



## Sex Hormones and Cardiometabolic Health: Role of Estrogen and Estrogen Receptors

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With increased life expectancy, women will spend over three decades of life postmenopause. The menopausal transition increases susceptibility to metabolic diseases such as obesity, diabetes, cardiovascular disease, and cancer. Thus, it is more important than ever to develop effective hormonal treatment strategies to protect aging women. Understanding the role of estrogens, and their biological actions mediated by estrogen receptors (ERs), in the regulation of cardiometabolic health is of paramount importance to discover novel targeted therapeutics. In this brief review, we provide a detailed overview of the literature, from basic science findings to human clinical trial evidence, supporting a protective role of estrogens and their receptors, specifically ER $\alpha$ , in maintenance of cardiometabolic health. In so doing, we provide a concise mechanistic discussion of some of the major tissue-specific roles of estrogens signaling through ER $\alpha$ . Taken together, evidence suggests that targeted, perhaps receptor-specific, hormonal therapies can and should be used to optimize the health of women as they transition through menopause, while reducing the undesired complications that have limited the efficacy and use of traditional hormone replacement interventions. (*Endocrinology* 158: 1095–1105, 2017)

**W**ith increased life expectancy, women are spending more time in the postmenopausal phase of life, characterized in part by ovarian insufficiency and reductions in circulating estrogens, than ever before. Whereas cardiovascular disease (CVD) remains the leading cause of mortality in both men and women in the United States, the risk among women escalates following menopause for reasons that remain incompletely understood (1). Impaired estrogen signaling has been viewed as a major causal factor (2, 3). Meanwhile, the mechanistic role of estrogens and estrogen receptors (ERs) in regulating women's cardiometabolic health

remains largely unknown; and, despite decades of research, there is still confusion about the best therapeutic approaches to preserve cardiometabolic health during and following menopause. Indeed, hormone therapies have been shown to exert both positive and negative effects on a variety of endpoints in settings of ovarian hormone insufficiency. Most of our current understanding of the biological actions of estrogens is based on studies of classic estrogen signaling through its main receptors, ER $\alpha$  and ER $\beta$ . Although classical estrogen signaling results in estrogen-mediated gene expression changes, it has become apparent that estrogen receptors

ISSN Print 0013-7227 ISSN Online 1945-7170  
Printed in USA

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Received 15 September 2016. Accepted 14 February 2017.

First Published Online 17 February 2017

Abbreviations: AF, activation function; CNS, central nervous system; CVD, cardiovascular disease; ER, estrogen receptor; ERE, estrogen response element; GPR30, G protein-coupled estrogen receptor 30; HFD, high-fat diet; HRT, hormone replacement therapy; KO, knockout; PPAR, peroxisome proliferator-activated receptor; SNP, single-nucleotide polymorphism; T2DM, type 2 diabetes.

activate both genomic and nongenomic (*i.e.*, rapid signaling) pathways (1, 4). In this review, emerging findings pointing to a central role for ER $\alpha$  signaling in cardiometabolic health will be discussed. Improved understanding regarding how ER $\alpha$  signaling impacts cellular metabolism and integrative physiology will advance our understanding of disease pathobiology and lay the foundation for improved preventive care and treatment strategies for the maintenance of cardiometabolic health of women throughout the life span. Given the brevity of this review and the ever-expanding body of data on the role of ERs in cardiometabolic health, we apologize for any important studies that we may have missed.

### Estrogen Receptors: Structure, Function, and Location

The ERs were among the first of the nuclear receptor superfamilies to be cloned, second only to the glucocorticoid receptor superfamily cloned in 1985 (5–7). Tissue-specific gene targets and mechanisms of action, including activation or repression of genes involved in the integrative regulation, are an area of intense investigation. There are 30 nuclear receptor genes, and they can be grouped into three subfamilies, as follows: (1) thyroid hormone and retinoic acid receptors, (2) orphan receptors, and (3) steroid hormone receptors. The cellular effects of estrogens are mediated predominately by two ERs: ESR1 (the gene that encodes ER $\alpha$ ) and ESR2 (the gene that encodes ER $\beta$ ). ESR1 was identified in 1958 (8), and ESR2 was first identified in the rat prostate and ovary in 1996 (9). Different splice variants of each receptor have been identified, and each exhibits distinct tissue-specific expression patterns and functions (10, 11). All ERs, with the exception of the ER $\alpha$ D3 isoform, are composed of six functional domains, from A to F, which contain the NH<sub>2</sub>-terminal domain, the DNA-binding domain, and the COOH-terminal ligand-binding domain. Two regions, named activation functions (AFs), have been identified as crucial for the transcriptional response of the ERs; the first one is localized at the NH<sub>2</sub>-terminal domain, whereas the second one is in the ligand-binding domain (12). ESR1 and ESR2 are structurally different in the ligand-binding pockets, which has facilitated the development of receptor-specific selective ligands (11). An important function of all ERs is to act as ligand-mediated transcriptional factors (11, 13–15).

