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Review

The role of estrogen in adipose tissue metabolism: insights into glucose homeostasis regulation

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Abstract. Adipose tissue is an organ with active endocrine function involved in the regulation of energy balance and glucose homeostasis *via* multiple metabolic signaling pathways targeting the brain, liver, skeletal muscle, pancreas, and other organs. There is increasing evidence demonstrating that the female sex hormone, estrogen, regulates adipose development and improves systemic glucose homeostasis in both males and females. The underlying mechanism linking estrogenic regulation in adipose tissue and systemic glucose metabolism has not been fully elucidated, but is thought to include interactions of estrogen receptor signaling events involving lipolytic and/or lipogenic enzyme activity, free fatty acid metabolism, and adipocytokine production. Thus, understanding the effects of estrogen replacement on adipose tissue biology and metabolism is important in determining the risk of developing obesity-related metabolic disorders in patients undergoing treatment for sex hormone deficiency. In this report, we review literature regarding the role of estrogens and their corresponding receptors in the control of adipose metabolism and glucose homeostasis in both rodents and humans. We also discuss the effects of selective estrogen receptor modulators on glucose metabolism.

Key words: Estrogen, Adipose tissue, Glucose homeostasis, Estrogen receptors, Selective estrogen receptor modulators

IT HAS BEEN WELL established that adipose tissue is a dynamic tissue which is involved in the regulation of glucose and lipid metabolism, energy homeostasis and inflammation. In addition, adipose tissue is a complex and highly active endocrine organ that is a major site of sex steroid metabolism and production of bioactive adipokines that act at both the local (autocrine/paracrine) and systemic (endocrine) level [1]. Thus, a functional failure of adipose tissue can cause changes in systemic energy delivery, impair glucose consumption, and affect the activation of self-regulatory mechanisms that regulate the whole body homeostasis system [2]. In addition, assessing the regional distribution of adipose tissue is critical during clinical examination of patients, particularly if they are obese [3]. The multiple endocrine abnormalities found in abdominal visceral obesity are more pronounced than in other obesity phenotypes [3].

At menopause, women begin to develop increased amounts of visceral fat with redistribution of body fat. The risk of developing obesity-related diseases is significantly lower in premenopausal women than that in men, but not anymore after menopause, indicating the significant role of the female estrogen hormone in adipogenesis and adipose metabolism [4]. Furthermore, since adipose tissue serves as a crucial integrator of glucose homeostasis, estrogen deficiency strongly contributes to impaired glucose metabolism and the development of type 2 diabetes accompanied by abnormal adipose function. In fact, visceral adiposity is associated with postmenopausal women and ovariectomized (OVX) rodents, a condition that can be reversed with 17β-estradiol (E2) treatment [5, 6]. In addition, E2 replacement therapy in postmenopausal women is associated with enhanced insulin action toward suppression of lipolysis [7], suggesting that increased lipolysis and subsequent free fatty acids (FFA) release from adipose tissue in E2-deficient women may contribute to the observed insulin resistance. Thus, it is important to understand the current knowledge relating to estrogenic regulations of glucose metabolism, particularly in adipose tissue. This review focuses on the role of estrogen in the regulation of adipose tissue biology and glucose homeostasis in rodents and humans.

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1. Clinical evidence of estrogen action in adipose tissue

It has been known that estrogen is a major regulator of adipose development and deposition in males as well as females [8]. In menopausal women, the loss of estrogen signaling produces a preferential increase in visceral fat, which results from both increased adipocyte size and number. A recent report showed that postmenopausal women have lower postprandial fatty acid oxidation, greater meal fatty acid, and direct FFA storage than premenopausal women [9]. Moreover, weight gain in postmenopausal women is strongly correlated with increases in serious health problems such as cardiovascular disease, type 2 diabetes, and breast cancer.

