THEMATIC REVIEW

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, ER α and ER β that belong to the nuclear receptor family of ligand-activated transcription factors.

ER α is mainly expressed in reproductive tissues, kidney, bone, white adipose tissue, and liver, whereas ER β 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 ER α and ER β with 95% amino acid identity. The D-domain is referred to as the

hinge domain and shows low conservation between ER α and ER β (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 17 β -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 ER α -selective agonist propyl-pyrazole-triol (PPT) and the ER β -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 ER α and ER β contain a potent AF-2 function, but unlike ER α , ER β 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

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(ERs) in the regulatory regions of estrogen target genes followed by activation of the transcriptional machinery at the transcription start site. Estrogen also modulates gene expression by a second mechanism in which ERs interact with other transcription factors, such as activating protein-1 and stimulating protein-1, through a process referred to as transcription factor cross talk. Estrogen may also elicit effects through non-genomic mechanisms, which involve the activation of downstream signaling cascades such as protein kinase A, protein kinase C, and mitogen-activated protein (MAP) kinase via membrane-localized ERs.

Recently, an orphan G protein-coupled receptor (GPR) 30 in the cell membrane was reported to mediate non-genomic and rapid estrogen signaling (Revankar *et al.* 2005, Thomas *et al.* 2005). GPR30 is structurally unrelated to ER α and ER β and the rapid effects from stimulation of this receptor include release of intracellular Ca²⁺ and subsequent activation of calcium-calmodulin-dependent kinases or activation of MAP kinase and phosphoinositide 3-kinase pathways.

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 ethnical 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 (PPAR γ , 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 ER α) 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 ER β) 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 ER α 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 ER α 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 ER α knockout mice are diabetogenic and obese with severe hepatic IR. Ovariectomy of ER α -deficient mice leads to normalized homeostasis of circulating glucose and insulin levels and reverses the obese phenotype, suggesting that ER β activity may result in a diabetogenic and adipogenic phenotype. In contrast, ER β knockout mice display improved insulin sensitivity and glucose tolerance without increased body fat content, suggesting that ER α 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.

ERα -/-		ERβ -/-		AR -/-		
	♀	♂	♀	♂	♀	♂
CNS	N.D food intake ↓ Energy expense	N.D food intake ↓ Energy expense	N.D food intake N.D energy expense	N.D food intake N.D energy expense	N.D food intake N.D energy expense	N.D food intake N.D energy expense
β cells	↓ Insulin production ↓ Insulin release ↑ Lipogenesis	↓ Insulin production ↓ Insulin release ↑ Lipogenesis	↓ Insulin release	↓ Insulin release	↓ Insulin production ↓ Insulin release	↓ Insulin production ↓ Insulin release
Skeletal muscle	↓ Insulin sensitivity	↓ Insulin sensitivity ↓ GLUT4	↑ Insulin sensitivity	↑ Insulin sensitivity ↑ GLUT4	↓ Insulin sensitivity	↓ Insulin sensitivity
Liver	↓ Insulin sensitivity ↑ Glucose production	↓ Insulin sensitivity ↑ Glucose production	↑ TG accumulation	↑ TG accumulation	↑ TG accumulation ↓ Insulin sensitivity	↑ TG accumulation ↓ Insulin sensitivity
WAT	↑ WAT mass ↑ Adipogenesis ↑ Lipogenesis ↓ Lipolysis	↑ WAT mass ↑ Adipogenesis ↑ Lipogenesis ↓ Lipolysis	↑ WAT mass (HFD) ↑ Adipogenesis (HFD) ↑ Lipogenesis (HFD)	↑ WAT mass (HFD) ↑ Adipogenesis (HFD) ↑ Lipogenesis (HFD)	↑ WAT mass ↑ Adipogenesis ↑ Lipogenesis	↑ WAT mass ↑ Adipogenesis ↑ Lipogenesis

Figure 1 Summary of the effects on metabolism observed in ER α , ER β , 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.

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).

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 is secreted from adipose tissue in direct proportion to fat content and crosses the blood–brain barrier to interact with leptin receptors (lepr) in the hypothalamus.

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).

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 α 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 estrogenic 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 α 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 α knockout mice display an obese phenotype with increased fat deposition in the absence of differences in food intake compared with wild-type mice. ER α 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 α -specific ligand PPT displayed an inhibitory effect on eating behavior and reduced body weight gain compared with vehicle-treated mice.