ESR1 is broadly expressed in the central nervous system (CNS) (16–18) and in peripheral tissues, including adipose tissue (19), skeletal muscle, the cardiovascular system, and immune cells (20); ESR2 is the predominant receptor in the ovary, lung, bladder, hematopoietic cells,

and gastrointestinal tract (20–22). In tissues in which both ERs are present, such as adipose tissue and the cardiovascular system, ERs are found in varying distribution in the different adipose tissue depots and blood vessel types (23–25). Recently, it was discovered that both ERs are also present in human fetal brown adipose tissue (26) with ESR1 being the more prominent receptor. ESR1 in the CNS influences adipose tissue distribution and locomotor activity (27). In the liver, ESR1 is significantly more expressed than ESR2, in which receptor activation is thought to be protective against insulin resistance in mice (28–30). Although both ERs, including splice variants (*e.g.*, ER $\alpha$  46 and 66), are expressed in the heart and the vascular wall (31), there are conflicting data regarding the localization of ESR2 (32). Both ERs are thought to be involved in the development of cardiac and vascular dysfunction.

There are some data suggesting that the cellular localization of receptors mediates their function and action (15, 33, 34). Importantly, estrogens mediate most of their biological effects through ERs at the level of gene regulation by interacting through its site-specific DNA and with other coregulatory proteins. In the nucleus, ERs up- or downregulate the expression of target genes by interacting through their site-specific DNA and with other coregulatory proteins that include coactivators and corepressors (35–37). The classical hormone/receptor paradigm includes ER monomers in the cytosol that form protein complexes with chaperone heat-shock proteins. Ligand-mediated activation of ER promotes dissociation from these complexes, and dimerization with other free monomers follows. Homodimers are most often formed; however, ER $\alpha$ /ER $\beta$  heterodimers have also been observed (38). ER dimers enter the nucleus, where they bind DNA either directly via estrogen response elements (ERE) in the promoter of target genes, or indirectly, through protein–protein tethering (39). A host of genes can be induced or repressed depending on the cell type, the presence of transcriptional cofactors, the type and concentration of the ligand, as well as the type of ER dimer formed (40). Splice variants, including the truncation of AF domains, are shown to manifest unique metabolic phenotypes and target gene signatures.

In 2000, pioneering work done by O'Malley and colleagues (37) demonstrated that ERs function as ligand-activated transcription factors. The *trans*-activation activity of ERs initiates through the ligand-bound receptor to its cognate, *cis*-acting enhancers, ERE (38). The consensus palindromic element ERE was this perfect ERE sequence that was shown to function in an orientation- and distance-independent manner, both of which are properties of an enhancer (7, 41). When ER directly interacts with the promoter/enhancer, binding to a full

ERE is apparently the dominant mode of interaction. The human full EREs have a 3-bp spacer between the two half sites, the exceptions being response elements in the human transforming growth factor- $\alpha$  promoter, with a 4-bp spacer, and in the promoter of the rat luteinizing hormone  $\beta$  gene, with a 5-bp spacer (41). Controversy still exists concerning ER DNA binding via ERE half sites, although a number of examples exist (42–45).