Adipose tissue changes in rodents caused by a lack of estrogen can be reversed with estrogen replacement. However, in postmenopausal women, the role of estrogen replacement in modulating adiposity is controversial. Chen *et al*. [10] reported that a 3-year intervention of estrogen with progestin in postmenopausal women (n = 835 total) maintained lean body mass and reduced upper-body fat distribution. Sites *et al*. [11] showed that estrogen treatment in early postmenopausal women (n = 51 total) reduced insulin sensitivity without affecting body composition or body fat distribution. Interestingly, in the Sites's study, insulin sensitivity was increased in women who had taken estrogen 1 year following discontinuation of hormone therapy to a level similar to those in women in a placebo group. Another retrospective study of 287 postmenopausal women reported that estrogen replacement for 18 months increased body fat percentage by decreasing lean body mass and by affecting upper-to-lower body fat distribution, without producing significant changes in overall weight [12].

Human and rodent adipocytes express the two types of estrogen receptors (ERs), ER-α and ER-β, indicating that estrogen signaling may occur through either of these ERs in adipose tissue [8]. Pedersen *et al*. [13] demonstrated that ER-α and ER-β mRNA are differentially expressed during the differentiation of human preadipocytes, in an adipose depot-specific manner, with a similar pattern of expression in both genders. These findings suggest that estrogen's action in adipose tissue metabolism may occur differently depending on the stage of adipocyte differentiation and adipose tissue localization.

Several clinical studies have demonstrated that estrogen could directly inhibit adipose deposition by decreasing lipogenesis. These studies demonstrate that E2 is a major suppressor of fasting lipoprotein lipase (LPL) [14, 15], an enzyme that regulates lipid uptake by adipocytes, and that E2 also represses LPL gene expression at the transcriptional level *via* an estrogen response element (ERE) found on the LPL promoter [16]. Moreover, estrogen therapy in postmenopausal women decreases the expression of genes involved in lipogenesis including acetyl-CoA carboxylate-α and -β, sterol regulatory element binding protein 1c, stearoyl-CoA desaturase, LPL, fatty acid synthase, fatty acid desaturase, and peroxisome proliferator-activated receptor-γ [17, 18].

2. Sexual differences in glucose metabolism through the action of estrogens in adipose tissue

Fat accumulation in different depots is sexually dimorphic: men accumulate more visceral fat, whereas women accumulate more subcutaneous fat and have a higher percentage of body fat [19]. Despite women having more fat mass, the prevalence of early abnormalities in glucose metabolism is 2-3 times higher in men than in women [20], and women are naturally protected against insulin resistance and type 2 diabetes mellitus during their ovulatory life [21]. These sexual differences in glucose metabolism and adipose development are closely related with sexual differences in insulin sensitivity in adipose tissue. In women, abdominal adipocytes have increased insulin sensitivity compared with those from men [19, 22]. Indeed, in females, intra-abdominal adipocytes are more insulin-sensitive than subcutaneous adipocytes and more insulin-sensitive than male adipocytes from either depot, with higher mRNA/protein expression levels of several genes involved in glucose and lipid metabolism [19]. These findings may account for the lower level of insulin resistance and diabetes risk observed in women.

Estrogens are potent regulators of body composition, energy balance, and glucose metabolism in both women and men [23-25]. Animals and humans lacking endogenous estrogen production exhibit insulin resistance, which can be restored by estrogen replacements at physiological concentrations [26-28]. In postmenopausal women, the estrogen level is dependent on age, body weight, and physical fitness [29], and the main source of estrogens is the conversion of the androgens testosterone and androstenedione into E2 and estrone,

respectively, which mainly takes place in adipose tissue [30]. A randomized, double-blind, placebo-controlled trial has demonstrated that hormone replacement in postmenopausal women with coronary heart disease, who are at high risk of developing type 2 diabetes mellitus, results in a 35% reduction in the incidence of diabetes at 4 years [31]. In the Diabetes Prevention Program, metformin treatment in prediabetic individuals reduced the incidence of diabetes by 31% at 3 years [32], proving that the antidiabetic effect of estrogen in women with high oxidative stress is significant.

In men, E2 production principally occurs in adipose tissue through the conversion of androgens by the activity of aromatase, the product of the CYP19 gene [21]. Although plasma estrogen levels in men are low compared to that in women, local concentrations may be much higher and physiologically relevant at the site of production and/or action, where levels may reach concentrations in the micromolar range [30]. There have been clinical evidences that the men who lack endogenous E2 secretion due to a mutation in the CYP19 gene [26, 33] or who have genetic ER-α deficiency [34] develop hypertriglyceridemia, glucose tolerance, and/or insulin resistance, indicating the importance of estrogen signaling in the maintenance of glucose homeostasis in men. Taken together, the disturbances in estrogen production and function in adipose tissue may adversely affect fat and glucose metabolism in both sexes.

