The diversity of sex steroid action: regulation of metabolism by estrogen signaling

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Abstract

The metabolic syndrome is a complex condition characterized by obesity, insulin resistance, decreased high-density lipoproteins, and hypertension associated with high risk of developing type 2 diabetes and cardiovascular disease. A major increase in the incidence of developing metabolic syndrome and related diseases is observed worldwide in association with a change toward a less active lifestyle and increased food consumption. Estrogen and the estrogen receptors (ERs) are well-known regulators of several aspects of metabolism, including glucose and lipid metabolism, and impaired estrogen signaling is associated with the development of metabolic diseases. This review will describe the key effects of estrogen signaling in metabolic and glucose sensing tissues, including the liver, pancreatic β cells, adipose tissue, and skeletal muscle. The impact on metabolic processes of impaired estrogen signaling and knock out of each ER subtype will also be discussed.

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Estrogen signaling

Estrogens exert their physiological effects through two estrogen receptor (ER) subtypes, ERα and ERβ that belong to the nuclear receptor family of ligand-activated transcription factors.

ERα is mainly expressed in reproductive tissues, kidney, bone, white adipose tissue, and liver, whereas ERβ is expressed in the ovary, prostate, lung, gastrointestinal tract, bladder, hematopoietic cells, and the central nervous system (CNS) (Matthews & Gustafsson 2003).

ERs share a common structure with other members of the nuclear receptor family. The N-terminal A/B domain is the most variable region with <20% amino acid identity between the two ERs and could confer subtype-specific actions on target genes. This region harbors the activation function-1 (AF-1) that is ligand independent and shows promoter- and cell-specific activity. The centrally located C-domain harbors the DNA binding domain, which is involved in DNA binding and receptor dimerization. This domain is highly conserved between ERα and ERβ with 95% amino acid identity. The D-domain is referred to as the hinge domain and shows low conservation between ERα and ERβ (30%). This domain has been shown to contain a nuclear localization signal. The C-terminal E-domain is the ligand binding domain (LBD) and the two subtypes display 59% conservation in this region. The LBD contains a hormone-dependent AF-2 and is responsible for ligand binding and receptor dimerization. The F-domain has <20% amino acid identity between the two ER subtypes and the functions of this domain remain undefined (Zhao et al. 2008).

The major physiological estrogen is 17β-estradiol (E2) that has a similar affinity for both ERs. In addition, ERs are activated by a range of ligands including selective ER modulators such as raloxifene and tamoxifen, the ERα-selective agonist propyl-pyrazole-triol (PPT) and the ERβ-selective agonist diarylpropionitrile, and many other compounds (Heldring et al. 2007). Like other nuclear receptors, ligand-bound ERs act as dimers to regulate transcriptional activation. Full transcriptional activity of the ERs is mediated through a synergistic action between the two activation domains, AF-1 and AF-2. Both ERα and ERβ contain a potent AF-2 function, but unlike ERα, ERβ seems to have a weaker corresponding AF-1 function and depends more on the ligand-dependent AF-2 for its transcriptional AF (Bryzgalova et al. 2006).

The classical estrogen signaling occurs through a direct binding of ER dimers to estrogen-responsive elements...
Estrogen signaling and metabolic syndrome

The metabolic syndrome refers to a group of interrelated metabolic abnormalities that include disturbed glucose homeostasis, insulin resistance (IR), increased body weight and abdominal fat accumulation, mild dyslipidemia, and hypertension. However, the exact mechanisms of the complex pathways leading to the metabolic syndrome are not known. Individuals with the metabolic syndrome have an increased risk of developing cardiovascular diseases (CVD) and type 2 diabetes (T2D). It is currently not known which mechanisms behind the development of the metabolic syndrome are primary and secondary; however, visceral obesity seems to be a major component. Accumulating evidence also points toward a strong inheritable genetic component for various parts of the metabolic syndrome. Several potential candidate genes have been suggested according to their biological relevance and many of them have been further associated with the metabolic syndrome in different ethnic populations. These candidate genes have been divided into clusters and include, among others, genes that cause monogenic obesity (leptin, melanocortin receptor genes), regulate free fatty acid (FFA) metabolism (adiponectin, lipases), affect insulin sensitivity (PPARY, insulin receptor substrates), affect lipid metabolism (CD36, apolipoprotein E), and are related to inflammation (tumor necrosis factor-α, C-reactive protein (CRP); reviewed by Song et al. (2006)).

