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Exploring the effects of estrogen deficiency and aging on organismal homeostasis during menopause

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Abstract

Sex hormone signalling declines during aging, from early mid-life through menopause, as a consequence of reduced circulating estrogens and decreased receptiveness to these hormones in target tissues. Estrogens preserve energy homeostasis and promote metabolic health via coordinated and simultaneous effects throughout the brain and body. Age-associated loss of estrogen production during menopause has been implicated in a higher risk for metabolic diseases and increased mortality. However, it remains unclear whether age-associated changes in homeostasis are dependent on reduced estrogen signalling during menopause. While menopausal hormone therapies containing estrogens can alleviate symptoms, concerns about the risks involved have contributed to a broad decline in the use of these approaches. Non-hormonal therapies have emerged that target tissues or pathways with varying levels of selectivity reduced risk. We summarize here the broad effects of estrogen loss on homeostasis during menopause, current and emerging therapies, and opportunities for understanding homeostatic disruptions associated with menopause.

Introduction

Aging is a principal risk factor for a number of diseases, including cancers, cardiovascular and neurodegenerative conditions[1]. Circulating sex hormones produced by the ovaries decline during aging (see Box 1 for an overview of estrogen production and signalling)[2]. Energy homeostasis can be broadly defined as the interplay between energy intake and energy expenditure. When imbalanced, changes in body weight and adiposity often occur, which can lead to metabolic dysfunction and increased risk of mortality and a variety of diseases. Energy homeostasis is maintained not only by peripheral cues from the body, but also by the integration of the central nervous system, specifically the hypothalamus[3]. Estrogens play a potent role in energy homeostasis across the lifespan and their decline during aging has been implicated in the increased risk for neurological and metabolic diseases [4]. The use of estrogens as a treatment to mitigate these effects has been

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implemented over the last century as menopausal hormone therapies (previously referred to as hormone replacement therapies) and sex hormone replacement. In this review we present an overview of the central and peripheral roles of estrogens in governing homeostasis across the lifespan. Unless otherwise stated, our reference to estrogens refers to the hormone family as a whole rather than one specific member. A number of studies cited in this review do not include how they determine sex or gender. We therefore interpret their definition of women to include those assigned female at birth, individuals who self-report as women or clinical studies focusing on cis women. We recognize that this excludes other individuals who go through menopause and women who do not go through menopause.

Estrogen deficiency disrupts organismal homeostasis

A notable change that occurs in estrogen signalling over human aging is the menopause transition, which is driven by changes in the follicular reserve of the ovaries. Ovarian aging is principally classified by a decline in both the quantity and quality of oocytes, with primordial follicles gradually reducing from millions at birth to within hundreds by perimenopause [19,20]. The depleted ovarian reserve and reduced production of estrogens and progestogens from the ovaries, results in wide-ranging changes in menstrual length and altered cycling of HPG axis hormones during perimenopause, followed by a cessation of menstruation and a gradual reduction of circulating estrogens to negligible levels post-menopause [21,22]. The capability to reproduce is lost after menopause at the average age of 51 [23]. Over this period changes in body composition occur including an increase in adiposity, although this increase in body fat is generally in line with the gradual increase in adiposity that occurs progressively across life [24,25]. The risk of cardiovascular events increases after menopause, as estrogens are thought to act protectively against cardiovascular disease by influencing arterial vasodilation and vascular tone [26,27]. By contrast, bone density shows a marked decline after menopause compared to earlier ages, with this reduction beginning many years before the onset of menopause [28,29] and the risk for fractures is increased post-menopause[30]. An increase in bone turnover with reduced bone formation results in the loss of trabecular bone and overall bone density [31]. Along with a loss of estrogens, a variety of hormones show dramatic changes in their circulating levels over and after menopause. For example, increased FSH is implicated in driving the loss of bone mass that co-occurs with lowered circulating estrogen [32]. However, estrogen-based menopausal hormone therapy (MHT) can ameliorate some of the changes in body composition that occur with menopause (discussed below), suggesting that estrogen signalling, both directly or via effects on other aspects of the HPG-axis (e.g. reducing FSH levels), is implicated in the changes in organismal homeostasis that occur over menopause (Figure 1).

Evidence of ovarian estrogen loss as the driver of changes in health during menopause is observable in comparisons of those who have undergone surgical removal of their ovaries for health conditions. Premenopausal research participants undergoing oophorectomy for benign health conditions have been compared to reference groups who have not undergone a surgical sterilization procedure, or who have undergone a hysterectomy with ovarian conservation [33,34]. Ovary removal has been associated with an increased cardiovascular disease incidence/severity [35], increased mortality [33,35], increased risk of bone fracture [36], increased risk of dementia[37], and increased anxiety, [38] although it has been noted

that population differences and the reasons for why people undergo a specific surgery could explain these relationships. Indeed, in large-scale comparisons (~25,000 individuals) of women who entered the Women's Health Initiative observational study, no long-term negative health consequences of ovarian removal were documented, although most went through surgery around the age of typical menopause [39]. Overall, studies have indicated that risk of impaired health with oophorectomy is greatest when ovaries are removed early in life, before the menopause transition begins. For example, worsening of vasomotor symptoms and a decline in sexual function and satisfaction have been reported in patients who underwent an oophrectomy while in perimenopause [40]. This is consistent with health consequences of natural menopause, which appear to be more profound when menopause occurs prematurely [41]. Thus, abrupt and/or early loss of ovarian estrogen production appears to be associated with the most profound health effects in humans, although changes in associated hormones or confounding disease states may explain these results.

