and gene expression changes," *Steroids*, vol. 70, no. 5–7, pp. 397–406, 2005.
- [66] A. Malyala, C. Zhang, D. N. Bryant, M. J. Kelly, and O. K. Rønnekleiv, "PI3K signaling effects in hypothalamic neurons mediated by estrogen," *Journal of Comparative Neurology*, vol. 506, no. 6, pp. 895–911, 2008.
- [67] L. Björnström and M. Sjöberg, "Mechanisms of estrogen receptor signaling: convergence of genomic and nongenomic actions on target genes," *Molecular Endocrinology*, vol. 19, no. 4, pp. 833–842, 2005.
- [68] M. Singh, "Ovarian hormones elicit phosphorylation of Akt and extracellular-signal regulated kinase in explants of the cerebral cortex," *Endocrine*, vol. 14, no. 3, pp. 407–415, 2001.
- [69] N. Vasudevan and D. W. Pfaff, "Non-genomic actions of estrogens and their interaction with genomic actions in the brain," *Frontiers in Neuroendocrinology*, vol. 29, no. 2, pp. 238–257, 2008.
- [70] C. J. Park, Z. Zhao, C. Glidewell-Kenney et al., "Genetic rescue of nonclassical ER α signaling normalizes energy balance in obese ER α -null mutant mice," *The Journal of Clinical Investigation*, vol. 121, no. 2, pp. 604–612, 2011.
- [71] R. A. Gorski, R. E. Harlan, C. D. Jacobson, J. E. Shryne, and A. M. Southam, "Evidence for the existence of a sexually dimorphic nucleus in the preoptic area of the rat," *Journal of Comparative Neurology*, vol. 193, no. 2, pp. 529–539, 1980.
- [72] D. W. Pfaff, M. McCarthy, S. Schwartz-Giblin, and L. M. Kow, "Cellular and molecular mechanism of female reproductive behavior," in *The Physiology of Reproduction*, E. Knobil and J. D. Neill, Eds., pp. 107–220, Raven Press, New York, NY, USA, 1994.
- [73] C. L. Sisk and D. L. Foster, "The neural basis of puberty and adolescence," *Nature Neuroscience*, vol. 7, no. 10, pp. 1040–1047, 2004.
- [74] Q. Gao, G. Mezei, Y. Nie et al., "Anorectic estrogen mimics leptin's effect on the rewiring of melanocortin cells and Stat3 signaling in obese animals," *Nature Medicine*, vol. 13, no. 1, pp. 89–94, 2007.
- [75] T. M. D'Eon, S. C. Souza, M. Aronovitz, M. S. Obin, S. K. Fried, and A. S. Greenberg, "Estrogen regulation of adiposity and fuel partitioning. Evidence of genomic and non-genomic regulation of lipogenic and oxidative pathways," *The Journal of Biological Chemistry*, vol. 280, no. 43, pp. 35983–35991, 2005.
- [76] H. Gao, G. Bryzgalova, E. Hedman et al., "Long-term administration of estradiol decreases expression of hepatic lipogenic genes and improves insulin sensitivity in ob/ob mice: a possible mechanism is through direct regulation of signal transducer and activator of transcription 3," *Molecular Endocrinology*, vol. 20, no. 6, pp. 1287–1299, 2006.
- [77] H. Shi and S. P. D. S. Kumar, "Sex differences in obesity-related glucose intolerance and insulin resistance," in *Glucose Tolerance*, S. Chackrewarthy, Ed., chapter 4, pp. 37–66, InTech, Rijeka, Croatia, 2012.
- [78] M. Österlund, G. J. M. Kuiper, J.-Å. Gustafsson, and Y. L. Hurd, "Differential distribution and regulation of estrogen receptor- α and - β mRNA within the female rat brain," *Molecular Brain Research*, vol. 54, no. 1, pp. 175–180, 1998.

- [79] I. Merchenthaler, M. V. Lane, S. Numan, and T. L. Dellovade, "Distribution of estrogen receptor α and β in the mouse central nervous system: in vivo autoradiographic and immunocytochemical analyses," *Journal of Comparative Neurology*, vol. 473, no. 2, pp. 270–291, 2004.
- [80] S. X. Simonian and A. E. Herbison, "Differential expression of estrogen receptor and neuropeptide Y by brainstem A1 and A2 noradrenergic neurons," *Neuroscience*, vol. 76, no. 2, pp. 517–529, 1997.
- [81] R. B. Simerly, C. Chang, M. Muramatsu, and L. W. Swanson, "Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an *in situ* hybridization study," *Journal of Comparative Neurology*, vol. 294, no. 1, pp. 76–95, 1990.
- [82] L. Zhu, Y. Yang, P. Xu et al., "Steroid receptor coactivator-1 mediates estrogenic actions to prevent body weight gain in female mice," *Endocrinology*, vol. 154, no. 1, pp. 150–158, 2012.
