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Review

REVIEW

Potential Mechanisms of Age Acceleration Caused by Estrogen Deprivation: Do Endocrine Therapies Carry the Same Risks?

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Abstract

Longer duration of endocrine therapy decreases breast cancer recurrence and mortality, but these benefits need to be weighed against potential risks to overall health. Notable side effects of endocrine therapy include cataracts, uterine cancer, thromboembolic events, osteoporosis and fracture risk, chronic musculoskeletal complaints, as well as vaginal dryness and discharge, and vasomotor symptoms. Estrogen deprivation in healthy women younger than 50 years undergoing bilateral oophorectomy has been shown to accelerate the development of diseases related to aging, including coronary artery disease, cardiac arrhythmias, stroke, dementia, and osteoporosis, raising concern that even less dramatic modulation of estrogen homeostasis may adversely affect health outcomes. Diminished available estrogen at the cellular and molecular level may facilitate mechanisms that underlie the aging process, often termed the hallmarks of aging. In this review, we describe estrogen's role in normal physiology across tissues, review the effects of estrogen deprivation on health outcomes in the setting of both surgical and natural menopause, and examine the hallmarks of aging with attention to the effects of estrogen and estrogen blockade on each molecular mechanism underlying the aging process.

Normal healthy aging is characterized by a progressive loss of physiologic function and increased risk of disease and death (1,2). Many of the long-term health consequences of normal aging, such as osteoporosis, heart disease, stroke, chronic musculoskeletal complaints, and cognitive impairment, are seen with reduced estrogen environments caused by surgical menopause, the natural menopausal transition, and breast cancer-directed endocrine therapy (ET) (3–10). Does this observation implicate the possibility of accelerated aging in association with ET?

Estrogen is a key regulator in development and tissue homeostasis across tissues throughout the life span (11). Estrogen is involved in regulating mechanisms required for tissue maintenance and repair, such as stem cell division (12), mitochondrial function (13), intracellular communication (14), and circadian rhythms (15). Alteration and dysregulation of these and other mechanisms are considered hallmarks of the natural aging process (2). ET perturbs hormonal homeostasis and interferes with the beneficial effects of estrogen earlier and more abruptly than the more gradual effects associated with the natural onset of menopause and postmenopausal decline in serum estradiol. Should this play a role in decisions regarding the use of extended ET from five years to 10 years (16–23)?

In this review, we consider how the application of ET for breast cancer may mimic aspects of normal aging and/or increase the likelihood of accelerated aging. We review the normal physiology and effects of estrogen in various tissues across the normal life span, with attention to the molecular, cellular, and organ-based changes that occur with development and aging. We will describe the role of estrogen as a modulator of inflammation in healthy aging women. We will review the molecular and physiologic changes of the normal aging process and describe evidence suggesting estrogen may be involved in each of these changes. We will review the effects of estrogen deprivation on health outcomes and accelerated aging

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phenotypes, comparing surgical and natural menopause in healthy women to the toxicities of ET in breast cancer survivors. Finally, we will discuss the role for future research on host factors, identifying subgroups of women who may be susceptible to accelerated aging as a result of ET.

Normal Physiology and Effects of Estrogen

Estrogen is synthesized in all vertebrates as well as some invertebrates, such as insects, suggesting an ancient evolutionary history (24). Estrogen is an important hormone throughout the life span of both women and men across organ systems. Many of the physiologic effects of estrogen are beneficial. Estrogen is a master regulator, with its actions occurring on varying time scales. Rapid events occur in seconds to minutes, intermediate events occur on the order of minutes to hours, and long events occur on the order of hours to days. In addition to its key roles in the female reproductive system, where it regulates ovarian, uterine, and mammary development and function, estrogen has beneficial physiologic effects across several organ systems. There are three major forms of estrogen: estrone (E1), estradiol (E2), and estriol (E3). Many of the effects of estrogen are synergistic with other hormones and pathways, such as insulin signaling pathways. The actions of estrogen are mediated by receptors on the cell membrane or in the nucleus. As a steroid hormone, estrogen readily diffuses across the cell membrane. Once inside the cell, estrogen binds to receptors that in turn modulate the expression of key genes. The ligand-associated nuclear factors ERα and ERβ were first identified in rat uterus in the 1960s (ERα) and in rat prostate in 1996 (ERβ). These soluble receptors shuttle between cytoplasm (5%) and the nucleus (95%) and bind *cis*-acting estrogen response elements in gene promoter and enhancer regions. ERβ is a dominant negative regulator of ERα and modulates transcription responses to estrogens (25). These receptors promote glucose transport and suppress inflammation. Estradiol is also active in mitochondria, regulating mitochondrial function and inducing mitochondrial genes. In addition to these effects, there are nongenomic effects of estrogen, via rapid cellular signaling cascades, mediated by membrane-associated forms of these receptors. The G-protein-coupled estrogen receptor (GPER, or GPR30), originally cloned in the late 1990s, is associated with most of the 17β estradiol-mediated rapid signaling events (11).

GPER is predominantly intracellular, with seven transmembrane domains (11,26). Its stimulation activates metalloproteinases, inducing the release of heparin-binding epidermal growth factor, which stimulates cAMP production, intracellular calcium mobilization, and activation of downstream molecules such as ERK1/2 (11,27) and PI3K/Akt/MAPK (11). GPER is also involved in expression of Bcl2, cyclin D2, and nerve growth factor, and mediates expression of c-Fos. Ligands of GPER include 17β-estradiol, estriol, estrone, phytoestrogens, xenoestrogens, and bisphenols, as well as contraceptives such as 17α-ethynyl estradiol, selective estrogen receptor modulators (SERMs) including tamoxifen and raloxifene, and selective estrogen receptor downregulators such as fulvestrant.

Physiologic Effects of Estrogen by Organ System

We briefly review the receptor distribution and function of estrogen in each human organ system in Table 1 to describe the physiological effects of a low estrogen environment. In each organ system, estrogen plays an important role in normal physiology as well as prevention of certain disease states. For example, given the key role of estrogen in hippocampal function and neuronal connectivity, it is not surprising that Alzheimer's dementia is more common in postmenopausal women than in men (15). It should be noted that men maintain higher levels of estrogen than women throughout the postmenopausal years due to the conversion of testosterone to estrogen, accounting for these gender differences with aging. Likewise, estrogen slows intestinal transit times, and premenopausal women have a ninefold higher rate of irritable bowel syndrome than men (25). As a third example, decreased levels of 17β-estradiol are associated with increased hip fracture risk after the menopausal transition. Finally, as estrogen regulates insulin secretion, it follows that obesity and insulin resistance are more common after the menopausal transition (75,76).

The role of estrogen on the immune system is complex, with anti-inflammatory and pro-inflammatory effects arising in different cells and tissues at varying levels of estrogen, and depending on the immune stimulus (77). T cells are inhibited by estrogen, including Th1 cell-mediated immunity active in autoimmune disease and Th2 antibody-mediated responses in allergic conditions (77). Estrogen activates B cells, stimulating B-cell antibody production, inhibiting CD8-mediated suppression of B cells, decreasing B-cell precursors, and enhancing mitogen stimulation of B cells leading to accumulation of IgM-secreting cells. The varying effects of estrogen on target organs and cell types depend on the concentration of estrogen, intracellular metabolism of estrogen, and variability of estrogen receptors in the microenvironment. For example, higher levels of ERβ relative to ERα are expressed in T cells in systemic lupus erythematosis and in macrophages in synovial tissue in rheumatoid arthritis. Oxidative stress has effects on estrogen expression, increasing ERβ expression in endothelial cells and in activated macrophages. By contrast, hypoxia decreases ERα expression (77).

