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G Protein-Coupled Estrogen Receptor in Energy Homeostasis and Obesity Pathogenesis

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Abstract

Obesity and its related metabolic diseases have reached a pandemic level worldwide. There are sex differences in the prevalence of obesity and its related metabolic diseases, with men being more vulnerable than women; however, the prevalence of these disorders increases dramatically in women after menopause, suggesting that sex steroid hormone estrogens play key protective roles against development of obesity and metabolic diseases. Estrogens are important regulators of several aspects of metabolism, including body weight and body fat, caloric intake and energy expenditure, and glucose and lipid metabolism in both males and females. Estrogens act in complex ways on their nuclear estrogen receptors (ERs) ER α and ER β and transmembrane ERs such as G protein-coupled estrogen receptor. Genetic tools, such as different lines of knockout mouse models, and pharmacological agents, such as selective agonists and antagonists, are available to study function and signaling mechanisms of ERs. We provide an overview of the evidence for the physiological and cellular actions of ERs in estrogen-dependent processes in the context of energy homeostasis and body fat regulation and discuss its pathology that leads to obesity and related metabolic states.

1. INTRODUCTION

1.1. Obesity is a risk factor for metabolic diseases

Despite attempts to increase awareness of the deleterious health consequences associated with obesity, there continues to be a significant increase in the obese population worldwide.¹ Obesity has been recognized as a major and an independent risk factor for metabolic syndrome, a group of interrelated metabolic abnormalities characterized by increased visceral fat accumulation, disturbed glucose homeostasis with hyperglycemia and insulin resistance, increased triglycerides and decreased high-density lipoprotein cholesterol, and hypertension.² The economic costs of caring for patients with obesity and its metabolic complications are enormously high.³ Although the pathophysiologic basis of obesity and metabolic syndrome is largely unknown, evidence suggests that modern diets with high fat content and sedentary lifestyle progressively lead to the accumulation of saturated fatty acids in nonadipose tissue. The subsequent metabolism of this ectopic fatty acid deposition leads to decreased insulin-stimulated glucose uptake in skeletal muscle, unsuppressed hepatic glucose production, and altered glucose-stimulated insulin release from pancreatic β -cells.⁴ Once diabetes is declared, hyperglycemia and elevated free fatty acids cooperate to generate major oxidative stress in tissues, further aggravating insulin resistance and deficiency.

Development of adverse metabolic complications has been attributed to increased body fat, not just body weight, and the fat distributed particularly in the abdominal visceral compartment, a source of bioactive mediators that directly contribute to metabolic

disorders.⁵ Intra-abdominal visceral adipose tissue carries a greater risk for the development of metabolic disorders than subcutaneous adipose tissue does. Therefore, body composition and body fat distribution are closely associated with metabolic state.

1.2. Sex differences in the development of obesity and metabolic diseases

There are important sex differences in the prevalence of obesity and metabolic diseases. The prevalence of obesity and its related metabolic diseases is particularly high among men and middle-aged women. Prior to menopause, women have much less obesity-related metabolic disorders than obese men. For example, hypercholesterolemia is more common in males, whose lipid profile is characterized by lower HDL, higher LDL and triglyceride levels, and larger VLDL particles. The incidence of liver steatosis is also higher in men.⁶ After menopause, however, women are at an increased risk of developing visceral obesity and metabolic syndrome⁷ due to the loss of - endogenous ovarian hormone production.

Risk for metabolic syndrome incidence generally relates more to the central distribution of fat with abdominal, omental, and visceral adiposity than to the total amount of fat, with individuals with large amounts of abdominal visceral fat having particularly high risk.^{8,9} Adipose tissue accumulation is sexually dimorphic.¹⁰ The amount and regional distribution of body fat is different between males and females, as males and females differ in terms of how and where they store body fat. Premenopausal women usually have a higher amount of body fat and more subcutaneous gluteal/femoral pattern of fat distribution, whereas men have a leaner body composition and have more fat is stored in the central intra-abdominal visceral depot.^{11,12} Because visceral fat is more likely to cause metabolic syndromes, incidence of obesity-related metabolic disorders is much lower in premenopausal women than men and women experiencing menopause.

1.3. Sex steroid estrogens play key roles in energy homeostasis and glucose regulation

1.3.1 Estrogens regulate energy homeostasis and body fat—The epidemiological and clinical evidence strongly suggests a major role for estrogens in the regulation of body weight and body fat. It is now evident that estrogens account for sexual dimorphism in body fat distribution and accretion. The differences in the fat distribution between premenopausal women and age-matched men are abolished between postmenopausal women and age-matched men.¹³ Estrogens exert important antiobesity effects in females. Postmenopausal women with decreased levels of estrogens are associated with an increased risk for developing obesity.¹⁴ Additionally, estrogens promote the accumulation of subcutaneous fat¹⁵ while visceral fat varies inversely with levels of estrogens.¹⁶ Consequently, the loss of endogenous estrogen production with menopause is associated with an increased risk to develop visceral obesity and symptoms of the metabolic syndrome.⁹ The increased visceral obesity in menopause women can be altered by exogenous hormone replacement therapy.^{17,18}

Studies using experimental animal models have provided important insights into the pathogenesis of human diseases and new therapeutic approaches. While metabolic diseases and obesity have been shown in a number of animal species, in the laboratory they are most commonly investigated in two species of rodents, rats (*Rattus norvegicus*) and mice (*Mus musculus*/*Mus domesticus*).

Reductions in circulating estrogens and estrogen signaling, which occurs following removal of ovaries (i.e., ovariectomy), transiently increase caloric intake, and result in increased adiposity,¹⁹ specifically visceral fat with no change of subcutaneous fat.²⁰ Interestingly, the transient hyperphagia induced by ovariectomy does not account for the development of obesity,²¹ as ovariectomized rats gain weight to a similar extent even when they are pair-fed

to 17 β -estradiol (E2)-treated rats,²² suggesting that endogenous estrogens regulate body weight homeostasis primarily by modulating energy expenditure.

E2 administration decreases energy intake and increases energy expenditure,²³ indicating that exogenous estrogens may promote a negative energy balance by influencing both energy intake and energy expenditure. The ovariectomy-induced increase in adiposity can be ameliorated by exogenous E2 administration.²⁰ Furthermore, peripheral or central administration of E2 to ovariectomized rats changes their body fat distribution to mirror that of intact females. Additionally, altering the sex hormone milieu in males with E2 administration increases subcutaneous fat deposition.²⁰ An important implication from these findings is that estrogens are critical determinates of body fat distribution and energy balance.

1.3.2 Estrogens regulate lipid metabolism—Epidemiological and prospective studies associate estrogens to several aspects of the metabolic syndrome (Fig. 6.1). There is increasing evidence both in humans and laboratory rodents suggesting that estrogens are not only involved in regulating adiposity and body fat distribution, but also directly involved in regulation of glucose and lipid metabolism. Thus, estrogen action seems to be implicated in adipose tissue regulation and prevention of obesity.

Estrogens suppress lipogenesis in adipose tissue, skeletal muscle, and liver.^{24,25} In order to understand the role of estrogens in liver, studies evaluated the consequences of hormone replacement therapy to postmenopausal women and the effects of estrogen-suppression therapy in breast cancer patients. Estrogens increase HDL and triglycerides, decrease LDL and total cholesterol, and decrease fasting insulin and glucose levels, suggesting beneficial effects of estrogen on lipid and glucose metabolism.²⁶ Recent studies demonstrate that the use of antiestrogen therapy, such as tamoxifen and ovariectomy, leads to abnormal lipid profile and liver steatosis.²⁷ Furthermore, studies reported that islet activation of estrogen receptors (ERs), estrogen receptor alpha (ER α), estrogen receptor beta (ER β), and G protein-coupled ER (GPER) by estrogens suppresses fatty acid synthase expression and enzymatic activity.²⁸ This effect reduces islet lipid accumulation in rodent and human β -cells and prevents β -cell failure in rodent models of type 2 diabetes.

Another essential argument for estrogens' antilipogenic roles on lipid metabolism comes from the study of men with estrogen deficiency. In both sexes, estrogens are biosynthesized by the cytochrome P450 enzyme complex called aromatase.²⁹ In men, estrogen secretion comes principally from the conversion of androgens in adipose tissue by aromatase. The aromatase-deficient patients, who cannot synthesize estrogens thus lack endogenous estrogen secretion secondary to missense mutations in the aromatase gene, show impaired liver functions, hepatic steatosis, and altered lipid profile,³⁰ as well as develop hypertriglyceridemia and insulin resistance.^{31,32} These metabolic abnormalities are improved or corrected by estrogen replacement therapy.^{30,32,33} In the E2-deficient aromatase knockout (ArKO) mice, insulin resistance develops due to hepatic steatosis.³⁴

1.4. Focus of this chapter

In both humans and rodent models, estrogens are important sex hormones central to health in both genders that have protective effects in many physiological processes in mammals, including but not limited to reproduction, cardiovascular health, metabolic systems, bone integrity, cognition, and behavior.³⁵ Given these widespread roles for estrogens, it is not surprising that estrogens are implicated in the development or progression of numerous diseases, which include but are not limited to obesity, insulin resistance, osteoporosis, cardiovascular disease, various types of cancer of reproductive tissues, and neurodegenerative diseases. Pertinent to this chapter is estrogens' regulation in energy

homeostasis, glucose and lipid metabolism, and insulin sensitivity (Fig. 6.1). Estrogens are protective against the development of visceral obesity and glucose intolerance, and thus contribute to the maintenance of insulin sensitivity. Deficiency in estrogen leads to development of visceral obesity and insulin resistance.

We discuss in this chapter the mechanisms of action by estrogens in metabolic processes, focusing on the central nervous system (CNS) and adipose tissues. We first introduce the estrogenic system, including the estrogens and their receptors. We then discuss the current knowledge and questions about estrogenic action in the brain and adipose tissue, and how these processes are involved in the regulation of energy homeostasis and lipid metabolism. We review studies that have investigated the roles of estrogens and their receptors in the regulation of such metabolism, focusing on GPER-dependent signaling and function. Controversies that have complicated our understanding of GPER, including interactions with nuclear and transmembrane ERs, will also be discussed.

2. ESTROGEN SIGNALING: ESTROGENS AND ERs

2.1. Estrogenic action through genomic and nongenomic signaling mechanisms

Estrogens are the best-characterized member of the family of steroid hormones that includes progesterone, testosterone, glucocorticoids, and mineralocorticoids. Human estrogens comprise a group of structurally related steroid molecules, namely 17 β -estradiol (E2), estrone, and estriol, which are the most important regulators of the female and male reproductive systems. Estrogens are synthesized from cholesterol by aromatase via a series of chemical reactions that are part of the steroidogenic pathways predominantly in the ovaries. Estrogens are important hormones in mammalian physiology exerting important functions on the regulation of female secondary sexual characteristics. In males, relatively small amount of estrogens are produced by Leydig and germ cells of the testis. Additionally, estrogens are synthesized at multiple discrete sites throughout the body from the liver, adrenal glands, and adipocytes,³⁶ where they have highly localized effects on various unrelated processes in many tissues.³⁷ Estrogens also interact with and exert regulatory effects in a variety of tissues in the body that do not secrete estrogen, including the CNS, cardiovascular system, immune system, breast, uterus, and bone.^{38–42}

Estrogenic actions are mediated by a number of different ERs (Fig. 6.2). The highly hydrophobic nature of estrogens allows them to pass through cellular membranes by passive diffusion⁴³ and also allows its concentration in cellular membranes.⁴⁴ Cellular responses to estrogens are mediated by receptors that initiate a complex array of cellular events upon ligand binding. These responses are divided into two broad categories. One is genomic responses that are characterized by changes in gene transcription and occur on the time frame of hours to days after estrogens bind to nuclear ERs, ER α and ER β . The other one is nongenomic rapid signaling events that occur within seconds to minutes of cell stimulation after estrogens bind to novel GPER⁴⁵ which is localized to the endoplasmic reticulum, membrane subpopulation ERs (mER α/β), or G protein-coupled receptor membrane ERs (Gq-mER), and mediates nongenomic estrogen signaling via cytosolic pathways involving second messengers.^{45,46} Thus, an organism's responses to estrogens are generally the result of a complex interaction between genomic and nongenomic signaling (Fig. 6.2). The genomic mechanisms are relatively well characterized, while the nongenomic estrogenic signaling is less well understood and is beginning to be explored.

2.2. Estrogenic genomic actions via nuclear ERs

Estrogens activate their nuclear ERs in target cells, acting as transcription factors to regulate the expression of target genes, ultimately controlling cell growth, differentiation, and homeostasis.⁴⁷ Two nuclear ERs located on distinct chromosomes have been identified.^{48–51}

The first described classical ER, today known as ER α , was discovered and characterized in 1973, on the basis of specific binding activity in rat uterus/vagina extracts.⁵² The classical nuclear ER was cloned in 1985,⁵¹ its DNA sequence was determined in 1986,⁵⁰ and the first crystal structure of an ER ligand-binding domain was described in 1997.⁵³ ER was renamed ER α when a second nuclear ER, ER β , was identified 10 years later.⁴⁹

The major physiological estrogen is E2 that has a similar affinity for both ERs. Pharmacological reagents have been developed that permit the investigation of the specific role of ERs. ERs are activated by a range of ligands, including selective ER modulators (SERM) such as raloxifene and tamoxifen; the ER α -selective agonist 4,4',4''-(4-propyl-[1H]-pyrazole-1,3,5-triyl) trisphenol (PPT), which has 410 times greater affinity for ER α than ER β ⁵⁴; and two commonly used ER β agonists, 2,3-bis(4-Hydroxyphenyl)-propionitrile (DPN) and 7-Bromo-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol (WAY200070), which show similar receptor specificity and neither is as selective for ER β as PPT is for ER α . DPN is 70-fold more selective for ER β than ER α ,⁵⁵ while the affinity of WAY200070 (compound 92) for ER β in mice is 68 times that for ER α .⁵⁶

Estrogens' genomic action on cell functions is via the activation of ER α and ER β , both of which belong to the nuclear hormone receptor family and are transcription factors. They have classically been described as ligand-activated transcription factors mediating long-term genomic effects in central and peripheral estrogen-regulated tissues. ER α and ER β exist primarily in the nucleus, where they are complexed with chaperones. Briefly, binding of estrogens to ER α and ER β leads to conformational changes, chaperone dissociation from their inhibitory protein complexes, and dimerization of the receptor, followed by receptor translocation to the nucleus to recognize and binding by receptor dimers to *cis*-acting estrogen response elements (EREs) on the promoters of hormonally regulated genes, where the recruitment of coactivators and/or corepressors leads to transcription of important estrogen responsive genes and alterations in the rate of gene expression.^{57–59} Estrogen also modulates gene expression by a non-ERE-mediated mechanism in which ER α and ER β dimers bind to other non-ERE promoter sites and interact with other transcription factors through protein–protein interactions, such as activating protein-1 and stimulating protein-1, Fos-Jun, and a number of coactivators to regulate estrogenic genomic actions, a process referred to as transcription factor cross talk. Furthermore, the ERs have some ligand-independent transcriptional responses and can also interact with other transcription factors in regulating gene expression.⁶⁰

Although ER α and ER β are highly homologous in their DNA- and ligand-binding domains, ER α and ER β lack homology in their transcriptional activation domains and their splice variants differ in their affinities for E2.^{60,61} Additionally, ER α and ER β differ in the tissue distribution.⁶² ER α is mainly expressed in reproductive tissues, kidney, bone, white adipose tissue, and liver, whereas ER β is expressed in the ovary, prostate, lung, gastrointestinal tract, bladder, hematopoietic cells, and the CNS.⁶³ These differences suggest that functional differences exist between the two subtypes ER α and ER β , which is supported by characterization of ER α and ER β KO mice.⁶⁴

Male and female ER α KO mice are diabetogenic and obese with severe hepatic insulin resistance induced by targeted deletion in the ER α gene.⁶⁵ Thus, ER α is believed to mediate most estrogenic effects on energy homeostasis and glucose regulation. The cause of obesity in ER α KO mice is unclear; however, overeating does not appear to be essential,^{65–68} and the increased adiposity in ER α KO mice is attributed primarily to decreased energy expenditure.^{65,67} While E2 replacement prevents ovariectomized wild type mice from developing hyperphagia and obesity, such protective effects are blocked in ovariectomized ER α KO mice.⁶⁶ Therefore, although no hyperphagia is observed in ER α KO mice,^{65–67}

ER α is clearly required to mediate normal satiation process because E2-induced hypophagia and CCK-induced satiation in wild type mice are blocked in ER α KO mice.⁶⁶

The role of ER β in the central regulation of feeding is less known. In ovariectomized rats, there is increased food intake, body weight, and abdominal fat accumulation, which can be reversed by E2. However, intracerebroventricular administration of E2 together with ER β antisense oligodeoxynucleotides abrogates the anorexic effects of E2. Ovariectomy of ER β KO mice leads to normalized homeostasis of circulating glucose and insulin levels and reverses the obese phenotype, suggesting that ER β activity may result in a diabetogenic and adipogenic phenotype, which is opposite to the effects of ER α . Additionally, ER β KO mice display improved insulin sensitivity and glucose tolerance without increased body fat content, suggesting that ER β also plays an important role in maintaining metabolic control.⁶⁹ These studies suggest opposite effects of E2 via ER α and ER β .⁷⁰

2.3. Estrogenic nongenomic actions via GPER

Estrogens are best known for their long-term transcriptional effects involving the genomic mechanism of action that typically takes several hours for the effect to be manifested due to the time needed for transcription and translation of estrogen-regulated genes.⁷¹ However, a growing body of evidence has emerged to describe rapid estrogen signal transduction events in various cellular systems,^{72,73} which occur within minutes and thus cannot be attributed to a genomic mechanism of action. These rapid effects have thus been designated as nongenomic effects of estrogen.^{38,74–78} The earliest reports for nongenomic effects of estrogens date back to 1967.⁷⁹ In 1977, Pietras and Szego discovered that estrogens bind to a cell surface receptor,⁸⁰ introducing the possibility that estrogenic signaling may involve nongenomic nontranscriptional mechanisms or indirect actions to influence gene expression.

Membrane-bound ERs have been described recently. GPER, previously known as G protein-coupled receptor 30 (GPR30), has been identified as an estrogen-binding intracellular membrane receptor.^{45,81–84} GPER expression is required for rapid cellular responses to E2.^{85,86} In addition, another estradiol-responsive, G protein-coupled membrane ER has been described (Gq-mER).⁸⁷ Even though its distribution is currently unknown, Gq-mER appears to be involved in maintaining homeostatic functions by reducing food intake and fat accumulation, as well as other functions such as increasing bone density.^{88–90} Finally, in 2002, the existence of an additional mER, named ER-X, was hypothesized.⁹¹ However, its gene sequence and amino acid structure are currently unknown.

It is noteworthy that membrane ERs have been the focus of a surge of studies over the past decade.⁹² A search in PubMed in May 2012 with the keywords “estrogen and nongenomic” yields 576 published papers since 1984, with 480 (83%) of these papers being published after 2000. This area has seen an explosion of interest in the past decade, and represents one of the fastest emerging areas in the field of estrogen research.

2.3.1 GPER distribution and expression in tissues—GPER is a seven transmembrane-domain G protein-coupled receptor. GPER was first identified and cloned by Carmechi *et al.* in 1997.⁸¹ Using a differential cDNA library screening technique, the investigators identified GPER as a differentially expressed gene in a screen of genes from an ER-positive breast cancer cell line (MCF-7) versus an ER-negative breast cancer cell line (MDA-MB-361).

Antibodies have been raised against GPER, facilitating a detailed analysis of its tissue distribution. Expression of GPER protein is not restricted to traditionally estrogen responsive tissues. Indeed, characterization of GPER using immunohistochemical analysis revealed ubiquitous expression of the receptor in normal and malignant human tissues.

GPER expression is tissue and cell type specific, with high levels of expression found in numerous human organs, including male and female reproductive systems, heart, intestine, ovary, CNS, pancreatic islets, adipose tissue, neurons, and inflammatory cells. GPER is moderately positive at skeletal/cardiac muscle, but is negative at most smooth muscle.^{83,93–105} GPER is expressed in many regions of the brain with high expression noted in the hypothalamic–pituitary axis, hippocampus, brainstem autonomic nuclei, cortex, and striatum.¹⁰⁶ Several primary breast cancers⁸¹ and lymphomas¹⁰⁷ also expressed GPER transcripts, although many others were negative. There are discrepancies in the reported expression levels in some tissues, such as the rodent embryonic and fetal tissues, placenta, lung, and liver.^{81,107,108} Therefore a thorough analysis of the regulation of GPER expression remains to be completed.