- [83] C. Ohlsson, N. Hellberg, P. Parini et al., "Obesity and disturbed lipoprotein profile in estrogen receptor- α -deficient male mice," *Biochemical and Biophysical Research Communications*, vol. 278, no. 3, pp. 640–645, 2000.
- [84] S. Musatov, W. Chen, D. W. Pfaff et al., "Silencing of estrogen receptor α in the ventromedial nucleus of hypothalamus leads to metabolic syndrome," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 7, pp. 2501–2506, 2007.
- [85] P. Zimmermann, "Estrogen-dependent changes in the functional interrelationships among neurons, ependymal cells and glial cells of the arcuate nucleus," *Cell and Tissue Research*, vol. 227, no. 1, pp. 113–128, 1982.
- [86] V. V. Chaban, A. J. Lakhter, and P. Micevych, "A membrane estrogen receptor mediates intracellular calcium release in astrocytes," *Endocrinology*, vol. 145, no. 8, pp. 3788–3795, 2004.
- [87] J. A. Mong and T. Blumstein, "Estradiol modulation of astrocytic form and function: implications for hormonal control of synaptic communication," *Neuroscience*, vol. 138, no. 3, pp. 967–975, 2006.
- [88] K. M. Dhandapani and D. W. Brann, "Estrogen-astrocyte interactions: implications for neuroprotection," *BMC Neuroscience*, vol. 3, no. 1, p. 6, 2002.
- [89] L. M. Garcia-Segura, F. Naftolin, J. B. Hutchison, I. Azcoitia, and J. A. Chowen, "Role of astroglia in estrogen regulation of synaptic plasticity and brain repair," *Journal of Neurobiology*, vol. 40, no. 4, pp. 574–584, 1999.
- [90] M. M. McCarthy, S. K. Amateau, and J. A. Mong, "Steroid modulation of astrocytes in the neonatal brain: implications for adult reproductive function," *Biology of Reproduction*, vol. 67, no. 3, pp. 691–698, 2002.
- [91] L. M. Garcia-Segura and M. M. McCarthy, "Minireview: Role of glia in neuroendocrine function," *Endocrinology*, vol. 145, no. 3, pp. 1082–1086, 2004.
- [92] P. Micevych, K. Sinchak, R. H. Mills, L. Tao, P. LaPolt, and J. K. H. Lu, "The luteinizing hormone surge is preceded by an estrogen-induced increase of hypothalamic progesterone in ovariectomized and adrenalectomized rats," *Neuroendocrinology*, vol. 78, no. 1, pp. 29–35, 2003.
- [93] C. García-Cáceres, E. Fuente-Martín, J. Argente, and J. A. Chowen, "Emerging role of glial cells in the control of body weight," *Molecular Metabolism*, vol. 1, no. 1-2, pp. 37–46, 2012.
- [94] P. J. Magistretti, "Neuron-glia metabolic coupling and plasticity," *Journal of Experimental Biology*, vol. 209, no. 12, pp. 2304–2311, 2006.
- [95] T. L. Horvath, B. Sarman, C. García-Cáceres et al., "Synaptic input organization of the melanocortin system predicts diet-induced hypothalamic reactive gliosis and obesity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 33, pp. 14875–14880, 2010.
- [96] C. García-Cáceres, E. Fuente-Martán, E. Burgos-Ramos et al., "Differential acute and chronic effects of leptin on hypothalamic astrocyte morphology and synaptic protein levels," *Endocrinology*, vol. 152, no. 5, pp. 1809–1818, 2011.
- [97] S. Tapia-González, L. M. García-Segura, M. Tena-Sempere et al., "Activation of microglia in specific hypothalamic nuclei and the cerebellum of adult rats exposed to neonatal overnutrition," *Journal of Neuroendocrinology*, vol. 23, no. 4, pp. 365–370, 2011.
- [98] C. van Kralingen, D. T. Kho, J. Costa, C. E. Angel, and E. S. Graham, "Exposure to inflammatory cytokines IL-1 β and TNF α induces compromise and death of astrocytes; implications for chronic neuroinflammation," *PLoS ONE*, vol. 8, no. 12, Article ID e84269, 2013.
- [99] T. Sartorius, M. Heni, O. Tschrüter et al., "Leptin affects insulin action in astrocytes and impairs insulin-mediated physical activity," *Cellular Physiology and Biochemistry*, vol. 30, no. 1, pp. 238–246, 2012.
- [100] J. P. Thaler, C.-X. Yi, E. A. Schur et al., "Obesity is associated with hypothalamic injury in rodents and humans," *The Journal of Clinical Investigation*, vol. 122, no. 1, pp. 153–162, 2012.
- [101] C.-H. Tang, D.-Y. Lu, R.-S. Yang et al., "Leptin-induced IL-6 production is mediated by leptin receptor, insulin receptor substrate-1, phosphatidylinositol 3-kinase, Akt, NF-kappaB, and p300 pathway in microglia," *The Journal of Immunology*, vol. 179, no. 2, pp. 1292–1302, 2007.