3. The role of estrogens in adipose tissue development

The development of adipose tissues is characterized by adipocyte differentiation (adipogenesis) and hypertrophy. Sex hormones and their cognate receptors play a critical role in adipocyte differentiation and function. In studies using ER knockout mouse models, the inhibitory effect of estrogen on adipose development, which is predominately mediated through ER-α, has been demonstrated [35, 36]. In these studies, ER-α knockout mice showed an increase in both adipocyte size and number with reduced energy expenditure and impaired glucose tolerance [35], whereas OVX ER-α knockout mice had decreased fat mass with improved glucose tolerance [36], suggesting that ER-β mediated effects on adipose metabolism are opposite to those mediated by ER-α. Moreover, deletion of aromatase, the key enzyme that catalyzes the biosynthesis of C_{18} estrogens from C_{19} steroids, increases adiposity with reduced spontaneous physical activity in both male and female mice [37]. Estrogen replacement in OVX animals and postmenopausal women prevents increases in visceral adiposity [38-40].

Despite data demonstrating that estrogen functions as a negative regulator of adipose development, it has also been suggested that E2 stimulates adipocyte proliferation and differentiation. Dieudonne *et al*. [41] reported that E2 enhanced the proliferative capacities of subcutaneous preadipocytes from female rats, but not from male rats. In human preadipocytes, E2 stimulates cell proliferation in a dose-dependent manner with a higher response to E2 in women than in men [42]. A more recent report showed that estrogen sulfotransferase (EST), a cytoplasmic enzyme that inactivates estrogens through sulfation of their hydroxyl group, inhibited adipocyte differentiation in 3T3-L1 cells, and overexpression of EST in the adipose tissue of female mice resulted in a smaller adipocyte size [43]. Taken together, estrogens may regulate adipose development with depot- and gender-specific differences, but their effects in the context of adipose tissue function remains unclear.

4. The regulations of glucose metabolism through estrogen signaling in adipose tissue

Adipocytes are highly specialized cells that maintain whole body energy homeostasis by regulating glucose and lipid metabolism [44]. Increased visceral fat is strongly associated with a series of metabolic complications including abnormal glucose metabolism [45, 46], hyperinsulinemia [47, 48], dyslipidemia [49], hypertension [50, 51]. A major contributor to these complications is the increased circulating FFA level coupled with the enhanced lipolytic activity in visceral fat cells. The excess production of adipocyte-derived FFA induces muscle insulin resistance through the inhibition of insulin-mediated glucose uptake and hepatic insulin resistance by affecting the insulin-mediated suppression of hepatic glucose production [44]. Additionally, the chronic exposure to FFA inhibits insulin secretion from pancreatic β-cells [52, 53] and led to β-cell apoptosis [54]. In human trials, an elevated circulating FFA was shown to inhibit insulin-stimulated glucose uptake in both healthy individuals [55] and patients with type 2 diabetes [56]. Thus, cir-