Epidemiological and prospective studies associate estrogen to several aspects of the metabolic syndrome. Studies on knockout mouse models have shed further light on the role of estrogen and its receptors in different tissues involved in metabolic processes (Fig. 1).

Genetic associations have also been described for polymorphisms of the ESR1 gene (coding for ERα) and several pathological conditions related to metabolism, including CVD, T2D, myocardial infarction, hypertension, venous thromboembolism, and lipoprotein metabolism (Schuit et al. 2004, Shearman et al. 2004, Yoshihara et al. 2009, Lamon-Fava et al. 2010). Polymorphisms in the ESR2 gene (coding for ERβ) have been associated with anorexia nervosa, bulimia nervosa, and premature coronary artery disease (Eastwood et al. 2002, Peter et al. 2005, Nilsson & Gustafsson 2010). The studies on genetic associations between polymorphisms of the ESR1 and ESR2 genes and the metabolic syndrome, however, are controversial as other studies do not confirm the previously obtained results (Goulart et al. 2009).

Adipose tissue accumulation is sexually dimorphic (Regitz-Zagrosek et al. 2006) and females have a higher percentage of body fat than males. The fat distribution is also different with females accumulating more subcutaneous fat and males accumulating more visceral fat (Nuutila et al. 1995, Crespo et al. 2002, Bonds et al. 2006). Estrogen deficiency or decline in estrogen levels after menopause often leads to dysregulation of metabolism. The onset of menopause is associated with several metabolic changes and postmenopausal women fall into the same risk category as men for development of atherosclerosis and myocardial infarction (Carr 2003). Other changes associated with low estrogen levels are IR, impaired glucose disposal, increased hepatic gluconeogenesis with subsequent glucose secretion, and increased levels of inflammatory markers.

Results from studies using aromatase-deficient patients and knockout animals confirm the relationship between estrogen levels and metabolic homeostasis. Male aromatase-deficient patients, as well as a male patient with loss of ERα function, display impaired glucose metabolism, IR, and hyperinsulinemia (Zirilli et al. 2008). In addition, the aromatase-deficient patients showed impaired liver functions, hepatic steatosis, and altered lipid profile (Maffei et al. 2004). Estrogen treatment of the male patient with loss of ERα function did not improve glucose homeostasis and hyperinsulinemia, whereas estrogen therapy of the aromatase-deficient patients led to improvement of the metabolic abnormalities (Maffei et al. 2004).

Female and male ERα knockout mice are diabetogenic and obese with severe hepatic IR. Ovariectomy of ERα-deficient mice leads to normalized homeostasis of circulating glucose and insulin levels and reverses the obese phenotype, suggesting that ERβ activity may result in a diabetogenic and adipogenic phenotype. In contrast, ERβ knockout mice display improved insulin sensitivity and glucose tolerance without increased body fat content, suggesting that ERα plays an important role in maintaining metabolic control (Nilsson & Gustafsson 2010).

The specific action of estrogen in different metabolic processes will be described in more detail in the following paragraphs.
A series of complex systems regulate energy homeostasis in order to keep energy levels and body weight stable (Miller 1982). Central brain circuits receive peripheral signals indicating satiety, energy levels, and energy stores. The hypothalamus is a key regulator of food intake and maintenance of energy homeostasis (Morton et al. 2006). The hypothalamus processes afferent signals from the gut and brain stem and efferent signals that modulate food intake and energy expenditure. The hypothalamus is subdivided into interconnecting nuclei, including the arcuate nucleus (ARC), paraventricular nucleus (PVN), ventromedial nucleus (VMN), dorsomedial nucleus, and lateral hypothalamic area (Simpson et al. 2009).