- [102] A. Matsumoto and Y. Arai, "Male-female difference in synaptic organization of the ventromedial nucleus of the hypothalamus in the rat," *Neuroendocrinology*, vol. 42, no. 3, pp. 232–236, 1986.
- [103] G. J. Morton, D. E. Cummings, D. G. Baskin, G. S. Barsh, and M. W. Schwartz, "Central nervous system control of food intake and body weight," *Nature*, vol. 443, no. 7109, pp. 289–295, 2006.
- [104] P. J. Shughrue, M. V. Lane, and I. Merchenthaler, "Comparative distribution of estrogen receptor- α and - β mRNA in the rat central nervous system," *Journal of Comparative Neurology*, vol. 388, no. 4, pp. 507–525, 1997.
- [105] B. E. Grayson, P. R. Levasseur, S. M. Williams, M. S. Smith, D. L. Marks, and K. L. Grove, "Changes in melanocortin expression and inflammatory pathways in fetal offspring of nonhuman primates fed a high-fat diet," *Endocrinology*, vol. 151, no. 4, pp. 1622–1632, 2010.
- [106] G. Barreto, M. Santos-Galindo, Y. Diz-Chaves et al., "Selective estrogen receptor modulators decrease reactive astrogliosis in the injured brain: effects of aging and prolonged depletion of ovarian hormones," *Endocrinology*, vol. 150, no. 11, pp. 5010–5015, 2009.
- [107] K. Yuri and M. Kawata, "The effect of estrogen on the estrogen receptor-immunoreactive cells in the rat medial preoptic nucleus," *Brain Research*, vol. 548, no. 1-2, pp. 50–54, 1991.
- [108] A. J. Bruce-Keller, J. L. Keeling, J. N. Keller, F. F. Huang, S. Camandola, and M. P. Mattson, "Antiinflammatory effects of estrogen on microglial activation," *Endocrinology*, vol. 141, no. 10, pp. 3646–3656, 2000.
- [109] E. Vegeto, S. Belcredito, S. Ghisletti, C. Meda, S. Etti, and A. Maggi, "The endogenous estrogen status regulates microglia reactivity in animal models of neuroinflammation," *Endocrinology*, vol. 147, no. 5, pp. 2263–2272, 2006.

- [110] E. Morselli, E. Fuente-Martin, B. Finan et al., "Hypothalamic PGC-1 α protects against high-fat diet exposure by regulating ER α ," *Cell Reports*, vol. 9, no. 2, pp. 633–645, 2014.
- [111] K. Saijo, J. G. Collier, A. C. Li, J. A. Katzenellenbogen, and C. K. Glass, "An ADIOL-ER β -CtBP transrepression pathway negatively regulates microglia-mediated inflammation," *Cell*, vol. 145, no. 4, pp. 584–595, 2011.
- [112] W.-F. Wua, X.-J. Tana, Y.-B. Daia, V. Krishnan, M. Warner, and J.-Å. Gustafsson, "Targeting estrogen receptor β in microglia and T cells to treat experimental autoimmune encephalomyelitis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 110, no. 9, pp. 3543–3548, 2013.
- [113] E. Hösl, K. Jurasin, W. Rühl, R. Lüthy, and L. Hösl, "Colocalization of androgen, estrogen and cholinergic receptors on cultured astrocytes of rat central nervous system," *International Journal of Developmental Neuroscience*, vol. 19, no. 1, pp. 11–19, 2001.
- [114] K. Sato, N. Matsuki, Y. Ohno, and K. Nakazawa, "Estrogens inhibit L-glutamate uptake activity of astrocytes via membrane estrogen receptor α ," *Journal of Neurochemistry*, vol. 86, no. 6, pp. 1498–1505, 2003.
- [115] J. Pawlak, M. Karolczak, A. Krust, P. Chambon, and C. Beyer, "Estrogen receptor- α is associated with the plasma membrane of astrocytes and coupled to the MAP/Src-kinase pathway," *Glia*, vol. 50, no. 3, pp. 270–275, 2005.
- [116] A. Quesada, H. E. Romeo, and P. Micevych, "Distribution and localization patterns of estrogen receptor- β and insulin-like growth factor-1 receptors in neurons and glial cells of the female rat substantia nigra: localization of ER β and IGF-1R in substantia nigra," *Journal of Comparative Neurology*, vol. 503, no. 1, pp. 198–208, 2007.
- [117] D. García-Ovejero, S. Veiga, L. M. García-Segura, and L. L. DonCarlos, "Glial expression of estrogen and androgen receptors after rat brain injury," *Journal of Comparative Neurology*, vol. 450, no. 3, pp. 256–271, 2002.