Estrogen replacement can extend lifespan in animal models

The direct effects of ovarian hormone loss on energy homeostasis have been studied in rodent models. Ovariectomy (OVX) in mice and rats is considered an obesogenic manipulation since this is associated with increased body weight and adiposity after surgery [42,43]. This is counteracted by treatment with 17βE2, highlighting the importance of estrogens in regulation of body weight in rodents [44,45]. Similar effects have been noted in clinical studies, with participants randomised to MHT containing estrogens over four years demonstrating a lower BMI in comparison with placebo participants [46]. Effects of OVX on lifespan and aging have been less consistent. If endogenous estrogen production has a positive effect on female survival then survival should be consistently reduced with OVX. Some studies show that OVX can reduce survival [46,47], others that OVX increases lifespan [47,48] with others demonstrating little change in survival [49,50]. Notably, many of these experiments were conducted prior to 1980 when deaths due to infectous disease where often reported in laboratory housed rodents and the average lifespan was much shorter, thus some results maybe complicated by effects of estrogens on the immune system.

There has been a more recent focus on the effects of direct estrogen treatment on lifespan in animal models. The Interventions Testing Program, funded by the National Institute of Aging, has reported that treatment with 17-alpha estradiol (17αE2), a steroisomer of 17βE2, can increase the lifespan of male mice by nearly 20%, although the same treatment has no effect on the survival of females [51,52]. Male mice treated with 17αE2 have improved glucose tolerance [53–55] and greater physical function and muscle mass at later ages [56], suggesting that this treatment slows various aspects of aging. The pathway through which 17αE2 is acting to influence aging is unclear, although 17αE2 treatment stimulates ERα expression in vitro, and the metabolic benefits of this treatment for mice on a high fat diet are lost in mice that do not express ERα in all tissues [57]. It has been assumed that at least a part of the metabolic and anti-aging benefits of this treatment are centrally mediated by ERα signalling in the hypothalamus, which regulates changes in feeding behaviour and energy expenditure (explained below). Indeed, mice that have a knockdown of pro-opiomelanocortin (*Pomc*), a precursor polypeptide that controls feeding behaviour, show a diminished response to 17αE2 for some traits [58]. However, a recent study has demonstrated that changes in body weight

and glucose tolerance with 17αE2 treatment when on a high fat diet ae maintained even when ERα expression is strongly reduced in the hypothalamus through loss of expression in either glutamatergic or GABAergic neurons [55]. It is possible that ERα signalling in multiple neuronal populations is required to drive metabolic changes in response to 17αE2, or that peripheral estrogen receptors are important in causing metabolic health benefits.

There has also been an interest in understanding why 17αE2 provides anti-aging benefits only to male and not female mice. Initial assumptions were that 17αE2 is replicating in male mice an anti-aging benefit that female mice receive from circulating 17βE2. Indeed, it is hypothesized that individuals sexed female on average live longer than individuals sexed male as a consequence of their exposure to ovarian estrogens and therefore understanding how 17αE2 acts to improve male survival could also help to explain sexual dimorphism in aging [59,60]. Suprisingly, however, the benefical effects of 17αE2 on glucose tolerance and physical function depend on male mice having intact testes, with castrated animals not showing the anti-aging benefits of 17αE2 treatment [53,56]. OVX female mice also show limited responsiveness to 17αE2 [53,56], suggesting that this treatment is not simply replacing the benefical effects that female animals gain from ovarian estrogen production. This work highlights the importance of estrogen signalling in male aging and a role for ERα in providing the health benefits of treatment, although the precise signalling pathways remain unclear. A similar 15% increase in lifespan has recently been reported in male mice treated with estriol, demonstrating that the lifespan response is not specific to 17αE2 and multiple estrogens can elicit the anti-aging response in male mice. Interestingly however, the same treatment caused a 6% reduction in female lifespan in mice that had inctact ovaries for the course of treatment [61].

While 17αE2 fails to influence aging in female mice, the impact of ovaries on female aging has been illustrated via ovarian transplant studies in CBA mice, which show ovarian failure relatively early in life compared to other mouse strains. In these studies OVX alone had a negligible effect on survival compared to controls, but surgical transplantation of young ovaries to middle-aged mice (11 months old) increased life-expectancy [62,63]. Female mice with transplanted ovaries also showed less evidence of cardiomyopathy at death compared to controls, and this benefit was greatest in animals that were still experiencing reproductive cycles at the time they received a young ovary [64]. This is consistent with the hypothesis that MHT may provide the greatest health benefits when initiated at the time of the menopause transition (discussed in further detail later). However, while it was initially assumed that the benefits of receiving young ovaries at mid-life was a consequence of increased exposure to ovarian hormones, particularly estradiol, more recent studies suggest that these benefits are hormone-independent and are a consequence of the transfer of somatic cells in ovaries. Selectively destroying germ cells from ovaries, which precludes follicle development and estrogen production, before transplanting them to recipients failed to block the lifespan effect and instead surprisingly increased the degree of lifespan extension [65]. Notably, this was a small study (n=6 per group) that only assessed changes in median lifespan in a model with early ovarian senescence and relatively short longevity. However, the results would suggest that other aspects of ovarian tissue, in addition to germline cells and associated estrogen production, may be important in the control of female lifespan.

Prior research in humans and animal models highlights a key role of ovarian hormones, and likely ovarian estradiol production, in the control of mortality and changes in energy homeostasis across the life course. However, to date there has been virtually no research into how estrogen signalling through specific receptors or tissues influences lifespan directly, and most results are complicated by the impact that broad manipulation of the ovary/circulating estrogens has on associated hormones (e.g. FSH, LH, activins). Instead, there has been a focus over the last decade on how estrogen signalling influences organismal energy homeostasis, which has increased the understanding of how estrogen signalling through specific receptors in specific cell types influences different aspects of activity, food intake and energy expenditure.