GPER expression is developmentally regulated during the development of murine female reproductive tissues. For example, in the mammary gland, GPER expression in elongating ducts is low during puberty but rises once sexual maturity is reached. GPER expression in the mammary ductal epithelia exhibits estrous-cycle-dependent changes and differentiation-dependent changes during pregnancy, lactation, and involution. Similarly, GPER expression in both luminal and glandular uterine epithelia is regulated during the estrous cycle. Finally, Roy and colleagues recently demonstrated that the distribution and level of expression of GPER in the hamster ovary also varies with the estrous cycle; both granulosa and theca cell layers are rich in GPER, and expression is reduced at estrous.¹⁰⁹

Interestingly, sexual dimorphisms for GPER expression and/or function have been described in the brain¹¹⁰ and in the pancreatic islets, where it is expressed at a much higher level in women than in men.⁹⁵ Accordingly, a role for GPER in the regulation of obesity-associated metabolic functions has been proposed.

These studies have provided a framework in which to interpret many of the reported functions of GPER, and suggest that GPER has been implicated to be involved in estrogen-dependent physiology of immune function and cardiovascular systems.^{47,93} For example, GPER is associated with diseases such as obesity, insulin resistance, and hormone-sensitive cancers,^{93,98,99} and GPER plays a role in estrogen-sensitive tumor growth in the absence of ER α / β .^{81,111}

2.3.2 GPER location in cells—GPER is expected to localize in the plasma membrane and its plasma membrane localization is reported by Thomas *et al.*⁸⁴ However, other groups reported GPER locations in the membrane of intracellular compartments. Revankar *et al.* used novel fluorescent estrogen derivatives (E2-Alexas) to directly visualize the cellular and subcellular binding properties of GPER. Confocal microscopy revealed that, whereas E2-Alexas detected ER α and ER β in the nucleus of cells, E2-Alexa binding to GPER occurred predominantly in the endoplasmic reticulum, with no detectable binding at the plasma membrane.⁴⁵ In addition, binding of the fluorescent E2-Alexas was directly proportional to expression of endogenous GPER on a cell-by-cell basis, strongly supporting the idea that expression of GPER protein directly generates estrogen-binding sites. In all cell lines where GPER expression has been observed, the E2-Alexa or antibody staining is colocalized in the endoplasmic reticulum, with staining also present in the Golgi apparatus and nuclear membrane. These results, although they are in contrast to the expected localization of GPER in the plasma membrane, clearly demonstrate that GPER is localized in the membrane of intracellular compartments, such as the endoplasmic reticulum and the Golgi complex.^{45,81,83–85,93}

2.3.3 GPER intracellular signaling—GPER binds E2 with high affinity to mediate subsequent nongenomic and rapid estrogen signaling via multiple pathways (Fig.

6.2).^{45,83–85} As a G protein-coupled receptor, GPER couples to G proteins and modulates second messenger pathways. Signaling mechanisms of this action include rapid increases in Ca^{2+} ,¹¹² cAMP^{79,113} and phosphoinositide-3 kinase (PI3K)/Akt and mitogen-activated protein kinase (MAPK) activation⁴⁷ (Fig. 6.2). cAMP and Ca^{2+} have emerged as the second messengers most frequently associated with GPER signaling. GPER is involved in the activation of downstream signaling cascades such as protein kinase A (PKA), protein kinase C, and MAPK via membrane-localized ERs⁸² and intracellular Ca^{2+} regulation.¹⁰⁶ The GPER couples to both a $\text{G}_{\alpha s}$ protein yielding stimulation of cyclic AMP (cAMP) production and a pertussis toxin (PTX)-sensitive $\text{G}_{\alpha i/o}$ protein in part yielding attenuation of cAMP production. Ding *et al.*¹¹⁴ have reported that E2-promoted vascular smooth muscle cell apoptosis is dependent on GPER-mediated PKA inhibition and PI3K activation, both of which are PTX sensitive. Furthermore, Maggiolini *et al.*⁸⁶ demonstrate GPER-mediated and PTX-sensitive c-fos upregulation in SkBr3 breast cancer cells, a cell line that has been shown to have GPER-dependent stimulation of cAMP production.⁸²

The rapid effects from stimulation of GPER also include release of intracellular Ca^{2+} . This signal has been described as phospholipase C-independent,⁴⁵ PTX-sensitive,^{45,105} and dependent on epidermal growth factor receptor (EGFR) activation.⁴⁵ Increased cytosolic Ca^{2+} subsequently activates Ca^{2+} -calmodulin-dependent kinases or activation of MAPK and PI3K pathways.

Filardo *et al.* demonstrated that in MCF-7 cells, which express $\text{ER}\alpha$, $\text{ER}\beta$, and GPER, estrogen mediated a rapid increase in MAPK phosphorylation but that in MDA-MB-231 breast cancer cells, which express $\text{ER}\beta$ but not $\text{ER}\alpha$ or GPER, this response was absent, suggesting a possible role for $\text{ER}\alpha$ or GPER. When MDA-MB-231 cells were transfected to express GPER, E2 stimulated both cAMP production and PTX-sensitive MAPK activation in the cells.⁸⁵ Additionally, they identified that GPER is involved in the estrogen-mediated activation of MAPK^{82,85,115} and PI3K⁴⁵ in SKBr3 breast cancer-derived cells that express only GPER but lack classical nuclear $\text{ER}\alpha$ and $\text{ER}\beta$. Together, these results strongly implicated GPER is essential in estrogen-mediated cellular responses.

Stimulation of GPER also leads to PI3K and PTX-sensitive Src activation, which in turn yields phosphorylation of Akt and SHC, respectively. While Akt phosphorylation has been linked to antiapoptotic and cardioprotective effects,¹¹⁶ SHC phosphorylation triggers cleavage of membrane-tethered heparin-bound EGF, presumably through activation of a metalloproteinase, resulting in transactivation of the EGF receptor.⁸⁵ However, there is a debate on whether the PI3K/Akt pathway lies downstream of $\text{G}_{\alpha i/o}$ or not. While several groups have shown that the GPER-coupled Src/SHC/EGF/MAPK pathway is PTX sensitive,^{85,114} and thus dependent on $\text{G}_{\alpha i/o}$ signaling, Revankar *et al.*⁴⁵ reported that PI3K/Akt activation is downstream of the Src/SHC/EGF/MAPK pathway but insensitive to PTX.

2.3.4 Pharmacologic tools—GPER agonists and antagonists—Elucidation of the biological roles of GPER has been facilitated by the identification of specific pharmacological reagents. GPER agonists, including G-1, ($\pm$)-1-[(3aR*, 4S*, 9bS*)-4-(6-Bromo-1,3-benzodioxol-5-yl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinolin-8-yl]-ethanone,¹¹⁷ and 5408–0877, which behaved similarly to G-1,¹¹⁸ and an antagonist G-15, (3aS*)-4R*, 9bR*-(6-Bromo-1,3-benzodioxol-5-yl)-3a,4,5,9b-3H-cyclopenta[c] quinoline,¹¹⁹ exhibit high affinity and high selectivity for GPER over classical $\text{ER}\alpha$ and $\text{ER}\beta$, with none of them showing affinity for $\text{ER}\alpha$ or $\text{ER}\beta$.^{117,119} These tools have revealed that GPER activation may have several beneficial effects in the metabolic system including stimulation of insulin release and protection against pancreatic β -cell apoptosis. Thus, GPER is emerging as a candidate therapeutic target in metabolic disease.

To demonstrate the selectivity of G-1 toward GPER as compared with classical ERs, Ca^{2+} mobilization in COS7 cells expressing either ER α or ER β revealed no response to G-1 compared with estrogen, which yielded a rapid response with both ER α and ER β . G-1 also activates transfected and endogenous GPER, resulting in PI3K activation. With the identification of the first GPER-selective agent, it is now possible to investigate the specific functions of GPER compared with those of ERs in complex systems expressing both receptor types.

The pharmacological agents are currently being widely employed to investigate GPER function and physiology in various systems.^{117,119} GPER agonists including G-1 and 5408-0877 and antagonist G-15 remain to be fully evaluated for unequivocal specificity for this receptor.

GPER can also be activated by tamoxifen and raloxifene, SERMs^{45,55,120,121} traditionally thought only to modulate the function of ER α and ER β .^{45,55,85} These studies provide experimental evidence that support the concept that GPER activation has inhibitory effects on food intake, body weight, and fat mass.^{122,123}

2.3.5 Genetic tools—GPER knockout mice—The availability of genetic tools (i.e., different mouse models lacking the GPER gene) and pharmacological agents (i.e., GPER-selective agonists and antagonists) has advanced our understanding on GPER. Four mouse models lacking the GPER gene (GPER knockout, GPER KO) have been used to study the pathophysiological role of this receptor.^{98,124–126}

GPER KO mice are found to associate with increased visceral adiposity^{100,127} whereas Mårtensson *et al.*⁹⁸ using a different GPER KO strategy found changes in body weight that were limited to GPER KO females. The same investigators found no effect of GPER deficiency on the antiobesity effects of E2.¹²⁸ By contrast, Isensee *et al.* found no effect of GPER deficiency on body weight in animals on either a normal or high-fat diet in another model, LacZ-GPER reporter mice, which represent a partial GPER deletion.^{125,129,130} Data obtained from different GPER KO models generated by different transgenic techniques do not show a homogenous phenotype under physiological conditions.¹²⁶ One group was unable to demonstrate an E2-dependent phenotype in GPER KO mice indicating that GPER might not be relevant for estrogen signaling,^{130,131} whereas other groups did.^{98,101,124} Such discrepancy would not exclude the possibility that E2-mediated effects might involve overlapping signaling of various ERs, and might be due to cross talk effects between GPER and other ERs¹³² as described below.

2.4. Cross talk between GPER and other ERs

2.4.1 Cross talk between membrane subpopulation ERs (mER α/β) and GPER

—Rapid estrogen effects are mediated by ERs localized to the plasma membrane, comprising subpopulations of the classical ERs, namely membrane subpopulations of ER α and ER β (mER α/β).^{47,57,133} Activation of mER α/β induces a variety of intracellular signaling cascades and mediates rapid estrogenic actions that involve cell-signaling mechanisms, mostly the phosphorylation of various kinases^{134,135} and the regulation of intracellular Ca^{2+} levels.^{136,137}

An mER α/β selective ligand, STX, [2-(4-hydroxyphenyl)-3-phenylpent-2-enoic acid [4-(2-dimethyl amino ethoxy)phenyl] amide, E-enantiomer], has been developed and used in studies on homeostasis.¹³⁸ Most behavioral investigations with this drug have utilized systemic administrations.^{139,140} Less commonly, direct brain infusions have attempted to identify its specific site of action.¹⁴¹

Membrane-associated full-length ER α and two truncated isoforms, ER α 46 kDa and ER α 36 kDa, exist that are at least in part responsible for nongenomic estrogenic signaling.^{142–144} Wang and colleagues have identified ER α 36 as a novel splice variant of the human ER.¹⁴⁵ In breast cancer cell lines lacking ER α , expression of ER α 36 is associated with improved survival.¹⁴⁶ It has been recently proposed that ER α 36 is the main effector and mediator of GPER-dependent effects mediated by estradiol and that its induction is dependent on GPER.¹⁴⁷ However, others found that regulation of ER α 36 or ER α 36 is not detectable despite effects of G-1 being observed.¹⁴⁸ A recent report demonstrating protective effects of G-1 in hypertensive rats in the kidney did not find any expression of ER α 36 while ER α 46 and ER α 66 were abundantly expressed.¹⁴⁸ At present, data—particularly *in vivo* data—are still scarce. A recent report found effects of GPER agonists tamoxifen and ICI 182,780 are dependent on ER α 36 in cancer cells.¹⁴⁹ Levin has proposed that GPER might act as “collaborator” of ER α —which it might be under certain conditions, as cross talk between both receptors has been described.^{150,151} Recently, it has been suggested that GPER signaling requires and might even solely occur through ER α 36.¹⁴³

Different experimental settings from *in vitro* observations, immortalized cells vs. experimental animals, cancer cell vs. normal kidney, may be applicable to certain but not all conditions. In addition, results from *in vitro* findings cannot be extrapolated to the *in vivo* functions of receptors and thus are only of limited relevance. Another aspect to consider is that, unlike GPER, rapid ER α 36-dependent signaling is also activated by estrone, estradiol, estriol, and estetrol, and that E2-induced MAPK activation is further potentiated by GPER agonists such as ICI or tamoxifen.¹⁴³ By contrast, the ER α 36 agonist estriol is an antagonist for GPER.¹⁵² Importantly, *in vivo* evidence for a role of ER α 36 in mediating the E2-mediated effects of GPER under physiological or disease conditions is still lacking.

2.4.2 Cross talk between EGFRs and GPER—Cellular signaling by GPER activates EGFRs via a G protein-dependent pathway, which involves Src and metalloproteinase (MMP) cleavage of proheparin-binding epidermal growth factor-like growth factor. Filardo and colleagues described GPER-mediated elevation of cAMP by estrogen as a mechanism to restore EGFR-activated MAPK to basal levels through PKA-dependent inhibition of Raf-1 activity.⁸² Two questions arose from this observation: (a) Do different ligands specifically activate the two receptors? (b) Do the two receptors utilize identical signaling pathways? To answer the first question, the same cells are treated with 4-hydroxytamoxifen, a well-described ER partial antagonist previously shown to activate MAPK via GPER. Stimulation with 4-hydroxytamoxifen resulted in no activation of PI3K in ER α -expressing cells but did result in PI3K activation in GPER-expressing cells, demonstrating for the first time in a side-by-side comparison the differential effects of tamoxifen on ER α and GPER. To answer the second question, the EGFR inhibitor AG1478 is employed. Two groups have shown independently that both ER α and GPER mediated EGFR transactivation.^{85,153} When EGFR phosphorylation is blocked, GPER-, but not ER α -mediated PI3K activation is inhibited. These studies demonstrated the close link in cellular signaling between GPER and EGFR.

2.4.3 Cross talk between classical ER and GPER—There appears to be complex interplay between ER α and GPER although such interplay has not yet been fully defined.^{93,150,154} Classical ERs may couple to G protein signaling via an unknown mechanism to mediate rapid estrogen signaling. The coexpression of ER α and ER β with GPER in breast cancer cells has suggested a possible role for interactions between these two receptors.^{132,155,156}

Indeed, functional cross talk has been demonstrated in cancer cells and uterine epithelial cells,¹⁵⁰ where GPER functions as inhibitor of ER α -dependent, estradiol-mediated cell growth.¹⁵¹ Functional cross talk between ERs is also evident from isolated vascular

preparations, where the E2-induced acute vasodilator response is only seen with ER α selective agonists but completely abrogated when ER α , ER β , and GPER are activated simultaneously by E2.¹⁵⁷ Navarro *et al.* demonstrated by coimmunoprecipitation studies that ER α interacts with G α i3 in immortalized GnRH neurons, and that estrogen-induced inhibition of cAMP can be blocked by pertussis toxin, a G protein signaling inhibitor.¹⁵⁸ Additionally, Kelly and coworkers^{87,159} have provided evidence of a G α q-coupled ER that regulates the PKC and PKA signaling pathway in hypothalamic neurons and is involved in hypothalamic control of energy homeostasis.

Mitochondrial protection in response to E2 unrelated to GPER¹⁶⁰ has been described for ER β .¹⁶¹ E2 effect on insulin is absent in GPER KO mice,^{28,98,101} and protective effects on pancreatic islets are comparable independent of whether selective activation of ER α , ER β , or GPER occurs.²⁸ Thus, possible cross talk between GPER and ERs (also possibly other steroid receptors) as well as redundancy of ER function must be considered when assessing possible pathogenic and therapeutic questions related to GPER activation or inhibition.¹³² This should also be considered when determining the specific activity of compounds or drugs targeting either one^{117,119} or several membrane ERs.¹⁶²

3. ESTROGENIC ACTION IN THE BRAIN: CENTRAL REGULATION OF ENERGY HOMEOSTASIS

3.1. Estrogenic action in the CNS

3.1.1 Estrogens directly act on hypothalamic areas involved in energy balance

—A series of complex systems regulate energy homeostasis in order to keep energy storage levels and body weight stable. Central brain circuits receive peripheral signals indicating satiety, energy levels, and energy stores. The brain is made aware of the metabolic state of the body through signals released from adipose tissue, skeletal muscle, liver, pancreas, and gut. There is a wide variety of these signals, including hormones (insulin, leptin, gut hormones), cytokines (IL-6, TNF- α), and nutrients (glucose, free fatty acids, lipids). The hypothalamus is a key regulator of food intake and maintenance of energy homeostasis.¹⁶³ The hypothalamus processes afferent signals from the gut and brain stem and efferent signals that modulate food intake and energy expenditure. Anorexigenic and orexigenic signals from the CNS then induce behavioral and metabolic changes targeting energy homeostasis and survival.

The main hypothalamic areas involved in food intake and satiety are the arcuate nucleus (ARC), the lateral hypothalamus (LH), the paraventricular nucleus (PVN), and the ventromedial hypothalamus (VMH).¹³⁴ Several melanocortin receptors composing the melanocortin system are expressed throughout these nuclei and are one of the main regulators of hunger and satiety.¹⁶⁴

Estrogens are major effectors for the regulation of energy balance and body fat. Energy homeostasis and feeding behavior in the hypothalamus follows the menstrual cycle, and food intake in women varies across the cycle with lowest daily food intake during the periovulatory period when estrogen levels are maximum.¹⁶⁵ In several mammals and in women, higher levels of E2 during the estrus of menstrual cycles and pregnancy lead to decreased food intake and fat accumulation. In addition to its central actions, E2 also regulates peripheral feedback hormones that influence feeding and meal size through their actions in the CNS.

In contrast, ovariectomy, antiestrogen treatment, and menopause lead to an increase in food consumption and meal size, a decrease in running wheel activities, and increased fat mass, which can be reversed by E2 replacement.¹⁶⁶ The importance of the brain for the observed

phenotypes has been demonstrated by studies showing that direct intracranial injections of E2 into the PVN leads to anorexia, reduced food intake, and body weight and increased running activities.^{167,168} These observations indicate that E2 has direct anorexigenic action in the hypothalamus.¹³⁴

3.1.2 Estrogens act on central melanocortin system—Estrogens act on central melanocortin system to regulate energy balance and body fat distribution. The melanocortin system is responsible for the opposing actions of these neurons; however, so far a clear relation between E2, the melanocortin system, and energy balance has not been established.^{169,170} In the ARC, several types of neurons and receptors of the melanocortin system (MC3R and MC4R) are involved in the regulation of appetite and respond to variations in glucose, lipids, leptin, and insulin. The proopiomelanocortin (POMC) neurons have an anorexigenic action and, when activated, reduce food intake through the release of two peptides, α -melanocyte stimulating hormone (α -MSH) and cocaine-and-amphetamine-regulated transcripts. The neuropeptide Y (NPY) neurons, on the other hand, release NPY hormone and agouti gene-related protein (AgRP), which prevent the binding of α -MSH to MC3R and MC4R, increasing food intake.¹⁶⁴ Whole animal studies in ovariectomized rats have reported that a significant reduction in food intake by E2 treatment compared to oil treatment was not detected after administration of the melanocortin receptor antagonist SHU919.

A recent study demonstrated that NPY/AgRP neurons are required to mediate the anorexigenic effects of estrogens. In this study, Xu and colleagues showed that hypothalamic expression of NPY and AgRP in wild type mice is tightly regulated across the estrus cycle, with the lowest levels during the estrus, which coincides with the plasma estrogen peak and feeding nadir.¹⁷¹ They further showed that central E2 administration suppresses fasting-induced c-Fos activation in NPY/AgRP neurons and blunts the refeeding response.¹⁷¹ Importantly, the cyclic changes in food intake and E2-induced anorexia are blunted in mice with degenerated NPY/AgRP neurons. This study indicates that NPY/AgRP neurons are functionally required for the cyclic changes in feeding across estrous cycles. Surprisingly, these authors also found that ER α is not expressed in NPY/AgRP neurons,¹⁷¹ suggesting that estrogen may regulate these neurons indirectly via presynaptic neurons that express ER α (e.g., POMC neurons). Indeed, POMC neurons coexpress ER α .^{172–174} In addition, estrogens regulate excitability of POMC neurons. Using electron microscopy, Horvath and colleagues have reported that the number of excitatory synaptic inputs to ARC POMC neurons rises as mice enter proestrus when estrogen levels are high.²³

Further, central E2 administration rapidly increases the excitatory synapses on POMC neurons, an effect that is also reflected by increased miniature excitatory postsynaptic current recorded from POMC neurons.²³ These synaptological rearrangements in POMC neurons are tightly paralleled with the effects of E2 on food intake and body weight.²³ Similarly, Ronnekleiv and colleagues reported that E2 administration in hypothalamic slices activates POMC neurons by rapidly uncoupling GABAB receptors from the G protein—gated inwardly rectifying K⁺ channels.¹⁷⁵ Importantly, female mice lacking ER α in POMC neurons only develop hyperphagia.¹⁷² These observations, together with the findings from the Horvath and Ronnekleiv groups, indicate that estrogen signals in POMC neurons are physiologically relevant in the regulation of food intake.

3.1.3 Estrogens act on nuclear and membrane ER in the CNS—Classical ER α and ER β and GPER are all highly expressed in the brain, their distribution being both overlapping and unique.⁶¹ The critical intracellular signals that mediate the anorexigenic effects of estrogens have been explored. Stimulation of nuclear and membrane ERs engages

genomic and nongenomic events that may mediate distinct functions of estrogens, as detailed below.