- [118] R. D. Spence, A. J. Wisdom, Y. Cao et al., "Estrogen mediates neuroprotection and anti-inflammatory effects during EAE through ER α signaling on astrocytes but not through ER β signaling on astrocytes or neurons," *The Journal of Neuroscience*, vol. 33, no. 26, pp. 10924–10933, 2013.
- [119] C. A. Lisciotto and J. I. Morrell, "Circulating gonadal steroid hormones regulate estrogen receptor mRNA in the male rat forebrain," *Molecular Brain Research*, vol. 20, no. 1-2, pp. 79–90, 1993.
- [120] H.-Y. Li, J. D. Blaustein, G. J. De Vries, and G. N. Wade, "Estrogen-receptor immunoreactivity in hamster brain: preoptic area, hypothalamus and amygdala," *Brain Research*, vol. 631, no. 2, pp. 304–312, 1993.
- [121] J. M. Meredith, C. J. Auger, and J. D. Blaustein, "Down-regulation of estrogen receptor immunoreactivity by 17 beta-estradiol in the guinea pig forebrain," *Journal of Neuroendocrinology*, vol. 6, no. 6, pp. 639–648, 1994.
- [122] C. L. Sisk and L. L. DonCarlos, "Estrogen receptor immunoreactivity in ferret brain is regulated by estradiol in a region-specific manner," *Brain Research*, vol. 688, no. 1-2, pp. 198–202, 1995.
- [123] C. A. Fox, L. R. Ross, R. J. Handa, and C. D. Jacobson, "Localization of cells containing estrogen receptor-like immunoreactivity in the Brazilian opossum brain," *Brain Research*, vol. 546, no. 1, pp. 96–105, 1991.
- [124] T. L. Dellovade, J. D. Blaustein, and E. F. Rissman, "Neural distribution of estrogen receptor immunoreactive cells in the female musk shrew," *Brain Research*, vol. 595, no. 2, pp. 189–194, 1992.
- [125] M. Gahr, "Distribution of sex steroid hormone receptors in the avian brain: functional implications for neural sex differences and sexual behaviors," *Microscopy Research and Technique*, vol. 55, no. 1, pp. 1–11, 2001.
- [126] M. Gahr, H.-R. Güttinger, and D. E. Kroodsmas, "Estrogen receptors in the avian brain: survey reveals general distribution and forebrain areas unique to songbirds," *Journal of Comparative Neurology*, vol. 327, no. 1, pp. 112–122, 1993.
- [127] F. J. C. M. van Eerdenburg, I. A. J. M. Daemen, E. M. van der Beek, and F. W. van Leeuwen, "Changes in estrogen- α receptor immunoreactivity during the estrous cycle in lactating dairy cattle," *Brain Research*, vol. 880, no. 1-2, pp. 219–223, 2000.
- [128] P. C. Goldsmith, J. E. Boggan, and K. K. Thind, "Estrogen and progesterone receptor expression in neuroendocrine and related neurons of the pubertal female monkey hypothalamus," *Neuroendocrinology*, vol. 65, no. 5, pp. 325–334, 1997.
- [129] A. E. Herbison, T. L. Horvath, F. Naftolin, and C. Leranth, "Distribution of estrogen receptor-immunoreactive cells in monkey hypothalamus: relationship to neurones containing luteinizing hormone-releasing hormone and tyrosine hydroxylase," *Neuroendocrinology*, vol. 61, no. 1, pp. 1–10, 1995.
- [130] M. M. Blurton-Jones, J. A. Roberts, and M. H. Tuszynski, "Estrogen receptor immunoreactivity in the adult primate brain: neuronal distribution and association with p75, trkA, and choline acetyltransferase," *Journal of Comparative Neurology*, vol. 405, no. 4, pp. 529–542, 1999.
- [131] F. P. M. Kruijver, R. Balesar, A. M. Espila, U. A. Unmehopa, and D. F. Swaab, "Estrogen receptor- α distribution in the human hypothalamus in relation to sex and endocrine status," *Journal of Comparative Neurology*, vol. 454, no. 2, pp. 115–139, 2002.
- [132] J. E. Donahue, E. G. Stopa, R. L. Chorsky et al., "Cells containing immunoreactive estrogen receptor- α in the human basal forebrain," *Brain Research*, vol. 856, no. 1-2, pp. 142–151, 2000.
- [133] M. K. Österlund, E. Keller, and Y. L. Hurd, "The human forebrain has discrete estrogen receptor α messenger RNA expression: high levels in the amygdaloid complex," *Neuroscience*, vol. 95, no. 2, pp. 333–342, 1999.
- [134] B. Parsons, T. C. Rainbow, D. W. Pfaff, and B. S. McEwen, "Hypothalamic protein synthesis essential for the activation of the lordosis reflex in the female rat," *Endocrinology*, vol. 110, no. 2, pp. 620–624, 1982.