In addition to its roles in cellular proliferation in normal physiology, estrogen is a critical mediator of breast and lung carcinogenesis and tumor development (11,78–80). In normal breast tissue, 17β-estradiol stimulates proliferation during puberty, menstrual cycling, and pregnancy. GPER is expressed in 50% of breast cancers, regardless of ER status, and its expression is correlated with increased tumor size, rate of metastases, and poor outcome (81). MCF7 cells have increased expression of GPER (82). Treatment with tamoxifen in breast tumors with high GPER expression leads to decreased survival (11). Tamoxifen stimulates proliferation via transactivation of the epidermal growth factor receptor (EGFR) (83). GPER is also expressed in germ cell tumors, including intratubular seminomas, and embryonal carcinomas (11). Expression of GPER in uterine and ovarian cancer predicts poor survival. Likewise, ERα and ERβ are present in both normal and cancerous lung epithelium (84–86), and play important roles in lung development and lung function (87), and in the pathogenesis and progression of non-small cell lung cancer (80,88–90). The incidence of lung cancer is rising in nonsmokers, and a higher percentage of women with lung cancer are nonsmokers (53% vs 15% of men with lung cancer) (91). In a large study from 1973 evaluating men after myocardial infarction treated with estrogen, there was an increased incidence of, and mortality from, lung cancer among the treated group compared with placebo (92). More recent results from a post hoc analysis of data from the Women's Health Initiative trial (93) and a retrospective chart review of 498 women with lung cancer (94) confirm an increase in lung cancer mortality among postmenopausal women who received

Table 1. Physiologic effects of estradiol by organ system, with receptor distribution and effects at each site*

Organ system	Receptors	Receptor distribution and function	Reference
Central nervous	ERα, ERβ, GPER	hypothalamic preoptic nucleus – thermoregulation suprachiasmatic nucleus – sleep, circadian rhythm prefrontal cortex – executive function thalamus – sensory integration basal forebrain – learning and memory amygdala – emotion and motivation raphe nucleus – serotinergic system, affect, and mood locus coeraleus – adrenergic system hippocampus – information processing, short-term memory, working memory, cholinergic activity arterial vessels and pericytes – vascular tone, endothelial function, oxidative stress and inflammatory response general – regulates glucose metabolism, increases neuronal connectivity, regulates synaptic plasticity	(11, 15, 28–30)
Peripheral nervous	GPER	spinal cord, dorsal root ganglia, visceral nerves – modulates pain sensation	(11, 15)
Cardiovascular	ERα, ERβ, GPER	myocardium, endothelial cells, smooth muscle – increases contractile function endothelial cells, smooth muscle – improves perfusion macrophages	(11, 31–42)
Gastrointenstinal	ERα, ERβ	enteric nerve cells – inhibits nitric oxide production smooth muscle – mediates smooth muscle contraction macrophages – slows intestinal transit times increases ileal glucose uptake	(25, 43–50)
Pancreas	ERα, ERβ, GPER	pancreatic islets – stimulates insulin secretion anti-inflammatory effects	(51–53)
Liver	ERα, ERβ, GPER	hepatocytes – regulates lipid biosynthesis and lipid transport anti-inflammatory effects	(11, 25, 32, 54–57)
Kidney	GPER	epithelial cells, renal tubules – regulates renin-angiotensin system regulates vascular function	(11, 58–62)
Bone and articular cartilage	ERα, ERβ, GPER	osteoblasts – stimulates osteoblastogenesis osteoclasts – regulates bone resorption and remodeling chrondrocytes – regulates differentiation	(11, 63–71)
Skin	ERα, ERβ	keratinocytes, basal cells – increases skin thickness basal cells, melanocytes – enhances vascularization melanocytes – increases melanin production	(72)
Eye	ERα, ERβ	Retina and retinal pigment epithelium – unclear role	(73, 74)

*ER = estrogen receptor; GPER = G-protein-coupled estrogen receptor.

hormone replacement therapy (HRT). By contrast, antiestrogens function to suppress signaling pathways for growth and tumor progression in lung cancer (80,95,96), and women treated with anti-estrogen therapy have reduced incidence of lung cancer and decreased mortality (89).

Hallmarks of Aging: Potential Molecular Mechanisms of Accelerated Aging with Premature Menopause and Endocrine Therapy for Breast Cancer

We briefly describe the biological features of mammalian aging (2) to facilitate a discussion of the potential effects of estrogen on these features. These include genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication (2). Cancer survivors who have been treated with chemotherapy and radiotherapy have been shown to have manifestations of several hallmarks of aging, including altered telomere length, senescent cells, epigenetic modifications, and microRNAs (97). We explore here the potential mechanisms of the effects of ET on the biologic aging process.

Stem Cell Exhaustion

Multiple animal studies have shown a role of estrogen in stimulating stem cell division, in hematopoietic stem cells, embryonal stem cells, and mesenchymal stem cells. Estrogen stimulates hematopoietic stem cell (HSC) division in female mice (12). Ovariectomy decreases HSC division and administration of estradiol increases HSC division in male and female mice (12). Estradiol enhances mouse embryonal stem cell proliferation, and this enhanced proliferation is associated with

store-operated calcium entry (SOCE) (98). Estradiol decelerates telomere attrition in mesenchymal stem cells and chondrocytes, but does not prevent somatic cells from replicative exhaustion or senescence (99). Interestingly tamoxifen inhibits estradiol-stimulated reduction in telomere shortening (99), suggesting that it could impact capacity for self-renewal.

Genomic Instability

Rodent cancer models demonstrate that estrogen signaling enhances genomic instability, promotes cell cycle entry, induces micronuclei, and increases DNA damage in cancer cells (100–104). Studies of ERα-negative breast cells reveal that estrogen and estrogen metabolites cause DNA double-strand breaks (DSB), and *BRCA1* is required to repair these DSBs (105). *BRCA1* also regulates estrogen metabolism and metabolite-mediated DNA damage by repressing the transcription of estrogen-metabolizing enzymes, such as *CYP1A1* in breast cells (105).

Altered Intercellular Communication

Animal and cellular studies have demonstrated a role for estrogen signaling in promoting intracellular communication, suggesting that reducing estrogen might lead to dysregulation of this communication and contribute to accelerated aging. Estrone increases intracellular cell-to-cell communication in prostate epithelial cells, inducing a twofold increase in connexin (14). Estrogen increases gap junctional intercellular communication (GJIC) and reduces proliferation in malignant human liver Huh7 cells (106). Furthermore, 17β-estradiol attenuates the inhibitory effect of metabolic inhibition of GJIC in rat cardiomyocytes via membrane-associated signaling (107). By contrast, estrogens have been shown to inhibit GJIC in mouse Leydig TM3 cells (108), suggesting that further study is needed to understand its role in different tissues.

Mitochondrial Dysfunction

Estrogens exert direct and indirect effects on mitochondrial function in a variety of tissues. Estrogens have cell-specific effects on many physiologic endpoints, including regulation of mitochondrial biogenesis and activity (13). ERα and ERβ reside within mitochondria and estrogen has both rapid and longer-term effects on mitochondrial function. Nuclear effects of estrogen on gene expression directly control mitochondrial biogenesis, oxygen consumption, mitochondrial DNA transcription, and apoptosis (13). ERα and ERβ mediate distinct pathways of vascular gene expression, including genes involved in mitochondrial electron transport and generation of reactive oxygen species (109). NRF-1, a key transcription factor regulating mitochondrial function, is regulated by ERα and ERβ in mouse aorta (110). Estrogen stimulates transcription of NRF-1 leading to increased mitochondrial biogenesis (111). Based on these findings, we postulate that impaired mitochondrial function may represent a mechanism of accelerated aging in premature menopause. Letrozole therapy combined with Aβ1–42 treatment in transgenic mice experiments leads to mitochondrial function impairment in hippocampal cells, which may represent the mechanism underlying the neurological deficits that arise in this setting (112). Estrogen protects against mitochondrial dysfunction at the early phase of ischemic injury (112).

Telomerase Expression

In a study of hematopoietic cells, sex hormones acting on the *TERT* gene increase telomerase activity (113). Administration of tamoxifen was able to inhibit this effect, reducing telomerase activity (113), and raising the question whether limiting estrogen accelerates aging via telomere shortening. Tamoxifen is a mixed agonist and antagonist of ERα and ERβ, and displays different outcomes in different tissues. In the uterus, tamoxifen acts as an estrogen agonist and induces growth in endometrial cells, causing proliferation and carcinogenesis. Likewise, in bone, estrogen acts as an agonist, and provides protection against menopausal bone loss. In breast cells, tamoxifen acts as an antagonist, and it inhibits growth of breast cancer cell lines. Tamoxifen inhibits hTERT mRNA expression differently in these two tissues (114). In MCF-7 cells, tamoxifen inhibits hTERT mRNA expression in the presence of estrogen. In contrast, in Ishikawa endometrial cells, tamoxifen activates the MAPK pathway, and activation of hTERT mRNA expression is effectively blocked by MEK inhibitors. Tamoxifen-induced activation of hTERT may be a component of estrogen agonistic function that is involved in endometrial carcinogenesis.