Even though ER α is a central player in the control of energy homeostasis, ER β is also likely to play an important role. ER β is expressed in the same hypothalamic nuclei as ER α , however, at lower levels with the highest expression in the PVN. Co-administration of E₂ and ER β 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 α 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 β 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. E₂-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 α -deficient mice exhibit an increased adipose tissue mass without displaying differences in energy intake, suggesting that ER α 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 α , which show 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 (Iverius & Brunzell 1988).

E₂ 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 α -deficient and wild-type mice revealed ER α -dependent increased expression of lipogenic genes and decreased expression of genes regulating lipid transport (Bryzgalova *et al.* 2006). E₂ treatment suppressed the expression of lipogenic genes, i.e. fatty acid synthase (Fasn), stearoyl-coenzyme A desaturase 1 (Scd1), and glycerol-3-phosphate acyltransferase (Gpm), in livers of leptin-deficient *Ob/Ob* mice (Gao *et al.* 2006).

Recent studies indicate that ER β -deficient female mice have a higher body weight under HFD feeding than wild-type littermates (Foryst-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 γ was negatively regulated by ER β , suggesting that PPAR γ could be a mediator of the metabolic effects observed in ER β knockout mice (Foryst-Ludwig *et al.* 2008). Female ER β -deficient mice on HFD displayed impaired food efficiency and increased respiratory quotient, which is an indication of disturbed fatty acid oxidation. PPAR γ DNA binding properties and target gene activation were markedly induced in the gonadal fat of the ER β -deficient mice and inhibition of adipose PPAR γ signaling reversed this metabolic phenotype (Foryst-Ludwig *et al.* 2008).

Cross talk between ER and PPAR γ has also been described earlier (Keller *et al.* 1995). PPAR γ , 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 γ activation in two different breast cancer cell lines (Wang & Kilgore 2002). Furthermore, ER β -specific ligands inhibit PPAR γ activation and the mechanism behind the inhibition is suggested to be a competition between PPAR γ and ER β for the common

co-activator PPAR γ 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 (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).

ER α 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 ER α -deficiency displays impaired glucose metabolism and, further, polymorphisms in the ER α gene have been associated with development of T2D and the metabolic syndrome (Yamada *et al.* 2002, Okura *et al.* 2003). The critical role of ER α in maintaining glucose homeostasis has been validated in *Ob/Ob* mice where treatment with the ER α -selective ligand PPT improved glucose tolerance and insulin sensitivity (Lundholm *et al.* 2008). Studies using euglycemic hyperinsulinemic clamps revealed that ER α knockout is associated with hepatic IR (Bryzgalova *et al.* 2008).

Estrogens are also known to regulate pancreatic β cell function through an ER α -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 ER α -deficient mice, when compared with islets derived from either ER β -deficient or wt mice (Alonso-Magdalena *et al.* 2008). However, ER β -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 ER α inducing and ER β inhibiting GLUT4 expression (Barros *et al.* 2006). Data show that ER α -deficient mice treated with the ER antagonist tamoxifen display increased GLUT4 expression in skeletal muscle, which could indicate a pro-diabetogenic effect of ER β (Barros *et al.* 2009). Targeted knock out of ER β in male mice has been shown to protect against diet-induced IR by increasing PPAR γ 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 (Martensson *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

Figure 2 Model showing estrogenic control of glucose homeostasis by regulatory actions in CNS, β cells, muscles, liver, and adipocytes.

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, Stepan *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 TNF α , 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 ovariectomized rats is associated with increased serum levels of TNF α and enhanced

artery sensitivity to vasoconstriction (Arenas *et al.* 2006). Administration of TNF α inhibitors or E₂ replacement is associated with a decrease in constriction of arteries, which suggests that upregulation of TNF α during estrogen deficiency may contribute to enhanced vascular constriction (Arenas *et al.* 2006).

Activation of NF κ B is known to mediate a variety of chronic inflammatory diseases, including CVD. Estrogen has been shown to inhibit NF κ B 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 NF κ B and NF κ B-induced expression of chemokines/cytokines. This occurs through a direct interaction between ER and NF κ B in the nucleus or by ER-mediated inhibition of upstream NF κ B signaling in the cytoplasm.

ER α activation has been shown to attenuate injury-induced vascular remodeling and studies on ER α knockout mice support these vascular protective effects (Karas *et al.* 1999, Bouchet *et al.* 2001). *In vitro* studies have shown that ER β also plays a protective role in injured arteries (Christian *et al.* 2006). *In vivo* evidence for a role of ER β in estrogen-induced vasoprotection was provided by showing that stimulation with an ER β -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 ER α and ER β . In addition, a newly discovered membrane-bound ER, GPR30, has been demonstrated to exert metabolic functions.