- [135] L. A. deGraffenried, T. A. Hopp, A. J. Valente, R. A. Clark, and S. A. W. Fuqua, "Regulation of the estrogen receptor α minimal promoter by Sp1, USF-1 and ER α ," *Breast Cancer Research and Treatment*, vol. 85, no. 2, pp. 111–120, 2004.
- [136] M. Koš, G. Reid, S. Denger, and F. Gannon, "Minireview: genomic organization of the human ER α gene promoter region," *Molecular Endocrinology*, vol. 15, no. 12, pp. 2057–2063, 2001.
- [137] M. E. Wilson and J. M. Westberry, "Regulation of oestrogen receptor gene expression: new insights and novel mechanisms," *Journal of Neuroendocrinology*, vol. 21, no. 4, pp. 238–242, 2009.
- [138] M. Kobayashi, H. Ishii, and Y. Sakuma, "Identification of novel splicing events and post-transcriptional regulation of human estrogen receptor α F isoforms," *Molecular and Cellular Endocrinology*, vol. 333, no. 1, pp. 55–61, 2011.
- [139] K. F. H. Grandien, A. Berkenstam, S. Nilsson, and J.-A. Gustafsson, "Localization of DNase I hypersensitivity sites in

- the human oestrogen receptor gene correlates with the transcriptional activity of two differentially used promoters," *Journal of Molecular Endocrinology*, vol. 10, no. 3, pp. 269–277, 1993.
- [140] J.-P. J. Issa, Y. L. Ottaviano, P. Celano, S. R. Hamilton, N. E. Davidson, and S. B. Baylin, "Methylation of the oestrogen receptor CpG island links ageing and neoplasia in human colon," *Nature Genetics*, vol. 7, no. 4, pp. 536–540, 1994.
- [141] J. S. Rosenblatt, C. K. Wagner, and J. I. Morrell, "Hormonal priming and triggering of maternal behavior in the rat with special reference to the relations between estrogen receptor binding and ER mRNA in specific brain regions," *Psychoneuroendocrinology*, vol. 19, no. 5–7, pp. 543–552, 1994.
- [142] J. M. Davidson, J. J. Chen, L. Crapo, G. D. Gray, W. J. Greenleaf, and J. A. Catania, "Hormonal changes and sexual function in aging men," *Journal of Clinical Endocrinology and Metabolism*, vol. 57, no. 1, pp. 71–77, 1983.
- [143] H. A. Zacur, "Hormonal changes throughout life in women," *Headache*, vol. 46, no. 2, pp. S50–S55, 2006.
- [144] J. C. Lovejoy, "The influence of sex hormones on obesity across the female life span," *Journal of Women's Health*, vol. 7, no. 10, pp. 1247–1256, 1998.
- [145] A. H. Lauber, C. V. Mobbs, M. Muramatsu, and D. W. Pfaff, "Estrogen receptor messenger RNA expression in rat hypothalamus as a function of genetic sex and estrogen dose," *Endocrinology*, vol. 129, no. 6, pp. 3180–3186, 1991.
- [146] A. H. Lauber, G. J. Romano, C. V. Mobbs, and D. W. Pfaff, "Estradiol regulation of estrogen receptor messenger ribonucleic acid in rat mediobasal hypothalamus: an in situ hybridization study," *Journal of Neuroendocrinology*, vol. 2, no. 5, pp. 605–611, 1990.
- [147] R. B. Simerly and B. J. Young, "Regulation of estrogen receptor messenger ribonucleic acid in rat hypothalamus by sex steroid hormones," *Molecular Endocrinology*, vol. 5, no. 3, pp. 424–432, 1991.
- [148] P. J. Shughrue, C. D. Bushnell, and D. M. Dorsa, "Estrogen receptor messenger ribonucleic acid in female rat brain during the estrous cycle: a comparison with ovariectomized females and intact males," *Endocrinology*, vol. 131, no. 1, pp. 381–388, 1992.
- [149] L. J. Young, P. K. Nag, and D. Crews, "Species differences in behavioral and neural sensitivity to estrogen in whiptail lizards: correlation with hormone receptor messenger ribonucleic acid expression," *Neuroendocrinology*, vol. 61, no. 6, pp. 680–686, 1995.
- [150] L. J. Young, P. K. Nag, and D. Crews, "Regulation of estrogen receptor and progesterone receptor messenger ribonucleic acid by estrogen in the brain of the whiptail lizard (*Cnemidophorus uniparens*)," *Journal of Neuroendocrinology*, vol. 7, no. 2, pp. 119–125, 1995.
- [151] L. L. DonCarlos, M. McAbee, D. S. Ramer-Quinn, and D. M. Stancik, "Estrogen receptor mRNA levels in the preoptic area of neonatal rats are responsive to hormone manipulation," *Developmental Brain Research*, vol. 84, no. 2, pp. 253–260, 1995.