Estrogen is a major effector for the regulation of energy balance, body weight, fat distribution, and appetite in mice (Dubuc 1985). Ovariectomized mice display an increase in food consumption, decreased running wheel activities, and increased fat mass, which can be reversed with estrogen replacement (Laudenslager et al. 1980). The importance of the brain for the observed phenotypes has been demonstrated by studies showing that direct injections of E₂ into PVN reduce food intake and body weight and increase running activities (Colvin & Sawyer 1969, Ahdieh & Wade 1982). Energy homeostasis and feeding behavior in the hypothalamus also follows the menstrual cycle, and food intake in women varies across the cycle with lowest daily food intake during the peri-ovulatory period when estrogen levels are maximum (Asarian & Geary 2006).

Leptin is one of the key metabolic hormones in central regulation of metabolism and transfers a catabolic signal to the brain to inhibit food intake and increase energy expenditure (Ahima et al. 1999, Elias et al. 1999, Elmquist et al. 1999). Leptin levels are higher in females compared with males (Shimizu et al. 1997). After puberty, estrogen modulates leptin synthesis and secretion via ER–dependent transcriptional mechanisms (Machinal et al. 1999). In addition, raised levels of estrogens have been associated with increased leptin sensitivity in the brain (Ainslie et al. 2001), although ovariectomy reduces the sensitivity to leptin when compared with intact females, an effect that can be restored by E₂ replacement. Furthermore, administration of E₂ in male rats increases the sensitivity to leptin (Clegg et al. 2006).

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**Figure 1** Summary of the effects on metabolism observed in ERα, ERβ, and aromatase knockout (ArKO) female and male mice. Reported effects in different metabolic tissues, i.e. central nervous system (CNS), pancreatic β cell, skeletal muscle, liver, and white adipose tissue (WAT), are indicated. ND, no difference; HFD, high-fat diet; TG, triglycerides.

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**Estrogen signaling and central regulation of metabolism**

A series of complex systems regulate energy homeostasis in order to keep energy levels and body weight stable (Miller 1982). Central brain circuits receive peripheral signals indicating satiety, energy levels, and energy stores. The hypothalamus is a key regulator of food intake and maintenance of energy homeostasis (Morton et al. 2006). The hypothalamus processes afferent signals from the gut and brain stem and efferent signals that modulate food intake and energy expenditure. The hypothalamus is subdivided into interconnecting nuclei, including the arcuate nucleus (ARC), paraventricular nucleus (PVN), ventromedial nucleus (VMN), dorsomedial nucleus, and lateral hypothalamic area (Simpson et al. 2009).

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Although there are several isoforms of the lepr, the b form (leprb) is the critical variant for regulating energy balance (Chen et al. 1996). It has been reported that leprb is co-localized with ER\(\alpha\) in the hypothalamus and estrogens have been reported to regulate the expression of leprb mRNA via an ERE in the lepr gene, suggesting interactions between these pathways for the regulation of energy homeostasis (Diano et al. 1998, Lindell et al. 2001). The exact mechanisms behind the estrogentic effects on leptin signaling and leprb levels are currently not well understood. How fluctuations in estrogen levels affect leptin signaling probably includes effects downstream of leprb transcription/translation (Lindell et al. 2001).

The neuronal circuits that control metabolism express both subtypes of ERs; however, which subtype is involved in the effects of estrogen on central regulation of energy homeostasis is controversial. ER\(\alpha\) has been shown to be expressed in the VMN, the ARC, and the PVN (Simerly et al. 1990, Simonian & Herbison 1997, Voisin et al. 1997). ER\(\alpha\) knockout mice display an obese phenotype with increased fat deposition in the absence of differences in food intake compared with wild-type mice. ER\(\alpha\) silencing in the VMN resulted in increased food intake and reduced energy expenditure by decreased physical activity and impaired thermogenic responses to feeding (Musatov et al. 2007). Furthermore, ovariectomized rodents treated with the ER\(\alpha\)-specific ligand PPT displayed an inhibitory effect on eating behavior and reduced body weight gain compared with vehicle-treated mice.

Even though ER\(\alpha\) is a central player in the control of energy homeostasis, ER\(\beta\) is also likely to play an important role. ER\(\beta\) is expressed in the same hypothalamic nuclei as ER\(\alpha\), however, at lower levels with the highest expression in the PVN. Co-administration of E\(2\) and ER\(\beta\) anti-sense oligodeoxynucleotides (ODN) into the third ventricle in the brain reduced the estrogenic inhibitory effects on food intake in ovariectomized rats, whereas administration of ER\(\alpha\) anti-sense ODN had no effect in this assay (Liang et al. 2002).