DNA Methylation

Estrogen may be an important regulator of DNA methylation in development, homeostasis, and carcinogenesis. Estrogen signaling regulates DNA methylation in spermatozoa in male adult rats, and also regulates methylating enzymes during adult rat spermatogenesis (115). Estrogen signaling is also associated with alterations in DNA methylation in breast cancer, where it mediates epigenetic repression of the metallothionein-1 gene cluster (116). Methylation of estrogen-related enhancers defines endocrine sensitivity in breast cancer (117). Estrogen is speculated to have a role in regulating epigenetic enzymes in the hypothalamus (118). DNA methyltransferase activity is significantly reduced in the hypothalamus of female rat pups treated with estradiol (119). Finally, estrogen enables inhibition of vascular soluble epoxide hydrolase expression via methylation (120), leading to an increase in vascular level of epoxyeicosatrienoic acids that possess cardioprotective properties, including vasodilation, resulting in lower blood pressure and improved blood supply to tissues (120).

The epigenetic clock, an estimate of biologic aging based on methylation levels at 353 5′-cytosine-phosphate-guanine-3′ sites, strongly correlates with chronologic age, is accelerated in disease states, and is predictive of frailty and mortality. Recently, the epigenetic age of blood has been shown to be significantly associated with earlier age at menopause, bilateral oophorectomy, and a longer time since menopause (121). These results suggest that the menopausal transition (MT) accelerates biologic aging as measured by the epigenetic clock, but further studies are needed to elucidate the mechanisms by which this acceleration takes place.

Nutrient Sensing and the Circadian Clock

Estrogen receptors, including ERα, ERβ, and GPER, are expressed in the suprachiasmatic nucleus, which regulates circadian rhythms (11,15). Recent evidence suggests that nuclear hormone receptors and their ligands act as "nutrient sensors," demonstrating crosstalk between circadian and metabolic pathways (122). Nuclear hormone receptors REV-ERBα and RAR-related

orphan receptor alpha regulate transcription of *BMAL1*, which activates transcription of the Period and Cytochrome genes. BMAL1 also activates genes involved in the production and utilization of nutrient metabolites, including the NAD+ biosynthetic enzyme NAMPT, and NAD+ is an important cofactor for the metabolic regulators *SIRT1* and *PARP1*, which mediate rhythms of oxidative metabolism. ERRα is an orphan nuclear receptor that acts as a nutrient sensor and also plays a role in the circadian rhythms of transcriptional activity. Although some SERMs (such as 4-hydroxytamoxifen) affect ERRβ and ERRγ, they have no effect on ERRα (122).

Protein Destabilization

When unfolded proteins accumulate within the endoplasmic reticulum (ER) of eukaryotic cells, an unfolded protein response occurs that either restores homeostasis or commits the cell to apoptosis. Recently, estrogen has been shown to mediate this stress response (123), suggesting that protein destabilization may mediate the effects of estrogen blockade on accelerated aging.

Deleterious Effects of Estrogen Deprivation in Healthy Women

There is compelling evidence suggesting that bilateral oophorectomy for a noncancerous condition before age 50 years leads to accelerated aging (3,4,124–132). A recent review (3) comparing ovarian conservation with removal at the time of benign hysterectomy lists deleterious effects of bilateral salpingo-oophorectomy (BSO), including increased risk of all-cause mortality in the Nurses' Health Study (128,129), the Breast Cancer Demonstration Project (130), the Rochester Epidemiologic Project in the Mayo Clinic Study of Oophorectomy and Aging (125), and the English Healthcare Registries (131), with an even higher risk in women undergoing oophorectomy before age 50 years who never used estrogen therapy (125,128,129). The Dutch breast cancer screening cohort further revealed a 2% decrease in mortality per year of delayed menopause (132). In addition, the Mayo Clinic Study of Oophorectomy and Aging revealed higher rates of stroke (126) and multimorbidity in women undergoing oophorectomy for a noncancer indication (4), with an increased risk of depression, hyperlipidemia, cardiac arrhythmias, coronary artery disease, arthritis, asthma, chronic obstructive pulmonary disease, and osteoporosis in women undergoing oophorectomy prior to age 46 years (4). An important limitation of the Mayo Clinic Study is the retrospective chart review design and potential bias in selection of control subjects. Their findings are contradicted by two prospective studies that did not show an increase in all-cause mortality (133), and a large prospective cohort study suggesting that BSO might not have an adverse effect on cardiovascular health, hip fracture risk, cancer risk, or overall mortality compared with hysterectomy and ovarian conservation (134).

Surgical menopause also leads to harmful effects on cognition. The Mayo Clinic study demonstrated a doubled long-term risk of cognitive impairment or dementia in women who underwent oophorectomy before menopause (124). The risk of cognitive impairment and dementia increased with younger age at the time of oophorectomy. This risk was eliminated in women who received HRT after surgery until 50 years of age, but those who received HRT and stopped before age 50 years had an elevated risk of cognitive impairment and dementia that increased with younger age of stopping this therapy. An extension of the Mayo Clinic study reported an association between surgical menopause and cognitive decline with Alzheimer's disease pathology (135). In this cohort study, earlier age at surgical menopause was associated with faster decline in global cognition, episodic memory, and semantic memory, and increased plaque burden. HRT begun within five years of surgery and continued for 10 years was associated with diminished decline in global cognition.

Although its effects are less dramatic, the natural MT has a great impact on health outcomes. Table 2 shows the deleterious effects of estrogen deprivation, comparing the dramatic acceleration of age-related conditions reported after surgical menopause with effects of the natural MT. Findings from the prospective Study of Women's Health Across the Nation (SWAN) showed that longitudinal changes in reproductive hormones during the MT are related to vasomotor symptoms (138), increased bone turnover markers (139), decreased bone mineral density (5,140,141), changes in weight and waist circumference and increased body fat (6,142), and increased cardiovascular fat (136). The gradual loss of oocytes and approach toward a threshold number of oocytes with natural menopause depends on the initial number of oocytes and the rate of loss. Age at menopause is associated with an increased risk of cardiovascular disease, stroke, and mortality (7,143). The mechanistic role of estrogen on cardiovascular health remains poorly understood (144). Emerging data suggest that HRT may have positive effects if received near the time of the MT (144–148). Natural menopause was not associated with cognitive decline in contrast to the observations with surgical menopause (135). However, SWAN reported a cross-sectional association between self-reported forgetfulness and being perimenopausal (149), and further demonstrated that objective changes in cognition during the MT were not accounted for by self-reported hot flashes, anxiety, depression, and sleep disturbance (8). Anxiety and depressive symptoms were associated with slow psychomotor speed (8) consistent with findings that ERβ mediates these effects on serotonin and hypothalamic-pituitary-adrenal axis function (8,149,150).

Toxicity and Benefits of Breast Cancer Endocrine Therapies

Table 3 shows the side effects of ET in pre- and postmenopausal women with breast cancer. Outcomes observed in randomized controlled trials (RCTs) in women undergoing adjuvant ET are helpful in weighing the risk of breast cancer recurrence with the risk of toxicity of ET. The risk of breast cancer recurrence and breast cancer death will decrease each year after early-stage breast cancer diagnosis for a given patient, whereas the risk of concurrent illness will increase, underlining the importance in paying close attention to observations of adverse outcomes. Concerning adverse events with long-term ET, including osteoporotic fractures and cardiovascular events with aromatase inhibitor (AI) therapy, as well as thromoembolic events, cataracts, and second cancers with tamoxifen, recapitulate age-related diseases, raising the question of common mechanisms and potentially increased vulnerability to these toxicities with advancing age. Here we describe outcomes of RCTs in the setting of adjuvant AI and tamoxifen therapy, and tamoxifen chemoprevention.