- [152] Y. Zhou, P. J. Shughrue, and D. M. Dorsa, "Estrogen receptor protein is differentially regulated in the preoptic area of the brain and in the uterus during the rat estrous cycle," *Neuroendocrinology*, vol. 61, no. 3, pp. 276–283, 1995.
- [153] S. Yamada, D. Noguchi, H. Ito, and K. Yamanouchi, "Sex and regional differences in decrease of estrogen receptor α -immunoreactive cells by estrogen in rat hypothalamus and midbrain," *Neuroscience Letters*, vol. 463, no. 2, pp. 135–139, 2009.
- [154] M. Koch, "Effects of treatment with estradiol and parental experience on the number and distribution of estrogen-binding neurons in the ovariectomized mouse brain," *Neuroendocrinology*, vol. 51, no. 5, pp. 505–514, 1990.
- [155] V. Malikov and M. D. Madeira, "Regulation of ER α protein expression by 17 β -estradiol in cultured neurons of hypothalamic ventromedial nucleus," *Neurochemical Research*, vol. 38, no. 1, pp. 82–89, 2013.
- [156] T. J. Brown, R. B. Hochberg, J. E. Zielinski, and N. J. MacLusky, "Regional sex differences in cell nuclear estrogen-binding capacity in the rat hypothalamus and preoptic area," *Endocrinology*, vol. 123, no. 4, pp. 1761–1770, 1988.
- [157] N. Devidze, J. A. Mong, A. M. Jasnow, L.-M. Kow, and D. W. Pfaff, "Sex and estrogenic effects on coexpression of mRNAs in single ventromedial hypothalamic neurons," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 40, pp. 14446–14451, 2005.
- [158] M. Mahavongtrakul, M. P. Kanjiya, M. Maciel, S. Kanjiya, and K. Sinchak, "Estradiol dose-dependent regulation of membrane estrogen receptor- α , metabotropic glutamate receptor-1a, and their complexes in the arcuate nucleus of the hypothalamus in female rats," *Endocrinology*, vol. 154, no. 9, pp. 3251–3260, 2013.
- [159] J. Godwin and D. Crews, "Sex differences in estrogen and progesterone receptor messenger ribonucleic acid regulation in the brain of little striped whiptail lizards," *Neuroendocrinology*, vol. 62, no. 3, pp. 293–300, 1995.
- [160] L. J. Young and D. Crews, "Comparative neuroendocrinology of steroid receptor gene expression and regulation: relationship to physiology and behavior," *Trends in Endocrinology and Metabolism*, vol. 6, no. 9–10, pp. 317–323, 1995.
- [161] C. Donaghue, B. R. Westley, and F. E. B. May, "Selective promoter usage of the human estrogen receptor- α gene and its regulation by estrogen," *Molecular Endocrinology*, vol. 13, no. 11, pp. 1934–1950, 1999.
- [162] G. Zaman, H. L. Jessop, M. Muzylak et al., "Osteocytes use estrogen receptor α to respond to strain but their ER α content is regulated by estrogen," *Journal of Bone and Mineral Research*, vol. 21, no. 8, pp. 1297–1306, 2006.
- [163] A. J. Murphy, P. M. Guyre, C. R. Wira, and P. A. Pioli, "Estradiol regulates expression of estrogen receptor ER α 46 in human macrophages," *PLoS ONE*, vol. 4, no. 5, Article ID e5539, 2009.
- [164] I. R. Pirwany, N. Sattar, I. A. Greer, C. J. Packard, and R. Fleming, "Supraphysiological concentrations of estradiol in menopausal women given repeated implant therapy do not adversely affect lipid profiles," *Human Reproduction*, vol. 17, no. 3, pp. 825–829, 2002.
- [165] J.-Z. Bao, C.-R. Ni, and W.-Q. Zheng, "Age-related effects of estrogen on the expression of estrogen receptor (ER) α and β mRNA in the ovariectomized (OVX) monkey hypothalamus," *Neuroscience Bulletin*, vol. 22, no. 2, pp. 97–102, 2006.
- [166] P. K. Sharma and M. K. Thakur, "Expression of estrogen receptor (ER) α and β in mouse cerebral cortex: effect of age, sex and gonadal steroids," *Neurobiology of Aging*, vol. 27, no. 6, pp. 880–887, 2006.
- [167] M. Marino, M. Pellegrini, P. La Rosa, and F. Acconcia, "Susceptibility of estrogen receptor rapid responses to xenoestrogens: physiological outcomes," *Steroids*, vol. 77, no. 10, pp. 910–917, 2012.
- [168] K. Yoon, S. J. Kwack, H. S. Kim, and B.-M. Lee, "Estrogenic endocrine-disrupting chemicals: molecular mechanisms of actions on putative human diseases," *Journal of Toxicology and Environmental Health, Part B: Critical Reviews*, vol. 17, no. 3, pp. 127–174, 2014.