**Estrogen signaling in lipogenesis/lipolysis**

Organisms store energy for later use during times of nutrient scarcity. Excess energy is stored as triacylglycerol (TAG) in lipid droplets produced by lipogenesis. When energy is required, TAGs are catabolized into FFAs via lipolytic pathways. There is substantial evidence associating T2D with excess intracellular lipids in non-adipose tissues. Intracellular lipids disrupt cellular function in insulin secreting pancreatic \(\beta\) cells and insulin-responsive cells, such as hepatocytes.

Sex steroids are known to regulate adipose tissue development and function and female mice have increased lipogenic capacities in adipocytes compared with male mice (Macotela et al. 2009). Interestingly, despite the increased lipogenesis, adipocytes from females are smaller than adipocytes from males. E\(2\)-treated ovariectomized mice display reduced lipogenesis in adipocytes (D'Eon et al. 2005).

When circulating levels of estrogen are raised above the physiological range, adipose tissue metabolism is altered resulting in reduced lipogenic rates and fat depot size. ER\(\alpha\)-deficient mice exhibit an increased adipose tissue mass without displaying differences in energy intake, suggesting that ER\(\alpha\) plays an important role in adipose tissue biology (Heine et al. 2000). This is further supported by studies on 3T3-L1 pre-/adipocytes with stably transfected ER\(\alpha\), which showed decreased triglyceride accumulation and reduced expression of lipoprotein lipase (LPL), the enzyme that catalyzes the conversion of triglycerides into FFA and glycerol (Homma et al. 2000). This is further supported by epidemiological observations that serum triglyceride levels increase in postmenopausal women and that the level of LPL activity is reduced by estrogen treatment (Ivierus & Brunnell 1988).

E\(2\) suppresses lipogenesis and TG accumulation in adipose tissue and liver in high-fat diet (HFD) fed and leptin-deficient female mice (Bryzgalova et al. 2008). Global gene expression analysis of livers from ER\(\alpha\)-deficient and wild-type mice revealed ER\(\alpha\)-dependent increased expression of lipogenic genes and decreased expression of genes regulating lipid transport (Bryzgalova et al. 2006). E\(2\) treatment suppressed the expression of lipogenic genes, i.e. fatty acid synthase (Fasn), stearoyl-coenzyme A desaturase 1 (Scd1), and glycerol-3-phosphate acyltransferase (Gpam), in livers of leptin-deficient Ob/Ob mice (Gao et al. 2006).

Recent studies indicate that ER\(\beta\)-deficient female mice have a higher body weight under HFD feeding than wild-type littermates (Fryost-Ludwig et al. 2008). This was reported to be a result from increased adipogenesis with subsequent increased mass of adipose tissue and improved insulin sensitivity. Furthermore, the key adipogenic and lipogenic factor PPAR\(\gamma\) was negatively regulated by ER\(\beta\), suggesting that PPAR\(\gamma\) could be a mediator of the metabolic effects observed in ER\(\beta\) knockout mice (Fryost-Ludwig et al. 2008). Female ER\(\beta\)-deficient mice on HFD displayed impaired food efficiency and increased respiratory quotient, which is an indication of disturbed fatty acid oxidation. PPAR\(\gamma\) DNA binding properties and target gene activation were markedly induced in the gonadal fat of the ER\(\beta\)-deficient mice and inhibition of adipose PPAR\(\gamma\) signaling reversed this metabolic phenotype (Fryost-Ludwig et al. 2008).

Cross talk between ER and PPAR\(\gamma\) has also been described earlier (Keller et al. 1995). PPAR\(\gamma\), together with its heterodimeric partner retinoid X receptor, has been shown to suppress ER-induced target gene expression via competitive binding to an estrogen response element site in the vitellogenin A2 promoter. Conversely, ER was reported to inhibit ligand-induced PPAR\(\gamma\) activation in two different breast cancer cell lines (Wang & Kilgore 2002). Furthermore, ER\(\beta\)-specific ligands inhibit PPAR\(\gamma\) activation and the mechanism behind the inhibition is suggested to be a competition between PPAR\(\gamma\) and ER\(\beta\) for the common
co-activator PPARγ coactivator 1 (Foryst-Ludwig et al. 2008, Yepuru et al. 2010).