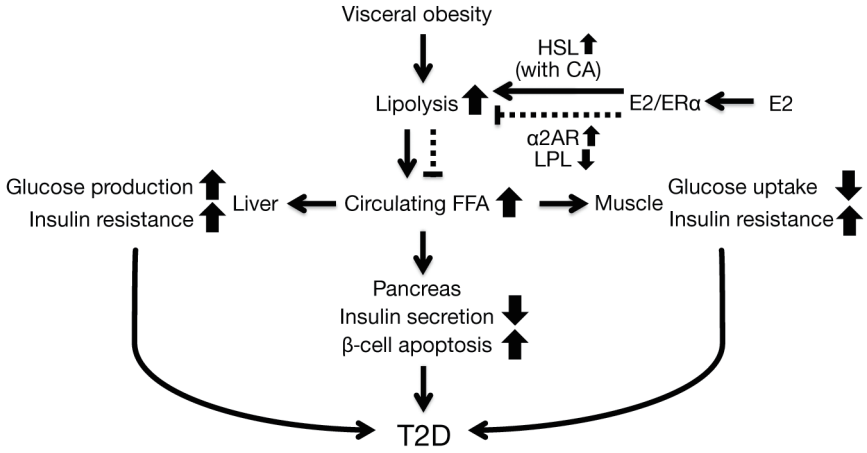

Fig. 1 A potential mechanism of estrogenic regulations of systemic glucose homeostasis through the suppression of circulating FFA E2 may attenuate adipose tissue lipolysis by the upregulation of α2AR and downregulation of LPL through ERα. A decreased circulating FFA resulting from E2 treatment may be a major contributor for the protection against systemic insulin resistance and T2D. Dashed lines indicate the inhibitory actions of E2 on adipose tissue lipolysis. E2, 17β-estradiol; ERα, estrogen receptor α; α2AR, α2-adrenergic receptor; LPL, lipoprotein lipase; HSL, hormone-sensitive lipase; CA, catecholamines; FFA, free fatty acids; T2D, type 2 diabetes.

culating FFA may directly contribute to the underlying pathophysiology of type 2 diabetes and development of insulin resistance [57].

Certain studies have reported greater serum FFA concentrations in estrogen-deficient women compared with postmenopausal women receiving estrogen treatment [58-60], suggesting that estrogen treatment has beneficial effects in postmenopausal women by preventing the accelerated delivery of FFA into the circulation. A potential mechanism of estrogen regulation of systemic glucose homeostasis through the suppression of circulating FFA is illustrated in Fig. 1. Estrogen may attenuate lipolysis occurring in adipose tissue through the up-regulation of α2-adrenergic receptors [61], resulting in decreased mobilization of FFA from adipose tissue. In fact, two months of E2 treatment significantly reduces whole body lipolysis in postmenopausal women, as assessed by whole body palmitate turnover [58]. Estrogen reduces the expression and activity of lipoprotein lipase, a key enzyme for FFA release from lipoprotein particles and the uptake of fat into the adipocytes [16, 18]. Estrogen may also enhance the expression of hormone sensitive lipase (HSL), an enzyme that regulates lipolysis in adipose tissue [62]. Although enhanced HSL expression and/or activity increases the release of FFA into the plasma, the effect of estrogen on HSL-mediated lipolysis may be dependent on the presence of catecholamines. Adipocytes isolated from E2-treated mice showed lower rates of basal lipolysis in the absence of catecholamines, but an approximate doubling of the lipolytic rate following stimulation by epinephrine [63].

As already mentioned, ER-α deficient mice exhibit an increased adipose tissue mass in the absence of differences in energy intake [35], suggesting that ER-α plays a key role in adipose tissue biology. Both female and male ER-α knockout mice show severe insulin resistance and impaired glucose tolerance, as well as adipocyte hyperplasia and hypertrophy [35, 36, 64]. Furthermore, polymorphisms in the ER-α gene are associated with the development of type 2 diabetes and adipose tissue mass [65]. These studies indicate that ER-α plays an important role in the maintenance of glucose metabolism through the regulation of adipose tissue biology. However, the role of ER-β in glucose metabolism and obesity is unclear. Targeted knockout of ER-β in male mice resulted in animals with a similar body weight and fat distribution, as well as lipid and insulin levels, when compared to control littermates [66]. However, under high-fat diet feeding, ER-β knockout female mice showed a higher weight gain with a strong activation of PPARγ in adipose tissue than their wild type littermates, suggesting anti-lipogenic action through ER-β activation [67]. Deletion of the ER-β gene also protected female mice against diet-induced insulin resistance and glucose intolerance

[67]. The beneficial regulations of glucose metabolism by estrogen in adipose tissue may occur through ERα-mediated responses, but further studies are required to determine whether the activation of ER-β in lipid and glucose metabolism pathways is detrimental.