Since the identification of a canonical ERE, several computational approaches have been undertaken to identify target genes based on the presence of EREs within promoter-proximal regions (46). For instance, for the 38 estrogen-responsive genes reviewed by Klinge (7), most of the functional EREs located within the promoters or 3'-untranslated regions are not the traditional consensus sequence. Thus, many target genes contain response elements that bear little similarity to consensus EREs. In one of the most comprehensive studies, Bourdeau and coworkers (47) screened for all EREs in the human and mouse genomes and identified in excess of >70,000 EREs within the human genome, >17,000 of which were within 15 kb of messenger RNA start sites. Elimination of EREs that were not conserved between the human and mouse genomes reduced the number of gene-proximal EREs to 660. A number of these sites were validated as genuine ER interaction sites, supporting the use of computational models to predict putative ER target genes to some degree (48). A variety of transcription factors, whose activity is controlled by protein phosphorylation events, are influenced by nongenomic ER action in a cell-specific context (29, 49). Membrane (*i.e.*, nongenomic) ERs are found in caveolae and lipid rafts, where they interact with other proteins to mediate rapid intracellular signaling pathways through proteins, including growth factor receptors (such as insulin-like growth factor-1), G proteins, and tyrosine kinases (such as SRC and RAS) (29, 49). ER $\alpha$  interaction with caveolin-1 (in caveolae) and nongenomic receptor activities can be controlled by receptor palmitoylation (41). Rapid, nongenomic, estrogenic signaling has also been shown to occur via a membrane-bound G protein-coupled estrogen receptor 30 (GPR30) (42). GPR30 is also involved in a wide range of physiological and pathological conditions, including some evidence for it playing a protective role in CVD (43).

### ERs and cardiometabolic risk: mechanistic evidence from animal studies

Much of the functional significance observed for ERs in mediating cardiometabolic risk has been obtained in rodents, where female mice carrying ESR1 mutations develop age-dependent vascular dysfunction and features of the metabolic syndrome, including obesity, glucose intolerance, and insulin resistance (44–46, 48),

reminiscent of the human condition (described below). Total body ER $\alpha$  knockout (KO) mice ( $\alpha$ ERKO) recapitulate a remarkable metabolic dysfunction similar to that observed in humans with rare inactivating receptor mutations and genetic polymorphisms in the receptor. Not only do these mice have increased adiposity caused by reductions in energy expenditure and increased food intake, but they also exhibit glucose intolerance, insulin resistance, and reduced endothelial-derived nitric oxide production (vasculoprotective molecule), thus demonstrating the critical role for ESR1 in regulating energy, metabolic, and vascular homeostasis (44, 50, 51). Additionally, ESR1 is required to mediate the beneficial metabolic actions of estradiol. Indeed, estradiol exerts a protective effect against high-fat diet (HFD)-induced glucose intolerance in wild-type but not  $\alpha$ ERKO mice (52).

Another interesting mouse model is the total body ESR1 AF deletion mutant. Male and female mice lacking the AF2 domain of ESR1 show a phenotype similar to ESR1 KO mice, suggesting that this region of the ER is involved in estrogen-mediated transcriptional activation. Additionally, the AF2 region, but not the AF1, appears essential in preventing insulin resistance and glucose intolerance in mice fed a regular chow diet or following exposure to HFD (53), and in preventing atherosclerosis in low-density lipoprotein receptor-deficient mice fed a hypercholesterolemic diet (54). Specifically, it was recently observed that mice deficient of AF1, when exposed to estradiol, still maintain estradiol-related vasculoprotective functions, including estradiol's ability to induce re-endothelialization capacity, the ability to produce nitric oxide, and the possibility of preventing atheroma generation (55); however, the specific actions of ER-AF regions still remain partially unknown because their function appears uniquely cell-type dependent (56).

ESR1 deletion from selected brain regions is shown to influence a variety of metabolic functions associated with cardiometabolic risk, including the following: body fat distribution, energy expenditure, reproduction, blood pressure, and food intake (57–60). In addition to expression and action of ER $\alpha$  in the CNS, ESR1 expression in the periphery is also shown to regulate metabolic homeostasis and insulin action. For example, ESR1 KO female mice have a lower insulin-stimulated glucose uptake in skeletal muscle tissue when compared with control/wild-type mice (48). Additionally, it was recently shown that muscle-specific ESR1 KO in female mice recapitulates a similar degree of metabolic dysfunction, as seen in the global null mouse model, including impaired muscle fatty acid oxidation, lipid accumulation, insulin resistance, and obesity (61). Muscle lipid accumulation and reduced muscle oxidative metabolism characteristic

of muscle-specific ESR1 KO mice were in part a consequence of mitochondrial dysfunction (61). Indeed, ESR1 promotes mitochondrial DNA replication, mitochondrial biogenesis, and the turnover of dysfunctional organelles (by a process called mitophagy) in skeletal muscle, and, when these processes are impaired due to ESR1 deficiency, the accumulation of dysfunctional mitochondria drives oxidative stress and tissue inflammation, molecular underpinnings involved in the development of insulin resistance and atherosclerosis (61, 62).