3.2. Estrogen acts on nuclear ERs in the CNS

Genomic activation of ERs results from estrogens binding to EREs in promoter regions of target genes.^{176,177} ER α is ubiquitously expressed in rodent brains. Areas of predominant ER α expression are the VMH and ARC.^{178–181} ER β is found in the same hypothalamic nuclei as ER α , but ER β expression is significantly reduced relative to ER α . ER β predominates in the suprachiasmatic, supraoptic, and PVN of the hypothalamus, the cerebellum, dorsal raphe, hippocampus, and cerebral cortex.^{68,179,182–184} ER α and ER β expression overlaps in the ARC, LH, medial amygdala, preoptic area, bed nucleus of the stria terminalis, nucleus of the solitary tract and periaqueductal gray.

Pertinent to the estrogenic action on melanocortin system is that both ER α and ER β have been identified in the ARC. Specifically, ER α is predominantly expressed in the POMC neurons, while both ER α and ER β are present in the NPY neurons.^{134,173} The PVN and VMH are also mediators of energy ingestion and expenditure and are interconnected with the ARC. The PVN is highly responsive to glucose, lipids, NPY, and AgRP, and its activation leads to increased food intake.^{134,185} The PVN is the hypothalamic region with the highest expression of ER β and is weakly ER α positive.¹⁸² Despite this apparent low level of ER α , in rats and mice, the ER α -selective agonist, PPT, rapidly results in a decrease in food intake and increase in expression of c-fos in the corticotrophin-releasing hormone-expressing cells in the PVN.¹⁸⁶ The VMH is ER α regulated and has an important function in the inhibition of food intake through anorexigenic signals from the ARC. Silencing of ER α in the VMH by RNA interference leads to hyperphagia, obesity, decreased glucose tolerance, and reduced energy expenditure.¹⁸⁷

The role of estrogens and ER α in the VMH in the regulation of energy balance has been reexamined using the ER α silencing approach.¹⁸⁷ In this study, ER α in the VMH is knocked down with an AAV-shRNA.¹⁸⁷ Animals with impaired ER α signaling in the VMH are less sensitive to E2-induced weight loss and develop obesity characteristic of increased visceral fat. The obesity syndrome is likely caused by decreased physical activity and impaired diet-induced thermogenesis, whereas food intake of these animals is not directly affected.

Xu *et al.* generated mice with ER α deleted in VMH SF1 neurons.¹⁷² They found that deletion of ER α in VMH SF1 neurons in female mice, while not affecting food intake, significantly reduces basal metabolic rate and diet-induced thermogenesis, which consequently results in increased body weight and adiposity. Interestingly, a significant increase in visceral fat deposition but not subcutaneous fat deposition is observed in these mutant females.¹⁷² Finally, they showed that the decreased energy expenditure and increased visceral fat distribution in mice lacking ER α in SF1 neurons presumably results from decreased sympathetic tone, as demonstrated by decreased plasma norepinephrine levels.¹⁷² These findings are largely consistent with those obtained from the VMH-specific ER α knockdown model. Collectively, these results support the hypothesis that ER α signaling in VMH neurons plays an important role in regulating energy expenditure and fat distribution.

To summarize, ER α and ER β are expressed in brain regions implicated in the regulation of appetite, satiety, and energy expenditure,^{49,68,178–180,183} which can explain the involvement of these receptors in changes of feeding behavior and energy expenditure and thus regulation of energy homeostasis. The differential distribution further suggests that, like for other

behaviors, the respective involvement of ER α and ER β in regulation of energy homeostasis may be different.

3.3. Estrogen acts on membrane ERs in the CNS

3.3.1 Identification of three membrane ERs in the CNS—Recent evidence suggests that estrogen has at least three different transmembrane receptors in the brain.^{115,188}

The first membrane ER identified and localized in the brain is the estrogen-binding G protein-coupled receptor GPER. Brain GPER expression in adult male and female rats was thoroughly characterized by Brailoiu *et al.* using immunohistochemical analysis.¹⁰⁶ GPER in the hypothalamus is localized primarily to the PVN and the supraoptic nucleus but is also found in ARC neurons.¹⁰⁶ GPER is also expressed in nonhypothalamic regions, including the anterior and posterior pituitary, striatum, hippocampus, and the autonomic nuclei of the brainstem including substantia nigra, area postrema, nucleus of the solitary tract (NTS), and dorsal motor nucleus of the vagus.¹⁰⁶ These hypothalamic and brainstem areas are typically associated with energy homeostasis function. GPER immunostaining exhibits a similar pattern in male and female mice,¹⁸⁹ suggesting that GPER expression in these autonomic areas is not regulated by estrogen status. In the NTS, GPER immunostaining is predominantly localized to neurons that coexpress ER α , whereas in the PVN, GPER displays moderate colocalization with nitric oxide synthase.¹⁹⁰ Consistent with this study, Sakamoto and coworkers have also shown expression of GPER mRNA and protein in the oxytocin neurons of the rat PVN and supraoptic nuclei.¹⁹¹

The second membrane ER is the putative G protein-coupled receptor membrane estrogen receptor (Gq-mER) functionally characterized by Kelly and coworkers.^{87,88} Although the Gq-mER has not been cloned, it has been functionally identified in at least three types of ARC neurons, including POMC, dopamine, and γ -aminobutyric acid (GABA) neurons.

The third membrane ER is membrane subpopulation of ER α and ER β has been identified. Ultrastructural electron microscopic studies have provided direct evidence of extranuclear localization of ERs in the brain.¹⁹² Milner *et al.*¹⁹² performed ultrastructural studies of ER α localization in the rat hippocampal formation and demonstrated that ER α , in addition to nuclear localization, is found in cytoplasm and plasma membrane of neurons, and in unmyelinated axons and axon terminals. Furthermore, approximately 25% of ER α is localized in dendritic spines, often associated with spine apparatus and/or polyribosomes, suggesting it may act to regulate Ca²⁺ availability, local protein translation, and synaptic growth. Using Western blot and estrogen-horseradish protein-FITC or estrogen-BSA-FITC conjugates, Marin *et al.* showed plasma membrane localization of ER α in SN56 septal-derived cholinergic neurons, and that the estrogen conjugates protected the cells against A-beta toxicity as effectively as estrogen.^{193,194} Xu *et al.*¹⁹⁵ transfected GFP-tagged human ER α in rat cortical neurons *in vitro* and determined its subcellular localization. The studies revealed that there was localization of the GFP-tagged human ER α in neurites of the cortical neurons and that the ER α appears to be directed to the neurites directly from its site of translation and not from nuclear stores. Furthermore, biotinylated membrane protein studies also support localization of ER α at the plasma membrane of rat cortical neurons. Finally, ultrastructural studies have also demonstrated that ER β is also localized at extranuclear sites such as cytoplasm, plasma membrane, dendritic spines, axons, and axon terminals in the rat hippocampus.¹⁹⁶ Thus, ER β could also have a role in mediating nongenomic actions of estrogen in the brain. We should also mention that a novel plasma membrane-associated ER called ER-X has been proposed as well in neurons of the brain, but attempts to clone it have so far been unsuccessful.⁹¹

3.3.2 Estrogenic nongenomic signaling in the CNS—Estrogens can initiate rapid signaling cascade events within minutes to affect cell physiology and activate gene transcription through membrane-initiated steroid signaling via nongenomic mechanisms at non-ERE transcription factor promoter sites. Instead, estrogens activate transcription via complexes with other transcription factors through protein–protein interactions, including CREB, STATs, Elk-1-SRF, ATF-2-Jun, and NFκB-inducing transcription via their respective promoter sites.¹⁹⁷

Reports of rapid E2 effects in the brain first began to appear in the literature over 40 years ago, when it was observed that estrogen rapidly modulated hypothalamic electrical activity.^{198–200} The latency of the estrogen effects was within minutes, and typically two types of effects were observed on hypothalamic neurons—some units had a decreased firing rate that lasted up to 150 min, whereas other units responded with a transitory increase in firing rates. The remaining units were unresponsive to E2.²⁰⁰ Kelly *et al.*^{201–204} showed that neurons from several hypothalamic regions displayed rapid electrophysiological responses to estrogen, including preoptic, ARC, and VMH neurons. These early observations set the stage for subsequent studies on the mechanisms underlying the rapid effects of estrogen in the brain. ERs in the plasma membrane are able to activate the MAPK cascade and PI3K pathway, causing a rise in intracellular Ca²⁺.^{205,206} ERs also activate PKB or Akt in neurons.^{207–209} The PI3K/Akt cascade mediates a variety of estrogens' central actions, including neuronal excitability, neuroprotection, reductions in inflammation, neurite outgrowth, and potentially body weight regulation.²¹⁰

The rapid effects of estrogen on Ca²⁺ and ion channel signaling and kinase activation suggest a potential membrane receptor-mediated effect of estrogen. One piece of evidence supporting a membrane receptor-mediated mechanism is the use of membrane impermeable conjugates of estrogen, such as estrogen-BSA and estrogen dendrimers. Similar to estrogen, estrogen-BSA was shown to rapidly enhance phosphorylation of MAPK in the human neuroblastoma SK-N-SHR cells,²¹¹ in rat hypothalamic neurons *in vitro*,²¹² and in the rat hippocampus *in vivo*.²¹³ Estrogen-BSA conjugates have also been shown to rapidly enhance PKA activation in hippocampal neurons,²¹⁴ increase intracellular Ca²⁺ levels within minutes in both astrocytes and neurons,^{215,216} and to rapidly modulate potassium channel activation in hypothalamic neurons.⁸⁷ Nevertheless, it should be pointed out that there has been some criticism of estrogen-BSA conjugates, in that some estrogen (3–5%) can be found in the free unconjugated form in commercially available estrogen-BSA conjugates.²¹⁷ For this reason, it is advisable that the estrogen-BSA be subjected to filtration in order to remove free estrogen, and that studies that fail to perform the filtration should be interpreted cautiously. Stevis *et al.*²¹⁷ reported that estrogen-BSA did not bind ERα or ERβ in binding studies; however, a subsequent study demonstrated that estrogen-BSA indeed binds to purified ERs *in vitro* and to membrane ERs in intact cells, albeit at a slower rate than estrogen.²¹⁸ Taken as a whole, the estrogen conjugate data suggest that rapid signaling effects of estrogen involve mediation via a membrane ER.

3.3.2.1 Rapid estrogen signaling in ER KO mice: One approach to determine the role of ERs in mediating rapid signaling effects of estrogen in the brain is to utilize mutant mice with one or both classical ERs knocked out. Using this approach, Chaban and Micevych demonstrated that rapid estrogen inhibition of ATP-induced Ca²⁺ signaling in dorsal root ganglion neurons is blocked by an ER antagonist, and that the effect of estrogen on Ca²⁺ signaling is lost in ERα KO mice.²¹⁹ Likewise, Herbison and colleagues demonstrated that rapid estrogen-induced phospho-CREB responses in hypothalamic neurons, where ERβ is densely localized, is preserved in ERα KO mice, but lost in ERβ KO mice.²²⁰

With regard to MAPK regulation, there are contradicting reports on the estrogen regulation of phospho-MAPK in ER KO mice. Singh *et al.*²²¹ reported that estrogen-induced phosphorylation of MAPK was not lost in cortical neurons from ER α knockout mice, suggesting that ER α may not mediate the rapid phospho-MAPK-enhancing effects of estrogen. In contrast, Abraham *et al.*²²⁰ reported that rapid estrogen induction of phosphorylation of MAPK in the medial preoptic nucleus was lost in both ER α and ER β knockout mice, suggesting that both ER α and ER β may mediate the MAPK regulatory effect of estrogen. Finally, estrogen can also rapidly induce c-Fos protein in the brain, an effect which has been shown to be lost in double ER α/β KO mice in several brain areas including the VMH.²²²

As a whole, results with the ER KO mice shed some light on involvement of ER α or ER β in rapid signaling effects of estrogen in the brain. Additionally, they suggest that the role of ER α or ER β in rapid estrogen signaling effects may differ from one brain region to another, and results in one region cannot be immediately extrapolated to another region or another kinase.

3.3.2.2 Rapid estrogen signaling in the brain via G protein-coupled receptors: G

protein-coupled receptors are vital membrane mediators for a host of central and peripheral signals that control energy homeostasis in the hypothalamus.²²³ Because G protein-coupled receptors target potassium channels to control neuronal excitability, they may account for one of the rapid mechanisms estrogen utilizes to control energy homeostasis. A candidate for an estrogenic mechanism recently identified is the control of neuronal excitability through a G protein-coupled receptor membrane ER (Gq-mER).^{87,88} The Gq-mER affects neuronal activity, alters metabolic processes such as body weight, and regulates energy balance.

The Gq-mER was initially characterized in female guinea pigs, but has also been functionally examined in both male and female ER α KO mice and ER β KO mice, and in male ER α/β double KO mice.⁸⁸ Estrogen through Gq-mER activates multiple signaling pathways including PI3K, phospholipase C (PLC), MAPK and PKA and PKC pathways.^{210,224,225} Briefly, the Gq-mER activates a Gq-PLC-PKA pathway that inhibits the activation of G protein-coupled inwardly rectifying potassium channels. The inhibition of the potassium channel activity will depolarize the POMC neuron and increase neuronal activity and potentially add another mechanism for estrogen to control energy homeostasis.

The Gq-mER involved in estrogenic membrane-initiated steroid signaling is thought to be ER α ,²²⁵ although recent data suggest that, in a transfected neuronal cell line, ER β is also associated with the membrane and is translocated to the membrane by estrogen to activate MAPK signaling.²²⁶ In the hypothalamus, estrogen membrane-initiated steroid signaling has been identified in the VMH to potentiate the effects of estrogenic nuclear-initiated steroid signaling during particular reproductive behaviors such as lordosis²²⁷ and may also affect estrogen's control of homeostasis via VMH neurons.

There is *in vivo* evidence suggesting a role for the activation of membrane-initiated estrogenic signaling events that affect energy homeostasis. E2 can attenuate food intake within 6 h of administration into the third ventricle via cannula after an overnight fast compared to saline in mice²²⁸ and between 4 and 14 h in fed rats.²³ Estrogen can activate signaling cascades initiated through Gq-mER that would rapidly alter neuronal activity and initiate changes in feeding behavior.^{87,88} Indeed, acute administration of estrogen to hypothalamic slices will alter neuronal excitability of many different types of relevant neurons through the modulation of various ion channels.^{229,230}

To elucidate this estrogen pathway, STX, a selective agonist for the Gq-mER, has been developed that has no binding capacity to classical ER.¹³⁸ STX is more potent than estrogen in attenuating the activation of K⁺ channels.^{87,88} STX is an excellent tool for the elucidation of the role of the Gq-mER in energy homeostasis and gene regulation.⁸⁹ The ability of STX to alter POMC neuronal excitability leads to the hypothesis that the putative Gq-mER has a role in energy homeostasis. Whole animal studies in which STX was systemically injected in ovariectomized female guinea pigs over a period of 4 weeks support this hypothesis. This study demonstrated a dose-dependent effect on the reduction in body weight gain by systemic STX treatment. Furthermore, the administration of systemic STX-generated new transcription in the ARC of these STX-treated female guinea pigs.⁸⁹ The genes regulated include NPY gene involved in the control of energy homeostasis and Cav3.1 gene involved in neuronal activity. Therefore, Gq-mER may function in the estrogenic control of energy homeostasis, presumably through activation of POMC neurons in the ARC, although other hypothalamic nuclei may be involved.⁸⁸

The molecular characterization of this Gq-mER, including cloning, localization, and regulation, is crucial for a full understanding of the role of the rapid, membrane-mediated effects of estrogen on energy homeostasis. Additionally, STX can be utilized to target the Gq-mER to determine whether Gq-mER actually contributes to the regulation of energy homeostasis in ERKO mice.

4. ESTROGEN ACTION IN ADIPOSE TISSUE: REGULATION OF LIPOGENESIS/LIPOLYSIS AND ADIPOSITY

Organisms store energy for later use during times of nutrient scarcity. Excess energy is stored as triacylglycerol (TG) in lipid droplets produced by lipogenesis. When energy is required, TG is catabolized into free fatty acids via lipolytic pathways. Obesity results from excess white adipose tissue, which is considered to be an endocrine organ and it is able to store and metabolize steroid hormones. ERs are expressed in adipose tissue, where they may influence adiposity and inflammation. Estrogens regulate both the metabolism and the location of white adipose tissue and play a role in adipose deposition, lipogenesis, lipolysis, and adipocyte proliferation.²³¹

4.1. Estrogenic action in adipose tissue prevents obesity

Obesity, high body fat percentage, and increased hip circumference are all strongly correlated to increased E2 levels.²³² Sex steroids are known to regulate lipid metabolism and are responsible for accumulation, distribution, and development and function of adipose tissues. The differences in body fat distribution suggest that the sex steroid hormones could have an effect on adipose tissues. Direct effects seem plausible, as sex steroid hormone receptors have now been found in adipose tissues. The amount of the receptor varies with the fat depot, with subcutaneous adipose tissue having a higher concentration of ERs than intra-abdominal adipose.

Estrogen activates lipogenesis under normal physiological conditions in lean animals but suppresses lipogenesis under pathologic conditions. In normal lean animals, increased intracellular E2 activates ER α and stimulates lipogenesis.²³³ Consequently, female mice with higher E2 level have greater lipogenic capacities in adipocytes compared with male mice.²³⁴ Specifically, E2 increase the deposition of subcutaneous fat on the hips, upper thighs, and lower abdomen, ectopic fat in the liver and muscle, and visceral fat in and about abdominal organs.²³⁵ As the enlarging fat cells produce more E2, they increase their leptin output,²³⁶ to cyclically upregulate 11 β -hydroxysteroid dehydrogenase,²³⁷ increase intracellular E2 production.²³⁸ This stimulation of lipogenesis by E2 is ER α -dependent, as

revealed by global gene expression analysis from ER α KO and wild type mice that demonstrated ER α dependent increased expression of lipogenic genes and decreased expression of genes regulating lipid transport.²³⁹ Interestingly, despite the increased lipogenesis, adipocytes from females are smaller than adipocytes from males.

It is noteworthy that androgen receptors (AR) are also expressed in adipose tissues.²⁴⁰ Subcutaneous adipose tissue has higher concentrations of ERs whereas visceral adipose tissue has higher concentrations of AR.²⁴¹ Additionally, subcutaneous adipose tissue has few AR, and estrogens downregulate AR expression in subcutaneous fat.¹² Adipose tissue-specific AR knockout mice have increased intra-adipose estrogens, which leads to increased subcutaneous obesity and hyperleptinemia.²⁴² The sexual dimorphism in adipose tissue distribution may partially explain the greater risk for the metabolic syndrome in men compared with premenopausal women.

Estrogens suppress lipogenesis under certain conditions leading to obesity. E2 suppresses the expression of lipogenic genes, i.e. fatty acid synthase, stearoyl-coenzyme A desaturase 1, and glycerol-3-phosphate acyltransferase, and thus suppresses lipogenesis and TG accumulation in adipose tissue and liver in high-fat diet fed, leptin-deficient *ob/ob* mice.²⁴³ When circulating levels of estrogen are raised above the physiological range due to enlarged adipocytes, adipose tissue metabolism is altered resulting in reduced lipogenic rates and fat depot size to prevent further expansion of adipose tissue. E2-treated ovariectomized mice display reduced lipogenesis in adipocytes.²⁴ This is further supported by epidemiological observations that serum triglyceride levels increase in postmenopausal women with increased adiposity and that estrogen hormone replacement therapy reduces the activity of lipoprotein lipase (LPL),²⁴⁴ the enzyme that catalyzes the conversion of triglycerides into free fatty acids and glycerol.²⁴⁵ Therefore, normal amount of estrogen favors homeostasis in adipose tissues and estrogenic action in adipose tissue prevents the development of obesity by decreasing the lipogenic activity in adipose tissue. However, decreases in the amount of estrogen, as occur with menopause in women and ageing or gonadectomy in rodents, usually result in central obesity with increased lipid accumulation and decreased lipid utilization at visceral fat.²⁴⁶

Genetic mouse models of either E2 deficiency by knockout of the aromatase gene (ArKO) or E2 resistance in ERKO have provided important information on the role of E2 signaling in adipose tissue physiology and support roles of E2 and ER in adipose tissue.