5. Estrogens, adipocytokines and metabolic syndromes

Adipose tissue is an endocrine organ that secretes a variety of signaling molecules known as adipocytokines that are involved in the pathogenesis of obesity-related diseases such as type 2 diabetes, cardiovascular disease, and inflammation. In a recent systematic review of diabetes, Maiorana *et al*. classified adipocytokines into two groups based on their putative physiological role: 'insulin resistance-inducing factors' such as resistin, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), plasminogen activator inhibitor-1 (PAI-1), angiotensinogen, adipsin, acylation-stimulating protein (ASP) and retinol binding protein-4 (RBP-4) and 'insulin-sensitizing factors' such as adiponectin, leptin, visfatin and omentin [44]. The metabolic effects of adipocytokines may be mediated by a common pathway involving the activation of 5'-AMP-activated protein kinase (AMPK), an enzyme that is involved in sensing of the energy status of cells [44].

It has been recently shown that estrogen may contribute to the prevention and/or improvement of obesity-related metabolic syndromes by altering the production of adipocytokines in rodents, monkeys, and humans [68-70]. Table 1 is a summary of human and animal studies on the estrogenic regulation of adipocytokines and metabolic disorders; the study designs, specific models used, and the main results. One of adipocytokines that shows a strong correlation with estrogen is leptin. Leptin is a key metabolic hormone involved in the central regulation of metabolism and transfers a catabolic signal to the brain to inhibit food intake and increase energy expenditure [71, 72]. The serum leptin level is higher in premenopausal women than in postmenopausal women and in men [73]. E2 increases ob gene expression and leptin secretion *via* ER-dependent transcriptional mechanisms *in vivo* and *in vitro* [74]. In addition, estrogen sensitizes leptin signaling, whereas estrogen deficiency causes central leptin insensitivity and an increase in hypothalamic neuropeptide Y [75]. More interestingly, estrogen directly affects energy homeostasis in leptin and leptin receptor mutant mice, suggesting that estrogen functions as a leptin mimetic [76]. Indeed, like leptin, estrogen triggers a robust increase in the number of excitatory inputs in arcuate nucleus pro-opiomelanocortin neurons [76].

Like leptin, adiponectin is also an insulin-sensitive hormone and plays a central role in lipid and glucose metabolism. However, unlike other adipokines, the plasma level of adiponectin is inversely correlated with body mass index and visceral adiposity [77, 78]. Thus, the plasma leptin to adiponectin ratio (LAR) has been proposed as a potentially useful measure of insulin resistance [79, 80]. Our previous study showed that estrogen treatment in OVX mice significantly decreased the LAR compared to OVX vehicle, reflecting the protective effect of estrogen in the development of insulin resistance in OVX mice [81]. In adipocytes, estrogen treatment suppresses adiponectin mRNA and protein expression, and this effect is blocked by treatment with an estrogen antagonist [82]. Serum adiponectin concentration shows a significant negative correlation with the serum levels of E2 and monocyte chemotactic protein-1 (MCP-1) in postmenopausal women [83]. Since MCP-1 plays an important role in the development of insulin resistance and induces adipocyte dedifferentiation [84], these findings indicate that estrogen may exert protective effects against insulin resistance and obesity through the regulation of adiponectin production.

There is evidence of spontaneous increases in the expression and secretion of the proinflammatory cytokines IL-1, IL-6, and TNF-α during estrogen deficiency in rodents and humans [85-87]. E2 treatment inhibits the expression and/or release of IL-1β, IL-6 or TNF-α in macrophages [88, 89] and whole-blood cultures [90]. The serum IL-6 level is lower in postmenopausal women undergoing hormonal replacement therapy [91, 92] and in estrogen-treated OVX mice [93] than that in untreated controls, indicating that estrogen may suppress adipocyte-derived IL-6 release. Indeed, adipose tissue is a major determinant of the circulating IL-6 [94], and it was recently reported that E2 treatment protects female OVX mice against adipocyte inflammation by suppressing IL-6 and TNF-α mRNA expressions [95]. Although further investigation is required, estrogen may also regulate retinol-binding protein 4 (RBP4) and lipocalin 2 levels that have recently been reported to function as important adipocyte-derived signals in the regulation of obesity-related insulin resistance [96-98]. Transgenic overexpression of human RBP4 or

Table 1 Clinical and animal researches regarding estrogens, adipocytokines, and metabolic effects