Estrogen deficiency results in coordinated metabolic disruptions across the brain and body

Strong evidence that estrogen signalling can benefit metabolic health comes from rodent studies. Similar to changes in caloric intake and physical activity across the menstrual cycle in humans [66,67], cyclic patterns of feeding, physical activity, and body temperature have been reported across the estrous cycle in rats [68,69] and mice [70–72]. Although these effects could be mediated by multiple hormones from the ovaries or from other signals produced in response to ovarian secretions, data from genetically engineered knockout mice point to a major role of estrogen signalling through estrogen receptor alpha (ERα). Eliminating ERα either globally or in the central nervous system leads to obesity due to increased feeding, reduced movement, and reduced thermogenesis in both males and females [73–75], pointing to a critical role for ERα signalling in energy balance.

Tissue-specific or cell-type-specific conditional knockout mouse models have begun to reveal the central mediators of estrogen's metabolic effects. Estrogens modulate food intake via ERα signalling in the arcuate nucleus [71,75] and the nucleus of the solitary tract [76], as well as through other estrogen receptors in the arcuate [77]. Central ERα signalling also has potent effects on energy expenditure. Estrogens modulate two types of energy expenditure via ERα signalling in the ventromedial nucleus of the hypothalamus [75,78–81] and the medial preoptic area [82,83]. Interestingly, the metabolic effects of estrogens via the hypothalamus (and the preoptic area, which is functionally related to the hypothalamus but has a distinct developmental origin [84]) are female-specific. ERα signalling outside of the hypothalamus, in the medial amygdala, alters physical activity in male mice [85]. Together these studies suggest that estrogens in the non-masculinized brain act on multiple regions in concert to maintain energy balance, and on distinct regions in the masculinized brain to exert a similar effect.

Aging is very likely to alter both estrogen signalling and cellular processes in the hypothalamus. The expression of ERα and ERβ decline with age in the brains of female rats, although this occurs in a region-specific manner [86,87]. These age-effects are also observable in OVX rats treated with estradiol which may suggest that old and young animals will show different signalling responses to estrogen treatment because they differ in receptor expression [86]. In support of this, previous research in rats has shown that old animals require greater estradiol doses to elicit memory improvements when compared to younger animals

[88]. More generally, many cellular processes within hypothalamic cells are altered with aging. Gene expression analyses comparing aged and young female mice identified differences in aging signatures within cell clusters with predicted roles in feeding and energy balance [89]. These signatures of hypothalamic aging may interact with the effects of estrogens on metabolism. Indeed, knocking out the G-protein coupled estrogen receptor (GPER) can interfere with metabolic health later in life. Female mice lacking GPER globally have higher adiposity [90] but the phenotype has been reported to emerge after 16 weeks of age [91].

Estrogen actions in peripheral tissues are also potent mediators of energy homeostasis, largely shown in rodents but also in human studies. Both ERs are expressed in the mouse liver, with ERα being the most abundant [92,93]. ERα in the liver plays an important role in glucose and insulin like growth factor (IGF-1) signalling in rodents [92,94]. Polymorphisms of *ESR1*, the gene that encodes ERα, are associated with increased plasma glucose levels and type II diabetes in human patients, demonstrating the important role of estrogen signalling in metabolic homeostasis [95]. ERα agonists can ameliorate steatosis in the liver of aromatase knockout male mice, while ablation of ERα in the liver leads to increased lipid and triglyceride accumulation in both male and female mice [94,96,97]. The potential of estrogen to enhance liver regeneration has been discussed for decades, with both estradiol treatment and ERα binding activity supporting the induction of regrowth post hepatectomy in rodents [98,99]. Interestingly, oral estrogen administration after menopause in humans with non-alcoholic fatty liver disease actually worsened the disease, however, transdermal routes reduced the prevalence [100]. It is likely that while estrogen signalling plays an important role in maintaining homeostasis in the liver, the delivery route and timing of treatment may influence its protective potential.

Adipose tissue is largely considered an endocrine gland with insulin sensitivity, immune responses and mitochondrial activity being labelled as the hallmarks of adipocyte physiology [4]. Adiposity deposition changes after menopause, redistributing from femoral and gluteal subcutaneous deposits to increased visceral adipose tissue. This elevated central adiposity can increase insulin resistance, lipolysis and lead to overall metabolic disease [4,101]. It is widely known that OVX in rodents induces weight gain, with mice showing an increase in abdominal fat after 12 weeks [102]. In OVX mice, fat deposition changes as a direct consequence of reduced estrogen signalling, with increased visceral and subcutaneous fat that is not due to a reduction in physical activity [103]. OVX also increases adiponectin levels in female rats, a hormone secreted by adipose tissue [104]. Global ERαKO mice and selective knockdown of ERα in adipose tissue and adipocytes significantly increases body fat, adipocyte size, tissue inflammation and fibrosis in both male and female animals [105].