Risks of AI Therapy

A recent meta-analysis examined 16 349 patients from seven RCTs who received initial adjuvant endocrine monotherapy,

Table 2. Deleterious effects of a low estrogen environment: comparing the pervasive consequences of surgical menopause to the subtle outcomes of natural menopause*

	Oophorectomy before age 50 years		Natural menopause	
Organ system	Effect	Reference	Effect	Reference
Central nervous	depression HR = 1.43, 95% CI = 1.24 to 1.66 anxiety HR = 1.25, 95% CI = 1.07 to 1.46 cognitive impairment or dementia HR = 1.46, 95% CI = 1.13 to 1.90	(4, 124)		
Cardiovascular	hyperlipidemia HR = 1.35, 95% CI = 1.21 to 1.50 arrhythmia HR = 1.16, 95% CI = 1.00 to 1.36	(4)	↑ cardiovascular fat 11.69% difference, 95% CI = 2.25 to 22	(136)
Pulmonary	COPD HR = 1.39, 95% CI = 1.15 to 1.68 Asthma HR = 1.39, 95% CI = 1.11 to 1.74	(4)		
Pancreas	Diabetes mellitus HR = 1.19, 95% CI = 1.04 to 1.38	(4)		
Bone	Arthritis HR = 1.36, 95% CI = 1.21 to 1.52	(4)	↓ BMD LS: 1.8–2.3% per year, $P<.001$ hip: 1.0–1.4% per year hip, $P=.002$	(137)

*HR = hazard ratio; CI = confidence interval; COPD = chronic obstructive pulmonary disease; BMD = bone mineral density; LS = lumbar spine.

Table 3. Side effects of endocrine therapy for pre- and postmenopausal women*

	Endocrine therapy: SERMs		Endocrine therapy: AIs	
Organ system	Effect	Refs	Effect	Refs
Cardiovascular	thromboembolic events OR = 1.73, 95% CI = 1.47 to 2.05 stroke (age ≥50 years) RR = 1.47, 95% CI = 0.97 to 2.21 pulmonary embolism RR = 2.15, 95% CI = 1.08 to 4.51	(10, 151)	cardiovascular events: MI, stroke, TIA, angina, thromboembolic events, arrhythmias, LV failure OR = 1.18, 95% CI = 1.0 to 1.4 dyslipidemia (AIs compared with Tam) OR = 2.36, 95% CI = 2.15 to 2.60	(9, 152)
Bone	↓ BMD (premenopausal) LS: ↓ 1.44% per year, $P<.001$ hip: ↓ NS ↑ BMD (postmenopausal) LS: ↑ 1.17% per year, $P<.005$ Hip: ↑1.71% per year, $P<.001$ ↓ fractures HR = 0.66, 95% CI = 0.59 to 0.73 RR = 0.68, 95% CI = 0.51 to 0.92	(10, 151, 153)	↑ fractures OR = 1.34, 95% CI = 1.16 to 1.55	(9)
Eye	cataracts RR = 1.21, 95% CI = 1.10 to 1.34	(151)		
Uterine	endometrial cancer HR = 1.64, 95% CI = 1.14 to 2.36	(10)		

*OR = odds ratio; CI = confidence interval; RR = risk ratio; HR = hazard ratio; MI = myocardial infarction; TIA = transient ischemic attack; LV = left ventricular; BMD = bone mineral density; LS = lumbar spine; Tam = tamoxifen.

and compared outcomes in those receiving extended ET to those receiving placebo (9). Enrolled patients were randomly assigned to receive five additional years of adjuvant ET vs placebo in four of these trials, three years of anastrozole versus no treatment in one trial, and longer vs shorter duration of AI therapy in the remaining two trials. Prolonged therapy with AIs is associated with a marginally increased risk of cardiovascular events (see Table 3), with 7% of patients receiving prolonged AI therapy having cardiovascular events compared with 6% in the group that did not receive extended AI therapy (9). There was no association between relative odds and median age at random assignment (9). Cardiovascular events included myocardial infarction, stroke, transient ischemic attack, new or worsening angina, angina leading to percutaneous coronary intervention or coronary artery bypass graft, thromboembolic events, supraventricular and ventricular arrhythmias, and left ventricular failure (9). Hypertension was not associated with longer duration of AI therapy (9). In another meta-analysis of adjuvant ET,

AI therapy was associated dyslipidemia and arterial hypertension (152). A recent pilot cross-sectional study of women with curative-intent breast cancer on an AI compared with healthy postmenopausal women revealed reductions in endothelial function in women on AIs, suggesting that these women are at increased risk of cardiovascular disease (154). However, the apparent increased risk of AIs compared with tamoxifen is due to a protective effect of tamoxifen for cardiovascular events, rather than an adverse effect of AIs (155).

Extended AI therapy beyond five years is also associated with increased odds of bone fractures (see Table 3) with fractures occurring in 6.3% of patients receiving prolonged AI therapy compared with 4.8% of patients in the control group (9). Again, older age at random assignment was not associated with increased relative odds of bone fracture. In four of the seven RCTs, occurrence of second cancers was reported, and no difference was observed between patients treated with prolonged AI therapy (2.2% patients with second cancers) and those not receiving extended therapy (2.4% patients). The percentage of patients who discontinued therapy for adverse events was 17% among patients receiving extended AI therapy compared with 13.4% in the control group (OR = 1.45, 95% confidence interval = 1.23 to 1.68, $P < .001$). Death without recurrence occurred in 3.9% of patients receiving prolonged AIs compared with 2.9% of those receiving placebo or no treatment, and was not associated with prolonged use of AIs (OR = 1.11, 95% confidence interval = 0.9 to 1.36, $P = .34$). Although disease-free survival was higher in patients receiving prolonged AI therapy, an exploratory analysis revealed no effect on overall survival (9).

Risks of Selective Estrogen Receptor Modulator Therapy

SERM therapies exhibit mixed proestrogen and antiestrogen effects. The unopposed proestrogen effects of tamoxifen mediate the most serious risks of SERM therapies, such as venous thromboembolism (151) and uterine cancer (10). The risk of uterine cancer increases with advancing age and duration of tamoxifen therapy (2.30 per 1000 women per year of tamoxifen use) (156). Endometrial polyps are much more common, occurring in 8–50% of women on tamoxifen (157). In premenopausal patients, tamoxifen disrupts the menstrual cycle, causing functional ovarian cysts (158).

A meta-analysis of four chemoprevention trials that reported on the effects of tamoxifen, including the NSABP P-1 (151), IBIS-1 (159), Royal Marsden Hospital Tamoxifen Prevention Trial (160), and the Italian Randomized Tamoxifen Prevention Trial (161), revealed a 38% reduction in invasive breast cancer, a significant reduction in vertebral fractures, and a significantly increased risk of thromboembolic events with all SERMs (10). None of these trials was individually powered to examine mortality outcomes, and the meta-analysis revealed no effect of any SERM on all-cause mortality (10). Adverse outcomes reported individually in these trials included increased incidence of cataracts (151), increased risk of invasive endometrial carcinoma in women aged 50 years or older receiving tamoxifen (151), increased vascular events and hypertriglyceridemia (161), and a nonstatistically significant increase in incidence rates of stroke (151). Increased side effects with tamoxifen include vasomotor symptoms (159,160), gynecological problems (160), including vaginal discharge, abnormal bleeding, endometrial polyps, and amenorrhea (159), thrush, ovarian cysts or lumps, nail changes and breast complaints (159), and bone loss in younger women (160).

Identifying Host Factors That Modulate Risks of Accelerated Aging With Endocrine Therapy

Making a recommendation for a prolonged course of ET in a breast cancer survivor requires a balanced discussion of risks, toxicities, and benefits. Weighing the risks of accelerated aging against the risks of breast cancer recurrence requires a careful assessment of the individual at risk. Factors that need to be considered include performance status, coexisting illnesses, cardiovascular and bone health, and tumor characteristics. The decision to direct or withhold ET may be clear in some patients with overt impairments, and less clear in others with preclinical frailty. For example, a postmenopausal patient with severe osteoporosis or debilitating arthritis may be directed toward tamoxifen. A perimenopausal woman with an inherited thrombophilia and history of life-threatening pulmonary embolism should not be given tamoxifen, with recommendations for ovarian suppression therapy and AI therapy following the MT. More concerning is a patient without known risk factors who develops ET toxicity that adversely affects health and contributes to comorbidity or competing causes of death. For example, a postmenopausal patient with stage 0 disease who has mild osteopenia, but is functional and independent, may experience accelerated bone loss on an AI, leading to hip fracture, causing the patient to become dependent and institutionalized. A premenopausal woman with hypertension and hypercholesterolemia exposed to 10 years of ET may potentially carry the risk of developing earlier age-related cardiovascular morbidity and mortality.