ER α 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, ER α further centrally regulates food intake and energy expenditures. ER β , 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 PPAR γ 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 ERs 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

- Ahdieh HB & Wade GN 1982 Effects of hysterectomy on sexual receptivity, food intake, running wheel activity, and hypothalamic estrogen and progesterin receptors in rats. *Journal of Comparative and Physiological Psychology* **96** 886–892. (doi:10.1037/0735-7036.96.6.886)
- Ahima RS, Bjorbaek C, Osei S & Flier JS 1999 Regulation of neuronal and glial proteins by leptin: implications for brain development. *Endocrinology* **140** 2755–2762. (doi:10.1210/en.140.6.2755)
- Ainslie DA, Morris MJ, Wittert G, Turnbull H, Proietto J & Thorburn AW 2001 Estrogen deficiency causes central leptin insensitivity and increased hypothalamic neuropeptide Y. *International Journal of Obesity and Related Metabolic Disorders* **25** 1680–1688. (doi:10.1038/sj.ijo.0801806)
- Alonso-Magdalena P, Ropero AB, Carrera MP, Cederroth CR, Baquie M, Gauthier BR, Nef S, Stefani E & Nadal A 2008 Pancreatic insulin content regulation by the estrogen receptor ER alpha. *PLoS ONE* **3** e2069. (doi:10.1371/journal.pone.0002069)
- Arenas IA, Xu Y & Davidge ST 2006 Age-associated impairment in vasorelaxation to fluid shear stress in the female vasculature is improved by TNF-alpha antagonism. *American Journal of Physiology: Heart and Circulatory Physiology* **290** H1259–H1263. (doi:10.1152/ajpheart.00990.2005)
- Asarian L & Geary N 2006 Modulation of appetite by gonadal steroid hormones. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **361** 1251–1263. (doi:10.1098/rstb.2006.1860)
- Bakir S, Mori T, Durand J, Chen YF, Thompson JA & Oparil S 2000 Estrogen-induced vasoprotection is estrogen receptor dependent: evidence from the balloon-injured rat carotid artery model. *Circulation* **101** 2342–2344.
- Balhuizen A, Kumar R, Amisten S, Lundquist I & Salehi A 2010 Activation of G protein-coupled receptor 30 modulates hormone secretion and counteracts cytokine-induced apoptosis in pancreatic islets of female mice. *Molecular and Cellular Endocrinology* **320** 16–24. (doi:10.1016/j.mce.2010.01.030)
- Barros RP, Machado UF, Warner M & Gustafsson JA 2006 Muscle GLUT4 regulation by estrogen receptors ERbeta and ERalpha. *PNAS* **103** 1605–1608. (doi:10.1073/pnas.0510391103)
- Barros RP, Gabbi C, Morani A, Warner M & Gustafsson JA 2009 Participation of ERalpha and ERbeta in glucose homeostasis in skeletal muscle and white adipose tissue. *American Journal of Physiology: Endocrinology and Metabolism* **297** E124–E133. (doi:10.1152/ajpendo.00189.2009)