White (WAT) and brown (BAT) adipose tissues have distinct metabolic functions, therefore it is important to discuss their roles separately when considering fluctuating estrogen levels. WAT is essential for glucose homeostasis, the secretion of leptin and adiponectin and energy storage, while BAT plays a major role in thermogenesis and energy expenditure [106–109]. Post-menopausal persons have a decreased core temperature and interestingly, a reduction in core temperature has been linked with large decreases in energy expenditure [110,111]. The conversion of WAT to BAT known as browning, which can happen in certain WAT depots, is generally considered protective against metabolic dysfunction, due to BAT's

capacity to consume glucose and fatty acids [112]. The role of estrogen signalling in beiging is somewhat controversial. The addition of 17βE2 to OVX rats leads to increased expression of thermogenesis markers such as the UCP-1 protein and increased temperature at interscapular BAT regions [78]. Selective activation of ERα in female mice induces beiging of WAT and stimulates UCP-1 expression, acting protectively against metabolic dysfunction [113]. In humans, decreased *ESR1* and *ESR2* mRNA levels have been reported in subcutaneous and visceral adipose tissue following bariatric surgery for the treatment of obesity, while *ESR1* and *ESR2* mRNA increased following surgery-induced weight loss [114]. Conversely, others have shown that female mice lacking ERα expression in adipocytes are resistant to HFD-induced obesity and have improved glucose tolerance, suggesting a protective effect of reduced estrogen signalling in the context of metabolic dysfunction [115]. These conflicting effects highlight the need to fully understand the effects of reduced estrogen signalling following menopause and the exact mechanisms underpinning changes in homeostasis and metabolic function.

Estrogens play an important role in the formation and maintenance of bone, which is variable across the lifespan. Estrogen receptors are expressed in osteoblasts (which promote bone formation), osteoclasts (which promote bone resorption) and osteocytes (mechanosensory cells) [116,117], allowing for estrogens to influence multiple aspects of bone homeostasis. Pre-menopause, estrogens act to limit osteoclast bone resorption and enhance bone formation by osteoblasts via the secretion of growth factors such as transforming growth factor-α [118]. After menopause, falling estrogen levels are associated with changes in the expression of estrogen responsive genes, shifting bone homeostasis from formation to resorption by inducing the secretion of IL-6 and osteoclastogenesis. These changes reduce bone mineral density and increases both fracture rates and osteoporosis risk [119–121]. Many factors play a role in the rate of bone loss and increased resorption post menopause, including body weight and age at entering menopause, illustrating how the effects of estrogens on bone can interact with health and aging [29,122].

Muscle physiology is also influenced by circulating estrogens, with skeletal muscle expressing ERα in both mice and humans [123,124]. ERα expression in muscle correlates with metabolic health in humans, in the context of female but not male physiology [125]. Estrogens increase muscle strength and responses to anabolic exercise. Aside from the age-related decline in muscle strength that is irrespective of sex, the decline in estrogens during menopause specifically appears to reduce muscle function. In rodent models, OVX and muscle-specific elimination of ERα leads to reduced insulin sensitivity and mitochondrial function in skeletal muscle [126,127]. Following MHT administration in humans, the data can be conflicting on its beneficial effects on muscle tissue. Isometric muscle strength, mobility and muscle power have all been reportedly improved, yet other studies have reported no improvements in skeletal muscle mass after estradiol implants [128,129].

Estrogen replacement can reverse homeostatic disruptions associated with menopause

The reduction of circulating estrogens during menopause is associated with a number of symptoms of disrupted homeostasis, such as hot flushes, cognitive impairment, vaginal atrophy, bone loss, sleep disturbances and mood alterations. Menopausal hormone therapy (MHT) is the standard treatment of care for the alleviation of these symptoms [101,130–133]. Multiple studies have shown that MHT containing estrogens is highly effective in improving hot flushes [134,135], vaginal atrophy [136], depression [137], metabolism [138], libido [139], sleep disturbances [140,141] and reducing osteoporosis risk [142,143] (Table 1).

Safety surrounding estrogens in MHT has been a contentious issue. Over 27,000 women were enrolled in the Women's Health Initiative study, which aimed to ascertain if estrogens were protective against coronary heart disease. The estrogen plus progestin trial was stopped in 2002 due to reported increases in coronary events, invasive breast cancer, stroke and venous thromboembolism, while the estrogen only arm for those with a hysterectomy was ended in 2004 due to reported increased risks of stroke and breast cancer [144]. Despite being one of the most prominent studies of MHT, there were many limitations including the advanced age of participants, a focus on relative rather than absolute risks and the predominantly white demographic of participants (Table 2). After initial findings were published, MHT use in the US fell by over 70% and reductions were also reported worldwide [145–151].

The phase of menopause at which estrogen therapy is commenced is important, known as the critical therapeutic window hypothesis or timing hypothesis, originally proposed from pioneering work in atherosclerosis in aging female monkeys [152]. If estrogens are given at or around the beginning of menopause, MHT can reduce the risk of cardiovascular disease [153]. However, there is accumulating evidence that estrogenic MHT does not improve cardiovascular disease which has already been established in older post-menopausal research participants, as shown in the ELITE study, a randomized, double-blinded, placebo-controlled trial looking at atherosclerotic plaque progression during menopause [154]. Similarly, estrogens can be protective against the progression of cognitive decline if started at or around menopause, whereas MHT does not seem to reduce already established cognitive impairment in later life [155].

It is also important to consider that MHT studies have historically been limited by the underrepresentation of many populations. The Women's Health Initiative for example had predominantly white participants and in their estrogen plus progestin arm, less than 6.5% of women were black, 5.5% were Hispanic, 0.3% were Native American and 2.3% identified as Asian/Pacific Islander [156]. Menopausal symptoms also vary among populations, with vasomotor symptoms reportedly more common [157] and more bothersome [158] in African American women than in white women.

MHT use is also disproportionate among groups. In the Carolina Breast Cancer Study, black women were less likely to use MHT overall but used unopposed estrogen formulations more than white women [159]. Purported risks for breast cancer following MHT has also varied

greatly between different populations [160–162]. Another limitation of several older MHT studies is the recent shift to using natural estrogens (such as estradiol) and progestogens (such as progesterone), along with other estrogen treatments that bypass the first pass effect of oral administration by the liver, including vaginal patches and creams. Earlier studies assessing risk factors of MHT use with cardiovascular events often used conjugated equine estrogens (CEEs) like Premarin or progestins (synthetic progestogens), whereas a recent review has suggested that estradiol may have a reduced risk in initiating cardiovascular disease in comparison with CEEs and act more protectively against bone fracture or cognitive decline [163].