Further research is needed to identify host factors that may predict a deleterious effect from prolonged ET. The ability to assess hallmarks of aging pre- and post-ET in a group of individuals of varying age and performance status could allow us to characterize the extent of accelerated aging that occurs with ET. If biological evidence for accelerated aging were found to occur, it would be important to investigate whether the degree of acceleration translates into functional decline and adverse outcomes. Given the large numbers of women who are receiving prolonged adjuvant ET for breast cancer, it will be critical to advance our understanding of the molecular mechanisms involved in accelerated aging with estrogen loss and the role of ET in the aging process. Through such research we may be able to better estimate the relative risks and benefits of prolonged ET in prevention and treatment of breast cancer in an aging population.

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References

1. Harman D. The aging process—major risk factor for disease and death. *Proc Natl Acad Sci USA*. 1991;88(12):5360–5363.
2. Lopez-Otin C, Blasco MA, Partridge L, et al. The hallmarks of aging. *Cell*. 2013;153(6):1194–1217.
3. Adelman MR, Sharp HT. Ovarian conservation vs removal at the time of benign hysterectomy. *Am J Obstet Gynecol*. 2018;218(3):269–279.
4. Rocca WA, Gazzuola-Rocca L, Smith CY, et al. Accelerated accumulation of multimorbidity after bilateral oophorectomy: a population-based cohort study. *Mayo Clin Proc*. 2016;91(11):1577–1589.
5. Sowers MR, Zheng H, Greendale GA, et al. Changes in bone resorption across the menopause transition: effects of reproductive hormones, body size, and ethnicity. *J Clin Endocrinol Metab*. 2013;98(7):2854–2863.
6. Thurston RC, Sowers MR, Sternfeld B, et al. Gains in body fat and vasomotor symptom reporting over the menopausal transition: the study of women's health across the nation. *Am J Epidemiol*. 2009;170(6):766–774.
7. van der Schouw YT, van der Graaf Y, Steyerberg EW, et al. Age at menopause as a risk factor for cardiovascular mortality. *Lancet*. 1996;347(9003):714–718.
8. Greendale GA, Wight RG, Huang MH, et al. Menopause-associated symptoms and cognitive performance: results from the study of women's health across the nation. *Am J Epidemiology*. 2010;171(11):1214–1224.
9. Goldvaser H, Barnes TA, Seruga B, et al. Toxicity of extended adjuvant therapy with aromatase inhibitors in early breast cancer: a systematic review and meta-analysis. *J Natl Cancer Inst*. 2018;110(1):31.
10. Cuzick J, Sestak I, Bonanni B, et al. Selective oestrogen receptor modulators in prevention of breast cancer: an updated meta-analysis of individual participant data. *Lancet*. 2013;381(9880):1827–1834.
11. Prossnitz ER, Barton M. The G-protein-coupled estrogen receptor GPER in health and disease. *Nat Rev Endocrinol*. 2011;7(12):715–726.
12. Nakada D, Oguro H, Levi BP, et al. Oestrogen increases haematopoietic stem-cell self-renewal in females and during pregnancy. *Nature*. 2014;505(7484):555–558.
13. Klinge CM. Estrogenic control of mitochondrial function and biogenesis. *J Cell Biochem*. 2008;105(6):1342–1351.
14. Carruba G, Webber MM, Quader ST, et al. Regulation of cell-to-cell communication in non-tumorigenic and malignant human prostate epithelial cells. *Prostate*. 2002;50(2):73–82.
15. Brinton RD, Yao J, Yin F, et al. Perimenopause as a neurological transition state. *Nat Rev Endocrinol*. 2015;11(7):393–405.
16. Davies C, Pan HC, Godwin J, et al. Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: ATLAS, a randomised trial. *Lancet*. 2013;381(9869):805–816.
17. Gray RG, Rea D, Handley K, et al. aTTom: long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years in 6,953 women with early breast cancer. *J Clin Oncol*. 2013;31(18_suppl):5.
18. Burstein HJ, Lacchetti C, Anderson H, et al. Adjuvant endocrine therapy for women with hormone receptor-positive breast cancer: American Society of Clinical Oncology Clinical practice guideline update on ovarian suppression. *J Clin Oncol*. 2016;34(14):1689–1701.
19. Goss PE, Ingle JN, Pritchard KI, et al. Extending aromatase-inhibitor adjuvant therapy to 10 years. *N Engl J Med*. 2016;375(3):209–219.
20. Goss PE, Ingle JN, Martino S, et al. Randomized trial of letrozole following tamoxifen as extended adjuvant therapy in receptor-positive breast cancer: updated findings from NCICCTG MA.17. *J Natl Cancer Inst*. 2005;97(17):1262–1271.
21. Mamounas EP, Jeong JH, Wickerham DL, et al. Benefit from exemestane as extended adjuvant therapy after 5 years of adjuvant tamoxifen: intention-to-treat analysis of the National Surgical Adjuvant Breast And Bowel Project B-33 trial. *J Clin Oncol*. 2008;26(12):1965–1971.
22. Gnant MSG, Greil R, Fitzal F, et al. A prospective randomized multi-center phase-III trial of additional 2 versus additional 5 years of anastrozole after initial 5 years of adjuvant endocrine therapy - results from 3,484 postmenopausal women in the ABCSG-16 trial. *Cancer Res*. 2018;78(4 supplement):GS3-01.
23. Blok EJ, van de Velde CJH, Meershoek-Klein Kranenbarg EM, et al. Optimal duration of extended letrozole treatment after 5 years of adjuvant endocrine therapy; results of the randomized phase III IDEAL trial (B00G 2006–05). *Cancer Res*. 2017;77(4 supplement):S1-04.
24. Mechoulam R, Brueggemeier RW, Denlinger DL. Estrogens in insects. *Experientia*. 1984;40(9):942–944.