- Bonds DE, Lasser N, Qi L, Brzyski R, Caan B, Heiss G, Limacher MC, Liu JH, Mason E, Oberman A *et al.* 2006 The effect of conjugated equine oestrogen on diabetes incidence: the Women's Health Initiative randomised trial. *Diabetologia* **49** 459–468. (doi:10.1007/s00125-005-0096-0)
- Bouchet L, Krust A, Dupont S, Chambon P, Bayard F & Arnal JF 2001 Estradiol accelerates reendothelialization in mouse carotid artery through estrogen receptor- α but not estrogen receptor- β . *Circulation* **103** 423–428.
- Bryzgalova G, Gao H, Ahren B, Zierath JR, Galuska D, Steiler TL, Dahlman-Wright K, Nilsson S, Gustafsson JA, Efendic S *et al.* 2006 Evidence that oestrogen receptor- α plays an important role in the regulation of glucose homeostasis in mice: insulin sensitivity in the liver. *Diabetologia* **49** 588–597. (doi:10.1007/s00125-005-0105-3)
- Bryzgalova G, Lundholm L, Portwood N, Gustafsson JA, Khan A, Efendic S & Dahlman-Wright K 2008 Mechanisms of antidiabetogenic and body weight-lowering effects of estrogen in high-fat diet-fed mice. *American Journal of Physiology. Endocrinology and Metabolism* **295** E904–E912. (doi:10.1152/ajpendo.90248.2008)
- Carr MC 2003 The emergence of the metabolic syndrome with menopause. *Journal of Clinical Endocrinology and Metabolism* **88** 2404–2411. (doi:10.1210/jc.2003-030242)
- Chen SJ, Li H, Durand J, Oparil S & Chen YF 1996 Estrogen reduces myointimal proliferation after balloon injury of rat carotid artery. *Circulation* **93** 577–584.
- Christian RC, Liu PY, Harrington S, Ruan M, Miller VM & Fitzpatrick LA 2006 Intimal estrogen receptor (ER) β , but not ER α expression, is correlated with coronary calcification and atherosclerosis in pre- and postmenopausal women. *Journal of Clinical Endocrinology and Metabolism* **91** 2713–2720. (doi:10.1210/jc.2005-2672)
- Clegg DJ, Brown LM, Woods SC & Benoit SC 2006 Gonadal hormones determine sensitivity to central leptin and insulin. *Diabetes* **55** 978–987. (doi:10.2337/diabetes.55.04.06.db05-1339)
- Colvin GB & Sawyer CH 1969 Induction of running activity by intracerebral implants of estrogen in ovariectomized rats. *Neuroendocrinology* **4** 309–320. (doi:10.1159/000121762)
- Crespo CJ, Smit E, Snelling A, Sempas CT & Andersen RE 2002 Hormone replacement therapy and its relationship to lipid and glucose metabolism in diabetic and nondiabetic postmenopausal women: results from the Third National Health and Nutrition Examination Survey (NHANES III). *Diabetes Care* **25** 1675–1680. (doi:10.2337/diacare.25.10.1675)
- Darabi M, Rabbani M, Ani M, Zarean E, Panjehpour M & Movahedian A 2010 Increased leukocyte ABCA1 gene expression in post-menopausal women on hormone replacement therapy. *Gynecological Endocrinology* (In press). (doi:10.3109/09513590.2010.507826)
- D'Eon TM, Souza SC, Aronovitz M, Obin MS, Fried SK & Greenberg AS 2005 Estrogen regulation of adiposity and fuel partitioning. Evidence of genomic and non-genomic regulation of lipogenic and oxidative pathways. *Journal of Biological Chemistry* **280** 35983–35991. (doi:10.1074/jbc.M507339200)
- Diano S, Kalra SP, Sakamoto H & Horvath TL 1998 Leptin receptors in estrogen receptor-containing neurons of the female rat hypothalamus. *Brain Research* **812** 256–259. (doi:10.1016/S0006-8993(98)00936-6)
- Dubuc PU 1985 Effects of estrogen on food intake, body weight, and temperature of male and female obese mice. *Proceedings of the Society for Experimental Biology and Medicine* **180** 468–473.
- Eastwood H, Brown KM, Markovic D & Pieri LF 2002 Variation in the ESR1 and ESR2 genes and genetic susceptibility to anorexia nervosa. *Molecular Psychiatry* **7** 86–89. (doi:10.1038/sj/mp/4000929)
- Elias CF, Aschkenasi C, Lee C, Kelly J, Ahima RS, Bjorbaek C, Flier JS, Saper CB & Elmquist JK 1999 Leptin differentially regulates NPY and POMC neurons projecting to the lateral hypothalamic area. *Neuron* **23** 775–786. (doi:10.1016/S0896-6273(01)80035-0)
- Elmquist JK, Elias CF & Saper CB 1999 From lesions to leptin: hypothalamic control of food intake and body weight. *Neuron* **22** 221–232. (doi:10.1016/S0896-6273(00)81084-3)
- Espenshade PJ & Hughes AL 2007 Regulation of sterol synthesis in eukaryotes. *Annual Review of Genetics* **41** 401–427. (doi:10.1146/annurev.genet.41.110306.130315)