Given that immune cells infiltrate and reside in almost every tissue, it is not surprising that immune cells were found to play an important role in regulating adiposity, metabolic, and vascular homeostasis. In recent years, extensive research has been performed to clarify the communication between immune cells (specifically macrophages) and adipocytes, to better understand the influence of immune cell infiltration on cardiometabolic risk. The Hevener laboratory observed increased chemoattractant signals in adipose tissue of myeloid-specific ESR1 KO mice, and this was paralleled by increased immune cell infiltration (63). Deletion of ESR1 from myeloid cells promoted increased body weight, glucose intolerance, insulin resistance, and altered plasma adipokine and cytokine levels in female mice (63). In macrophages, ESR1 controls inflammation and oxidative metabolism and regulates the expression of a variety of genes and signaling networks involved in regulating metabolic homeostasis and providing atherosclerosis protection (63). Secreted factors from ESR1 KO macrophages were shown to promote increased lipid storage, inflammation, and impaired insulin action in a variety of cell types in culture. *In vivo*, female mice with a hematopoietic deletion of ESR1 showed increased susceptibility for atherosclerosis with a twofold increase in atherosclerotic lesion area when fed a Western diet (63). In summary, these tissue-selective and cell-specific deletion studies have allowed us to more elegantly interrogate the functions of ER $\alpha$  to better understand the tissue-specific actions of this hormone receptor in controlling cardiometabolic risk. Figure 1 provides a summary of the phenotype of ESR1 KO and tissue-specific ESR1 KO male mice.

Fewer studies have investigated the effects of ESR2 mutations compared with those that have investigated ESR1 mutations. In contrast to the findings from animals with mutations in ESR1, whole-body ESR2/ER $\beta$  KO mice (ERKO) have not been shown to exhibit impaired insulin sensitivity, glucose intolerance, or increased body weight or adiposity compared with wild-type mice (50), further supporting the notion that ESR1 is more important in driving the positive cardiometabolic actions of estrogens. However,  $\beta$ ERKO do develop sustained

systolic and diastolic hypertension with aging (64). Evidence suggests that ESR2 may play a compensatory role in driving adipose tissue accumulation in the context of a HFD via inhibition of peroxisome proliferator-activated receptor (PPAR) $\gamma$ , a transcription factor controlling broad adipocyte-specific gene expression (65). Interestingly, in that study, the effect of ESR2 to augment PPAR signaling in adipose tissue was accompanied by reduced accumulation of triglycerides and preserved insulin sensitivity in liver and skeletal muscle (65). Conversely, in an additional study, authors demonstrated that protein levels of ESR2 are increased, whereas PPAR- $\gamma$  decreased, in visceral adipose tissues of rats following ovariectomy (66), thus suggesting that declines in circulating estrogen levels, mimicked in rats following ovariectomy, might increase adiposity in postmenopausal women, as well as increase metabolic and cardiovascular disease risk when compared with ovarian-intact rodents or cycling women.

Although the animal data supporting a role for ESR1 as a critical regulator in energy homeostasis are overwhelming, it is important to remember that most studies to date have only altered the expression levels of one of the receptors and not controlled for the actions of the other. Indeed, significantly less attention has focused on the role of ESR2, which has also been shown to mediate some metabolically protective effects of estradiol (28). Furthermore, it will be important to determine whether there are coactivating or coinhibitory roles influenced by heterodimerization of receptors or alternative mechanisms for activating/suppressing gene targets.