25. Bottner M, Thelen P, Jarry H. Estrogen receptor beta: tissue distribution and the still largely enigmatic physiological function. *J Steroid Biochem Mol Biol*. 2014;139:245–251.
26. Revankar CM, Cimino DF, Sklar LA, et al. A transmembrane intracellular estrogen receptor mediates rapid cell signaling. *Science*. 2005;307(5715):1625–1630.
27. Edwin F, Wiepz GJ, Singh R, et al. A historical perspective of the EGF receptor and related systems. *Methods Mol Biol*. 2006;327:1–24.
28. Maki PM, Resnick SM. Longitudinal effects of estrogen replacement therapy on PET cerebral blood flow and cognition. *Neurobiol Aging*. 2000;21(2):373–383.
29. Rasgon NL, Geist CL, Kenna HA, et al. Prospective randomized trial to assess effects of continuing hormone therapy on cerebral function in postmenopausal women at risk for dementia. *PLOS One*. 2014;9(3):e89095.
30. Rasgon NL, Silverman D, Siddarth P, et al. Estrogen use and brain metabolic change in postmenopausal women. *Neurobiol Aging*. 2005;26(2):229–235.
31. Patel VH, Chen J, Ramanjaneya M, et al. G-protein coupled estrogen receptor 1 expression in rat and human heart: protective role during ischaemic stress. *Int J Mol Med*. 2010;26(2):193–199.
32. Takada Y, Kato C, Kondo S, 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.
33. Holm A, Baldetorp B, Olde B, et al. The GPER1 agonist G-1 attenuates endothelial cell proliferation by inhibiting DNA synthesis and accumulating cells in the S and G2 phases of the cell cycle. *J Vasc Res*. 2011;48(4):327–335.
34. Haas E, Bhattacharya I, Brailoiu E, et al. Regulatory role of G protein-coupled estrogen receptor for vascular function and obesity. *Circ Res*. 2009;104(3):288–291.
35. Haas E, Meyer MR, Schurr U, et al. Differential effects of 17 beta-estradiol on function and expression of estrogen receptor alpha, estrogen receptor beta, and GPR30 in arteries and veins of patients with atherosclerosis. *Hypertension*. 2007;49(6):1358–1363.
36. 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.
37. Delbeck M, Golz S, Vonk R, et al. Impaired left-ventricular cardiac function in male GPR30-deficient mice. *Mol Med Rep*. 2011;4(1):37–40.
38. Deschamps AM, Murphy E. Activation of a novel estrogen receptor, GPER, is cardioprotective in male and female rats. *Am J Physiol Heart Circ Physiol*. 2009;297(5):H1806–H1813.
39. Deschamps AM, Murphy E, Sun JH. Estrogen receptor activation and cardioprotection in ischemia reperfusion injury. *Trends Cardiovasc Med*. 2010;20(3):73–78.
40. Bopassa JC, Eghbali M, Toro L, 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.
41. Weil BR, Manukyan MC, Herrmann JL, et al. Signaling via GPR30 protects the myocardium from ischemia/reperfusion injury. *Surgery*. 2010;148(2):436–443.
42. Filice E, Recchia AG, Pellegrino D, et al. A new membrane G protein-coupled receptor (GPR30) is involved in the cardiac effects of 17beta-estradiol in the male rat. *J Physiol Pharmacol*. 2009;60(4):3–10.
43. Kawano N, Koji T, Hishikawa Y, et al. Identification and localization of estrogen receptor alpha- and beta-positive cells in adult male and female mouse intestine at various estrogen levels. *Histochem Cell Biol*. 2004;121(5):399–405.
44. Datta S, Hey VM, Pleuvry BJ. Effects of pregnancy and associated hormones in mouse intestine, in-vivo and in-vitro. *Pflugers Arch*. 1974;346(2):87–95.
45. Beckett EA, McCloskey C, O'Kane N, et al. Effects of female steroid hormones on A-type K+ currents in murine colon. *J Physiol (Lond)*. 2006;573(pt 2):453–468.
46. Hogan AM, Collins D, Sheehan K, et al. Rapid effects of phytoestrogens on human colonic smooth muscle are mediated by oestrogen receptor beta. *Mol Cell Endocrinol*. 2010;320(1–2):106–110.
47. Harnish DC, Albert LM, Leathurby Y, et al. Beneficial effects of estrogen treatment in the HLA-B27 transgenic rat model of inflammatory bowel disease. *Am J Physiol Gastrointest Liver Physiol*. 2004;286(1):G118–G125.
48. ten Bolscher M, Netelenbos JC, Barto R, et al. Estrogen regulation of intestinal calcium absorption in the intact and ovariectomized adult rat. *J Bone Miner Res*. 1999;14(7):1197–1202.
49. Tuo BG, Wen GR, Wei JQ, et al. Estrogen regulation of duodenal bicarbonate secretion and sex-specific protection of human duodenum. *Gastroenterology*. 2011;141(3):854–863.
50. Ba ZF, Chaudry IH. Role of estrogen receptor subtypes in estrogen-induced organ-specific vasorelaxation after trauma-hemorrhage. *Am J Physiol Heart Circ Physiol*. 2008;295(5):H2061–H2067.
51. Nadal A, Alonso-Magdalena P, Soriano S, et al. The role of oestrogens in the adaptation of islets to insulin resistance. *J Physiol (Lond)*. 2009;587(pt 21):5031–5037.
52. Nadal A, Rovira JM, Laribi O, et al. Rapid insulinotropic effect of 17 beta-estradiol via a plasma membrane receptor. *Faseb J*. 1998;12(13):1341–1348.
53. Ropero AB, Soria B, Nadal A. A nonclassical estrogen membrane receptor triggers rapid differential actions in the endocrine pancreas. *Mol Endocrinol*. 2002;16(3):497–505.
54. Carmeci C, Thompson DA, Ring HZ, 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.
55. Feng Y, Gregor P. Cloning of a novel member of the G protein-coupled receptor family related to peptide receptors. *Biochem Biophys Res Commun*. 1997;231(3):651–654.
56. Kvingedal AM, Smeland EB. A novel putative G-protein-coupled receptor expressed in lung, heart and lymphoid tissue. *FEBS Lett*. 1997;407(1):59–62.