Reference	Model[a]	Estrogen treatment			Results	
		Dose[b]	Ad.[c]	Duration	Adipocytokine[d]	Metabolic effect[e]
Clinical study						
Karakus *et al*. [116]	Postmenopausal women	E2 (1 mg) + DRSP (2 mg)/d	Oral	6 mo	Leptin(S) ↑	Insulin(S) Δ, glucose(S) Δ LDL chol.(S) ↓
Tanco *et al*. [117]	Postmenopausal women	E2 (1 mg) + DRSP (1, 2, or 3 mg)/d	Oral	2 y	Adiponectin(S) ↓ in dose-dependent manner with DRSP IL-6(S) Δ	BF Δ Insulin(S) and glucose(S) ↑ with 1 or 3 mg DRSP
Castelo-Branco *et al*. [118]	Postmenopausal women	E2 (350 μg) + NET (50, 175, or 550μg)/d	IS	52 wk	Leptin(S) ↑ with all HRT after 24 weeks	Total and LDL chol.(S) ↓, HDL chol.(S) ↑, and glucose(S) Δ with all HRT
Cooper *et al*. [119]	Postmenopausal women	CE (0.625 mg) + MA (2.5 mg)/d	Oral	1 y	IL-6(S), leptin(S), and adiponectin(S) Δ	BF Δ Glucose(S) ↓
Im *et al*. [120]	Postmenopausal women	CE (0.625 mg) or E2 (2 mg) + PR/d	Oral	> 5 y	Adiponectin(S) ↓ with all HRT	Fasting glucose ↓, fasting insulin Δ, HDL chol. ↑, and LDL chol. ↓ with all HRT
Gower *et al*. [121]	Postmenopausal women	CE (0.625 mg) + MA (2.5 mg)/d	Oral	1 mo - 6 y	Leptin(S) Δ	BF ↓ Fasting insulin and glucose Δ
Bednarek-Tupikowska *et al*. [122]	Postmenopausal women	E2 (50 μg/d)	TP	4 mo	Leptin(S) Δ	Blood pressure ↓ BMI Δ
Rizzo *et al*. [123]	Postmenopausal women	E2 (1 mg) + DRSP (2 mg) or DG (5 mg)/d	Oral	6 mo	IL-6(S) ↓ with E2+DRSP	BW ↓ and fasting glucose ↓ with all HRT BMI ↓ with E2+DRSP
Salbach *et al*. [124]	Postmenopausal women	E2 (50 μg/d)	TP	6 mo	Leptin(S) Δ	BMI Δ
Elbers *et al*. [125]	Postmenopausal women	E2 (2 mg/d)	Oral	2 mo	Leptin(S) ↑	BMI Δ, BF Δ Fasting insulin Δ
Animal study						
Kafkas *et al*. [69]	OVX rats	E2 (2 μg/kg/d or 20 μg/kg/d)	SI	6 wk	Adiponectin(S) ↓ with low E2 Leptin(S) ↓ with high E2	BW ↓ with high E2
Shaw *et al*. [70]	Normal and obese monkeys	E2 (30mcg) + levonorgestrel (150mcg)	Oral	6 mo	Adiponectin(S) ↑ in obese Resistin(S) ↑ in normal	BW ↓ in obese delta-like protein 1(S) Δ
Kim *et al*. [81]	OVX mice	E2 (0.5mg/pellet, 90-d release) or CE (2.5 mg/kg) w/wo BZA (3 mg/kg)	IM (E2) or oral (CE and BZA)	8 wk	Leptin(S), adiponectin(S), and RBP4(S) ↓ with all HRT LCN2(S) ↑ with E2 or E2+BZA	Visceral adiposity ↓, Liver/muscle TG ↓, Glucose tolerance ↑, Insulin sensitivity ↑ with all HRT
Zuckerman *et al*. [89]	LPS-stimulated female mice	17α-estradiol (1 μg – 1 mg/kg) w/wo TAM	Oral	4 d	TNF(S) ↑ and IL-6(S) ↓ with 17α-estradiol TNF(S) Δ with 17α-estradiol+TAM	
Stubbins *et al*. [95]	OVX mice	E2 pellet (5 μg/d)	IM	10 wk	TNFα(G) ↓ and IL-6(G) Δ	BW, BF, and hepatic lipid ↓ Insulin sensitivity ↑
Xu *et al*. [126]	OVX rats	E2 (25 μg/kg/twice a week)	SI	12 wk	Fasting leptin (S) ↓ Leptin(G) ↓, MCP-1(G) ↓ Adiponectin(G) ↑	BW, BF, and total chol.(S) ↓ Glucose tolerance ↑
Babaei *et al*. [38]	OVX rats	E2 (30 μg/kg/fifth a week)	SI	8 wk	Adiponectin(S) Δ	BW ↓, visceral fat ↓ Insulin sensitivity Δ
Rachon *et al*. [127]	OVX rats	E2B (10 mg/kg diet)	Dietary supplementation	6 wk	Leptin(S) ↓	BW ↓, Glucose tolerance ↑ Chol. (total, HDL, and LDL) ↓
Sanchez-Mateos *et al*. [128]	OVX rats	E2 (25 μg/kg/twice a week) w/wo melatonin (20 μg/mL)	SI (E2) or drinking water (melatonin)	7 wk	Leptin(S) ↓ with all HRT	BW ↓, total chol.(S) ↓, and glucose(S) Δ with all HRT
Zoth *et al*. [129]	OVX rats	E2 (4 μg/kg/d) w/wo exercise	OP	6 wk	Leptin(S) ↓ by E2 -w/wo exercise	BW ↓, BF ↓, total chol.(S) ↓, and insulin(S) Δ by E2 w/wo exercise