ArKO, ER α KO, and mice with double knockout of both ER α and ER β exhibit an increased white adipose tissue mass but not brown adipose tissue mass,^{65,67,247} whereas knockout of the ER β gene alone does not develop obesity,²⁴⁸ suggesting that ER α plays an important role in adipogenesis. In addition, ArKO mice and ER α KO mice show increase in adipocyte size (hypertrophy) and number (hyperplasia), display dyslipidemia including an increase in serum cholesterol and a change in the lipoprotein profile, and insulin resistance, implicating a role for ER α in adipocyte growth and proliferation. By studying estrogen replacement in ovariectomized ER α and/or ER β knockout mice, Lindberg *et al.*²⁴⁹ demonstrated a reduction of fat in these animals with estrogen replacement mediated mainly by ER α . These studies demonstrate the importance of E2 signaling through the ER α in adipocyte differentiation and metabolism.^{65,247,250} This is further supported by studies on 3T3-L1 preadipocytes with stably transfected ER α , which show decreased triglyceride accumulation and reduced expression of LPL. The observation from ArKO mice is consistent with men with aromatase deficiency who have central obesity, elevated blood lipids, and severe insulin resistance.²⁵¹

ER β KO female mice have a higher body weight under HFD feeding than wild type littermates.²⁵² This could be a result from increased adipogenesis with subsequent increased mass of adipose tissue and improved insulin sensitivity. Furthermore, the key adipogenic and lipogenic factor PPAR γ is negatively regulated by ER β , suggesting that PPAR γ could be a mediator of the metabolic effects observed in ER β KO mice.²⁵² Female ER β KO mice on HFD displayed impaired food efficiency and increased respiratory quotient, which is an indication of disturbed fatty acid oxidation. PPAR γ DNA-binding properties and target gene activation are markedly induced in the gonadal fat of the ER β KO mice and inhibition of adipose PPAR γ signaling reversed this metabolic phenotype.²⁵² In summary, both ER isoforms participate in the antilipogenic actions of estrogens.

Estrogens carry out their action directly on adipose tissues by a combination of genomic (receptor DNA interactions) and nongenomic (membrane receptors) mechanisms in estrogen regulation of adipose accretion and metabolism.^{253,254} In the genomic mechanism, estrogens bind to their receptors and the steroid–receptor complex regulates the transcription of given genes. LPL is a key adipose protein regulated by estrogens in a classical genomic mechanism. Second messenger pathways via the cAMP and phosphoinositide cascades are modulated by membrane receptors for estrogens in a nongenomic mechanism. See below for details.

4.2. Estrogenic action on adipogenesis via genomic mechanism

4.2.1 Classical nuclear receptors ER α and ER β in adipose tissue—The mechanism for the regulation of the amount and distribution of adipose tissues by estrogens is not completely clear. One possible mechanism would be the regulation of key proteins in adipose tissues at the genomic level by transcriptional means. This would require ERs to be present in adipose tissues.

Many groups have demonstrated that ER α and ER β mRNA and protein are expressed in human adipose tissue.^{240,255–260} In initial experiments, it was not possible to detect ER in subcutaneous or omental fat from normal women.^{261,262} Later, low levels of ER have been found in human adipose tissues with increased sensitivity of molecular techniques.

Price & O'Brien²⁵⁸ demonstrated by PCR the presence of ER mRNA in human subcutaneous abdominal tissue. Mizutani *et al.*²⁶⁰ identified ER protein and ER mRNA in subcutaneous abdominal tissue. The ER protein had a molecular weight of 65 kDa, which is similar to the ER from other tissues including reproductive tissues. Human subcutaneous and visceral adipose tissues express both ER α and ER β . Expression of ER α is similar in subcutaneous gluteal and subcutaneous abdominal adipose tissues, and is similar between men and women.²⁵⁶ The ER is higher in concentration in subcutaneous fat than in visceral fat in both males and females. These differences in concentrations of ER in visceral adipose tissue and subcutaneous adipose tissue may offer a possible explanation why men have a more central accumulation of fat, whereas women have a more gluteal/femoral accumulation.

Pedersen *et al.* demonstrated by ligand binding and RT-PCR the presence of ER in human adipose tissue and mature adipocytes.²⁶³ ER α and ER β are present in cytosols of adipose tissues from both men and women. Cytosols from visceral adipose tissue and subcutaneous abdominal tissue of women showed about the same amount of ER binding, whereas cytosol of subcutaneous abdominal tissue from men showed a higher amount of binding than those from visceral adipose tissue. By immunohistochemistry and RT-PCR analyses, ER α and ER β are detected in both stromal cells and adipocytes from subcutaneous and omental adipose tissues.²⁵⁷ A higher amount of ER mRNA was found in adipocytes than in adipose stromal cells. In mature human adipocytes, ER α is the more highly expressed isoform. ER β

is expressed in breast and subcutaneous abdominal tissues,²⁴⁰ and it appears to be more highly expressed in women than in men.²⁵⁹ Interestingly, only ER α , not ER β , is expressed in human preadipocytes by RT-PCR, although the expression of ER α in preadipocytes is very low compared with that in mature adipocytes.²⁶⁴

E2 treatment regulates ER α and ER β in stromal cells from subcutaneous and omental adipose tissues. In adipocytes from subcutaneous adipose tissue, E2 treatment decreases the expression of ER α , while increases ER β expression. In omental adipocytes, the decrease in the expression of ER α with E2 treatment is not observed.

Laboratory rodents have been used to study the relationship of estrogens to obesity. Obesity in laboratory rodents is easily induced by overeating and reduced energy expenditure, and the animals develop many of the same deleterious effects, such as insulin resistance and hyperinsulinemia, as seen in human obesity. The major advantage of working with mice is the development of techniques that allow for the manipulation of their genome to generate transgenic mice that lack functional ER.²⁶⁵ Both ERs are expressed in rodent white adipose tissue^{256,266–269} with a predominance of ER α ²⁷⁰ and colocalization of ER α and ER β in the same nuclei of some cells,²⁷¹ whereas only ER α but not ER β mRNA has been identified in brown adipose tissue.²⁷² Additionally, brown adipocytes from male rats have higher ER α expression compared to those of female rats, suggesting that this receptor could have a role in thermogenesis.²⁷²

One of the first indication that ERs exists in adipose tissues was reported by Wade & Gray.²⁶⁶ They showed estrogen-specific, high affinity [³H]-E2 binding in cytosolic extracts of ovariectomized rat adipose tissues with similar properties to ER in reproductive tissue, but at a much lower concentration. Binding was highest in parametrial fat and lowest in inguinal fat. In a subsequent paper, Gray & Wade²⁷³ showed estrogen-binding sites in male rat adipose tissues. Gray *et al.*²⁷⁴ also demonstrated binding of injected [³H]-E2 in the nuclei in adipose tissues. Using ovariectomized and adrenalectomized rats for conditions of low endogenous sex steroid production, Rebuffe-Scrive²⁷⁵ demonstrated cytoplasmic E2 binding in adipose tissues from these animals that decreased dramatically after estrogen administration. Pedersen *et al.*²⁷⁶ showed nuclear binding of [³H]-E2 in isolated rat adipocytes. The binding is specific for E2 and showed regional variation. Using immunogold electron microscopic localization, Echeverria *et al.*²⁷⁷ demonstrated significant labeling of ER in adipose cells from various locations of both male and female rats. A higher density of label is found in the nucleus, but some label was observed in the cytoplasm of the adipose cells. All these results support the notion that estrogens may have a direct effect on adipose tissues via its binding to their receptors.

ER knockout mice have abnormalities in their adipose tissues.²⁷⁸ Indeed, ER α is now considered a candidate gene for obesity.²⁷⁹ In male mice, deletion of ER α causes progressive increase in epididymal, perirenal, and inguinal adipose tissue with advancing age. Epididymal and perirenal adipocyte size is increased and adipocyte number is greater in the fat pads. Female ER α KO mice have increased fat, adipocyte size, number, and leptin and cholesterol levels and smaller LDL particles. Both males and females are insulin resistant with impaired glucose tolerance.⁶⁵ E2 administered to ovariectomized mice fed a high-fat diet preserved glucose tolerance and insulin sensitivity and improved glucose tolerance in wild type but not in ER α KO mice, suggesting that targeting of the ER α could represent a strategy to reduce the impact of high-fat diet-induced insulin resistance.²⁸⁰

ERs play roles on GLUT4 function in adipose tissue. The administration of tamoxifen to ER α KO or ER β KO mice resulted in different responses on GLUT4 expression. In ER α KO mice, tamoxifen did not interfere with GLUT4 expression, whereas treatment of ER β

KO mice with tamoxifen substantially decreased it, suggesting that ER α is the main regulator of GLUT4 expression in adipose tissue.²⁷¹ Ovariectomy of ER β KO mice prevented accumulation of triglycerides and reduced body fat and adipocyte size, and preserved regular insulin signaling in liver and skeletal muscle, and improved whole body insulin sensitivity and glucose tolerance, suggesting that ER α and ER β have opposite functions on lipid and glucose metabolism, with ER β involved in adipogenesis.^{252,281} An imbalance between ER α and ER β in adipose tissue may have important implications for the development of metabolic diseases.

4.2.2 Estrogenic genomic action on LPL in adipose tissue—In the classical mechanism of action of sex steroid hormones, the steroid enters the cell and binds with high affinity to a specific receptor. The steroid–receptor complex then undergoes a conformation change and binds to a specific response element on the DNA. Once bound, it regulates the transcription of a given gene. Transcription may be up- or downregulated depending upon the gene, the steroid, and the coregulatory proteins. Many genes in adipose tissues are transcriptionally regulated by sex steroid hormones. An ERE is present in the LPL genome and thus *LPL* gene is a direct transcriptional target of estrogen. LPL is the key protein involved in lipid deposition.

LPL is the key enzyme for the hydrolysis of circulating triglycerides into free fatty acids and glycerol. Its highest activity is located in adipose tissues, heart, and skeletal muscle, and it plays a major role in adipocyte lipid storage and muscle fuel supply and hence the regulation of lean muscle mass and obesity. It is synthesized and secreted by the adipocyte and then transported to the capillary endothelium, where it hydrolyses the circulating triglycerides into free fatty acids, which in turn are stored in the adipose tissues. LPL in adipocytes is activated by insulin. During feasting, increased insulin activates LPL to promote adipogenesis.

Although the mechanisms responsible for estrogen's regulation of adipogenesis remain unclear,²⁵⁵ they may involve estrogens' suppression of LPL, a lipogenic enzyme that regulates the metabolism of plasma triglycerides to free fatty acids and increases lipid storage by adipocytes. Specifically, the effect of estrogens on LPL activity may be concentration dependent, as Palin *et al.* found that a high concentration (10^{-7} mol L⁻¹) of estrogen reduced LPL expression in isolated adipocytes from subcutaneous abdominal fat of women, while lower estrogen concentrations increased LPL expression.²⁸² Consistent with this notion, ovariectomy of female rats results in increased adipose tissue LPL while estrogen replacement decreases the LPL activity.^{283–288} E2 administration to male rats also significantly reduces adipocyte LPL activity²⁸⁹ to suppress adipogenesis.

In obese women, plasma LPL activity has an inverse correlation with plasma E2 levels.²⁴⁴ The administration of estrogens alone or estrogen–progestin in women decreases LPL activity and increases serum triglyceride concentrations.^{290–292} A transdermal E2 patch placed in the gluteal region of women decrease LPL in adipose tissue from beneath the patch as compared to tissue beneath the placebo patch.²⁹³ However, there is no difference in LPL mRNA levels between these two groups, suggesting that a possible posttranscriptional modification of LPL led to the difference. In women, LPL levels increase after menopause.²⁹⁴ In addition to inhibiting fatty acid uptake (lipogenesis), estrogen also increases fatty acid and glycerol release (lipolysis) by increasing expression of hormone-sensitive lipase (HSL), which catalyzes the breakdown of stored TG and the release of fatty acids.^{282,295} However, estrogen has also been shown to inhibit lipolysis. Estrogen treatment of postmenopausal women increases expression of the α 2A-adrenergic receptor in subcutaneous adipose tissue. Activation of this receptor inhibits lipolysis; therefore, increased expression due to estrogen would inhibit lipolysis.

This increase was recapitulated *in vitro* using cultured human adipose tissue fragments incubated with E2 and was correlated with inhibition of lipolysis,²⁹⁶ suggesting a direct role for ER in regulating lipolysis. The complex relationship between the ERs and adipogenesis remains to be elucidated and may provide critical information for the prevention and treatment of obesity.

4.3. Estrogenic nongenomic action via second messengers in adipose tissue

Another possible mechanism would be the regulation of second messengers at the cell membrane by nongenomic effects. Although transcriptional regulation by steroid hormones has been the most studied mechanism of steroid action, recent studies have shown that a nongenomic mechanism also exists for steroid hormone action.²⁹⁷ This would require a sex steroid receptor to be present in the membrane. There is also evidence for rapid nongenomic estrogen actions via a membrane-bound ER in adipocytes.^{112,298} Nongenomic actions of steroids are generally rapid responses occurring in less than 10 min, while transcriptional regulation requires several hours to days.^{197,299,300}

In the nongenomic mechanism, estrogens bind to their receptors in the plasma membrane, and second messengers are formed to carry out their action. Estrogens appear to regulate various components of the membrane-signaling systems, including both the cAMP cascade and the phosphoinositide cascade.^{301,302}

Activation of the cAMP cascade by sex steroid hormones would activate HSL leading to lipolysis in adipose tissues. In the phosphoinositide cascade, diacylglycerol and inositol 1,4,5-trisphosphate are formed as second messengers ultimately causing the activation of protein kinase C. Their activation appears to be involved in the control of preadipocyte proliferation and differentiation.

Sex steroid membrane receptors have been characterized in many different tissues,¹¹² but little is known about the receptors in adipose tissues. Anwar *et al.*²⁵⁷ showed immunostaining for both ER α and ER β in the cellular membranes of subcutaneous abdominal and omental human adipose tissues. Dos Santos *et al.*²⁹⁸ found immunoreactive ER α in the membrane fraction from cultured rat adipocytes, but not in the membrane fraction from preadipocytes.

These plasma membrane ERs are associated with caveolae endocytic vesicles. Caveolin-1 coimmunoprecipitate with ER α and bind directly to ER α .³⁰³ Caveolin-1 has also been reported to bind to endoplasmic reticulum membranes.³⁰⁴

4.3.1 cAMP cascade—Early studies showed an increase in cAMP and lipolysis within 5 min when isolated rat adipocytes were treated with E2³⁰⁵ and adenylate cyclase activity in fat cell mass increased by E2 treatment of ovariectomized rats.³⁰⁶ This response to estrogen appears to be depot specific as ovariectomy caused a decrease in cAMP production in rat parametrial adipocytes, but not in femoral subcutaneous adipocytes.³⁰⁷ These studies with rats would suggest that estrogen increased cAMP in adipose tissues, resulting in stimulation of HSL and increased lipolysis.

In humans, estrogens also appear to decrease lipolysis in adipose tissues. Lindberg *et al.* found that women who were given oral ethinyl E2 had a decrease in noradrenaline-stimulated lipolysis in subcutaneous abdominal adipocytes.³⁰⁸ To study the effect of estrogens on lipolysis in women, Pedersen *et al.* used adipocytes that were isolated from adipose tissue fragments, which were incubated *in vitro* with or without estradiol.²⁹⁶ A decrease in adrenaline-stimulated lipolysis was observed in adipocytes from subcutaneous E2-treated adipose tissue fragments. E2 also increased the number of antilipolytic α 2A-

adrenergic receptors in subcutaneous adipocytes. These results suggested that estrogen lowered lipolysis by increasing the number of antilipolytic α 2A-adrenergic receptors. In contrast, no effect of estrogen was observed in adipocytes from intra-abdominal fat. This difference in the E2 effect on α 2A-adrenergic receptors between subcutaneous fat and intra-abdominal fat may be one factor to explain how estrogen maintains the gynoid fat pattern in women. This increase in α 2A-adrenergic receptors with estrogen in subcutaneous fat from women may also explain the difference in response to estrogen between humans and rats as adipose tissue from rats do not have α 2A-adrenergic receptors.³⁰⁸

4.3.2 Phosphoinositide cascade—The phosphoinositide cascade also appears to be regulated by sex steroid hormones. Protein kinase C content of rat subcutaneous and parametrial fat cells are reduced by ovariectomy and restored to normal by E2 and progesterone treatment.³⁰⁹ Lacasa *et al.*³¹⁰ demonstrated that ovariectomy increased proliferation, MAPK activity, and c-fos protein in preadipocytes from rat perirenal fat depots. Treatment of the ovariectomized animals with E2 and progesterone reversed the increased proliferation and c-fos protein induction, but not the MAPK activation. The ovarian status had no effect on these parameters in subcutaneous preadipocytes, indicating that this regulation is depot specific. Recently, Dos Santos *et al.*²⁹⁸ found that estrogen activated p42/p44 MAPK by phosphorylation in rat white adipocytes through a rapid nongenomic pathway. This pathway was postulated to involve membrane ER α , G protein, protein kinase C, raf, and MAPK kinase (MEK). The phosphoinositide cascade appears to be involved in adipose proliferation and differentiation.

5. ESTROGENS INTERACT WITH LEPTIN

5.1. Estrogen interact with leptin in the CNS

Leptin, the product of the obese gene, is a key adipocytokine hormone that is normally released by adipose tissue in direct proportion to fat content and is related to the amount and distribution of body fat.³¹¹ It is a potent anorexigenic and catabolic hormone in central regulation of metabolism by crossing the blood–brain barrier to interact with leptin receptors (lepr) in the hypothalamus. Although there are several isoforms of the lepr, the b form (leprb) is the critical variant for regulating energy balance.³¹² Leprb has been identified in the ARC, PVN, and VMH, where they colocalize with ER α .³¹³ Leptin transfers a catabolic signal to the brain to inhibit food intake and increase energy expenditure.^{314–316} Thus, leptin plays a key role in the regulation of body fat and energy homeostasis. Estrogen not only modulates leptin receptor leprb mRNA in the ARC and VMH via an ERE in the lepr gene, but also increases hypothalamic sensitivity to leptin, altering peripheral fat distribution.³¹⁷ In addition, raised levels of estrogens have been associated with increased leptin sensitivity in the brain,³¹⁸ although ovariectomy reduces the sensitivity to leptin when compared with intact females, an effect that can be restored by E2 replacement. Furthermore, administration of E2 in male rats increases the sensitivity to leptin.²⁰ Thus, interactions between E2 and leptin exist for the regulation of energy homeostasis.^{313,319}

5.2. Estrogen interact with leptin in adipose tissue

Leptin levels are higher in females compared with males.³²⁰ After puberty, estrogen modulates leptin synthesis and secretion via ER-dependent transcriptional mechanisms in adipose tissue.³²¹ The presence of rising intracellular bioactive E2 stimulates leptin production by fat cells.²³⁶ In support of this, Hardie *et al.*³²² reported a correlation between serum leptin and estrogen levels. As estrogen levels decrease with menopause, a decrease in leptin levels might be expected with menopause.^{320,323,324} Additionally, increase in leptin with estrogen replacement therapy has been reported.^{324,325} Thomas *et al.*³²⁶ found that bioavailable estrogen levels in postmenopausal women who were not on

hormone replacement therapy are significantly and directly related to serum leptin levels, but in premenopausal women or in women on hormone replacement therapy, variations in estrogen level are not associated with the higher leptin levels, suggesting a possible threshold effect. The most convincing evidence that the sex steroid hormones affect blood leptin levels comes from studies on cross-sex administration of sex steroid hormones to transsexuals. Elbers *et al.*³²⁷ were able to show that estrogen and antiandrogen administration to male-to-female transsexuals greatly increased the serum leptin levels and testosterone administration to female-to-male transsexuals decreased the serum leptin levels. They concluded that the sex steroid milieu plays an important role in the regulation of blood leptin levels.

Studying human omental adipose tissue *in vitro*, Casabiell *et al.*³²⁸ demonstrated that leptin secretion was higher in tissue from women than in tissue from men. Dexamethasone and E2 stimulated leptin secretion in fat from women, but not in fat from men. The increased E2^{237,329} and the increased leptin³³⁰ both upregulate aromatase to further increase extra-glandular E2 production,²³⁷ to increase subcutaneous fat deposition³³¹ and to potentiate the action of E2 on ER α ³³² and transmembrane GPER.³³³

As in humans, female rats have higher circulating levels of leptin than do males.^{334–336} Ovariectomy of female rats has been shown by some investigators to lower circulating leptin levels, and estrogen replacement in these animals has been found to return the levels back to normal.^{320,337} Other investigators, however, found no effect on circulating leptin levels after ovariectomy and estrogen replacement.^{321,334–336,338,339} These discrepancies could be caused by the time of the blood samples, as Chu *et al.*³⁴⁰ showed that leptin levels declined during the first 7 weeks after ovariectomy. Subsequently, its concentration increased, and by the end of 13 weeks the value was higher than the level before ovariectomy. The fluctuation of serum leptin levels with ovariectomy was eliminated by estrogen replacement. These discrepancies could also be caused by weight gain in the ovariectomized animals, as leptin levels are related to the amount of fat, which was not always controlled in these experiments.

Ovariectomy has also been reported by some authors to decrease leptin gene expression in white adipose tissues of rats, and this decrease can be reversed by estrogen replacement.^{320,321,337,339} In contrast, other authors have reported no effect of estrogen replacement on leptin gene expression in white adipose tissues.^{336,338} These discrepancies could be caused by differences in leptin secretion and regulation in various adipose sites, as Shimizu *et al.*³²⁰ showed that although leptin gene expression decreased in subcutaneous and retroperitoneal white adipose tissues of ovariectomized rats as compared to controls, leptin gene expression actually increased in mesenteric white adipose tissues of the ovariectomized rats.