57. Owman C, Blay P, Nilsson 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.
58. Hazell GGJ, Yao ST, Roper JA, 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.
59. Sanden C, Broselid S, Cornmark L, et al. G protein-coupled estrogen receptor 1/g protein-coupled receptor 30 localizes in the plasma membrane and traffics intracellularly on cytokeratin intermediate filaments. *Mol Pharmacol*. 2011;79(3):400–410.
60. Lafferty AR, Torpy DJ, Stowasser M, et al. A novel genetic locus for low renin hypertension: familial hyperaldosteronism type II maps to chromosome 7 (7p22). *J Med Genet*. 2000;37(11):831–835.
61. Lindsey SH, Cohen JA, Brosnihan KB, et al. Chronic treatment with the G protein-coupled receptor 30 agonist G-1 decreases blood pressure in ovariectomized mRen2.Lewis rats. *Endocrinology*. 2009;150(8):3753–3758.
62. Gilliam-Davis S, Cohen JA, Lindsey SH, et al. The estrogen receptor GPR30 is renoprotective in diabetic hypertensive female rats. *Hypertension*. 2010;56(5): E56–E56.
63. Martensson UE, Salehi SA, Windahl S, 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.
64. Bland R. Steroid hormone receptor expression and action in bone. *Clin Sci*. 2000;98(2):217–240.
65. Imai Y, Kondoh S, Kouzmenko A, et al. Minireview: osteoprotective action of estrogens is mediated by osteoclastic estrogen receptor-alpha. *Mol Endocrinol*. 2010;24(5):877–885.
66. Heino TJ, Chagin AS, Savendahl L. The novel estrogen receptor G-protein-coupled receptor 30 is expressed in human bone. *J Endocrinol*. 2008;197(2): R1–R6.
67. Chagin AS, Savendahl L. GPR30 estrogen receptor expression in the growth plate declines as puberty progresses. *J Clin Endocrinol Metab*. 2007;92(12): 4873–4877.
68. Ohlsson C, Vandenput L. The role of estrogens for male bone health. *Eur J Endocrinol*. 2009;160(6):883–889.
69. Carani C, Qin K, Simoni M, et al. Effect of testosterone and estradiol in a man with aromatase deficiency. *N Engl J Med*. 1997;337(2):91–95.
70. Smith EP, Boyd J, Frank GR, et al. Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. *N Engl J Med*. 1994;331(16):1056–1061.
71. Smith EP, Specker B, Bachrach BE, et al. Impact on bone of an estrogen receptor-alpha gene loss of function mutation. *J Clin Endocrinol Metab*. 2008; 93(8):3088–3096.
72. Ohata C, Tadokoro T, Itami S. Expression of estrogen receptor beta in normal skin, melanocytic nevi and malignant melanomas. *J Dermatol*. 2008; 35(4):215–221.
73. Ogueta SB, Schwartz SD, Yamashita CK, et al. Estrogen receptor in the human eye: influence of gender and age on gene expression. *Invest Ophthalmol Vis Sci*. 1999;40(9):1906–1911.
74. Munaut C, Lambert V, Noel A, et al. Presence of oestrogen receptor type beta in human retina. *Br J Ophthalmol*. 2001;85(7):877–882.
75. Meyer MR, Clegg DJ, Prossnitz ER, et al. Obesity, insulin resistance and diabetes: sex differences and role of oestrogen receptors. *Acta Physiol (Oxf)*. 2011;203(1):259–269.
76. Faulds MH, Zhao C, Dahlman-Wright K, et al. The diversity of sex steroid action: regulation of metabolism by estrogen signaling. *J Endocrinol*. 2012; 212(1):3–12.
77. Straub RH. The complex role of estrogens in inflammation. *Endocr Rev*. 2007; 28(5):521–574.
78. Russo J, Ao X, Grill C, et al. Pattern of distribution of cells positive for estrogen receptor alpha and progesterone receptor in relation to proliferating cells in the mammary gland. *Breast Cancer Res Treat*. 1999;53(3):217–227.
79. Cordera F, Jordan VC. Steroid receptors and their role in the biology and control of breast cancer growth. *Semin Oncol*. 2006;33(6):631–641.
80. Kazmi N, Marquez-Garban DC, Aivazyan L, et al. The role of estrogen, progesterone and aromatase in human non-small-cell lung cancer. *Lung Cancer Manag*. 2012;1(4):259–272.
81. Filardo EJ, Graeber CT, Quinn JA, 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.
82. Jordan VC, Lewis-Wambi J, Kim H, et al. Exploiting the apoptotic actions of oestrogen to reverse antihormonal drug resistance in oestrogen receptor positive breast cancer patients. *Breast*. 2007;16(2 Supplement): S105–S113.
83. Ignatov A, Ignatov T, Roessner A, et al. Role of GPR30 in the mechanisms of tamoxifen resistance in breast cancer MCF-7 cells. *Breast Cancer Res Treat*. 2010;123(1):87–96.
84. Koehler KF, Helguero LA, Haldosen LA, et al. Reflections on the discovery and significance of estrogen receptor beta. *Endocr Rev*. 2005;26(3):465–478.
85. Gustafsson JA. Estrogen receptor beta – a new dimension in estrogen mechanism of action. *J Endocrinol*. 1999;163(3):379–383.
86. Kaiser U, Hofmann J, Schilli M, et al. Steroid-hormone receptors in cell lines and tumor biopsies of human lung cancer. *Int J Cancer*. 1996;67(3):357–364.
87. Patrone C, Cassel TN, Pettersson K, et al. Regulation of postnatal lung development and homeostasis by estrogen receptor beta. *Mol Cell Biol*. 2003; 23(23):8542–8552.
88. Bardin A, Boulle N, Lazennec G, et al. Loss of ERbeta expression as a common step in estrogen-dependent tumor progression. *Endocr Relat Cancer*. 2004;11(3):537–551.
89. Bouchardy C, Benhamou S, Schaffar R, et al. Lung cancer mortality risk among breast cancer patients treated with anti-estrogens. *Cancer*. 2011; 117(6):1288–1295.
90. Miki Y, Abe K, Suzuki S, et al. Suppression of estrogen actions in human lung cancer. *Mol Cell Endocrinol*. 2011;340(2):168–174.
91. Wakelee HA, Chang ET, Gomez SL, et al. Lung cancer incidence in never smokers. *J Clin Oncol*. 2007;25(5):472–478.
92. The Coronary Drug Project. Findings leading to discontinuation of the 2.5-mg day estrogen group. The coronary Drug Project Research Group. *JAMA*. 1973;226(6):652–657.
93. Chlebowski RT, Prabhaker R, Tagawa T. Breast cancer chemoprevention: targeting the estrogen receptor. *Am J Hematol Oncol*. 2016;12(1):6–13.
94. Ganti AK, Sahmoun AE, Panwalkar AW, et al. Hormone replacement therapy is associated with decreased survival in women with lung cancer. *J Clin Oncol*. 2006;24(1):59–63.
95. Pietras RJ, Marquez DC, Chen HW, et al. Estrogen and growth factor receptor interactions in human breast and non-small cell lung cancer cells. *Steroids*. 2005;70(5–7):372–381.
96. Weinberg OK, Marquez-Garban DC, Fishbein MC, et al. Aromatase inhibitors in human lung cancer therapy. *Cancer Res*. 2005;65(24):11287–11291.
97. Cupit-Link MC, Kirkland JL, Ness KK, et al. Biology of premature ageing in survivors of cancer. *Esmo Open*. 2017;2(5):e000250.
98. Wong CK, So WY, Law SK, et al. Estrogen controls embryonic stem cell proliferation via store-operated calcium entry and the nuclear factor of activated T-cells (NFAT). *J Cell Physiol*. 2012;227(6):2519–2530.
99. Breu A, Sprinzing B, Merkl K, et al. Estrogen reduces cellular aging in human mesenchymal stem cells and chondrocytes. *J Orthop Res*. 2011;29(10): 1563–1571.
100. Paquette B. Enhancement of genomic instability by 17 beta-estradiol in minisatellite sequences of X-ray-transformed mouse 10T1/2 cells. *Carcinogenesis*. 1996;17(6):1221–1225.
101. Williamson LM, Lees-Miller SP. Estrogen receptor alpha-mediated transcription induces cell cycle-dependent DNA double-strand breaks. *Carcinogenesis*. 2011;32(3):279–285.
102. Lin C, Yang L, Tanasa B, et al. Nuclear receptor-induced chromosomal proximity and DNA breaks underlie specific translocations in cancer. *Cell*. 2009; 139(6):1069–1083.
103. Tubbs A, Nussenzweig A. Endogenous DNA damage as a source of genomic instability in cancer. *Cell*. 2017;168(4):644–656.
104. Li JJ, Weroha SJ, Davis MF, et al. ER and PR in renomedullary interstitial cells during Syrian hamster estrogen-induced tumorigenesis: evidence for receptor-mediated oncogenesis. *Endocrinology*. 2001;142(9):4006–4014.
105. Savage KI, Matchett KB, Barros EM, et al. BRCA1 deficiency exacerbates estrogen-induced DNA damage and genomic instability. *Cancer Res*. 2014; 74(10):2773–2784.
106. Carruba G, Cocciadiferro L, Bellavia V, et al. Intercellular communication and human hepatocellular carcinoma. *Ann N Y Acad Sci*. 2004;1028(1): 202–212.
107. Chung TH, Wang SM, Wu JC. 17β-estradiol reduces the effect of metabolic inhibition on gap junction intercellular communication in rat cardiomyocytes via the estrogen receptor. *J Mol Cell Cardiol*. 2004;37(5):1013–1022.
108. Iwase Y, Fukata H, Mori C. Estrogenic compounds inhibit gap junctional intercellular communication in mouse Leydig TM3 cells. *Toxicol Appl Pharmacol*. 2006;212(3):237–246.
109. O'Lone R, Knorr K, Jaffe IZ, et al. Estrogen receptors alpha and beta mediate distinct pathways of vascular gene expression, including genes involved in mitochondrial electron transport and generation of reactive oxygen species. *Mol Endocrinol*. 2007;21(6):1281–1296.
110. Scarpulla RC. Nuclear control of respiratory gene expression in mammalian cells. *J Cell Biochem*. 2006;97(4):673–683.
111. Mattingly KA, Ivanova MM, Riggs KA, et al. Estradiol stimulates transcription of nuclear respiratory factor-1 and increases mitochondrial biogenesis. *Mol Endocrinol*. 2008;22(3):609–622.
112. Chang PK, Boridy S, McKinney RA, et al. Letrozole potentiates mitochondrial and dendritic spine impairments induced by beta amyloid. *J Aging Res*. 2013; 2013:538979.
113. Calado RT, Yewdell WT, Wilkerson KL, et al. Sex hormones, acting on the TERT gene, increase telomerase activity in human primary hematopoietic cells. *Blood*. 2009;114(11):2236–2243.
114. Wang Z, Kyo S, Maida Y, et al. Tamoxifen regulates human telomerase reverse transcriptase (hTERT) gene expression differently in breast and endometrial cancer cells. *Oncogene*. 2002;21(22):3517–3524.
115. Dumasia K, Kumar A, Deshpande S, et al. Estrogen signaling, through estrogen receptor beta, regulates DNA methylation and its machinery in male germ line in adult rats. *Epigenetics*. 2017;12(6):476–483.