Total body GPR30 KO mice manifest a phenotype characterized by moderate obesity associated with reduced energy expenditure (67), impaired glucose tolerance (68). Specifically, deletion of G protein-coupled ER increased atherosclerosis progression, total and low-density lipoprotein cholesterol levels, and inflammation, while reducing vascular nitric oxide bioactivity in rodents following exposure to a high-fat atherogenic diet (69). GPR30 KO female mice have increased adipose tissue mass with aging (67). Interestingly, GPR30 KO females are less responsive to estradiol replacement following ovariectomy when compared with wild-type mice (67), suggesting a potential role for GPR30 in modulating estrogen sensitivity. In addition, female GPR30 KO mice are more susceptible to perturbations in glucose homeostasis as a consequence of estradiol insufficiency, and this phenotype is due to defects in pancreatic function (70). Although estradiol preserved pancreatic  $\beta$ -cell mass in female mice following streptozotocin-induced type-1 diabetes, this beneficial effect of estrogen was lost in GPR30 KO animals (70). Recently, it was shown that GPR30 agonist treatment induces vasodilation in female

rats, although the mechanism mediating this effect is unclear (71). Together, these findings demonstrate extensive roles for GPR30 in mediating estrogenic effects in the maintenance of glucose homeostasis and vascular function.

### ERs and cardiometabolic risk: mechanistic evidence from human studies

It is clear that estrogen loss in women leads to a cluster of cardiometabolic abnormalities that greatly increase risk of diseases, including CVD, type 2 diabetes, and certain forms of cancer. Consistently, in rodents fed a HFD, ovariectomy promotes CVD and weight gain and reduces insulin sensitivity, effects that are prevented by selective activation of ESR1 with 4,4',4''-(4-propyl-[1H]-pyrazole-1,3,5-triyl) (72). Surprisingly little is known, however, about the role that ERs play in age-related cardiometabolic disease pathology in humans. What makes research on the role of ER signaling in cardiometabolic disease in humans difficult is the fact that ER expression is not static, and the balance and levels of ERs (ER $\alpha$  isoforms and ER $\beta$ ) can change with aging, disease, and prolonged estrogen deficiency, all of which can alter the response to estrogen (73, 74). For example, ERs have been reported to be lower in postmortem coronary arteries from postmenopausal women compared with premenopausal women and lower in atherosclerotic coronary arteries compared with normal coronary arteries regardless of menopausal state (75).





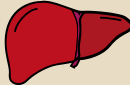

Although hormone replacement therapy (HRT) is sometimes highly effective at mitigating the negative cardiometabolic consequences of estrogen loss, it has also been associated with adverse cardiometabolic effects, such as an increase in insulin resistance and cardiac events (76, 77). Time lapse between ovarian failure and onset of therapy (*i.e.*, duration of estrogen deficiency) is thought to play a role in determining who benefits from hormone therapy (78). Indeed, experiments in ovariectomized rats showed that early-onset, but not late-onset estrogen replacement therapy prevents oxidative stress and alteration in brain glucose uptake caused by estrogen withdrawal and by the increase in the ER $\alpha$ / $\beta$  ratio (79). In humans, clinical data now suggest that HRT might be protective in preventing menopausal symptoms when used in women close to menopause transition, whereas null or adverse effect occurs when HRT is initiated in older women after the onset of menopause (12, 80–83).

Another current limitation associated with HRT is it lacks tissue specificity, and therefore acts on different tissues in the body ranging from the brain and adipose tissue (where it exerts positive effects) to the breast, endometrium, and endothelium (where it might exert toxic effects) (84). Furthermore, the precise mechanism(s) by

which HRT imparts its effects on tissues has not been fully elucidated, but it most likely involves differences in receptor biochemistry (78).

ESR1 expression is reduced in endothelial cells harvested from the antecubital veins of healthy estrogen-deficient postmenopausal compared with premenopausal women (85), as well as in skeletal muscle from women with the metabolic syndrome. Reductions in endothelial cell ER $\alpha$  have been associated with impaired endothelial function (85), the key antecedent for atherosclerosis development. Moreover, skeletal muscle ESR1 expression was inversely correlated with adiposity and fasting insulin, surrogate markers of cardiometabolic health (*i.e.*, low muscle ESR1 expression levels are associated with metabolic dysfunction). Thus, maintenance of vascular or skeletal ER $\alpha$  expression, or activation of muscle ESR1, could serve as an effective means to improve cardiometabolic health and combat diseases associated with metabolic dysfunction (61). Observational studies by Nilsson *et al.* (86) support this idea, showing reduced ESR1 expression in adipose tissue from obese women suffering from metabolic dysfunction.