Non-hormonal alternatives to MHT

The approval of fezolinetant by the FDA has been the most recent non-hormonal treatment for menopause, effective in reducing moderate to severe vasomotor symptoms [164]. When estrogens in the circulation are low, GnRH neurons are stimulated to release LH in a pulsatile fashion, which is often correlated with a rise in skin temperature. This release of LH is thought to be mediated by kisspeptin expressing neurons, which also co-express neurokinin B in the hypothalamus [165,166]. Kisspeptin, neurokinin B and dynorphin (KNDY) neurons within the hypothalamus modulate hot flush activity [167]. Therefore Fezolinetant, a highly selective treatment that antogonizes the neurokinin B receptor, has emerged as an effective treatment for vasomotor symptoms resulting from low levels of circulating estrogens during menopause. Other treatments include selective serotonin reuptake inhibitors (SSRIs) for the treatment depressive symptoms, however they are generally not effective for weight gain or severe vasomotor symptoms [168]. Phytoestrogens are a compound found in plants which have similar properties to estradiol [169]. While some studies have shown their efficacy against hot flushes, there have also been reports of altered HPG axis signalling and adverse effects on reproductive organs [169,170].

Selective estrogen receptor modulators (SERMS) are synthetic, nonsteroidal ligands that can act either as estrogen receptor agonists or antagonists depending on the target gene or tissue, where they compete against estrogens and cause confirmational changes to ligand-receptor structural complexes [171]. SERMS currently approved by the FDA include Tamoxifen, Raloxifene and Bazedoxifene. A significant limitation of these ligands however, is the lack of an ideal SERM which acts agonistically in tissues where estrogenic action is desirable, such as muscle, bone or even the brain, but inhibits estrogenic action in reproductive tissues [172]. Tamoxifen is a commonly used SERM to treat invasive breast cancer which functions as an antagonist and agonist in the breast and uterus respectively, with the latter increasing the risk of endometrial hyperplasia [173]. Tamoxifen also causes hot flushes in over 70% of patients, but the mechanism is poorly understood [174]. Raloxifene is a SERM prescribed to treat osteoporosis due to its agonistic actions in bone, but can also increase the risk of deep vein thrombosis or further exacerbate stroke risk in patients with an already elevated cardiovascular disease risk profile. Raloxifene also is not effective for treating hot flushes and may also exasperate vasomotor symptoms. [175–177].

There are currently no SERMS approved for the treatment of hot flushes, however bazedoxifene can be useful when prescribed in conjunction with a conjugated equine

estrogen in what is known as a tissue selective estrogen complex [178]. This is generally suitable when the uterus is intact and it is greater than a year since the last menstrual period. The clinical benefit of using tissue selective estrogen complexes over traditional MHT include the prevention of bone loss while avoiding proliferation of breast or endometrium tissue due its estrogen antagonistic properties, removing the need for a progestogen in combination with a conjugated equine estrogen [179]. Ospemifene, a SERM similar in structure to tamoxifen, was approved by the FDA in the last decade for the treatment of vulvovaginal atrophy and acts agonistically in vaginal epithelium, with minimal effects on the endometrium [180]. However, clinical trials involving ospemifene ran for only 52 weeks, therefore the longer acting effects of this SERM are unknown [181].

Opportunities and the future for targeting estrogen receptors in MHT

The past two decades have seen an increased application of genetic manipulations to understand the roles of specific estrogen receptor signalling pathways in different tissues, cell types and neuronal populations, predominantly using rodents as a model. This has revealed profound impacts of estrogen receptor signalling at specific neurons on whole body energy expenditure and homeostasis, and at the same time important local actions of estrogens in tissues like liver and fat that also play key roles in energy metabolism and storage. Despite this knowledge, estrogen-related hormone treatments for menopause have barely progressed over the same period, with the only major new treatment for menopause symptoms targeting kisspeptin expressing neurons, that likely manifest only a subset of physiological changes that occur with the menopause transition. The development of treatments that can ameliorate the constellation of homeostatic changes, without stimulating tissues that might increase cancer or cardiovascular disease risk, could provide major improvements in menopausal care.

One strategy to specifically target estrogens is the use of estrogen associated prodrugs that are metabolized to produce bioactive estrogens in certain tissues, while reducing off target effects. Given the role of the brain in mediating many of the effects of estrogens in health and homeostasis, prodrugs have been created that target bioactive estrogens specifically to central nervous system (CNS). Currently under investigation is the novel prodrug of 17βE2, 10β,17β-dihydroxyestra-1,4-dien-3-one (DHED). DHED is an inert derivative of 17βE2 which is enzymatically converted to estradiol exclusively within the brain by a CNS specific enzyme and has a short acting half-life, leading to brain-specific estrogenic activity without actions in the periphery. DHED has already improved symptoms in animal models of CNS regulated conditions such as cognitive decline, stroke, hot flush and depression [182,183]. Whether DHED has the potential to exert protective effects against symptoms originating in the periphery such as bone loss remains to be investigated. Therefore, the generation of prodrugs or other pharmacological agents which selectively target estrogens or their signalling pathways may pave the way for effective alternative hormonal treatments, while reducing off- target effects.