116. Jadhav RR, Ye ZQ, Huang RL, et al. Genome-wide DNA methylation analysis reveals estrogen-mediated epigenetic repression of metallothionein-1 gene cluster in breast cancer. *Clin Epigenetics*. 2015;7(1):13.
117. Stone A, Zotenko E, Locke WJ, et al. DNA methylation of oestrogen-regulated enhancers defines endocrine sensitivity in breast cancer. *Nat Commun*. 2015;6(1):7758.
118. Stevenson TJ. Environmental and hormonal regulation of epigenetic enzymes in the hypothalamus. *J Neuroendocrinol*. 2017;29(5).
119. Nugent BM, Wright CL, Shetty AC, et al. Brain feminization requires active repression of masculinization via DNA methylation. *Nat Neurosci*. 2015;18(5):690.
120. Yang YM, Sun D, Kandhi S, et al. Estrogen-dependent epigenetic regulation of soluble epoxide hydrolase via DNA methylation. *Proc Natl Acad Sci USA*. 2018;115(3):613–618.
121. Levine ME, Lu AT, Chen BH, et al. Menopause accelerates biological aging. *Proc Natl Acad Sci USA*. 2016;113(33):9327–9332.
122. Peek CB, Ramsey KM, Marcheva B, et al. Nutrient sensing and the circadian clock. *Trends Endocrinol Metab*. 2012;23(7):312–318.
123. Raina K, Noblin DJ, Serebrenik YV, et al. Targeted protein destabilization reveals an estrogen-mediated ER stress response. *Nat Chem Biol*. 2014;10(11): 957–962.
124. Rocca WA, Bower JH, Maraganore DM, et al. Increased risk of cognitive impairment or dementia in women who underwent oophorectomy before menopause. *Neurology*. 2007;69(11):1074–1083.
125. Rocca WA, Grossardt BR, de Andrade M, et al. Survival patterns after oophorectomy in premenopausal women: a population-based cohort study. *Lancet Oncol*. 2006;7(10):821–828.
126. Rocca WA, Grossardt BR, Miller VM, et al. Premature menopause or early menopause and risk of ischemic stroke. *Menopause*. 2012;19(3):272–277.
127. Rocca WA, Ulrich LG. Oophorectomy for whom and at what age? Primum non nocere. *Maturitas*. 2012;71(1):1–2.
128. Parker WH, Feskanich D, Broder MS, et al. Long-term mortality associated with oophorectomy compared with ovarian conservation in the Nurses' Health Study. *Obstet Gynecol*. 2013;121(4):709–716.
129. Parker WH, Broder MS, Chang E, et al. Ovarian conservation at the time of hysterectomy and long-term health outcomes in the Nurses' Health Study. *Obstet Gynecol*. 2009;113(5):1027–1037.
130. Gierach GL, Pfeiffer RM, Patel DA, et al. Long-term overall and disease-specific mortality associated with benign gynecologic surgery performed at different ages. *Menopause*. 2014;21(6):592–601.
131. Mytton J, Evison F, Chilton PJ, et al. Removal of all ovarian tissue versus conserving ovarian tissue at time of hysterectomy in premenopausal patients with benign disease: study using routine data and data linkage. *BMJ*. 2017;356(8002):j372.
132. Ossewaarde ME, Bots ML, Verbeek AL, et al. Age at menopause, cause-specific mortality and total life expectancy. *Epidemiology*. 2005;16(4):556–562.
133. Duan L, Xu X, Koebnick C, et al. Bilateral oophorectomy is not associated with increased mortality: the California Teachers Study. *Fertil Steril*. 2012; 97(1):111–117.
134. Jacoby VL, Grady D, Wactawski-Wende J, et al. Oophorectomy vs ovarian conservation with hysterectomy: cardiovascular disease, hip fracture, and cancer in the Women's Health Initiative Observational Study. *Arch Intern Med*. 2011;171(8):760–768.
135. Bove R, Secor E, Chibnik LB, et al. Age at surgical menopause influences cognitive decline and Alzheimer pathology in older women. *Neurology*. 2014; 82(3):222–229.
136. El Khoudary SR, Shields KJ, Janssen I, et al. Cardiovascular fat, menopause, and sex hormones in women: the SWAN Cardiovascular Fat ancillary study. *J Clin Endocrinol Metab*. 2015;100(9):3304–3312.
137. Finkelstein JS, Brockwell SE, Mehta V, et al. Bone mineral density changes during the menopause transition in a multiethnic cohort of women. *J Clin Endocrinol Metab*. 2008;93(3):861–868.
138. Randolph JF, Sowers MF, Bondarenko I, et al. The relationship of longitudinal change in reproductive hormones and vasomotor symptoms during the menopausal transition. *J Clin Endocrinol Metab*. 2005;90(11):6106–6112.
139. Sowers MR, Greendale GA, Bondarenko I, et al. Endogenous hormones and bone turnover markers in pre- and perimenopausal women: SWAN. *Osteoporos Int*. 2003;14(3):191–197.
140. Sowers MR, Finkelstein JS, Ettinger B, et al. The association of endogenous hormone concentrations and bone mineral density measures in pre- and perimenopausal women of four ethnic groups: SWAN. *Osteoporos Int*. 2003; 14(1):44–52.
141. Sowers MR, Jannausch M, McConnell D, et al. Hormone predictors of bone mineral density changes during the menopausal transition. *J Clin Endocrinol Metab*. 2006;91(4):1261–1267.
142. Gold EB, Crawford SL, Shelton JF, et al. Longitudinal analysis of changes in weight and waist circumference in relation to incident vasomotor symptoms: the Study of Women's Health Across the Nation (SWAN). *Menopause*. 2017;24(1):9–26.
143. Lisabeth LD, Beiser AS, Brown DL, et al. Age at natural menopause and risk of ischemic stroke: the Framingham heart study. *Stroke*. 2009;40(4): 1044–1049.
144. Clegg D, Hevener AL, Moreau KL, et al. Sex hormones and cardiometabolic health: role of estrogen and estrogen receptors. *Endocrinology*. 2017;158(5): 1095–1105.
145. Arnal JF, Fontaine C, Abot A, et al. Lessons from the dissection of the activation functions (AF-1 and AF-2) of the estrogen receptor alpha in vivo. *Steroids*. 2013;78(6):576–582.
146. Hodis HN, Mack WJ. The timing hypothesis and hormone replacement therapy: a paradigm shift in the primary prevention of coronary heart disease in women. Part 1: comparison of therapeutic efficacy. *J Am Geriatr Soc*. 2013; 61(6):1005–1010.
147. Hodis HN, Mack WJ. The timing hypothesis and hormone replacement therapy: a paradigm shift in the primary prevention of coronary heart disease in women. Part 2: comparative risks. *J Am Geriatr Soc*. 2013;61(6):1011–1018.
148. Salpeter SR, Buckley NS, Liu H, et al. The cost-effectiveness of hormone therapy in younger and older postmenopausal women. *Am J Med*. 2009; 122(1):42–52.
149. Gold EB, Sternfeld B, Kelsey JL, et al. Relation of demographic and lifestyle factors to symptoms in a multi-racial/ethnic population of women 40-55 years of age. *Am J Epidemiol*. 2000;152(5):463–473.
150. Lund TD, Rovis T, Chung WC, et al. Novel actions of estrogen receptor-beta on anxiety-related behaviors. *Endocrinology*. 2005;146(2):797–807.
151. Fisher B, Costantino JP, Wickerham DL, et al. Tamoxifen for the prevention of breast cancer: current status of the National Surgical Adjuvant Breast and Bowel Project P-1 study. *J Natl Cancer Inst*. 2005;97(22):1652–1662.
152. Amir E, Seruga B, Niraula S, et al. Toxicity of adjuvant endocrine therapy in postmenopausal breast cancer patients: a systematic review and meta-analysis. *J Natl Cancer Inst*. 2011;103(17):1299–1309.
153. Powles TJ, Hickish T, Kanis JA, et al. Effect of tamoxifen on bone mineral density measured by dual-energy x-ray absorptiometry in healthy premenopausal and postmenopausal women. *J Clin Oncol*. 1996;14(1):78–84.
154. Blaes A, Beckwith H, Florea N, et al. Vascular function in breast cancer survivors on aromatase inhibitors: a pilot study. *Breast Cancer Res Treat*. 2017; 166(2):541–547.
155. Khosrow-Khavar F, Filion KB, Al-Qurashi S, et al. Cardiotoxicity of aromatase inhibitors and tamoxifen in postmenopausal women with breast cancer: a systematic review and meta-analysis of randomized controlled trials. *Ann Oncol*. 2017;28(3):487–496.
156. Fisher BJ, Perera FE, Cooke AL, et al. Long-term follow-up of axillary node-positive breast cancer patients receiving adjuvant tamoxifen alone: patterns of recurrence. *Int J Radiat Oncol Biol Phys*. 1998;42(1):117–123.
157. Lahti E, Blanco G, Kauppila A, et al. Endometrial changes in postmenopausal breast cancer patients receiving tamoxifen. *Obstet Gynecol*. 1993;81(5(pt 1)): 660–664.
158. Cohen I, Rosen DJ, Altaras M, et al. Tamoxifen treatment in premenopausal breast cancer patients may be associated with ovarian overstimulation, cystic formations and fibroid overgrowth. *Br J Cancer*. 1994;69(3):620–621.
159. Cuzick J, Forbes J, Edwards R, et al. First results from the International Breast Cancer Intervention Study (IBIS-I): a randomised prevention trial. *Lancet*. 2002;360(9336):817–824.
160. Powles T, Eeles R, Ashley S, et al. Interim analysis of the incidence of breast cancer in the Royal Marsden Hospital tamoxifen randomised chemoprevention trial. *Lancet*. 1998;352(9122):98–101.
161. Veronesi U, Maisonneuve P, Costa A, et al. Prevention of breast cancer with tamoxifen: preliminary findings from the Italian randomised trial among hysterectomised women. Italian Tamoxifen Prevention Study. *Lancet*. 1998; 352(9122):93–97.