In humans, genetic variations in the genes coding for ER $\alpha$  and ER $\beta$  have been associated with a myriad of diseases, including cardiovascular disease and premature ischemic heart disease (87–89). The Insulin Resistance Atherosclerosis Family Study identified a positive association between single-nucleotide polymorphisms (SNPs) in ESR1 and the metabolic syndrome, type 2 diabetes (T2DM), and adiposity (obesity) (90). The correlation between SNPs in ESR1 and the metabolic syndrome was confirmed in Japanese and Chinese women who were part of the Study of Women's Health Across the Nation (91). Specifically, in this study they were able to identify an association between SNPs in ESR1 and insulin sensitivity. The *XbaI* polymorphism of ESR1 has been correlated with the onset of the metabolic syndrome in Egyptian women; however, the mechanism associated with this polymorphism and insulin resistance has not been clearly identified (92). The presence of *XbaI* polymorphism of ESR1 with the *PvuII* C allele contributes to a metabolic and reproductive phenotype that is also observed in women with polycystic ovarian syndrome (93), an endocrine disorder caused by excessive androgen production, which increases the risk of insulin resistance, impaired glucose tolerance, T2DM, obesity, and dyslipidemia, and which eventually leads to early onset of CVD (94, 95). The same polymorphisms have also been considered as potential risk factors for the development of CVD, with the *XbaI* and/or *PvuII* C polymorphisms positively correlating with the onset of cardiovascular dysfunction. Indeed, the risk of developing a more severe form of coronary artery disease is higher in postmenopausal

	MALES	FEMALES
<b>Total Body ER<math>\alpha</math> KO</b> 	<b>Obesity</b> <b>Insulin Resistance</b> <b>Dysregulated Glucose Homeostasis</b>	<b>Obesity</b> <b>Insulin Resistance</b> <b>Dysregulated Glucose Homeostasis</b>
<b>Brain ER<math>\alpha</math> KO</b> 	- Nestin Cre <b>Obesity</b> <b>Hyperphagia</b> ↓ Heat Production ↓ Locomotion - POMC Cre <b>No Reported Phenotype</b>  - SF1 Cre <b>No Reported Phenotype</b>  - Medial Amigdala <b>No Known Phenotype</b>	- Nestin Cre <b>Obesity</b> <b>Hyperphagia</b> ↓ Energy Expenditure ↓ Locomotion - POMC Cre ↑ Food Intake ↓ Energy Expenditure ↓ Fertility - SF1 Cre <b>Obesity</b> <b>(Increased Gonadal Fat Pad)</b> <b>Dysregulated Glucose Homeostasis</b> ↓ Energy Expenditure - Medial Amigdala ↑ Blood Pressure
<b>Adipocyte ER<math>\alpha</math> KO</b> 	<b>Adipose Tissue Inflammation and Fibrosis</b> ↑ Adipocyte Size <b>Insulin Resistance</b>	<b>Obesity</b>
<b>Skeletal Muscle ER<math>\alpha</math> KO</b> 	<b>No Known Phenotype</b>	<b>Lipid Accumulation in Muscle Tissue</b> <b>Oxidative Stress (Mitochondrial Dysfunction)</b> <b>Insulin Resistance</b>
<b>Liver ER<math>\alpha</math> KO</b> 	<b>No Reported Phenotype</b>	<b>No Reported Phenotype</b>
<b>Hematopoietic Cells ER<math>\alpha</math> KO</b> 	<b>No Known Phenotype</b>	<b>Obesity</b> <b>Insulin Resistance</b> <b>Dysregulated Glucose Homeostasis</b> ↑ Pro-inflammatory Cytokines

**Figure 1.** Tissue-specific effects of estrogen receptor deletion. Total body ER $\alpha$  deletion in mice promotes obesity and tissue-specific metabolic dysfunction. To better understand the involvement of ER $\alpha$  in the development of metabolic disease, in the last two decades, tissue-specific ER $\alpha$  knockout mice have been generated.

women carrying the *XbaI* and/or *PvuII* C polymorphisms (96). A similar association was previously reported also in men with coronary artery disease (87, 89, 97). However, despite these findings, there are some data that have found opposite results that may be related to differences

in genetic background as well as the age of the study cohort (98). Given these disparities, additional information is required to better understand how these polymorphisms influence cardiometabolic outcomes. Polymorphisms in *ESR2* have also been associated with the onset of

cardiometabolic diseases in humans. In particular, the polymorphism *AluI* promotes obesity and is associated with increased cholesterol levels in women (99). Lastly, there appears to be an additive and adverse effect of *ESR1* and *ESR2* polymorphisms, which results in compromised metabolic function (100).