In summary, it appears that both ER isoforms participate in the anti-lipogenic actions of estrogens.

**Estrogen signaling in glucose homeostasis and insulin sensitivity**

Circulating levels of glucose are controlled by two hormones, insulin and glucagon. In response to high glucose levels, pro-insulin is released from pancreatic β cells and converted to the active form. Insulin then stimulates the uptake and storage of glucose in skeletal muscles and adipose tissue or as glycogen through glycogenesis in the liver. When insulin binds to the insulin receptor, a signaling phosphorylation cascade starts, which leads to translocation of vesicles containing glucose transporter 4 (GLUT4) to the plasma membrane facilitating glucose entry into the cells (Zhou et al. 1999).

T2D is characterized by high blood glucose in the context of IR and relative insulin deficiency. IR is defined as impairment in insulin action on glucose metabolism and is manifested in several tissues. Glucose uptake is reduced in muscle and adipose tissue, whereas hepatic IR results in reduced glycogen synthesis and storage and a failure to suppress glucose production and subsequent release into the circulation.

Studies on humans and rodents link estrogen to the regulation of glucose homeostasis (Fig. 2). Premenopausal women are more insulin sensitive with associated improved glucose tolerance, are more resistant to develop IR compared with men, and display increased expression of GLUT4 and glucose uptake (Kuhl et al. 2005, Macotela et al. 2009). Hormone replacement therapy (HRT) has been shown to improve insulin sensitivity and to lower blood glucose in healthy postmenopausal women and to reduce the incidence of T2D in postmenopausal women with coronary heart diseases (Crespo et al. 2002, Kanaya et al. 2003).

Importantly, men with aromatase deficiency, who cannot synthesize estrogen hormones, display impairment in glucose metabolism and IR (Morishima et al. 1995). Additional rodent studies link estrogen to anti-diabetic effects. Intact female mice are protected against hyperglycemia and ArKO mice are insulin resistant (Jones et al. 2000). Estrogen deficiency is strongly linked to the development of IR and subsequent manifestations in various metabolic tissues (see Fig. 3 for an overview).

ERα has been shown to be involved in the maintenance of glucose metabolism in several tissues including liver, skeletal muscle, adipose tissue, pancreatic β cells, and CNS. A man identified with ERα-deficiency displays impaired glucose metabolism and, further, polymorphisms in the ERα gene have been associated with development of T2D and the metabolic syndrome (Yamada et al. 2002, Okura et al. 2003). The critical role of ERα in maintaining glucose homeostasis has been validated in Ob/Ob mice where treatment with the ERα-selective ligand PPT improved glucose tolerance and insulin sensitivity (Lundholm et al. 2008). Studies using euglycemic hyperinsulinemic clamps revealed that ERα knockout is associated with hepatic IR (Bryzgalova et al. 2008).

Estrogens are also known to regulate pancreatic β cell function through an ERα-dependent mechanism. A recent study suggests that long-term estrogen exposure increases insulin levels, insulin target gene expression, and insulin release without changing β cell mass in mice (Alonso-Magdalena et al. 2008). Estrogen-dependent insulin release in cultured pancreatic islets was reduced in ERα-deficient mice, when compared with islets derived from either ERβ-deficient or wt mice (Alonso-Magdalena et al. 2008). However, ERβ-deficient mice show mild pancreatic islet hyperplasia with delayed first-phase IR (Barros et al. 2009).

The two subtypes of ER have been shown to have opposing effects in the muscle, with ERα inducing and ERβ inhibiting GLUT4 expression (Barros et al. 2006). Data show that ERα-deficient mice treated with the ER antagonist tamoxifen display increased GLUT4 expression in skeletal muscle, which could indicate a pro-diabetogenic effect of ERβ (Barros et al. 2009). Targeted knock out of ERβ in male mice has been shown to protect against diet-induced IR by increasing PPARγ signaling in adipose tissue (Foryst-Ludwig et al. 2008, Barros et al. 2009).