Machinal *et al.*³²¹ also observed that ovariectomy resulted in a 25% decrease in leptin gene expression in isolated perirenal adipocytes and a small but insignificant decrease in subcutaneous adipocytes. Therefore, the regional distribution of adipose tissues may greatly influence leptin levels and its regulation by sex steroid hormones. Basal leptin production is equal in epididymal fat fragments from males and parametrial fat fragments from females. E2 increased leptin secretion in both male and female adipose fragments, but the effect was greater in female adipose tissue. Parametrial fat from ovariectomized rats had lower leptin secretion compared to shamoperated controls, and E2 treatment of ovariectomized rats maintained a normal leptin secretion in fat fragments.

Sex steroid hormone regulation of leptin production has also been studied in normal and mutant mice. Nedvidkova *et al.* showed a decrease of serum leptin levels in estrogen-treated

male mice.³⁴¹ However, in female mice, Pelleymounter *et al.* reported that ovariectomy or E2 administration did not alter leptin levels.³⁴² Kronfeld-Schor *et al.* demonstrated that subcutaneous implants of E2 or corticosterone into lactating mice stimulated leptin secretion rates of omental adipose tissue *in vitro*, and corticosterone, but not estradiol, stimulated leptin secretion rates when added to isolated omental adipose tissue.³⁴³ From this study, the authors suggest that E2 could be acting indirectly on the omental adipose tissue possibly through an increase in plasma corticosterone levels.

In ER α KO male mice, Ohlsson *et al.* demonstrated an enhanced serum leptin level as compared to controls that was not seen in ER β KO mice.⁶⁷ Jones *et al.* showed an elevation of leptin levels in ArKO male and female mice.²⁴⁷ However, both the ER α KO mice and ArKO mice had an increase in fat mass, which could account for the increased leptin levels.

In summary, estrogens appear to increase leptin production. However, many discrepancies exist in the data. If sex steroid hormones are going to directly regulate the production of leptin, an ERE should be present in the leptin gene. After reviewing the cDNA sequence of the leptin gene, Shimizu *et al.*³²⁰ concluded that this gene had a consensus sequence of the ERE in its promoter region. These results suggest that the leptin gene has an ERE; that leptin promoter activation may depend upon coactivators present in leptin-producing cells; and that different effects of estrogen may be due to the type of ER expressed in target tissue.

6. CONCLUSIONS

Obesity and its associated health-related problems have become so prevalent in developed countries that many programs and strategies have been developed to prevent and/or treat this affliction, but without much success.^{344,345} Although sex steroid hormones have a major role in lipid metabolism and deposition in adipose tissues, they or their derivatives have not been extensively used to prevent or treat obesity.

There is abundant evidence that estrogen can utilize both genomic and nongenomic signaling mechanisms to regulate energy homeostasis, body fat, and glucose regulation. Estrogens exert rapid nongenomic effects in the brain and adipose tissues to regulate second messengers and kinase signaling pathways, and play physiologically important roles in estrogen-induced regulation of homeostasis.

One area where sex steroid hormones appear to have a positive effect on obesity is in hormone replacement therapy in older women. Menopause in women causes a large decrease in endogenous estrogens, which leads to central obesity and an increased risk in metabolic disorders. A relationship of central obesity and its complications to the decrease in sex steroid hormones is unclear. If it is related, hormone replacement therapy in postmenopausal women should reduce the occurrence of these problems.³⁴⁶ Several studies were able to show that hormone replacement therapy did decrease central obesity in postmenopausal women and hence prevent at least some of the problems of menopause.^{18,347–354} In contrast, other studies have not been able to confirm the decrease in central obesity with hormone treatment.^{17,355,356} In contrast, a recent report showed that hormone replacement therapy in postmenopausal women results in an increased risk for coronary heart disease, stroke, thromboembolic events, and breast cancer,³⁵⁷ suggesting caution when using sex steroid treatment. For the amelioration of obesity by sex steroid hormones, it would be helpful to develop compounds that would be specific for adipose tissues and that would not have the adverse side effects of estrogen. Considerable time was devoted to the need for SERMs that are adipose tissue specific and have pathways related to lipid metabolism for safe intervention in a number of endocrine-regulated conditions.³⁵⁸ SERMs, such as estren³⁵⁹ and CP-336,156,³⁶⁰ that were shown to prevent bone loss in

animal models without serious side effects, and CP-336,156 that was shown to prevent the increase in body fat in ovariectomized rats, may be an option for obesity regulation in the future.

Regulation of some key proteins in adipose tissues by estrogens, such as downregulation of LPL in visceral adipose tissue, may also be a mechanism for the treatment and/or prevention of obesity.³⁶¹ Estrogens are known to decrease LPL activity at least in gluteal adipose tissue, which may be important in body fat distribution.²⁹²

Leptin is another adipose protein that could be a target for regulation by estrogens to control central obesity. The extension of leptin therapy in experimental animals to humans has been only partially successful.^{362,363} In patients with a genetic defect of leptin production, leptin therapy greatly reduces the obesity in these individuals.³⁶⁴ However, these individuals only represent an extremely small percentage of the obese population, and in the common diet-induced obesity, leptin therapy remains questionable because leptin levels are very high in obese individuals. The high leptin levels in obese individuals might suggest that exogenous leptin therapy would be ineffective in decreasing fat stores. It is possible that regulation of endogenous leptin by sex steroid hormones would be more effective than exogenous leptin.

By gaining a more thorough understanding of estrogen's effects on the CNS and peripheral adipose tissues, and a better grasp of the signaling cascade involved, we will gain new insights that lead to better diagnostic tools and improved therapeutics in humans.

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ABBREVIATIONS

α-MSH	α -melanocyte stimulating hormone
AgRP	agouti-related protein
ARC	arcuate nucleus
cAMP	cyclic AMP
CNS	central nervous system
E2	17 β -estradiol
EGFRs	epidermal growth factor receptors
ERs	estrogen receptors
Era	estrogen receptor alpha
ERβ	estrogen receptor beta
EREs	estrogen response elements
GLUT4	glucose transporter 4
GPOR	G protein-coupled estrogen receptor
HSL	hormone-sensitive lipase
LH	lateral hypothalamus
LPL	lipoprotein lipase

mER	membrane subpopulation estrogen receptors
NPY	neuropeptide Y
PI3K	phosphoinositide-3 kinases
PKA	protein kinase A
PKB	protein kinase B (Akt)
POMC	pro-opiomelanocortin
PTX	pertussis toxin
PVN	paraventricular nucleus
SERM	selective estrogen receptor modulators
STZ	streptozotocin
VMH	ventromedial hypothalamus

REFERENCES

1. James WPT. The epidemiology of obesity: the size of the problem. *J Intern Med.* 2008; 263(4):336–352. [PubMed: 18312311]
2. Cummings DE, Schwartz MW. Genetics and Pathophysiology of Human Obesity. *Annu Rev Med.* 2003; 54(1):453–471. [PubMed: 12414915]
3. Alberti K, Zimmet P, Shaw J. IDF epidemiology task force consensus group: the metabolic syndrome—a worldwide definition. *Lancet.* 2005; 366:1059–1062. [PubMed: 16182882]
4. McGarry JD. Banting lecture 2001: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. *Diabetes.* 2002; 51(1):7–18. [PubMed: 11756317]
5. Xu H, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest.* 2003; 112(12):1821–1830. [PubMed: 14679177]
6. Kolovou G, et al. Apolipoprotein E gene polymorphism and gender. *Ann Clin Lab Sci.* 2009; 39(2):120–133. [PubMed: 19429797]
7. Ford ES. Prevalence of the metabolic syndrome defined by the International Diabetes Federation among adults in the U.S. *Diabetes Care.* 2005; 28(11):2745–2749. [PubMed: 16249550]
8. Kannel WB, et al. Regional obesity and risk of cardiovascular disease; the Framingham study. *J Clin Epidemiol.* 1991; 44(2):183–190. [PubMed: 1995775]
9. Lee CG, et al. Adipokines, inflammation, and visceral adiposity across the menopausal transition: a prospective study. *J Clin Endocrinol Metab.* 2009; 94(4):1104–1110. [PubMed: 19126626]
10. Regitz-Zagrosek V, Lehmkuhl E, Weickert M. Gender differences in the metabolic syndrome and their role for cardiovascular disease. *Clin Res Cardiol.* 2006; 95(3):136–147. [PubMed: 16598526]
11. Björntorp P. Body fat distribution, insulin resistance, and metabolic diseases. *Nutrition.* 1997; 13(9):795–803. [PubMed: 9290093]
12. Björntorp P. Hormonal control of regional fat distribution. *Hum Reprod.* 1997; 12(Suppl. 1):21–25. [PubMed: 9403318]
13. Kotani K, et al. Sexual dimorphism of age-related changes in whole-body fat distribution in the obese. *Int J Obes Relat Metab Disord.* 1994; 18(4):207–212. [PubMed: 8044194]
14. Carr MC. The emergence of the metabolic syndrome with menopause. *J Clin Endocrinol Metab.* 2003; 88(6):2404–2411. [PubMed: 12788835]
15. Krotkiewski M, et al. Impact of obesity on metabolism in men and women. Importance of regional adipose tissue distribution. *J Clin Invest.* 1983; 72(3):1150–1162. [PubMed: 6350364]
16. Bouchard C, Despres J-P, Mauriege P. Genetic and nongenetic determinants of regional fat distribution. *Endocr Rev.* 1993; 14(1):72–93. [PubMed: 8491156]

17. Ryan AS, Nicklas BJ, Berman DM. Hormone replacement therapy, insulin sensitivity, and abdominal obesity in postmenopausal women. *Diabetes Care*. 2002; 25(1):127–133. [PubMed: 11772913]
18. Samaras K, et al. 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*. 1999; 22(9):1401–1407. [PubMed: 10480500]
19. Blaustein JD, Wade GN. Ovarian influences on the meal patterns of female rats. *Physiol Behav*. 1976; 17(2):201–208. [PubMed: 1033580]
20. Clegg DJ, et al. Gonadal hormones determine sensitivity to central leptin and insulin. *Diabetes*. 2006; 55(4):978–987. [PubMed: 16567519]
21. Wallen WJ, Belanger MP, Wittnich C. Sex hormones and the selective estrogen receptor modulator tamoxifen modulate weekly body weights and food intakes in adolescent and adult rats. *J Nutr*. 2001; 131(9):2351–2357. [PubMed: 11533278]
22. Roy EJ, Wade GN. Role of food intake in estradiol-induced body weight changes in female rats. *Horm Behav*. 1977; 8(3):265–274. [PubMed: 881167]
23. Gao Q, et al. Anorectic estrogen mimics leptin's effect on the rewiring of melanocortin cells and Stat3 signaling in obese animals. *Nat Med*. 2007; 13(1):89–94. [PubMed: 17195839]
24. D'Eon TM, et al. Estrogen regulation of adiposity and fuel partitioning Evidence of genomic and non-genomic regulation of lipogenic and oxidative pathways. *J Biol Chem*. 2005; 280(43):35983–35991. [PubMed: 16109719]
25. Gao H, 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. *Mol Endocrinol*. 2006; 20(6):1287–1299. [PubMed: 16627594]
26. Espeland MA, et al. Effect of postmenopausal hormone therapy on lipoprotein(a) concentration. PEPI Investigators. Postmenopausal estrogen/progestin interventions. *Circulation*. 1998; 97(10):979–986. [PubMed: 9529266]
27. Redig AJ, Munshi HG. Care of the cancer survivor: metabolic syndrome after hormone-modifying therapy. *Am J Med*. 2010; 123(1):87.e1–87.e6. [PubMed: 20102997]
28. Tiano JP, et al. Estrogen receptor activation reduces lipid synthesis in pancreatic islets and prevents β cell failure in rodent models of type 2 diabetes. *J Clin Invest*. 2011; 121(8):3331–3342. [PubMed: 21747171]
29. Simpson ER, et al. Estrogen—the good, the bad, the unexpected. *Endocr Rev*. 2005; 26(3):322–330. [PubMed: 15817672]
30. Maffei L, et al. Dysmetabolic syndrome in a man with a novel mutation of the aromatase gene: effects of testosterone, alendronate, and estradiol treatment. *J Clin Endocrinol Metab*. 2004; 89(1):61–70. [PubMed: 14715828]
31. Morishima A, et al. Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. *J Clin Endocrinol Metab*. 1995; 80(12):3689–3698. [PubMed: 8530621]
32. Bilezikian JP, et al. Increased bone mass as a result of estrogen therapy in a man with aromatase deficiency. *N Engl J Med*. 1998; 339(9):599–603. [PubMed: 9718379]
33. Rochira V, et al. 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. *Diabet Med*. 2007; 24(12):1491–1495. [PubMed: 17976198]
34. Takeda K, et al. Progressive development of insulin resistance phenotype in male mice with complete aromatase (CYP19) deficiency. *J Endocrinol*. 2003; 176(2):237–246. [PubMed: 12553872]
35. Deroo BJ, Korach KS. Estrogen receptors and human disease. *J Clin Invest*. 2006; 116(3):561–570. [PubMed: 16511588]
36. Hess R. Estrogen in the adult male reproductive tract: a review. *Reprod Biol Endocrinol*. 2003; 1(1):52. [PubMed: 12904263]

37. Baquedano MS, et al. Identification and developmental changes of aromatase and estrogen receptor expression in prepubertal and pubertal human adrenal tissues. *J Clin Endocrinol Metab.* 2007; 92(6):2215–2222. [PubMed: 17405842]
38. Kelly, MJ.; Qiu, J.; Rønnekleiv, OK. Gerald, L. Vitamins & hormones. Academic Press; 2005. Estrogen signaling in the hypothalamus; p. 123–145.
39. McEwen BS, Alves SE. Estrogen actions in the central nervous system. *Endocr Rev.* 1999; 20(3): 279–307. [PubMed: 10368772]
40. Teede HJ. Sex hormones and the cardiovascular system: effects on arterial function in women. *Clin Exp Pharmacol Physiol.* 2007; 34(7):672–676. [PubMed: 17581228]
41. Turner RT, Riggs BL, Spelsberg TC. Skeletal effects of estrogen. *Endocr Rev.* 1994; 15(3):275–300. [PubMed: 8076582]
42. Gruber CJ, et al. Production and actions of estrogens. *N Engl J Med.* 2002; 346(5):340–352. [PubMed: 11821512]
43. Muller RE, et al. Studies on the mechanism of estradiol uptake by rat uterine cells and on estradiol binding to uterine plasma membranes. *Adv Exp Med Biol.* 1979; 117:401–421. [PubMed: 474287]
44. Jackson V, Chalkley R. The binding of estradiol-17 beta to the bovine endometrial nuclear membrane. *J Biol Chem.* 1974; 249(5):1615–1626. [PubMed: 4856342]
45. Revankar CM, et al. A transmembrane intracellular estrogen receptor mediates rapid cell signaling. *Science.* 2005; 307(5715):1625–1630. [PubMed: 15705806]
46. Nadal A, et al. Nongenomic actions of estrogens and xenoestrogens by binding at a plasma membrane receptor unrelated to estrogen receptor α and estrogen receptor β . *Proc Natl Acad Sci U S A.* 2000; 97(21):11603–11608. [PubMed: 11027358]
47. Meyer MR, et al. Non-genomic regulation of vascular cell function and growth by estrogen. *Mol Cell Endocrinol.* 2009; 308(1–2):9–16. [PubMed: 19549587]
48. Walter P, et al. Cloning of the human estrogen receptor cDNA. *Proc Natl Acad Sci U S A.* 1985; 82(23):7889–7893. [PubMed: 3865204]
49. Kuiper GG, et al. Cloning of a novel receptor expressed in rat prostate and ovary. *Proc Natl Acad Sci U S A.* 1996; 93(12):5925–5930. [PubMed: 8650195]
50. Greene G, et al. Sequence and expression of human estrogen receptor complementary DNA. *Science.* 1986; 231(4742):1150–1154. [PubMed: 3753802]
51. Green S, et al. Human oestrogen receptor cDNA: sequence, expression and homology to v-erb-A. *Nature.* 1986; 320(6058):134–139. [PubMed: 3754034]
52. Jensen EV, DeSombre ER. Estrogen–receptor interaction. *Science.* 1973; 182(4108):126–134. [PubMed: 4354173]
53. Brzozowski AM, et al. Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature.* 1997; 389(6652):753–758. [PubMed: 9338790]
54. Stauffer SR, et al. Pyrazole ligands: structure–affinity/activity relationships and estrogen receptor- α -selective agonists. *J Med Chem.* 2000; 43(26):4934–4947. [PubMed: 11150164]
55. Meyer MR, et al. Dilation of epicardial coronary arteries by the G protein-coupled estrogen receptor agonists G-1 and ICI 182,780. *Pharmacology.* 2010; 86(1):58–64. [PubMed: 20639684]
56. Malamas MS, et al. Design and synthesis of aryl diphenolic azoles as potent and selective estrogen receptor-beta ligands. *J Med Chem.* 2004; 47(21):5021–5040. [PubMed: 15456246]
57. Hammes SR, Levin ER. Extranuclear steroid receptors: nature and actions. *Endocr Rev.* 2007; 28(7):726–741. [PubMed: 17916740]
58. Hall JM, Couse JF, Korach KS. The multifaceted mechanisms of estradiol and estrogen receptor signaling. *J Biol Chem.* 2001; 276(40):36869–36872. [PubMed: 11459850]
59. Nilsson S, et al. Mechanisms of estrogen action. *Physiol Rev.* 2001; 81(4):1535–1565. [PubMed: 11581496]
60. Thomas C, Gustafsson J-Å, et al. The different roles of ER subtypes in cancer biology and therapy. *Nat Rev Cancer.* 2011; 11(8):597–608. [PubMed: 21779010]
61. Handa RJ, et al. Roles for oestrogen receptor b in adult brain function. *J Neuroendocrinol.* 2012; 24(1):160–173. [PubMed: 21851428]

62. Kuiper GGJM, et al. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. *Endocrinology*. 1997; 138(3):863–870. [PubMed: 9048584]
63. Matthews J, Gustafsson J-Å, et al. Estrogen signaling: a subtle balance between ER alpha and ER beta. *Mol Interv*. 2003; 3(5):281–292. [PubMed: 14993442]
64. Hewitt S, Korach K. Oestrogen receptor knockout mice: roles for oestrogen receptors alpha and beta in reproductive tissues. *Reproduction*. 2003; 125(2):143–149. [PubMed: 12578528]
65. Heine PA, et al. Increased adipose tissue in male and female estrogen receptor- α knockout mice. *P Natl Acad Sci USA*. 2000; 97(23):12729–12734.
66. Geary N, et al. Deficits in E2-dependent control of feeding, weight gain, and cholecystokinin satiation in ER- α null mice. *Endocrinology*. 2001; 142(11):4751–4757. [PubMed: 11606440]
67. Ohlsson C, et al. Obesity and disturbed lipoprotein profile in estrogen receptor- α deficient male mice. *Biochem Biophys Res Commun*. 2000; 278(3):640–645. [PubMed: 11095962]
68. Shughrue PJ, Lane MV, Merchenthaler I. Comparative distribution of estrogen receptor- α and - β mRNA in the rat central nervous system. *J Comp Neurol*. 1997; 388(4):507–525. [PubMed: 9388012]
69. Nilsson S, Gustafsson JA. Estrogen receptors: therapies targeted to receptor subtypes. *Clin Pharmacol Ther*. 2011; 89(1):44–55. [PubMed: 21124311]
70. Liang YQ, et al. Estrogen receptor beta is involved in the anorectic action of estrogen. *Int J Obes Relat Metab Disord*. 2002; 26(8):1103–1109. [PubMed: 12119576]
71. O'Lone R, et al. Genomic targets of nuclear estrogen receptors. *Mol Endocrinol*. 2004; 18(8):1859–1875. [PubMed: 15031323]
72. Edwards DP. Regulation of signal transduction pathways by estrogen and progesterone. *Annu Rev Physiol*. 2005; 67(1):335–376. [PubMed: 15709962]
73. Losel RM, et al. Nongenomic steroid action: controversies, questions, and answers. *Physiol Rev*. 2003; 83(3):965–1016. [PubMed: 12843413]
74. Brann DW, Hendry LB, Mahesh VB. Emerging diversities in the mechanism of action of steroid hormones. *J Steroid Biochem Mol Biol*. 1995; 52(2):113–133. [PubMed: 7873447]
75. Manavathi B, Kumar R. Steering estrogen signals from the plasma membrane to the nucleus: two sides of the coin. *J Cell Physiol*. 2006; 207(3):594–604. [PubMed: 16270355]
76. Moriarty K, Kim KH, Bender JR. Minireview: estrogen receptor-mediated rapid signaling. *Endocrinology*. 2006; 147(12):5557–5563. [PubMed: 16946015]
77. Mhyre AJ, Dorsa DM. Estrogen activates rapid signaling in the brain: role of estrogen receptor α and estrogen receptor β in neurons and glia. *Neuroscience*. 2006; 138(3):851–858. [PubMed: 16343790]
78. Boonyaratanakornkit V, Edwards DP. Receptor mechanisms mediating non-genomic actions of sex steroids. *Semin Reprod Med*. 2007; 25(03) 139,153.
79. Szego CM, Davis JS. Adenosine 3',5'-monophosphate in rat uterus: acute elevation by estrogen. *Proc Natl Acad Sci U S A*. 1967; 58(4):1711–1718. [PubMed: 4295833]
80. Pietras RJ, Szego CM. Specific binding sites for oestrogen at the outer surfaces of isolated endometrial cells. *Nature*. 1977; 265(5589):69–72. [PubMed: 834244]
81. Carmeci C, et al. Identification of a gene (GPR30) with homology to the G-protein-coupled receptor superfamily associated with estrogen receptor expression in breast cancer. *Genomics*. 1997; 45(3):607–617. [PubMed: 9367686]
82. Filardo EJ, et al. Estrogen action via the G protein-coupled receptor, GPR30: stimulation of adenylyl cyclase and cAMP-mediated attenuation of the epidermal growth factor receptor-to-MAPK signaling axis. *Mol Endocrinol*. 2002; 16(1):70–84. [PubMed: 11773440]
83. Prossnitz ER, et al. Estrogen signaling through the transmembrane G protein-coupled receptor GPR30. *Annu Rev Physiol*. 2008; 70(1):165–190. [PubMed: 18271749]
84. Thomas P, et al. Identity of an estrogen membrane receptor coupled to a G protein in human breast cancer cells. *Endocrinology*. 2005; 146(2):624–632. [PubMed: 15539556]
85. Filardo EJ, et al. Estrogen-induced activation of Erk-1 and Erk-2 requires the G protein-coupled receptor homolog, GPR30, and occurs via trans-activation of the epidermal growth factor receptor through release of HB-EGF. *Mol Endocrinol*. 2000; 14(10):1649–1660. [PubMed: 11043579]