Treatments that stimulate or inhibit specific pathways downstream of estrogen receptors may also be an alternative option to broaden therapeutic options with estrogens. For example, pathway preferential estrogens (often referred to as PaPes) have been developed

that have a lower binding affinity for ERα than 17βE2. Consequently, binding of pathway preferential estrogens is insufficient to sustain nuclear-pathway activity of ERα, but is still effective to stimulate the extranuclear pathway. Pathway preferential estrogens have been shown to reduce adiposity, body weight and triglyceride levels in mice, without causing proliferation of mammary, thymus or uterine tissue, as well as exerting protective effects on cardiovascular epithelium in OVX mice [184,185].

Another possibility is the incorporation of other estrogens currently not widely used in MHT. 17αE2 has already been discussed as a lifespan extending drug preferentially favouring male mice [51,54]. Continuing to understand the sexually dimorphic patterns underpinning the signalling of this estrogen could help uncover male-specific mechanisms which have the potential to be beneficially targeted during female aging. Estetrol (E4) produced by the fetus during pregnancy is thought to have more tissue-specific and favourable actions on organs such as the liver [186]. E4 has also been shown to improve vasomotor symptoms in rodent hot flush models [187], was promising in a phase I clinical trial, and is currently under further development for use against hot flushes [188]. Estriol (E3) also has a role for reducing menopausal symptoms, however a number of studies are now quite dated. E3 is metabolised from estradiol and has been shown to improve climactic symptoms while not inducing proliferation of the endometrium or breast tissue in a Japanese cohort [189]. Transvaginal estriol has also proven effective in treating vaginal atrophy [190]. Estriol has also been shown as a safe method of MHT in a phase III clinical trial in those with ER positive breast cancer [191]. The more widespread incorporation of alternative estrogens to those commonly used may therefore be at the forefront of future MHT treatments.

Conclusions

Estrogen signalling has widespread and coordinated effects across the brain and body to maintain energy homeostasis. In addition to preserving energy balance, ovarian estrogens have the potential to alter lifespan, either directly or indirectly. These intercalated effects of estrogens are disrupted during aging, which is associated with several metabolic perturbations and increased mortality. While MHT can alleviate some of these symptoms and provide overall benefit, studies involving MHT risk profiles have had severe limitations and future studies encompassing more appropriate groups are needed before conclusive thoughts on estrogen use at midlife and through aging can be critically assessed. Newer non-hormonal alternatives have the exciting potential to provide more nuanced therapeutic options, targeting tissues or pathways with varying levels of selectivity or specificity. A move toward more precise therapies may limit the benefits to certain tissues or side effects, but also provide an opportunity to minimize risks. Ultimately, understanding the effects of estrogens across many tissues and life stages will be foundational for counteracting the suite of homeostatic disruptions associated with menopause.

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Box 1: An overview of Estrogen Actions

Estrogens are members of the steroid family of hormones derived from cholesterol following a series of metabolic conversions [5]. The three main forms of endogenous estrogens are estrone (E1) estradiol (E2) and estriol (E3). The predominant endogenous circulating estrogen in cycling females is 17-beta estradiol (17βE2), produced by the ovaries [6]. Estradiol is also synthesized in the testes and is produced by the aromatization of androgens in other tissues [7]. Estrogenic effects in the control of both female and male reproduction have been well documented, but estrogens also play key roles in cardiovascular, neuroendocrine, skeletal and metabolism physiology [8].

The molecular actions of most estrogens are mediated by estrogen receptors (ERs). The classical ERs include estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ), members of the nuclear receptor family. While both receptors share many conserved domains, ligand binding to either receptor can have distinct physiological roles, depending on the tissue or site of interaction [9]. ERs are expressed in various tissues throughout the body, including in reproductive (uterus, ovaries, breast, vagina, prostate and epididymis) and non-reproductive (lungs, heart, liver, colon and brain) organs [10]. Within the brain of multiple species, ERα is expressed in the amygdala, hippocampus, cortex, midbrain, brainstem, pituitary gland, preoptic area and the hypothalamus [11–13]. ERβ is also expressed throughout the brain, with abundant levels in the hippocampus and hypothalamus [14].

The classical mechanism of estrogen signalling involves the following:

- Diffusion of estrogens across the plasma membrane
- Subsequent binding to the N terminus of the estrogen receptor in the cytoplasm
- Confirmational change of the ligand-receptor complex
- Translocation of the ligand-receptor complex to the nucleus
- Transcription of targeted genes by binding to DNA sequences known as estrogen response elements (EREs) [15,16].

Estrogens can also signal by binding to membrane bound G-protein coupled receptors (GPERs), increasing intracellular calcium and activating signalling cascades such as MAPK [17,18].