Interestingly, recent studies show that GPR30 knockout mice display impaired glucose tolerance, suggesting that the membrane-bound estrogen-responsive GPR30 has anti-diabetic properties (Martenson et al. 2009, Balhuizen et al. 2010).

**Estrogen signaling in cholesterol homeostasis**

Biosynthesis of cholesterol is directly regulated by cholesterol levels, although the homeostatic mechanisms involved are only partially understood. Increased food consumption leads
Estrogen deficiency

Insulin resistance

Pancreatic β cells
Muscle
Liver
Adipose tissue

Impaired insulin secretion
Impaired glucose uptake
Increased gluconeogenesis
Increased lipogenesis
Increased VLDL production
Decreased insulin clearance

Increased TG accumulation
Increased adipocyte size

Inflammation

Figure 3 Overview of IR induced by estrogen deficiency and subsequent disturbances in metabolic tissues.

to a decrease in endogenous cholesterol production, whereas low food intake has the opposite effect. The master regulators of cholesterol homeostasis are the sterol regulatory element-binding proteins (SREBP) 1 and 2 (reviewed by Espenshade & Hughes (2007)), which stimulate the transcription of cholesterogenic genes, like the low-density lipoprotein (LDL) receptor and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. The former binds circulating LDL cholesterol, which prevents cholesterol accumulation around the membrane surface and prevents atherosclerosis. Activation of HMG-CoA reductase, which is the rate-limiting enzyme in the mevalonate pathway, leads to an increase in the endogenous production of cholesterol.

Estrogen is known to decrease plasma LDL cholesterol and increase plasma high-density lipoprotein (HDL) cholesterol (Hong et al. 1992, Nabulsi et al. 1993, Darabi et al. 2010). The decrease in plasma LDL is a result of increased hepatic LDL receptor expression, which increases the clearance of plasma LDL and the secretion of cholesterol into the bile. A recent publication shows that estrogen-treated diabetic rats display a reduced lipase activity resulting in decreased total cholesterol concentration by 53% while the HDL cholesterol levels increased, contributing to a favorable blood lipid homeostasis (Hamden et al. 2011). This could partly be explained by increased gene expression levels of the ATP binding cassette A1, the key enzyme in the reverse cholesterol process where cholesterol is incorporated into HDL particles.

Estrogen increases the risk for the formation of cholesterol gallstones by promoting hepatic secretion of biliary cholesterol that induces an increase in cholesterol saturation of bile (reviewed by Wang et al. (2009)). Also, estrogen significantly enhances the activity of HMG–CoA reductase, the rate-limiting enzyme in hepatic cholesterol biosynthesis, under high dietary cholesterol loads, suggesting that there could be an increased delivery of cholesterol to bile from de novo synthesis in the liver (Everson et al. 1991, Wang et al. 2006a). Studies report that estrogen could increase the capacity of dietary cholesterol to induce cholesterol supersaturation of bile and high doses of estrogen augment intestinal cholesterol absorption leading to the overproduction of bile and the formation of cholesterol gallstones (Henriksson et al. 1989, Uhler et al. 1998).

Estrogen signaling in metabolic inflammatory processes

Impaired glucose tolerance and T2D are characterized by a low-grade inflammatory state. Several cytokines derived from adipocytes contribute to IR by impairing insulin signaling pathways. Changes in glucose homeostasis may be influenced by adipokines, such as adiponectin, leptin, and resistin. Leptin and resistin are known to increase in correlation to body fat and resistin has been shown to be associated with IR (Friedman & Halaas 1998, Steppan et al. 2001).

Postmenopausal women have increased circulating markers of inflammation compared with premenopausal women. Specifically, CRP and interleukin 6 (IL6) are associated with increased risk of developing CVD in elder women (Wang et al. 2006b). CRP is also associated with increased visceral fat and decreased glucose disposal in postmenopausal women. HRT increases CRP but decreases other circulating markers of inflammation. The authors speculate that the raised level of CRP is a sign of hepatic effects rather than a generalized pro-inflammatory response (Oger et al. 2001).