Incidence of insulin resistance and T2DM increases midlife in women (101–105). However, it is unclear whether menopause *per se* increases insulin resistance because prospective studies that have followed women across the menopause transition have not always reported declines in insulin sensitivity (106, 107). In support of a role for estrogens in protecting against insulin resistance, bilateral oophorectomy in women nearing menopausal age (range 40 to 54 years) reduced insulin sensitivity (estimated from oral glucose tolerance testing) (108). One mechanism by which loss of gonadal function may contribute indirectly to insulin resistance is through loss of ER expression or function secondary to prolonged estrogen deficiency. Although not all prospective studies have observed an association between natural menopause and diabetes risk (109, 110), surgical menopause has been strongly linked to an increased diabetes risk (111, 112). The increased risk associated with surgical menopause may be attributable to the more abrupt loss of sex steroids, compared with the slower decline that occurs during the natural menopause transition, supporting the notion that ovarian hormone loss is the major driver of metabolic dysfunction after menopause. Indeed, large randomized clinical trials consistently support a benefit of estrogen-based HRT on reducing fasting glucose, insulin, and incidence of new-onset T2DM after menopause (113–117). Moreover, secondary analysis of large randomized clinical trials suggests that initiating estrogen-based hormonal therapy early after menopause compared with later in menopause impacts hormonal therapy benefits and disease risk (117–121). A recent study by Van Pelt and colleagues (122) demonstrated that estrogen increased insulin-stimulated glucose disposal when administered to early postmenopausal women (*i.e.*, within 6 years of menopause), whereas estrogens reduced insulin action when administered to late postmenopausal women (*i.e.*, >10 years past menopause) who had never previously used HRT. The underlying mechanisms conferring the discordant effects of estrogen action on glucose disposal in early and late postmenopausal women are not yet known, but are most likely related to changes in ER with increasing age or duration of estrogen deficiency. Additional well-controlled studies in women are needed to elucidate tissue-specific changes in ERs and the underlying mechanisms of estrogen action *in vivo*.

## Conclusions and Future Directions

Due to loss of the protective effects of estrogen, menopause significantly increases cardiometabolic risk, promoting increased incidence of CVD and T2DM, leading causes of death among aging women (3). Mechanistically, the adverse cardiometabolic effects of estrogen loss are due to impaired ER action; thus, ER signaling is an area of active investigation. As discussed in this work, estrogen action goes beyond its classical signaling through receptors  $\alpha$  and  $\beta$ , with the GPR30 and other membrane-bound forms of the ERs also serving as important mediators of cardiometabolic protective effects of estrogen. Nonetheless, most research has focused on the classical ERs. Research has shown that loss of ER $\alpha$  signaling in particular increases adiposity (23), insulin resistance, and inflammation (123), a metabolic milieu that often precedes atherosclerosis development. These added insults lead to a vicious cycle of oxidative stress and inflammation within the vascular wall that can modulate estrogen–ER signaling, impair vascular function, and initiate atherosclerosis.

As women are living longer than any other time in history, it is critically important to elucidate therapeutic agents that can prevent and mitigate the cardiometabolic dysfunction that often precedes disease onset. In this regard, ERs may serve as prime therapeutic targets. However, more work is necessary to better understand ER signaling, which is exceedingly complex. Critical gaps remain in understanding how timing of hormone loss, aging, ER density, adiposity, pharmacological factors, and dietary factors affect ER biochemistry and the results of receptor agonization. Other novel therapeutic options may involve selective ER modulators or even tissue-specific targeting of the ERs.

## Acknowledgments

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This work was supported by Fondo Nacional de Desarrollo Científico y Tecnológico FONDECYT Grant 1160820 (to E.M.).

Disclosure Summary: The authors have nothing to disclose.

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