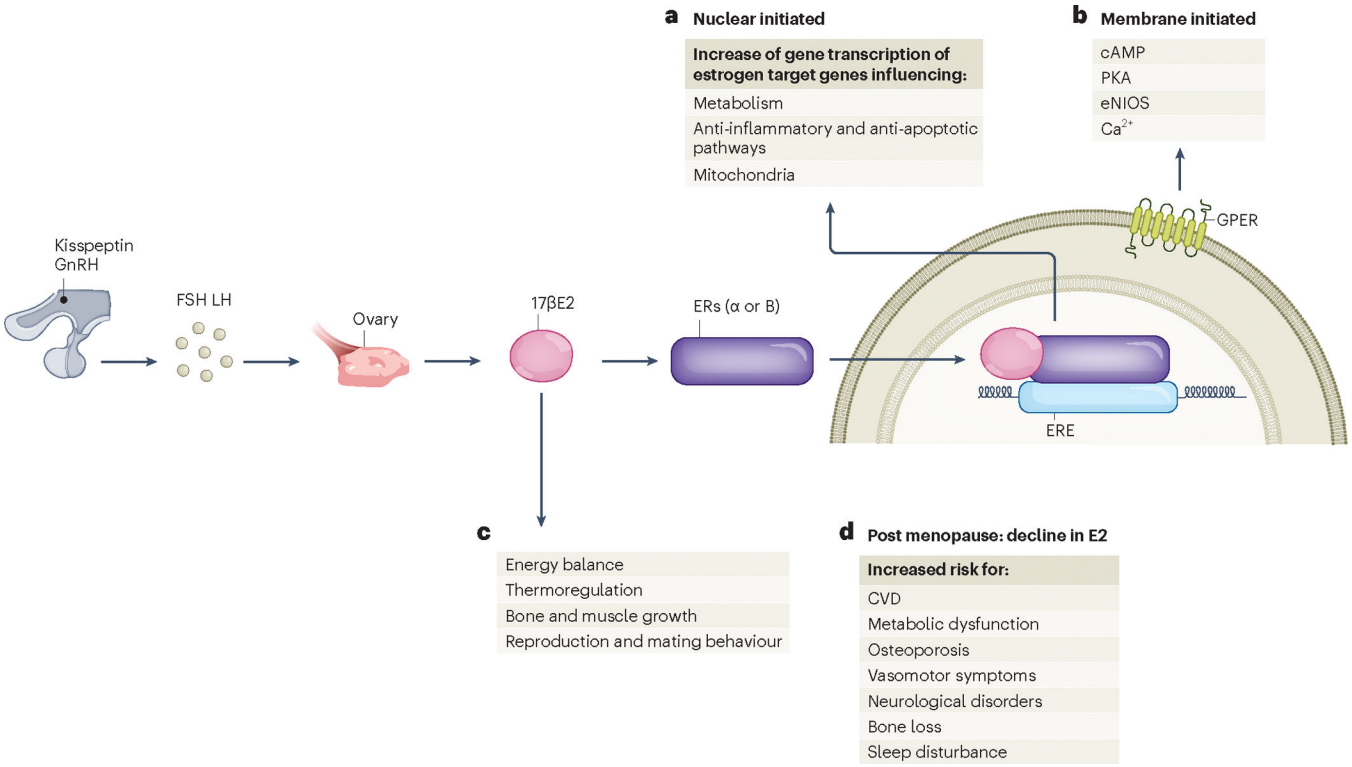

Figure 1:

Overview of estrogen signalling and physiological effects. 17-Beta Estradiol (17βE2) is the predominant circulating estrogen produced by the ovary following the release of FSH and LH from the anterior pituitary. a) 17βE2 binds to classical estrogen receptors, translocates to the nucelus and binds to estrogen response elements (EREs) which increases gene transcription influencing metabolism, anti-inflammatory pathways and mitochondrial activation by the nuclear initiated pathway. b) 17βE2 binding to membrane bound G protein coupled receptors initiate calcium signalling, cAMP, PKA and eNOS pathways. c) Estrogen signalling plays a role in energy balance, thermoregulation, bone and muscle growth and is essential for reproductive behaviours. d) reduced circulating estrogens post menopause increases the risk for cardiovascular disease (CVD), metabolic dysfunction, thermodysregulatinon, osteoporosis, neurological disorders, bone degeneration and sleep loss.

Table 1 |

MHT containing estrogens can alleviate a number of menopausal symptoms, such as sleep disturbances, vasomotor symptoms, mood disturbances, vaginal atrophy, osteoporosis, metabolic disruption and reduced libido

Menopausal Hormone Therapy (MHT)	Non-Hormonal Treatment Options
Effective in mediating:	**Includes:**
CNS derived symptoms including: Sleep disturbances, hot flushes, mood alterations, brain fog	**Neurokinin B Receptor Antagonists:** Fezolinetant - blocks neurokinin B receptor and KDNY neuron activity that stimulates thermoregulatory centres in hypothalamus, reducing hot flushes
Libido: By acting on brain regions responsible for sexual function Increases vaginal tone and blood flow	**SERMs:** Ospemifene - reduces vaginal atrophy Raloxifene - prevents osteoporosis
Vaginal Atrophy: Maintains vaginal epithelium Can improve sexual intercourse	**SSRIs:** Depressive symptoms and anxiety, vasomotor symptoms (off-label)
Osteoporosis Risk: Maintains bone density by limiting resorption	
Protection against metabolic dysfunction: Improves metabolism via mechanisms including mitochondrial protection	

Data from refs. 129,180–185. Non-hormonal options for menopausal symptoms include fezolinetant, a neurokinin B receptor antagonist, which reduces KNDY neuronal activity and reduces hot flushes[186]. Certain SERMs act agonistically or antagonistically on ERs in target tissues to improve symptoms, such as raloxifene on bone to improve osteoporosis and ospemifene on the vaginal epithelium to treat urinogenital symptoms. SSRIs, selective serotonin reuptake inhibitors.