[a] OVX, ovariectomized
[b] E2, 17β-estradiol; DRSP, drospirenone; NET, norethisterone; MA, medroxyprogesterone acetate; CE, conjugated estrogens; PR, progestogen; DG, dydrogesterone; BZA, bazedoxifene; TAM, tamoxifen; E2B, estradiol-3 benzoate.
[c] IS, intranasal spray; TP, transdermal patch; SI, subcutaneous injection; IM, subcutaneous implantation; OP, osmotic pump.
[d] (S), serum; (G), gene expression in adipose tissue; ↑, increase; ↓, decrease; Δ, no significant change; HRT, hormone replacement therapy.
[e] BMI, body mass index; BW, body weight; BF, body fat; TG, triglyceride

injection of recombinant RBP4 in normal mice results in insulin resistance [96]. Conversely, genetic deletion of RBP4 enhances insulin sensitivity [96]. Tan *et al*. [97] showed that E2 increased RBP4 mRNA expression, protein levels and secretion in human subcutaneous and omental adipose tissue explants. In addition, Fried *et al*. [98] recently reported that lipocalin 2 deficiency may cause dysregulation of systemic and adipose lipid metabolism by affecting the production and action of estrogens. This hypothesis is based on the observation that lipocalin 2 deficiency results in decreased serum E2 levels and estrogen receptor-α activity [99]. Conversely, our previous study showed that E2 treatment increases the serum lipocalin 2 level in OVX mice [81]. These data suggest that there is a positive correlation between estrogen and lipocalin 2 production and/or action in controlling adipose lipid metabolism. Taken together, estrogen contributes to the prevention of metabolic syndromes and inflammation through the regulation of the production of key adipocytokines involved in controlling energy homeostasis and insulin resistance.

6. The effects of selective estrogen receptor modulators on glucose metabolism

Selective estrogen receptor modulator (SERM) is a synthetic non-steroidal agent that exhibits ER agonistic or antagonistic activities depending on the target tissue. A novel approach to postmenopausal therapy is the use of tissue-selective estrogen complex (TSEC) treatment, which pairs a SERM with one or more estrogens. The goals of TSEC are to provide the benefits of estrogens, such as the reduction of hot flashes and vulvar–vaginal atrophy, preventing menopausal osteoporosis events, and protecting the endometrium and breast tissue from estrogen stimulation.

The action mechanism of SERM has not been precisely described, but their importance as an advance in postmenopausal treatments has been proven. Tamoxifen, a first-generation SERM, acts as an ER-α antagonist in breast and as an ER-α agonist in bone. As a result, tamoxifen is used for the treatment of postmenopausal breast cancer while simultaneously preventing osteoporosis [100]. However, tamoxifen reverses E2 protection of β-cell survival *in vitro*, and exacerbates the predisposition to insulin-deficient diabetes in female mice [101]. Thus, tamoxifen acts as an ER-α antagonist in β-cells with regard to antiapoptotic protection. In fact, tamoxifen therapy associated with an increased risk in the development of diabetes in breast cancer survivors [102].