- [169] K. J. Bailey, "Diurnal progesterone rhythms in the female mouse," *Journal of Endocrinology*, vol. 112, no. 1, pp. 15–21, 1987.
- [170] P. E. Chappell and J. E. Levine, "Stimulation of gonadotropin-releasing hormone surges by estrogen. I. Role of hypothalamic progesterone receptors," *Endocrinology*, vol. 141, no. 4, pp. 1477–1485, 2000.
- [171] O. K. Rønnekleiv and M. J. Kelly, "Diversity of ovarian steroid signaling in the hypothalamus," *Frontiers in Neuroendocrinology*, vol. 26, no. 2, pp. 65–84, 2005.
- [172] S. J. Legan, G. A. Coon, and F. J. Karsch, "Role of estrogen as initiator of daily LH surges in the ovariectomized rat," *Endocrinology*, vol. 96, no. 1, pp. 50–56, 1975.
- [173] M. C. Freeman, K. C. Dupke, and C. M. Croteau, "Extinction of the estrogen-induced daily signal for LH release in the rat: a role for the proestrous surge of progesterone," *Endocrinology*, vol. 99, no. 1, pp. 223–229, 1976.
- [174] M. Warembourg, A. Jolivet, and E. Milgrom, "Immunohistochemical evidence of the presence of estrogen and progesterone receptors in the same neurons of the guinea pig hypothalamus and preoptic area," *Brain Research*, vol. 480, no. 1-2, pp. 1–15, 1989.
- [175] J. D. Blaustein and T. J. Brown, "Progesterone decrease the concentration of hypothalamic and anterior pituitary estrogen receptors in ovariectomized rats," *Brain Research*, vol. 304, no. 2, pp. 225–236, 1984.
- [176] W. L. Kraus, K. E. Weis, and B. S. Katzenellenbogen, "Determinants for the repression of estrogen receptor transcriptional activity by ligand-occupied progesterone receptors," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 63, no. 4–6, pp. 175–188, 1997.
- [177] W. L. Kraus, K. E. Weis, and B. S. Katzenellenbogen, "Inhibitory cross-talk between steroid hormone receptors: differential targeting of estrogen receptor in the repression of its transcriptional activity by agonist- and antagonist-occupied progesterone receptors," *Molecular and Cellular Biology*, vol. 15, no. 4, pp. 1847–1857, 1995.
- [178] A. S. Clark, B. Nock, H. H. Feder, and E. J. Roy, " α 1-noradrenergic receptor blockade decreases nuclear estrogen receptor binding in guinea pig hypothalamus and preoptic area," *Brain Research*, vol. 330, no. 1, pp. 197–199, 1985.
- [179] B. Nock and H. H. Feder, "Noradrenergic transmission and female sexual behavior of guinea pigs," *Brain Research*, vol. 166, no. 2, pp. 369–380, 1979.
- [180] F. J. Steyn, G. M. Anderson, and D. R. Grattan, "Expression of ovarian steroid hormone receptors in tuberoinfundibular dopaminergic neurones during pregnancy and lactation," *Journal of Neuroendocrinology*, vol. 19, no. 10, pp. 788–793, 2007.
- [181] P. E. Mann and J. A. Babb, "Neural steroid hormone receptor gene expression in pregnant rats," *Molecular Brain Research*, vol. 142, no. 1, pp. 39–46, 2005.
- [182] W. Fan, T. Yanase, M. Nomura et al., "Androgen receptor null male mice develop late-onset obesity caused by decreased energy expenditure and lipolytic activity but show normal insulin sensitivity with high adiponectin secretion," *Diabetes*, vol. 54, no. 4, pp. 1000–1008, 2005.
- [183] H.-Y. Lin, Q. Xu, S. Yeh, R.-S. Wang, J. D. Sparks, and C. Chang, "Insulin and leptin resistance with hyperleptinemia in mice lacking androgen receptor," *Diabetes*, vol. 54, no. 6, pp. 1717–1725, 2005.
- [184] R. J. Handa, J. E. Kerr, L. L. DonCarlos, R. F. McGivern, and G. Hejna, "Hormonal regulation of androgen receptor messenger RNA in the medial preoptic area of the male rat," *Molecular Brain Research*, vol. 39, no. 1-2, pp. 57–67, 1996.
- [185] F. He, F. Tai, Y. Zhang, and X. Zhang, "Effects of castration on aggression and levels of serum sex hormones and their central receptors in mandarin voles (*Microtus mandarinus*)," *Journal of Comparative Physiology A*, vol. 198, no. 5, pp. 347–362, 2012.
- [186] D. Wu and A. C. Gore, "Changes in androgen receptor, estrogen receptor alpha, and sexual behavior with aging and testosterone in male rats," *Hormones and Behavior*, vol. 58, no. 2, pp. 306–316, 2010.