Table 2 |

Key MHT studies that have investigated the safety of hormone use for menopausal symptoms, the efficacy of unopposed versus combined therapies and the timing hypothesis of treatment initiation

Study Name	Year Begun (data collection/ recruitment)	Type of Study	Key research question	Sample Size	Hormone(s) used	Key Conclusions	Limitations
Women's Health Initiative[132, 187]	1993	Long term observational clinical trial, community prevention study. Over 40 centres across the United States with additional satellite centres	Evaluate HRT effects on CVD, cancer and osteoporosis.	>27,000	Conjugated equine estrogen (CEE)+ medroxyprogesterone acetate Or CEE only	Estrogen only: Increased risk in ischaemic stroke Estrogen plus progestin: increased risk in coronary heart disease, breast cancer, reduced osteoporosis fractures and colorectal cancer[132].	Predominantly white participants, study initiated in women who were recruited post menopause, emphasis of relative risk versus absolute risk[188, 189]
Heart and Estrogen/ progestin Replacement Study[190, 191]	1994	Randomized, blinded, placebo-controlled secondary prevention trial. 20 centres throughout the United States	Evaluate safety of estrogen plus progesterone against recurring heart disease.	2736	CEE+ medroxyprogesterone acetate Or CEE only	No overall reduction in coronary heart disease with HRT in women with already established coronary heart disease.	Predominantly white participants, >80% had minimum of high school education, only studied post- menopausal women.
Study of Women's Health Across the Nation[192]	1994	Randomized, double-blind, placebo-controlled trial. 7 centres throughout United States	Multi- ethnic study investigating physiological and psychological changes during menopause, beginning at the premenopausal stage.	>3000	Premenopausal women, not taking hormones	Transition to late perimenopause is when symptoms tend to be strongest (vasomotor symptoms, bone loss, depression, inflammation, sleep)[15, 145, 193, 194].	Despite being multi-ethnic, white participants were the biggest group, not all regions of US were represented ie south east[192].
ELITE[195]	2004	Double blinded, placebo-controlled trial, recruitment largely based in California.	Determine if HRT's ability to reduce atherosclerosis is dependant on time of treatment initiation.	643	Oral 17βE2 only (hysterectomised people) or 17βE2 + vaginal micronized progesterone gel (uterus intact).	When HRT initiated at time of menopause or <6 years after, CVD reduced. Supports Critical Therapeutic Window Hypothesis.	Large proportion of participants were college graduates, largest proportion of participants were white.
KRONOS EARLY ESTROGEN PREVENTION STUDY[196]	2005	Randomised, double-blind, placebo-controlled trial, 9 centres across United States	To investigate HRT effects on cardiovascular outcomes on recently post- menopausal women	727	Oral CEE or transdermal 17βE2.	No increased risk reported on atherosclerosis progression after 4 years of either treatment.	Central Europeans comprised majority of participants (81%)[196].
Danish Osteoporosis Prevention Study (DOPS)[197]	1990	Prospective, investigator initiated trial, conducted at 4 centres in Denmark	Investigate if HRT is effective as a primary preventative against osteoporotic fractures	2016	Oral 17βE2 + norethisterone acetate (intact) or oral 17βE2 (hysterectomy)	When HRT was initiated early after menopause, mortality, heart failure and myocardial infarction was reduced, with no increase risk	Lack of placebo may have introduced bias[199]. Caucasian Danish participants only[200].

Study Name	Year Begun (data collection/ recruitment)	Type of Study	Key research question	Sample Size	Hormone(s) used	Key Conclusions	Limitations
						in stroke, cancer or venous thromboembolism[198]	
THE ESTROGEN IN THE PREVENTION OF ATHEROSCLEROSIS TRIAL[201]	2001	Randomized, double blind, placebo-controlled, single centre, (California) carotid artery ultrasound trial	Investigate the efficacy of unopposed estrogen to reduce atherosclerosis progression in post -menopausal women without CVD	222	Unopposed micronized 17βE2 or placebo.	17βE2 significantly reduced CVD.	White women were most abundant (59%), >83% had greater than high school education.
ESTHER[202]	1999	Multi-centre (8 hospitals) case control study in France	To investigate whether the route of administration of estrogen plays a role in VTE risk in people with a prothrombotic mutation		Oral or transdermal estrogen	Oral, but not transdermal estrogen, increases VTE risk.	Only included postmenopausal women. Only included women in the vicinity of the hospitals ie from a small geographical area.
THE ESTROGEN REPLACEMENT AND ATHEROSCLEROSIS TRIAL[203]	2000	Randomized, double-blind, placebo-controlled clinical trial at 5 clinical sites in the United States	Investigate the role of estrogen and/or estrogen plus progesterone on the progression of already developed coronary artery atherosclerosis in women	309	CEE only, CEE+ medroxyprogesterone acetate, or placebo	Neither treatment affected progression of the disease, therefore should not be used as a preventative if the disease has already been established.	Age of women in study, questions surrounding relevance of cardiovascular endpoints, duration of menopause prior to ET or EPT commencement[204].
Women's Angiographic Vitamin and Estrogen Trial[205]	2002	Randomised, double blind trial 7 clinical centres in the United States and Canada	Investigate if estrogen or estrogen plus progestin therapy, either alone or in combination with antioxidant supplements, are effective in reducing 15–75% stenosis	423	CEE (hysterectomised participants) CEE+ medroxyprogesterone acetate (, intact participants) or placebo.	Neither treatment displayed improvement of condition.	Adherence to medications in the follow up portion varied between HRT assigned and vitamin assigned groups[206].
ESTONIAN POSTMENOPAUSAL HORMONE THERAPY TRIAL[207]	1991	Blinded and unblinded HRT or placebo trial, 3 clinical centres in Estonia	Investigate HRT on symptoms reported by women and quality of life	1823	CEE+ medroxyprogesterone acetate or placebo (blinded) CEE+ medroxyprogesterone acetate Placebo or no treatment (non-blinded)	Decreased sleeping problems and vasomotor symptoms with hormone therapy but increases in vaginal bleeding. No reported increase in quality of life. [28].	Study centres were located in the two largest cities in Estonia, therefore rural populations may not have been represented[208].

A meta-analysis of clinical trials with cardiovascular outcomes can be found in ref. 209. HRT, hormone replacement therapy; VTE, venous thromboembolism; ET, estrogen therapy; EPT, estrogen plus progestogen therapy.

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