Increased consumption of HFD, rich in saturated fatty acids, increases inflammation by activating toll-like receptor 4 (Shi et al. 2006). It is becoming increasingly evident that chronic activation of pro-inflammatory pathways may at least partly be responsible for obesity-induced IR and T2D (Kahn & Flier 2000, Wellen & Hotamisligil 2003, 2005). For example, the pro-inflammatory cytokines TNFα, IL6, and CRP are elevated in individuals diagnosed with IR and T2D (Shoelson et al. 2007, de Luca & Olefsky 2008) and elevated in muscle and liver upon HFD challenges (Shi et al. 2006). Suppression of pro-inflammatory responses represents a promising strategy to combat obesity and associated metabolic disorders. Female rats and mice are relatively protected from HFD-induced inflammatory responses (Gallou-Kabani et al. 2007, Payette et al. 2009) and several studies show that E2 plays a role in reducing the inflammatory response in adipose, cardiovascular, and neural in vitro systems (reviewed by Ghisletti et al. (2005) and Turgeon et al. (2006)).

Estrogen exerts an early anti-inflammatory effect in the rat vascular injury model in a sexually dimorphic manner (Chen et al. 1996, Bakir et al. 2000). A mechanism that has been implicated in the anti-inflammatory/vasoprotective role of E2 is by local inhibition of CRP in injured arteries (Wang et al. 2005). Studies in humans, as well as animal models of atherosclerotic disease, show that CRP is expressed in the injured vasculature and the extent of the lesion correlates with the level of CRP expression, which provides support for a functional role in the injury response for the CRP protein. Estrogen deficiency in ovariecctomized rats is associated with increased serum levels of TNFα and enhanced
artery sensitivity to vasoconstriction (Arenas et al. 2006). Administration of TNFα inhibitors or E2 replacement is associated with a decrease in constriction of arteries, which suggests that upregulation of TNFα during estrogen deficiency may contribute to enhanced vascular constriction (Arenas et al. 2006).

Activation of NFKB is known to mediate a variety of chronic inflammatory diseases, including CVD. Estrogen has been shown to inhibit NFKB signaling by a variety of mechanisms in an ER-dependent manner, where both ER isoforms seem to be of importance. Studies on vascular cells show that estrogenic activation of ER inhibits the DNA binding activity of NFKB and NFKB-induced expression of chemokines/cytokines. This occurs through a direct interaction between ER and NFKB in the nucleus or by ER-mediated inhibition of upstream NFKB signaling in the cytoplasm.

ERα activation has been shown to attenuate injury-induced vascular remodeling and studies on ERα knockout mice support these vascular protective effects (Karas et al. 1999, Brouchet et al. 2001). In vitro studies have shown that ERβ also plays a protective role in injured arteries (Christian et al. 2006). In vivo evidence for a role of ERβ in estrogen-induced vasoprotective effects was shown by demonstrating that stimulation with an ERβ-specific agonist inhibits neointima formation, which is the first step in the development of atherosclerosis, in wild-type mice (Krom et al. 2007).

Conclusions

The metabolic syndrome and its various manifestations, including obesity and diabetes, is one of the main causes of morbidity and mortality worldwide. The syndrome has already reached epidemic proportions with an increasing prevalence. Several epidemiological and prospective studies have linked estrogen and the ERs to various aspects of metabolic disease, yet the underlying molecular mechanisms are still unclear.

The onset of menopause dramatically increases the risk for women to develop disease states coupled to the metabolic syndrome, such as obesity, CVD, and T2D. These risks are reduced on HRT demonstrating the importance of functional estrogen signaling in metabolic tissues.

This review underlines the molecular and physiological mechanisms behind estrogen actions in regulation of metabolism with focus on ERα and ERβ. In addition, a newly discovered membrane-bound ER, GPR30, has been demonstrated to exert metabolic functions.

ERα seems to play a protective role in insulin and glucose metabolism, with actions on the liver, adipose tissue, muscle, and pancreatic β cells. In addition, ERα further centrally regulates food intake and energy expenditures. ERβ, on the other hand, has the potential to negatively influence insulin and glucose metabolism by impairment of the function of adipose tissue, probably through augmented PPARγ signaling, and declined expression of GLUT4 in the muscle.

The major concern in using therapies targeting ER in treatment of the metabolic syndrome is the risk of developing hormone-dependent cancer. Further studies are needed to identify and develop new ligands that target ERα in selective metabolic tissues but lack the mitogenic effects in others, like ovaries and breast.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References


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