86. Maggiolini M, et al. The G protein-coupled receptor GPR30 mediates c-fos upregulation by 17beta-estradiol and phytoestrogens in breast cancer cells. *J Biol Chem.* 2004; 279(26):27008–27016. [PubMed: 15090535]
87. Qiu J, et al. Rapid signaling of estrogen in hypothalamic neurons involves a novel G-protein-coupled estrogen receptor that activates protein kinase C. *J Neurosci.* 2003; 23(29):9529–9540. [PubMed: 14573532]
88. Qiu J, et al. A G-protein-coupled estrogen receptor is involved in hypothalamic control of energy homeostasis. *J Neurosci.* 2006; 26(21):5649–5655. [PubMed: 16723521]
89. Roepke TA, et al. Genes associated with membrane-initiated signaling of estrogen and energy homeostasis. *Endocrinology.* 2008; 149(12):6113–6124. [PubMed: 18755790]
90. Roepke TA, et al. Contribution of a membrane estrogen receptor to the estrogenic regulation of body temperature and energy homeostasis. *Endocrinology.* 2010; 151(10):4926–4937. [PubMed: 20685867]
91. Toran-Allerand CD, et al. ER-X: a novel, plasma membrane-associated, putative estrogen receptor that is regulated during development and after ischemic brain injury. *J Neurosci.* 2002; 22(19):8391–8401. [PubMed: 12351713]
92. Wang Z, et al. Identification, cloning, and expression of human estrogen receptor- α 36, a novel variant of human estrogen receptor- α 66. *Biochem Biophys Res Commun.* 2005; 336(4):1023–1027. [PubMed: 16165085]
93. Prossnitz ER, Barton M. Signaling, physiological functions and clinical relevance of the G protein-coupled estrogen receptor GPER. *Prostaglandins Other Lipid Mediat.* 2009; 89(3–4):89–97. [PubMed: 19442754]
94. O'Dowd BF, et al. Discovery of three novel G-protein-coupled receptor genes. *Genomics.* 1998; 47(2):310–313. [PubMed: 9479505]
95. Balhuizen A, et al. Activation of G protein-coupled receptor 30 modulates hormone secretion and counteracts cytokine-induced apoptosis in pancreatic islets of female mice. *Mol Cell Endocrinol.* 2010; 320(1–2):16–24. [PubMed: 20122988]
96. Hugo ER, et al. Bisphenol A at environmentally relevant doses inhibits adiponectin release from human adipose tissue explants and adipocytes. *Environ Health Perspect.* 2008; 116(12):1642–1647. [PubMed: 19079714]
97. Kanda N, Watanabe S. 17[beta]-estradiol inhibits oxidative stress-induced apoptosis in keratinocytes by promoting Bcl-2 expression. *J Invest Dermatol.* 2003; 121(6):1500–1509. [PubMed: 14675202]
98. Mårtensson UEA, et al. Deletion of the G protein-coupled receptor 30 impairs glucose tolerance, reduces bone growth, increases blood pressure, and eliminates estradiol-stimulated insulin release in female mice. *Endocrinology.* 2009; 150(2):687–698. [PubMed: 18845638]
99. Nadal A, et al. The role of oestrogens in the adaptation of islets to insulin resistance. *J Physiol.* 2009; 587(21):5031–5037. [PubMed: 19687125]
100. Haas E, et al. Regulatory role of G protein-coupled estrogen receptor for vascular function and obesity. *Circ Res.* 2009; 104(3):288–291. [PubMed: 19179659]
101. Liu S, et al. Importance of extranuclear estrogen receptor- α and membrane G protein-coupled estrogen receptor in pancreatic islet survival. *Diabetes.* 2009; 58(10):2292–2302. [PubMed: 19587358]
102. Blasko E, et al. Beneficial role of the GPR30 agonist G-1 in an animal model of multiple sclerosis. *J Neuroimmunol.* 2009; 214(1–2):67–77. [PubMed: 19664827]
103. Noel SD, et al. Involvement of G protein-coupled receptor 30 (GPR30) in rapid action of estrogen in primate LHRH neurons. *Mol Endocrinol.* 2009; 23(3):349–359. [PubMed: 19131510]
104. Rettew JA, McCall SH, Marriott I. GPR30/GPER-1 mediates rapid decreases in TLR4 expression on murine macrophages. *Mol Cell Endocrinol.* 2010; 328(1–2):87–92. [PubMed: 20654686]
105. Terasawa E, Noel SD, Keen KL. Rapid action of oestrogen in luteinising hormone-releasing hormone neurones: the role of GPR30. *J Neuroendocrinol.* 2009; 21(4):316–321. [PubMed: 19207808]
106. Brailoiu E, et al. Distribution and characterization of estrogen receptor G protein-coupled receptor 30 in the rat central nervous system. *J Endocrinol.* 2007; 193(2):311–321. [PubMed: 17470522]

107. Owman C, et al. Cloning of human cDNA encoding a novel heptahelix receptor expressed in Burkitt's lymphoma and widely distributed in brain and peripheral tissues. *Biochem Biophys Res Commun.* 1996; 228(2):285–292. [PubMed: 8920907]
108. Takada Y, et al. Cloning of cDNAs encoding G protein-coupled receptor expressed in human endothelial cells exposed to fluid shear stress. *Biochem Biophys Res Commun.* 1997; 240(3): 737–741. [PubMed: 9398636]
109. Wang C, Prossnitz ER, Roy SK. Expression of G protein-coupled receptor 30 in the hamster ovary: differential regulation by gonadotropins and steroid hormones. *Endocrinology.* 2007; 148(10):4853–4864. [PubMed: 17640985]
110. Canonaco M, et al. A sexually dimorphic distribution pattern of the novel estrogen receptor G-protein-coupled receptor 30 in some brain areas of the hamster. *J Endocrinol.* 2008; 196(1):131–138. [PubMed: 18180324]
111. Wang D, et al. G protein-coupled receptor 30 in tumor development. *Endocrine.* 2010; 38(1):29–37. [PubMed: 20960099]
112. Pietras R, Nemere I, Szego C. Steroid hormone receptors in target cell membranes. *Endocrine.* 2001; 14(3):417–427. [PubMed: 11444440]
113. Aronica SM, Kraus WL, Katzenellenbogen BS. Estrogen action via the cAMP signaling pathway: stimulation of adenylate cyclase and cAMP-regulated gene transcription. *Proc Natl Acad Sci U S A.* 1994; 91(18):8517–8521. [PubMed: 8078914]
114. Ding Q, et al. Estradiol-mediated ERK phosphorylation and apoptosis in vascular smooth muscle cells requires GPR 30. *Am J Physiol Cell Physiol.* 2009; 297(5):C1178–C1187. [PubMed: 19741198]
115. Filardo E, et al. Activation of the novel estrogen receptor G protein-coupled receptor30 (GPR30) at the plasma membrane. *Endocrinology.* 2007; 148(7):3236–3245. [PubMed: 17379646]
116. Weil BR, et al. Signaling via GPR30 protects the myocardium from ischemia/reperfusion injury. *Surgery.* 2010; 148(2):436–443. [PubMed: 20434187]
117. Bologa CG, et al. Virtual and biomolecular screening converge on a selective agonist for GPR30. *Nat Chem Biol.* 2006; 2(4):207–212. [PubMed: 16520733]
118. Broughton BRS, Miller AA, Sobey CG. Endothelium-dependent relaxation by G protein-coupled receptor 30 agonists in rat carotid arteries. *Am J Physiol Heart Circ Physiol.* 2010; 298(3):H1055–H1061. [PubMed: 20061543]
119. Dennis MK, et al. In vivo effects of a GPR30 antagonist. *Nat Chem Biol.* 2009; 5(6):421–427. [PubMed: 19430488]
120. Chow RWY, Handelsman DJ, Ng MKC. Minireview: rapid actions of sex steroids in the endothelium. *Endocrinology.* 2010; 151(6):2411–2422. [PubMed: 20392826]
121. Lin BC, et al. Stimulating the GPR30 estrogen receptor with a novel tamoxifen analogue activates SF-1 and promotes endometrial cell proliferation. *Cancer Res.* 2009; 69(13):5415–5423. [PubMed: 19549922]
122. Meli R, et al. Estrogen and raloxifene modulate leptin and its receptor in hypothalamus and adipose tissue from ovariectomized rats. *Endocrinology.* 2004; 145(7):3115–3121. [PubMed: 15059958]
123. Baptista T, et al. Tamoxifen prevents sulpiride-induced weight gain in female rats. *Pharmacol Biochem Behav.* 1997; 57(1–2):215–222. [PubMed: 9164575]
124. Wang C, et al. GPR30 contributes to estrogen-induced thymic atrophy. *Mol Endocrinol.* 2008; 22(3):636–648. [PubMed: 18063692]
125. Isensee J, et al. Expression pattern of G protein-coupled receptor 30 in LacZ reporter mice. *Endocrinology.* 2009; 150(4):1722–1730. [PubMed: 19095739]
126. Otto C, et al. GPR30 does not mediate estrogenic responses in reproductive organs in mice. *Biol Reprod.* 2009; 80(1):34–41. [PubMed: 18799753]
127. Ford J, et al. GPR30 deficiency causes increased bone mass, mineralization, and growth plate proliferative activity in male mice. *J Bone Miner Res.* 2011; 26(2):298–307. [PubMed: 20734455]
128. Windahl SH, et al. The role of the G protein-coupled receptor GPR30 in the effects of estrogen in ovariectomized mice. *Am J Physiol EndocrinolMetab.* 2009; 296(3):E490–E496.

129. Olde B, Leeb-Lundberg LMF. GPR30/GPER1: searching for a role in estrogen physiology. *Trends Endocrinol Metab.* 2009; 20(8):409–416. [PubMed: 19734054]
130. Langer G, et al. A critical review of fundamental controversies in the field of GPR30 research. *Steroids.* 2010; 75(8–9):603–610. [PubMed: 20034504]
131. Ohlsson C, Vandenput L. The role of estrogens for male bone health. *Eur J Endocrinol.* 2009; 160(6):883–889. [PubMed: 19304870]
132. Prossnitz ER, Barton M. The G-protein-coupled estrogen receptor GPER in health and disease. *Nat Rev Endocrinol.* 2011; 7(12):715–726. [PubMed: 21844907]
133. Pedram A, et al. Functional estrogen receptors in the mitochondria of breast cancer cells. *Mol Biol Cell.* 2006; 17(5):2125–2137. [PubMed: 16495339]
134. Roepke TA. Oestrogen modulates hypothalamic control of energy homeostasis through multiple mechanisms. *J Neuroendocrinol.* 2009; 21(2):141–150. [PubMed: 19076267]
135. Zhang C, Kelly MJ, Rønnekleiv OK. 17 β b-Estradiol rapidly increases K(ATP) activity in GnRH via a protein kinase signaling pathway. *Endocrinology.* 2010; 151(9):4477–4484. [PubMed: 20660067]
136. Kenealy BP, et al. STX, a novel nonsteroidal estrogenic compound, induces rapid action in primate GnRH neuronal calcium dynamics and peptide release. *Endocrinology.* 2011; 152(8):3182–3191. [PubMed: 21628385]
137. Kuo J, et al. Membrane estrogen receptors stimulate intracellular calcium release and progesterone synthesis in hypothalamic astrocytes. *J Neurosci.* 2010; 30(39):12950–12957. [PubMed: 20881113]
138. Tobias SC, et al. Synthesis and biological evaluation of SERMs with potent nongenomic estrogenic activity. *ChemMedChem.* 2006; 1(5):565–571. [PubMed: 16892393]
139. Clipperton Allen AE, et al. Agonistic behavior in males and females: effects of an estrogen receptor beta agonist in gonadectomized and gonadally intact mice. *Psychoneuroendocrinology.* 2010; 35(7):1008–1022. [PubMed: 20129736]
140. Clipperton-Allen AE, et al. Effects of an estrogen receptor alpha agonist on agonistic behaviour in intact and gonadectomized male and female mice. *Psychoneuroendocrinology.* 2011; 36(7):981–995. [PubMed: 21247705]
141. Chang Y-J, et al. Estrogen modulates sexually dimorphic contextual fear extinction in rats through estrogen receptor β . *Hippocampus.* 2009; 19(11):1142–1150. [PubMed: 19338017]
142. Chen Z, et al. Estrogen receptor α mediates the nongenomic activation of endothelial nitric oxide synthase by estrogen. *J Clin Invest.* 1999; 103(3):401–406. [PubMed: 9927501]
143. Kang L, et al. Involvement of estrogen receptor variant ER- α 36, not GPR30, in nongenomic estrogen signaling. *Mol Endocrinol.* 2010; 24(4):709–721. [PubMed: 20197310]
144. Chambliss KL, et al. Non-nuclear estrogen receptor α signaling promotes cardiovascular protection but not uterine or breast cancer growth in mice. *J Clin Invest.* 2010; 120(7):2319–2330. [PubMed: 20577047]
145. Wang Z, et al. A variant of estrogen receptor- α , hER- α 36: transduction of estrogen and antiestrogen-dependent membrane-initiated mitogenic signaling. *Proc Natl Acad Sci U S A.* 2006; 103(24):9063–9068. [PubMed: 16754886]
146. Pelekanou V, et al. ER α 36, a new variant of the ER α is expressed in triple negative breast carcinomas and has a specific transcriptomic signature in breast cancer cell lines. *Steroids.* 2012; 77:928–934. [PubMed: 22198466]
147. Wallacides A, et al. Estrogens promote proliferation of the seminoma-like Tcam-2 cell line through a GPER-dependent ER α 36 induction. *Mol Cell Endocrinol.* 2012; 350(1):61–71. [PubMed: 22138413]
148. Lindsey SH, et al. Estrogen receptor GPR30 reduces oxidative stress and proteinuria in the salt-sensitive female mRen2. Lewis rat. *Hypertension.* 2011; 58(4):665–671. [PubMed: 21844484]
149. Zhang X, et al. Estrogen receptor-alpha 36 mediates mitogenic antiestrogen signaling in ER-negative breast cancer cells. *PLoS One.* 2012; 7(1):e30174. [PubMed: 22276155]
150. Albanito L, et al. G protein-coupled receptor 30 (GPR30) mediates gene expression changes and growth response to 17 β -estradiol and selective GPR30 ligand G-1 in ovarian cancer cells. *Cancer Res.* 2007; 67(4):1859–1866. [PubMed: 17308128]

151. Gao F, et al. GPR30 activation opposes estrogen-dependent uterine growth via inhibition of stromal ERK1/2 and estrogen receptor alpha (ER α) phosphorylation signals. *Endocrinology*. 2011; 152(4):1434–1447. [PubMed: 21303939]
152. Lappano R, et al. Estriol acts as a GPR30 antagonist in estrogen receptor-negative breast cancer cells. *Mol Cell Endocrinol*. 2010; 320(1–2):162–170. [PubMed: 20138962]
153. Razandi M, et al. Proximal events in signaling by plasma membrane estrogen receptors. *J Biol Chem*. 2003; 278(4):2701–2712. [PubMed: 12421825]
154. Vivacqua A, et al. G protein-coupled receptor 30 expression is up-regulated by EGF and TGF alpha in estrogen receptor alpha-positive cancer cells. *Mol Endocrinol*. 2009; 23(11):1815–1826. [PubMed: 19749156]
155. Huang GS, et al. Co-expression of GPR30 and ER β and their association with disease progression in uterine carcinosarcoma. *Am J Obstet Gynecol*. 2010; 203(3):242.e1–242.e5. [PubMed: 20605134]
156. Filardo EJ, et al. Distribution of GPR30, a seven membrane-spanning estrogen receptor, in primary breast cancer and its association with clinicopathologic determinants of tumor progression. *Clin Cancer Res*. 2006; 12(21):6359–6366. [PubMed: 17085646]
157. Tica AA, et al. G protein-coupled estrogen receptor 1-mediated effects in the rat myometrium. *Am J Physiol Cell Physiol*. 2011; 301(5):C1262–C1269. [PubMed: 21865584]
158. Navarro CE, et al. Regulation of cyclic adenosine 3',5'-monophosphate signaling and pulsatile neurosecretion by Gi-coupled plasma membrane estrogen receptors in immortalized gonadotropin-releasing hormone neurons. *Mol Endocrinol*. 2003; 17(9):1792–1804.
159. Rønnekleiv OK, Kelly MJ. Diversity of ovarian steroid signaling in the hypothalamus. *Front Neuroendocrinol*. 2005; 26(2):65–84. [PubMed: 16009409]
160. Bopassa JC, et al. A novel estrogen receptor GPER inhibits mitochondria permeability transition pore opening and protects the heart against ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol*. 2010; 298(1):H16–H23. [PubMed: 19880667]
161. Jayachandran M, et al. Loss of estrogen receptor b decreases mitochondrial energetic potential and increases thrombogenicity of platelets in aged female mice. *Age (Dordr)*. 2010; 32(1):109–121. [PubMed: 19908165]
162. Lappano R, et al. MIBE acts as antagonist ligand of both estrogen receptor alpha and GPER in breast cancer cells. *Breast Cancer Res*. 2012; 14(1):R12. [PubMed: 22251451]
163. Morton GJ, et al. Central nervous system control of food intake and body weight. *Nature*. 2006; 443(7109):289–295. [PubMed: 16988703]
164. Pillot B, et al. Role of hypothalamic melanocortin system in adaptation of food intake to food protein increase in mice. *PLoS One*. 2011; 6(4):e19107. [PubMed: 21544212]
165. Asarian L, Geary N. Modulation of appetite by gonadal steroid hormones. *Philos T Roy Soc B*. 2006; 361(1471):1251–1263.
166. Laudenslager ML, et al. Energy balance in ovariectomized rats with and without estrogen replacement. *Am J Physiol*. 1980; 238(5):R400–R405. [PubMed: 7377378]
167. Ahdieh HB, Wade GN. Effects of hysterectomy on sexual receptivity, food intake, running wheel activity, and hypothalamic estrogen and progesterin receptors in rats. *J Comp Physiol Psychol*. 1982; 96(6):886–892. [PubMed: 7153386]
168. Colvin GB, Sawyer CH. Induction of running activity by intracerebral implants of estrogen in ovariectomized rats. *Neuroendocrinology*. 1969; 4(4–5):309–320. [PubMed: 4894216]
169. Polidori C, Geary N. Estradiol treatment fails to affect the feeding responses to melanocortin-3/4 receptor agonism or antagonism in ovariectomized rats. *Peptides*. 2002; 23(9):1697–1700. [PubMed: 12217431]
170. Rocha M, et al. Physiologic estradiol levels enhance hypothalamic expression of the long form of the leptin receptor in intact rats. *J Nutr Biochem*. 2004; 15(6):328–334. [PubMed: 15157938]
171. Olofsson LE, Pierce AA, Xu AW. Functional requirement of AgRP and NPY neurons in ovarian cycle-dependent regulation of food intake. *Proc Natl Acad Sci U S A*. 2009; 106(37):15932–15937. [PubMed: 19805233]
172. Xu Y, et al. Distinct hypothalamic neurons mediate estrogenic effects on energy homeostasis and reproduction. *Cell Metab*. 2011; 14(4):453–465. [PubMed: 21982706]