- [187] E. L. Ding, Y. Song, V. S. Malik, and S. Liu, "Sex differences of endogenous sex hormones and risk of type 2 diabetes: a systematic review and meta-analysis," *Journal of the American Medical Association*, vol. 295, no. 11, pp. 1288–1299, 2006.
- [188] S. Bhasin and J. G. Buckwalter, "Testosterone supplementation in older men: a rational idea whose time has not yet come," *Journal of Andrology*, vol. 22, no. 5, pp. 718–731, 2001.
- [189] A. Corbould, "Effects of androgens on insulin action in women: is androgen excess a component of female metabolic syndrome?" *Diabetes/Metabolism Research and Reviews*, vol. 24, no. 7, pp. 520–532, 2008.
- [190] D. M. Kelly and T. H. Jones, "Testosterone and obesity," *Obesity Reviews*, vol. 16, no. 7, pp. 581–606, 2015.
- [191] A. Dunaif and A. Thomas, "Current concepts in the polycystic ovary syndrome," *Annual Review of Medicine*, vol. 52, pp. 401–419, 2001.
- [192] E. Diamanti-Kandarakis, "Role of obesity and adiposity in polycystic ovary syndrome," *International Journal of Obesity*, vol. 31, no. 2, pp. S8–S13, 2007.
- [193] F. S. Silva, M. O. Sóter, M. F. Sales et al., "Estrogen receptor α gene (ESR1) PvuII and XbaI polymorphisms are associated to metabolic and proinflammatory factors in polycystic ovary syndrome," *Gene*, vol. 560, no. 1, pp. 44–49, 2015.
- [194] X. Nectaria, L. Leandros, G. Ioannis, and T. Agathocles, "The importance of ER α and ER β gene polymorphisms in PCOS," *Gynecological Endocrinology*, vol. 28, no. 7, pp. 505–508, 2012.
- [195] F. M. Zurvarra, N. R. Salvetti, J. I. Mason, M. M. L. Velazquez, N. S. Alfaro, and H. H. Ortega, "Disruption in the expression and immunolocalisation of steroid receptors and steroidogenic enzymes in letrozole-induced polycystic ovaries in rat," *Reproduction, Fertility and Development*, vol. 21, no. 7, pp. 827–839, 2009.
- [196] C. Bian, Y. Zhao, Q. Guo, Y. Xiong, W. Cai, and J. Zhang, "Aromatase inhibitor letrozole downregulates steroid receptor coactivator-1 in specific brain regions that primarily related to memory, neuroendocrine and integration," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 141, pp. 37–43, 2014.
- [197] A. Tchernof, A. Desmeules, C. Richard et al., "Ovarian hormone status and abdominal visceral adipose tissue metabolism," *Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 7, pp. 3425–3430, 2004.
- [198] K. Samaras, C. S. Hayward, D. Sullivan, R. P. Kelly, and L. V. Campbell, "Effects of postmenopausal hormone replacement therapy on central abdominal fat, glycemic control, lipid metabolism, and vascular factors in type 2 diabetes: a prospective study," *Diabetes Care*, vol. 22, no. 9, pp. 1401–1407, 1999.
- [199] C. K. Sites, M. Brochu, A. Tchernof, and E. T. Poehlman, "Relationship between hormone replacement therapy use with body fat distribution and insulin sensitivity in obese postmenopausal women," *Metabolism: Clinical and Experimental*, vol. 50, no. 7, pp. 835–840, 2001.

- [200] M. Gambacciani, M. Ciaponi, B. Cappagli, L. De Simone, R. Orlandi, and A. R. Genazzani, "Prospective evaluation of body weight and body fat distribution in early postmenopausal women with and without hormonal replacement therapy," *Maturitas*, vol. 39, no. 2, pp. 125–132, 2001.
- [201] D. Kritz-Silverstein and E. Barrett-Connor, "Long-term postmenopausal hormone use, obesity, and fat distribution in older women," *The Journal of the American Medical Association*, vol. 275, no. 1, pp. 46–49, 1996.
- [202] J. A. Kanaley, C. Sames, L. Swisher et al., "Abdominal fat distribution in pre- and postmenopausal women: the impact of physical activity, age, and menopausal status," *Metabolism: Clinical and Experimental*, vol. 50, no. 8, pp. 976–982, 2001.
- [203] A. S. Ryan, B. J. Nicklas, and D. M. Berman, "Hormone replacement therapy, insulin sensitivity, and abdominal obesity in postmenopausal women," *Diabetes Care*, vol. 25, no. 1, pp. 127–133, 2002.
- [204] H. D. Nelson, L. L. Humphrey, P. Nygren, S. M. Teutsch, and J. D. Allan, "Postmenopausal hormone replacement therapy: scientific review," *The Journal of the American Medical Association*, vol. 288, no. 7, pp. 872–881, 2002.



Hindawi
Submit your manuscripts at
<http://www.hindawi.com>