Raloxifene, another SERM, is approved by the US FDA for the treatment of postmenopausal osteoporosis because of its ER-agonist activity in bone [103]. In OVX mice, raloxifene showed an estrogen agonistic effect on fat mass homeostasis through modification of leptin level and leptin receptor expression, which was similar to the effects seen with E2 replacement, suggesting that in rodents, raloxifene acts as an ER agonist in hypothalamic neurons and fat [104]. In addition, in cultured INS-1 insulin-secreting cells, raloxifene acts as an ER agonist and prevents lipid accumulation by itself and in the presence of E2 [105]. However, clinical researches with postmenopausal women have shown that raloxifene treatment does not affect glycemic controls or indices of β-cell function and insulin sensitivity in healthy and diabetic postmenopausal women [106, 107]. In contrast to estrogen's effect in maintaining insulin sensitivity, short-term treatment with raloxifene decreases insulin sensitivity in healthy nondiabetic postmenopausal women [108]. Additionally, a 12-month raloxifene treatment was associated with reduced insulin sensitivity in women with postmenopausal osteoporosis, but without modification of plasma glucose concentration and tolerance [109].

Bazedoxifene, a third generation SERM, has been evaluated as a novel TSEC with particularly conjugated estrogens (CE) to obtain favorable effects on the menopausal symptoms without stimulating breast or uterus. Duavee®, a drug combination of bazedoxifene and CE, is the first TSEC approved by the US FDA (3 October 2013) for the treatment of vasomotor symptoms and the prevention of osteoporosis after menopause. Both preclinical and clinical studies demonstrated that bazedoxifene is more tissue selective than other SERMs in the context of their agonistic and antagonistic activity on estrogen target tissues [110]. Bazedoxifene-CE combination therapy in postmenopausal women and rodents results in a significant increase in bone mineral density [111, 112] and a decrease in the frequency of hot flashes [113, 114] while protecting the endometrium. In a postmenopausal monkey model, this TSEC treatment did not affect the adiposity and plasma lipid profile, but bazedoxifene was found to attenuate the atheroprotective effects of CE [115]. A molecular study of bazedoxifene function on energy and glucose homeostasis in OVX mice demonstrated that

bazedoxifene and CE improve hepatic lipid metabolism *via* partially independent pathways [81]. In this same study, bazedoxifene was shown to improve glucose and lipid homeostasis in an ERα-dependent manner and did not reverse CE's effects on systemic insulin action [81]. Further studies employing hepatocyte- and muscle-specific conditional ER-α knockout mice are needed to more clearly identify the mechanisms underlying the metabolic regulatory effect of bazedoxifene.

7. Conclusions

Estrogens are crucial hormonal regulators of systemic energy homeostasis in both sexes, and there is increasing evidence that E2 treatment exhibits a protective effect in humans and rodents against the development of obesity-related metabolic disorders such as type 2 diabetes *via* the regulation of adipose tissue metabolism. E2 seems to display the beneficial actions on insulin and glucose metabolism through ERα-mediated responses in adipose tissue. The potential antidiabetic actions of E2 in adipose tissue involve: 1) the suppression of adipose lipolysis resulting in decreased circulating FFA level and 2) the modulation of expression and/or secretion of adipocytokines for the improvement of insulin sensitivity.

Although estrogen therapy effectively offsets menopausal symptoms including adiposity and diabetes risk in postmenopausal women and animal models, the use of estrogen alone is not suitable due to the risk of the development of hormone-dependent cancer. A novel approach to postmenopausal therapy is TSEC that pairs a SERM with estrogens, and this therapy may provide tissue-selective ER activation with endometrial safety. TSEC represents a promising therapeutic option for menopausal symptoms such as osteoporosis and hot flashes. However, there is a lack of information regarding the alterations that may occur in adipose tissue-related metabolic signaling. Further studies are needed to determine how SERM affect estrogenic regulations of adipose tissue metabolism and to develop an optimal TSEC combination with an improved metabolic function and tolerability profile.

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