173. de Souza FSJ, et al. The estrogen receptor α colocalizes with proopiomelanocortin in hypothalamic neurons and binds to a conserved motif present in the neuron-specific enhancer nPE2. *Eur J Pharmacol.* 2011; 660(1):181–187. [PubMed: 21211522]
174. Miller MM, et al. Effects of age and long-term ovariectomy on the estrogen–receptor containing subpopulations of beta-endorphin-immunoreactive neurons in the arcuate nucleus of female C57BL/6J mice. *Neuroendocrinology.* 1995; 61(5):542–551. [PubMed: 7617132]
175. Malyala A, et al. PI3K signaling effects in hypothalamic neurons mediated by estrogen. *J Comp Neurol.* 2008; 506(6):895–911. [PubMed: 18085586]
176. Pappas T, Gametchu B, Watson C. Membrane estrogen receptors identified by multiple antibody labeling and impeded-ligand binding. *FASEB J.* 1995; 9(5):404–410. [PubMed: 7896011]
177. Razandi M, et al. Cell membrane and nuclear estrogen receptors (ERs) originate from a single transcript: studies of ER α and ER β expressed in Chinese hamster ovary cells. *Mol Endocrinol.* 1999; 13(2):307–319. [PubMed: 9973260]
178. Merchenthaler I, et al. Distribution of estrogen receptor α and β in the mouse central nervous system: in vivo autoradiographic and immunocytochemical analyses. *J Comp Neurol.* 2004; 473(2):270–291. [PubMed: 15101093]
179. Österlund M, et al. Differential distribution and regulation of estrogen receptor- α and - β mRNA within the female rat brain. *Mol Brain Res.* 1998; 54(1):175–180. [PubMed: 9526077]
180. Simerly RB, et al. Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an in situ hybridization study. *J Comp Neurol.* 1990; 294(1):76–95. [PubMed: 2324335]
181. Shima N, Yamaguchi Y, Yuri K. Distribution of estrogen receptor β mRNA-containing cells in ovariectomized and estrogen-treated female rat brain. *Anat Sci Int.* 2003; 78(2):85–97. [PubMed: 12828421]
182. Laflamme N, et al. Expression and neuropeptidergic characterization of estrogen receptors (ER α and ER β) throughout the rat brain: anatomical evidence of distinct roles of each subtype. *J Neurobiol.* 1998; 36(3):357–378. [PubMed: 9733072]
183. Mitra SW, et al. Immunolocalization of estrogen receptor β in the mouse brain: comparison with estrogen receptor α . *Endocrinology.* 2003; 144(5):2055–2067. [PubMed: 12697714]
184. Shughrue PJ, et al. Comparative distribution of estrogen receptor- α (ER- α) and β (ER- β) mRNA in the rat pituitary, gonad, and reproductive tract. *Steroids.* 1998; 63(10):498–504. [PubMed: 9800279]
185. Horvath TL. Synaptic plasticity in energy balance regulation. *Obesity (Silver Spring).* 2006; 14(S8):228S–233S. [PubMed: 17021372]
186. Thammacharoen S, et al. Divergent effects of estradiol and the estrogen receptor- [alpha] agonist PPT on eating and activation of PVN CRH neurons in ovariectomized rats and mice. *Brain Res.* 2009; 1268:88–96. [PubMed: 19281799]
187. Musatov S, et al. Silencing of estrogen receptor α in the ventromedial nucleus of hypothalamus leads to metabolic syndrome. *Proc Natl Acad Sci U S A.* 2007; 104(7):2501–2506. [PubMed: 17284595]
188. Kelly MJ, et al. Estrogen modulation of G-protein-coupled receptor activation of potassium channels in the central nervous system. *Ann N Y Acad Sci.* 2003; 1007(1):6–9. [PubMed: 14993035]
189. Hazell GGJ, et al. Localisation of GPR30, a novel G protein-coupled oestrogen receptor, suggests multiple functions in rodent brain and peripheral tissues. *J Endocrinol.* 2009; 202(2):223–236. [PubMed: 19420011]
190. Spary EJ, Maqbool A, Batten TFC. Oestrogen receptors in the central nervous system and evidence for their role in the control of cardiovascular function. *J Chem Neuroanat.* 2009; 38(3): 185–196. [PubMed: 19505570]
191. Sakamoto H, et al. Expression of G protein-coupled receptor-30, a G protein-coupled membrane estrogen receptor, in oxytocin neurons of the rat paraventricular and supraoptic nuclei. *Endocrinology.* 2007; 148(12):5842–5050. [PubMed: 17872373]
192. Milner TA, et al. Ultrastructural evidence that hippocampal alpha estrogen receptors are located at extranuclear sites. *J Comp Neurol.* 2001; 429(3):355–371. [PubMed: 11116225]

193. Marin R, et al. An oestrogen membrane receptor participates in estradiol actions for the prevention of amyloid- α peptide 1–40-induced toxicity in septal-derived cholinergic SN56 cells. *J Neurochem.* 2003; 85(5):1180–1189. [PubMed: 12753077]
194. Marin R, et al. An ICI 182,780-sensitive, membrane-related estrogen receptor contributes to estrogenic neuroprotective actions against amyloid-beta toxicity. *Ann N Y Acad Sci.* 2003; 1007(1):108–116. [PubMed: 14993045]
195. Xu Y, et al. Neurite-localized estrogen receptor- α mediates rapid signaling by estrogen. *J Neurosci Res.* 2003; 74(1):1–11. [PubMed: 13130501]
196. Milner TA, et al. Ultrastructural localization of estrogen receptor β immunoreactivity in the rat hippocampal formation. *J Comp Neurol.* 2005; 491(2):81–95. [PubMed: 16127691]
197. Kelly MJ, Levin ER. Rapid actions of plasma membrane estrogen receptors. *Trends Endocrinol Metab.* 2001; 12(4):152–156. [PubMed: 11295570]
198. Hukshorn CJ, et al. The effect of pegylated recombinant human leptin (PEG-OB) on weight loss and inflammatory status in obese subjects. *Int J Obes Relat Metab Disord.* 2002; 26(4):504–509. [PubMed: 12075577]
199. Bueno J, Pfaff DW. Single unit recording in hypothalamus and preoptic area of estrogen-treated and untreated ovariectomized female rats. *Brain Res.* 1976; 101(1):67–78. [PubMed: 1244221]
200. Yagi K. Changes in firing rates of single preoptic and hypothalamic units following an intravenous administration of estrogen in the castrated female rat. *Brain Res.* 1973; 53(2):343–352. [PubMed: 4706033]
201. Kelly MJ, Moss RL, Dudley CA. The effect of ovariectomy on the responsiveness of preoptic-septal neurons to microelectrophoresed estrogen. *Neuroendocrinology.* 1978; 25(4):204–211. [PubMed: 652126]
202. Kelly MJ, Moss RL, Dudley CA. Differential sensitivity of preoptic-septal neurons to microelectrophoresed estrogen during the estrous cycle. *Brain Res.* 1976; 114(1):152–157. [PubMed: 986858]
203. Kelly MJ, Moss RL, Dudley CA. The effects of microelectrophoretically applied estrogen, cortisol and acetylcholine on medial preoptic-septal unit activity throughout the estrous cycle of the female rat. *Exp Brain Res.* 1977; 30(1):53–64. [PubMed: 563341]
204. Kelly MJ, et al. The specificity of the response of preoptic-septal area neurons to estrogen: 17α -estradiol versus 17β -estradiol and the response of extrahypothalamic neurons. *Exp Brain Res.* 1977; 30(1):43–52. [PubMed: 563340]
205. Balthazart J, Baillien M, Ball GF. Phosphorylation processes mediate rapid changes of brain aromatase activity. *J Steroid Biochem Mol Biol.* 2001; 79(1–5):261–277. [PubMed: 11850233]
206. Sutter-Dub M-T. Rapid non-genomic and genomic responses to progestogens, estrogens, and glucocorticoids in the endocrine pancreatic B cell, the adipocyte and other cell types. *Steroids.* 2002; 67(2):77–93. [PubMed: 11755172]
207. Ivanova T, et al. Rapid stimulation of the PI3-kinase/Akt signalling pathway in developing midbrain neurones by oestrogen. *J Neuroendocrinol.* 2002; 14(1):73–79. [PubMed: 11903815]
208. Singh M. Ovarian hormones elicit phosphorylation of akt and extracellular-signal regulated kinase in explants of the cerebral cortex. *Endocrine.* 2001; 14(3):407–415. [PubMed: 11444439]
209. Wilson ME, Liu Y, Wise PM. Estradiol enhances Akt activation in cortical explant cultures following neuronal injury. *Mol Brain Res.* 2002; 102(1–2):48–54. [PubMed: 12191493]
210. Vasudevan N, Pfaff DW. Non-genomic actions of estrogens and their interaction with genomic actions in the brain. *Front Neuroendocrinol.* 2008; 29(2):238–257. [PubMed: 18083219]
211. Watters JJ, et al. 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.* 1997; 138(9):4030–4033. [PubMed: 9275096]
212. Carrer HF, Cambiasso MJ, Gorosito S. Effects of estrogen on neuronal growth and differentiation. *J Steroid Biochem Mol Biol.* 2005; 93(2–5):319–323. [PubMed: 15860276]
213. Kuroki Y, et al. Putative membrane-bound estrogen receptors possibly stimulate mitogen-activated protein kinase in the rat hippocampus. *Eur J Pharmacol.* 2000; 400(2–3):205–209. [PubMed: 10988335]

214. Shingo AS, Kito S. Estradiol induces PKA activation through the putative membrane receptor in the living hippocampal neuron. *J Neural Transm.* 2005; 112(11):1469–1473. [PubMed: 16245068]
215. Beyer C, Raab H. Nongenomic effects of oestrogen: embryonic mouse midbrain neurones respond with a rapid release of calcium from intracellular stores. *Eur J Neurosci.* 1998; 10(1): 255–262. [PubMed: 9753134]
216. Micevych PE, et al. Estradiol stimulates progesterone synthesis in hypothalamic astrocyte cultures. *Endocrinology.* 2007; 148(2):782–789. [PubMed: 17095591]
217. Stevis PE, et al. Differential effects of estradiol and estradiol-BSA conjugates. *Endocrinology.* 1999; 140(11):5455–5458. [PubMed: 10537181]
218. Taguchi Y, Koslowski M, Bodenner D. Binding of estrogen receptor with estrogen conjugated to bovine serum albumin (BSA). *Nuclear Receptor.* 2004; 2(1):5. [PubMed: 15318942]
219. Chaban VV, Micevych PE. Estrogen receptor- α mediates estradiol attenuation of ATP-induced Ca^{2+} signaling in mouse dorsal root ganglion neurons. *J Neurosci Res.* 2005; 81(1):31–37. [PubMed: 15952176]
220. Ábrahám IM, et al. Critical in vivo roles for classical estrogen receptors in rapid estrogen actions on intracellular signaling in mouse brain. *Endocrinology.* 2004; 145(7):3055–3061. [PubMed: 14976146]
221. Singh M, et al. Estrogen-induced activation of mitogen-activated protein kinase in cerebral cortical explants: convergence of estrogen and neurotrophin signaling pathways. *J Neurosci.* 1999; 19(4):1179–1188. [PubMed: 9952396]
222. Dominguez-Salazar E, Shetty S, Rissman EF. Rapid neural Fos responses to oestradiol in oestrogen receptor α double knockout mice. *J Neuroendocrinol.* 2006; 18(3):195–202. [PubMed: 16454803]
223. Schiöth HB. G protein-coupled receptors in regulation of body weight. *CNS Neurol Disord Drug Targets.* 2006; 5(3):241–249. [PubMed: 16787226]
224. Björnström L, Sjöberg M. Mechanisms of estrogen receptor signaling: convergence of genomic and nongenomic actions on target genes. *Mol Endocrinol.* 2005; 19(4):833–842. [PubMed: 15695368]
225. Levin ER. Integration of the extranuclear and nuclear actions of estrogen. *Mol Endocrinol.* 2005; 19(8):1951–1959. [PubMed: 15705661]
226. Sheldahl LC, et al. Estrogen induces rapid translocation of estrogen receptor β , but not estrogen receptor α , to the neuronal plasma membrane. *Neuroscience.* 2008; 153(3):751–761. [PubMed: 18406537]
227. Vasudevan N, Kow LM, Pfaff D. Integration of steroid hormone initiated membrane action to genomic function in the brain. *Steroids.* 2005; 70(5–7):388–396. [PubMed: 15862822]
228. Qiu J, et al. Serotonin 5-hydroxytryptamine $2C$ receptor signaling in hypothalamic proopiomelanocortin neurons: role in energy homeostasis in females. *Mol Pharmacol.* 2007; 72(4):885–896. [PubMed: 17622577]
229. Kelly MJ, et al. Rapid effects of estrogen on G protein-coupled receptor activation of potassium channels in the central nervous system (CNS). *J Steroid Biochem Mol Biol.* 2002; 83(1–5):187–193. [PubMed: 12650715]
230. Kow LM, et al. Acute estradiol application increases inward and decreases outward whole-cell currents of neurons in rat hypothalamic ventromedial nucleus. *Brain Res.* 2006; 1116(1):1–11. [PubMed: 16942760]
231. Cooke PS, Naaz A. Role of estrogens in adipocyte development and function. *Exp Biol Med.* 2004; 229(11):1127–1135.
232. Group EHBCC. Body Mass Index, Serum Sex Hormones, and Breast Cancer Risk in Postmenopausal Women. *J Natl Cancer Inst.* 2003; 95(16):1218–1226. [PubMed: 12928347]
233. Iguchi T, et al. Developmental effects: oestrogen-induced vaginal changes and organotin-induced adipogenesis. *Int J Androl.* 2008; 31(2):263–268. [PubMed: 18248399]
234. Macotela Y, et al. Sex and depot differences in adipocyte insulin sensitivity and glucose metabolism. *Diabetes.* 2009; 58(4):803–812. [PubMed: 19136652]

235. Dyck DJ, Heigenhauser GJF, Bruce CR. The role of adipokines as regulators of skeletal muscle fatty acid metabolism and insulin sensitivity. *Acta Physiol (Oxf)*. 2006; 186(1):5–16. [PubMed: 16497175]
236. Tanaka M, et al. Effects of estrogen on serum leptin levels and leptin mRNA expression in adipose tissue in rats. *Horm Res*. 2001; 56(3–4):98–104. [PubMed: 11847470]
237. Dieudonné M-N, et al. Sex steroids and leptin regulate 11 β -hydroxysteroid dehydrogenase I and P450 aromatase expressions in human preadipocytes: sex specificities. *J Steroid Biochem Mol Biol*. 2006; 99(4–5):189–196. [PubMed: 16621515]
238. Thornton MJ, et al. The modulation of aromatase and estrogen receptor alpha in cultured human dermal papilla cells by dexamethasone: a novel mechanism for selective action of estrogen via estrogen receptor beta? *J Invest Dermatol*. 2006; 126(9):2010–2018. [PubMed: 16691199]
239. Bryzgalova G, et al. Evidence that oestrogen receptor- α plays an important role in the regulation of glucose homeostasis in mice: insulin sensitivity in the liver. *Diabetologia*. 2006; 49(3):588–597. [PubMed: 16463047]
240. Crandall DL, et al. Identification of estrogen receptor β RNA in human breast and abdominal subcutaneous adipose tissue. *Biochem Biophys Res Commun*. 1998; 248(3):523–526. [PubMed: 9703958]
241. Lu SF, et al. Androgen receptor in mouse brain sex: differences and similarities in autoregulation. *Endocrinology*. 1998; 139(4):1594–1601. [PubMed: 9528939]
242. Yu I-C. Hyperleptinemia without obesity in male mice lacking androgen receptor in adipose tissue. *Endocrinology*. 2008; 149(5):2361–2368. [PubMed: 18276764]
243. Bryzgalova G, et al. Mechanisms of antidiabetogenic and body weight-lowering effects of estrogen in high-fat diet-fed mice. *Am J Physiol Endocrinol Metab*. 2008; 295(4):E904–E912. [PubMed: 18697913]
244. Iverius PH, Brunzell JD. Relationship between lipoprotein lipase activity and plasma sex steroid level in obese women. *J Clin Invest*. 1988; 82(3):1106–1112. [PubMed: 3417867]
245. Homma H, et al. Estrogen suppresses transcription of lipoprotein lipase gene. Existence of a unique estrogen response element on the lipoprotein lipase promoter. *J Biol Chem*. 2000; 275(15):11404–11411. [PubMed: 10753956]
246. Wohlers LM, Spangenburg EE. 17 β -estradiol supplementation attenuates ovariectomy-induced increases in ATGL signaling and reduced perilipin expression in visceral adipose tissue. *J Cell Biochem*. 2010; 110(2):420–427. [PubMed: 20336671]
247. Jones MEE, et al. Aromatase-deficient (ArKO) mice have a phenotype of increased adiposity. *Proc Natl Acad Sci U S A*. 2000; 97(23):12735–12740. [PubMed: 11070087]
248. Couse JF, Korach KS. Estrogen receptor null mice: what have we learned and where will they lead us? *Endocr Rev*. 1999; 20(3):358–417. [PubMed: 10368776]
249. Lindberg M, et al. Estrogen receptor specificity for the effects of estrogen in ovariectomized mice. *J Endocrinol*. 2002; 174(2):167–178. [PubMed: 12176656]
250. Misso ML, et al. Cellular and molecular characterization of the adipose phenotype of the aromatase-deficient mouse. *Endocrinology*. 2003; 144(4):1474–1480. [PubMed: 12639931]
251. Jones MEE, et al. Of mice and men: the evolving phenotype of aromatase deficiency. *Trends Endocrinol Metab*. 2006; 17(2):55–64. [PubMed: 16480891]
252. Foryst-Ludwig A, et al. Metabolic actions of estrogen receptor beta (ER β) are mediated by a negative cross-talk with PPAR γ . *PLoS Genet*. 2008; 4(6):e1000108. [PubMed: 18584035]
253. Luconi M, Forti G, Baldi E. Genomic and nongenomic effects of estrogens: molecular mechanisms of action and clinical implications for male reproduction. *J Steroid Biochem Mol Biol*. 2002; 80(4–5):369–381. [PubMed: 11983484]
254. Pedram A, et al. Integration of the non-genomic and genomic actions of estrogen Membrane-initiated signaling by steroid to transcription and cell biology. *J Biol Chem*. 2002; 277(52):50768–50775. [PubMed: 12372818]
255. Mayes JS, Watson GH. Direct effects of sex steroid hormones on adipose tissues and obesity. *Obes Rev*. 2004; 5(4):197–216. [PubMed: 15458395]

256. Pedersen SB, et al. Demonstration of estrogen receptor subtypes α and β in human adipose tissue: influences of adipose cell differentiation and fat depot localization. *Mol Cell Endocrinol.* 2001; 182(1):27–37. [PubMed: 11500236]
257. Anwar A, et al. Site-specific regulation of oestrogen receptor- α and - β by oestradiol in human adipose tissue. *Diabetes Obes Metab.* 2001; 3(5):338–349. [PubMed: 11703424]
258. Price TM, O'Brien SN. Determination of estrogen receptor messenger ribonucleic acid (mRNA) and cytochrome P450 aromatase mRNA levels in adipocytes and adipose stromal cells by competitive polymerase chain reaction amplification. *J Clin Endocrinol Metab.* 1993; 77(4): 1041–1045. [PubMed: 8408452]
259. Dieudonné MN, et al. Evidence for functional estrogen receptors α and β in human adipose cells: regional specificities and regulation by estrogens. *Am J Physiol Cell Physiol.* 2004; 286(3):C655–C661. [PubMed: 14761887]
260. Mizutani T, et al. Identification of estrogen receptor in human adipose tissue and adipocytes. *J Clin Endocrinol Metab.* 1994; 78(4):950–954. [PubMed: 8157726]
261. Rebuffé-Scrive M, et al. Steroid hormone receptors in human adipose tissues. *J Clin Endocrinol Metab.* 1990; 71(5):1215–1219. [PubMed: 2229280]
262. Brönnegård M, et al. Lack of evidence for estrogen and progesterone receptors in human adipose tissue. *J Steroid Biochem Mol Biol.* 1994; 51(5–6):275–281. [PubMed: 7826889]
263. Pedersen SB, et al. Identification of oestrogen receptors and oestrogen receptor mRNA in human adipose tissue. *Eur J Clin Invest.* 1996; 26(4):262–269. [PubMed: 8732482]
264. Joyner J, Hutley L, Cameron D. Estrogen receptors in human preadipocytes. *Endocrine.* 2001; 15(2):225–230. [PubMed: 11720251]
265. Lubahn DB, et al. Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene. *Proc Natl Acad Sci U S A.* 1993; 90(23):11162–11166. [PubMed: 8248223]
266. Wade GN, Gray JM. Cytoplasmic 17 β -[3H] estradiol binding in rat adipose tissues. *Endocrinology.* 1978; 103(5):1695–1701. [PubMed: 748011]
267. Watson GH, et al. Biochemical and immunological characterization of oestrogen receptor in the cytosolic fraction of gluteal, omental and perirenal adipose tissues from sheep. *J Endocrinol.* 1993; 139(1):107–115. [PubMed: 8254283]
268. Mayes JS, et al. Regional differences and up-regulation of progesterone receptors in adipose tissues from oestrogen-treated sheep. *J Endocrinol.* 1996; 148(1):19–25. [PubMed: 8568467]
269. McCann J, et al. Subcellular distribution and glycosylation pattern of androgen receptor from sheep omental adipose tissue. *J Endocrinol.* 2001; 169(3):587–593. [PubMed: 11375129]
270. Rodriguez-Cuenca S, et al. Depot differences in steroid receptor expression in adipose tissue: possible role of the local steroid milieu. *Am J Physiol Endocrinol Metab.* 2005; 288(1):E200–E207. [PubMed: 15367392]
271. Barros RPA, et al. Participation of ER α and ER β in glucose homeostasis in skeletal muscle and white adipose tissue. *Am J Physiol Endocrinol Metab.* 2009; 297(1):E124–E133. [PubMed: 19366879]
272. Rodriguez-Cuenca S, et al. Sex steroid receptor expression profile in brown adipose tissue Effects of hormonal status. *Cell Physiol Biochem.* 2007; 20(6):877–886. [PubMed: 17982270]
273. Gray JM, Wade GN. Cytoplasmic estrogen, but not progestin, binding sites in male rat adipose tissues. *Am J Physiol.* 1980; 239(4):E237. [PubMed: 7425116]
274. Gray JM, Dudley SD, Wade GN. In vivo cell nuclear binding of 17 β -[3H]estradiol in rat adipose tissues. *Am J Physiol.* 1981; 240(1):E43–E46. [PubMed: 7457597]
275. Rebuffé-Scrive M. Sex steroid hormones and adipose tissue metabolism in ovariectomized and adrenalectomized rats. *Acta Physiol Scand.* 1987; 129(4):471–477. [PubMed: 3296658]
276. Pedersen SB, et al. Nuclear estradiol binding in rat adipocytes Regional variations and regulatory influences of hormones. *Biochim Biophys Acta.* 1991; 1093(1):80–86. [PubMed: 1646650]
277. Echeverría OM, et al. Immuno-electron microscopic localization of estradiol receptor in cells of male and female reproductive and non-reproductive organs. *Biol Cell.* 1994; 81(3):257–265. [PubMed: 7696978]

278. Cooke PS, et al. The role of estrogen and estrogen receptor- α in male adipose tissue. *Mol Cell Endocrinol.* 2001; 178(1–2):147–154. [PubMed: 11403904]
279. Casazza K, Page GP, Fernandez JR. The association between the rs2234693 and rs9340799 estrogen receptor alpha gene polymorphisms and risk factors for cardiovascular disease: a review. *Biol Res Nurs.* 2010; 12(1):84–97. [PubMed: 20702456]
280. Riant E, et al. Estrogens protect against high-fat diet-induced insulin resistance and glucose intolerance in mice. *Endocrinology.* 2009; 150(5):2109–2117. [PubMed: 19164473]
281. Naaz A, et al. Effect of ovariectomy on adipose tissue of mice in the absence of estrogen receptor alpha (ER α): a potential role for estrogen receptor beta (ER β). *Horm Metab Res.* 2002; 34(11/12) 758,763.
282. Palin SL, et al. 17Beta-estradiol and anti-estrogen ICI:Compound 182,780 regulate expression of lipoprotein lipase and hormone-sensitive lipase in isolated subcutaneous abdominal adipocytes. *Metabolism.* 2003; 52(4):383–388. [PubMed: 12701046]
283. Pedersen SB, et al. Effects of in vivo estrogen treatment on adipose tissue metabolism and nuclear estrogen receptor binding in isolated rat adipocytes. *Mol Cell Endocrinol.* 1992; 85(1–2):13–19. [PubMed: 1526313]
284. Hamosh M, Hamosh P. The effect of estrogen on the lipoprotein lipase activity of rat adipose tissue. *J Clin Invest.* 1975; 55(5):1132–1135. [PubMed: 1123425]
285. Kim HJ, Kalkhoff RK. Sex steroid influence on triglyceride metabolism. *J Clin Invest.* 1975; 56(4):888–896. [PubMed: 1159092]
286. Wilson DE, et al. Estrogen treatment and gonadal function in the regulation of lipoprotein lipase. *Atherosclerosis.* 1976; 24(3):491–499. [PubMed: 971348]
287. Steingrimsdottir L, Brasel J, Greenwood MR. Hormonal modulation of adipose tissue lipoprotein lipase may alter food intake in rats. *Am J Physiol.* 1980; 239(2):E162–E167. [PubMed: 7406041]
288. Ramirez I. Estradiol-induced changes in lipoprotein lipase, eating, and body weight in rats. *Am J Physiol.* 1981; 240(2):E533–E538. [PubMed: 7235008]
289. Ellis GS, et al. Effects of estradiol on lipoprotein lipase activity and lipid availability in exercised male rats. *J Appl Physiol.* 1994; 77(1):209–215. [PubMed: 7961235]
290. Hazzard WR, et al. Studies on the mechanism of increased plasma triglyceride levels induced by oral contraceptives. *N Engl J Med.* 1969; 280(9):471–474. [PubMed: 5763701]
291. Fabian E, et al. Lipoprotein lipase and postheparin esterase activity and postheparin increase in free fatty acids after the short-term administration of a sequential contraceptive. *Am J Obstet Gynecol.* 1971; 109(8):1212–1213. [PubMed: 5554852]
292. Ence TJ, et al. Heparin metabolism and heparin-released lipase activity during longterm estrogen-progestin treatment. *Metabolism.* 1976; 25(2):139–145. [PubMed: 1250155]
293. Price TM, et al. Estrogen regulation of adipose tissue lipoprotein lipase— possible mechanism of body fat distribution. *Am J Obstet Gynecol.* 1998; 178(1, part 1):101–107. [PubMed: 9465811]
294. Urabe M, et al. Effect of Estrogen Replacement Therapy on Hepatic Triglyceride Lipase, Lipoprotein Liase and lipids Including Apolipoprotein E in Climacteric and Elderly Women. *Endocr J.* 1996; 43(6):737–742. [PubMed: 9075616]
295. Benz V, et al. Sexual dimorphic regulation of body weight dynamics and adipose tissue lipolysis. *PLoS ONE.* 2012; 7(5):e37794. [PubMed: 22662224]
296. Pedersen SB, et al. Estrogen controls lipolysis by up-regulating α 2A-adrenergic receptors directly in human adipose tissue through the estrogen receptor α Implications for the female fat distribution. *J Clin Endocrinol Metab.* 2004; 89(4):1869–1878. [PubMed: 15070958]
297. Simoncini T, Genazzani A. Non-genomic actions of sex steroid hormones. *Eur J Endocrinol.* 2003; 148(3):281–292. [PubMed: 12611608]
298. Dos Santos EG, et al. Rapid nongenomic E2 effects on p42/p44 MAPK, activator protein-1, and cAMP response element binding protein in rat white adipocytes. *Endocrinology.* 2002; 143(3): 930–940. [PubMed: 11861515]
299. Falkenstein E, et al. Multiple actions of steroid hormones—a focus on rapid, nongenomic effects. *Pharmacol Rev.* 2000; 52(4):513–556. [PubMed: 11121509]

300. Schmidt BMW, et al. Rapid, nongenomic steroid actions: a new age? *Front Neuroendocrinol.* 2000; 21(1):57–94. [PubMed: 10662536]
301. Segars JH, Driggers PH. Estrogen action and cytoplasmic signaling cascades Part I: membrane-associated signaling complexes. *Trends Endocrinol Metab.* 2002; 13(8):349–354. [PubMed: 12217492]
302. Driggers PH, Segars JH. Estrogen action and cytoplasmic signaling pathways Part II: the role of growth factors and phosphorylation in estrogen signaling. *Trends Endocrinol Metab.* 2002; 13(10):422–427. [PubMed: 12431838]
303. Schlegel A, et al. Caveolin-1 Potentiates Estrogen Receptor α (ER α) Signaling. *J Biol Chem.* 1999; 274(47):33551–33556. [PubMed: 10559241]
304. Schlegel A, Arvan P, Lisanti MP. Caveolin-1 binding to endoplasmic reticulum membranes and entry into the regulated secretory pathway are regulated by serine phosphorylation. Protein sorting at the level of the endoplasmic reticulum. *J Biol Chem.* 2001; 276(6):4398–4408. [PubMed: 11078729]
305. Mitznegg P, Estler CJ, Schubert E. Effect of estradiol on lipolysis and adenosine 3',5'-monophosphate accumulation in isolated rat adipocytes. *Biochem Pharmacol.* 1974; 23(16):2337–2339. [PubMed: 4368617]
306. Pasquier Y-N, Pecquery R, Giudicelli Y. Increased adenylate cyclase catalytic activity explains how estrogens “in vivo” promote lipolytic activity in rat white fat cells. *Biochem Biophys Res Commun.* 1988; 154(3):1151–1159. [PubMed: 2457367]
307. Lacasa D, et al. Influence of ovariectomy and regional fat distribution on the membranous transducing system controlling lipolysis in rat fat cells. *Endocrinology.* 1991; 128(2):747–753. [PubMed: 1846586]
308. Lindberg U-B, et al. Regional adipose tissue metabolism in postmenopausal women after treatment with exogenous sex steroids. *Horm Metab Res.* 1990; 22(06) 345,351.
309. Lacasa D, et al. Influence of ovarian status and regional fat distribution on protein kinase C in rat fat cells. *J Endocrinol.* 1994; 140(2):275–282. [PubMed: 8169560]
310. Lacasa D, et al. Control of rat preadipocyte adipose conversion by ovarian status: regional specificity and possible involvement of the mitogen-activated protein kinase-dependent and c-fos signaling pathways. *Endocrinology.* 1997; 138(7):2729–2734. [PubMed: 9202210]
311. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. *Nature.* 1998; 395(6704):763–770. [PubMed: 9796811]
312. Chen S-J, et al. Estrogen reduces myointimal proliferation after balloon injury of rat carotid artery. *Circulation.* 1996; 93(3):577–584. [PubMed: 8565178]
313. Diano S, et al. Leptin receptors in estrogen receptor-containing neurons of the female rat hypothalamus. *Brain Res.* 1998; 812(1–2):256–259. [PubMed: 9813356]
314. Ahima RS, et al. Regulation of neuronal and glial proteins by leptin: implications for brain development. *Endocrinology.* 1999; 140(6):2755–2762. [PubMed: 10342866]
315. Elias CF, et al. Leptin differentially regulates NPY and POMC neurons projecting to the lateral hypothalamic area. *Neuron.* 1999; 23(4):775–786. [PubMed: 10482243]
316. Elmquist JK, Elias CF, Saper CB. From lesions to leptin: hypothalamic control of food intake and body weight. *Neuron.* 1999; 22(2):221–232. [PubMed: 10069329]
317. Chakraborty S, et al. Stereological analysis of estrogen receptor expression in the hypothalamic arcuate nucleus of ob/ob and agouti mice. *Brain Res.* 2008; 1217:86–95. [PubMed: 18502406]
318. Ainslie DA, et al. Estrogen deficiency causes central leptin insensitivity and increased hypothalamic neuropeptide Y. *Int J Obes Relat Metab Disord.* 2001; 25(11):1680–1688. [PubMed: 11753591]
319. Lindell K, et al. Leptin receptor 5' untranslated regions in the rat: relative abundance, genomic organization and relation to putative response elements. *Mol Cell Endocrinol.* 2001; 172(1–2):37–45. [PubMed: 11165038]
320. Shimizu H, et al. Estrogen increases in vivo leptin production in rats and human subjects. *J Endocrinol.* 1997; 154(2):285–292. [PubMed: 9291839]

321. Machinal F, et al. In vivo and in vitro ob gene expression and leptin secretion in rat adipocytes: evidence for a regional specific regulation by sex steroid hormones. *Endocrinology*. 1999; 140(4):1567–1574. [PubMed: 10098489]
322. Hardie L, et al. Circulating leptin in women: a longitudinal study in the menstrual cycle and during pregnancy. *Clin Endocrinol*. 1998; 53(2):87–89.
323. Rosenbaum M, et al. Effects of gender, body composition, and menopause on plasma concentrations of leptin. *J Clin Endocrinol Metab*. 1996; 81(9):3424–3427. [PubMed: 8784109]
324. Konukoglu D, Serin Ö, Ercan M. Plasma leptin levels in obese and non-obese postmenopausal women before and after hormone replacement therapy. *Maturitas*. 2000; 36(3):203–207. [PubMed: 11063902]
325. Elbers JMH, et al. Effects of administration of 17 β -oestradiol on serum leptin levels in healthy postmenopausal women. *Clin Endocrinol*. 1999; 51(4):449–454.
326. Thomas T, et al. Relationship of serum leptin levels with body composition and sex steroid and insulin levels in men and women. *Metabolism*. 2000; 49(10):1278–1284. [PubMed: 11079816]
327. Elbers JMH, et al. Effects of sex steroid hormones on regional fat depots as assessed by magnetic resonance imaging in transsexuals. *Am J Physiol Endocrinol Metab*. 1999; 276(2):E317–E325.
328. Casabiell X, et al. Gender differences in both spontaneous and stimulated leptin secretion by human omental adipose tissue in vitro: dexamethasone and estradiol stimulate leptin release in women, but not in men. *J Clin Endocrinol Metab*. 1998; 83(6):2149–2155. [PubMed: 9626154]
329. Santner SJ, et al. Aromatase activity and expression in breast cancer and benign breast tissue stromal cells. *J Clin Endocrinol Metab*. 1997; 82(1):200–208. [PubMed: 8989259]
330. Geisler J, et al. Total body aromatization in postmenopausal breast cancer patients is strongly correlated to plasma leptin levels. *J Steroid Biochem Mol Biol*. 2007; 104(1–2):27–34. [PubMed: 17350249]
331. Shin J-H, et al. The ratio of estrogen receptor α to estrogen receptor β in adipose tissue is associated with leptin production and obesity. *Steroids*. 2007; 72(6–7):592–599. [PubMed: 17509633]
332. Catalano S, et al. Leptin induces, via ERK1/ERK2 signal, functional activation of estrogen receptor α in MCF-7 cells. *J Biol Chem*. 2004; 279(19):19908–19915. [PubMed: 14985328]
333. Vecchione C, et al. Leptin effect on endothelial nitric oxide is mediated through Akt–endothelial nitric oxide synthase phosphorylation pathway. *Diabetes*. 2002; 51(1):168–173. [PubMed: 11756337]
334. Pinilla L, et al. Regulation of serum leptin levels by gonadal function in rats. *Eur J Endocrinol*. 1999; 140(5):468–473. [PubMed: 10229915]
335. Watanobe H, Suda T. A detailed study on the role of sex steroid milieu in determining plasma leptin concentrations in adult male and female rats. *Biochem Biophys Res Commun*. 1999; 259(1):56–59. [PubMed: 10334915]
336. Wu-Peng S, et al. Effects of exogenous gonadal steroids on leptin homeostasis in rats. *Obes Res*. 1999; 7(6):586–592. [PubMed: 10574518]
337. Brann DW, et al. Regulation of leptin gene expression and secretion by steroid hormones. *Steroids*. 1999; 64(9):659–663. [PubMed: 10503725]
338. Luukkaa V, et al. Effects of estrous cycle and steroid replacement on the expression of leptin and uncoupling proteins in adipose tissue in the rat. *Gynecol Endocrinol*. 2001; 15(2):103–112. [PubMed: 11379006]
339. Yoneda N, et al. The influence of ovariectomy on ob gene expression in rats. *Horm Metab Res*. 1998; 30(05):263–265. [PubMed: 9660086]
340. Chu S-C, et al. Fluctuation of serum leptin level in rats after ovariectomy and the influence of estrogen supplement. *Life Sci*. 1999; 64(24):2299–2306. [PubMed: 10374920]
341. Nedvídková J, Haluzík M, Schreiber V. The decrease of serum leptin levels in oestrogen-treated male mice. *Physiol Res*. 1997; 46(4):291–294. [PubMed: 9728495]
342. Pelleymounter MA, Baker MB, McCaleb M. Does estradiol mediate leptin's effects on adiposity and body weight? *Am J Physiol Endocrinol Metab*. 1999; 276(5):E955–E963.

343. Kronfeld-Schor N, et al. Steroid-dependent up-regulation of adipose leptin secretion in vitro during pregnancy in mice. *Biol Reprod.* 2000; 63(1):274–280. [PubMed: 10859269]
344. Halpern A, Mancini MC. Treatment of obesity: an update on anti-obesity medications. *Obes Rev.* 2003; 4(1):25–42. [PubMed: 12608525]
345. Swinburn B, Egger G. Preventive strategies against weight gain and obesity. *Obes Rev.* 2002; 3(4):289–301. [PubMed: 12458974]
346. Tchernof A, et al. Menopause, central body fatness, and insulin resistance: effects of hormone-replacement therapy. *Coron Artery Dis.* 1998; 9(8):503–512. [PubMed: 9847982]
347. Gambacciani M, et al. Body weight, body fat distribution, and hormonal replacement therapy in early postmenopausal women. *J Clin Endocrinol Metab.* 1997; 82(2):414–417. [PubMed: 9024228]
348. Haarbo J, et al. Postmenopausal hormone replacement therapy prevents central distribution of body fat after menopause. *Metabolism.* 1991; 40(12):1323–1326. [PubMed: 1961129]
349. Espeland MA, et al. Effect of postmenopausal hormone therapy on body weight and waist and hip girths. Postmenopausal Estrogen-Progestin Interventions Study Investigators. *J Clin Endocrinol Metab.* 1997; 82(5):1549–1556. [PubMed: 9141548]
350. Samaras K, et al. Tobacco smoking and oestrogen replacement are associated with lower total and central fat in monozygotic twins. *Int J Obes.* 1998; 22(2):149–156.
351. Hänggi W, et al. Differential impact of conventional oral or transdermal hormone replacement therapy or tibolone on body composition in postmenopausal women. *Clin Endocrinol.* 1998; 48(6):691–699.
352. Gambacciani M, et al. Prospective evaluation of body weight and body fat distribution in early postmenopausal women with and without hormonal replacement therapy. *Maturitas.* 2001; 39(2): 125–132. [PubMed: 11514111]
353. Sites CK, et al. Relationship between hormone replacement therapy use with body fat distribution and insulin sensitivity in obese postmenopausal women. *Metabolism.* 2001; 50(7):835–840. [PubMed: 11436191]
354. Chmouliovsky L, et al. Beneficial effect of hormone replacement therapy on weight loss in obese menopausal women. *Maturitas.* 1999; 32(3):147–153. [PubMed: 10515671]
355. Kritz-Silverstein D, Barrett-Connor E. Long-term postmenopausal hormone use, obesity, and fat distribution in older women. *JAMA.* 1996; 275(1):46–49. [PubMed: 8531286]
356. Kanaley JA, et al. Abdominal fat distribution in pre- and postmenopausal women: the impact of physical activity, age, menopausal status. *Metabolism.* 2001; 50(8):976–982. [PubMed: 11474488]
357. Nelson HD, et al. Postmenopausal hormone replacement therapy: scientific review. *JAMA.* 2002; 288(7):872–881. [PubMed: 12186605]
358. Smith CL, O'Malley BW. Coregulator function: a key to understanding tissue specificity of selective receptor modulators. *Endocr Rev.* 2004; 25(1):45–71. [PubMed: 14769827]
359. Kousteni S, et al. Reversal of bone loss in mice by nongenotropic signaling of sex steroids. *Science.* 2002; 298(5594):843–846. [PubMed: 12399595]
360. Ke HZ, et al. Effects of CP-336,156, a new, nonsteroidal estrogen agonist/antagonist, on bone, serum cholesterol, uterus and body composition in rat models. *Endocrinology.* 1998; 139(4): 2068–2076. [PubMed: 9528995]
361. McCarty MF. Modulation of adipocyte lipoprotein lipase expression as a strategy for preventing or treating visceral obesity. *Medical Hypotheses.* 2001; 57(2):192–200. [PubMed: 11461172]
362. Mantzoros CS, Flier JS. Leptin as a therapeutic agent—trials and tribulations. *J Clin Endocrinol Metab.* 2000; 85(11):4000–4002. [PubMed: 11095422]
363. Lee DW, et al. Leptin and the treatment of obesity: its current status. *Eur J Pharmacol.* 2002; 440(2–3):129–139. [PubMed: 12007531]
364. Farooqi IS, et al. Effects of recombinant leptin therapy in a child with congenital leptin deficiency. *N Engl J Med.* 1999; 341(12):879–884. [PubMed: 10486419]

Figure 6.1.

Overview of estrogenic control of energy homeostasis, glucose, and lipid metabolism by regulatory actions in the CNS, adipose tissue, pancreatic β cells, skeletal muscles, and liver. Deficiency in estrogenic action leads to opposite events and subsequent disturbances in metabolic tissues. ER α , ER β , and GPER play roles to regulate energy balance and glucose homeostasis. ER α and ER β generally mediate opposing effects, while GPER generally has similar roles as ER α in the brain, adipose tissues, and pancreas.

Figure 6.2.

Schematic overview of estradiol-mediated genomic signaling pathway via ER α/β and rapid nongenomic signaling pathways via GPER, Gq-mER, and membrane subpopulation mER α/β .