- Everson GT, McKinley C & Kern F Jr 1991 Mechanisms of gallstone formation in women. Effects of exogenous estrogen (Premarin) and dietary cholesterol on hepatic lipid metabolism. *Journal of Clinical Investigation* **87** 237–246. (doi:10.1172/JCI114977)
- Foryst-Ludwig A, Clemenz M, Hohmann S, Hartge M, Sprang C, Frost N, Krikov M, Bhanot S, Barros R, Morani A *et al.* 2008 Metabolic actions of estrogen receptor β (ER β) are mediated by a negative cross-talk with PPAR γ . *PLoS Genetics* **4** e1000108. (doi:10.1371/journal.pgen.1000108)
- Friedman JM & Halaas JL 1998 Leptin and the regulation of body weight in mammals. *Nature* **395** 763–770. (doi:10.1038/27376)
- Gallou-Kabani C, Vige A, Gross MS, Rabes JP, Boileau C, Larue-Achagiotis C, Tome D, Jais JP & Junien C 2007 C57BL/6J and A/J mice fed a high-fat diet delineate components of metabolic syndrome. *Obesity* **15** 1996–2005. (doi:10.1038/oby.2007.238)
- Gao H, Bryzgalova G, Hedman E, Khan A, Efendic S, Gustafsson JA & Dahlman-Wright K 2006 Long-term administration of estradiol decreases expression of hepatic lipogenic genes and improves insulin sensitivity in ob/ob mice: a possible mechanism is through direct regulation of signal transducer and activator of transcription 3. *Molecular Endocrinology* **20** 1287–1299. (doi:10.1210/me.2006-0012)
- Ghisletti S, Meda C, Maggi A & Vegeto E 2005 17 β -estradiol inhibits inflammatory gene expression by controlling NF- κ B intracellular localization. *Molecular and Cellular Biology* **25** 2957–2968. (doi:10.1128/MCB.25.8.2957-2968.2005)
- Goulart AC, Zee RY, Pradhan A & Rexrode KM 2009 Associations of the estrogen receptors 1 and 2 gene polymorphisms with the metabolic syndrome in women. *Metabolic Syndrome and Related Disorders* **7** 111–117. (doi:10.1089/met.2008.0030)
- Hamden K, Jaouadi B, Zarai N, Rebai T, Carreau S & Elfeki A 2011 Inhibitory effects of estrogens on digestive enzymes, insulin deficiency, and pancreas toxicity in diabetic rats. *Journal of Physiology and Biochemistry* **67** 121–128. (doi:10.1007/s13105-010-0056-0)
- Heine PA, Taylor JA, Iwamoto GA, Lubahn DB & Cooke PS 2000 Increased adipose tissue in male and female estrogen receptor- α knockout mice. *PNAS* **97** 12729–12734. (doi:10.1073/pnas.97.23.12729)
- Heldring N, Pike A, Andersson S, Matthews J, Cheng G, Hartman J, Tujague M, Strom A, Treuter E, Warner M *et al.* 2007 Estrogen receptors: how do they signal and what are their targets. *Physiological Reviews* **87** 905–931. (doi:10.1152/physrev.00026.2006)
- Henriksson P, Einarsson K, Eriksson A, Kelter U & Angelin B 1989 Estrogen-induced gallstone formation in males. Relation to changes in serum and biliary lipids during hormonal treatment of prostatic carcinoma. *Journal of Clinical Investigation* **84** 811–816. (doi:10.1172/JCI114240)
- Homma H, Kurachi H, Nishio Y, Takeda T, Yamamoto T, Adachi K, Morishige K, Ohmichi M, Matsuzawa Y & Murata Y 2000 Estrogen suppresses transcription of lipoprotein lipase gene. Existence of a unique estrogen response element on the lipoprotein lipase promoter. *Journal of Biological Chemistry* **275** 11404–11411. (doi:10.1074/jbc.275.15.11404)
- Hong MK, Romm PA, Reagan K, Green CE & Rackley CE 1992 Effects of estrogen replacement therapy on serum lipid values and angiographically defined coronary artery disease in postmenopausal women. *American Journal of Cardiology* **69** 176–178. (doi:10.1016/0002-9149(92)91300-S)
- Iverius PH & Brunzell JD 1988 Relationship between lipoprotein lipase activity and plasma sex steroid level in obese women. *Journal of Clinical Investigation* **82** 1106–1112. (doi:10.1172/JCI113667)
- Jones ME, Thorburn AW, Britt KL, Hewitt KN, Wreford NG, Proietto J, Oz OK, Leury BJ, Robertson KM, Yao S *et al.* 2000 Aromatase-deficient (ArKO) mice have a phenotype of increased adiposity. *PNAS* **97** 12735–12740. (doi:10.1073/pnas.97.23.12735)
- Kahn BB & Flier JS 2000 Obesity and insulin resistance. *Journal of Clinical Investigation* **106** 473–481. (doi:10.1172/JCI10842)
- Kanaya AM, Vittinghoff E, Shlipak MG, Resnick HE, Visser M, Grady D & Barrett-Connor E 2003 Association of total and central obesity with mortality in postmenopausal women with coronary heart disease. *American Journal of Epidemiology* **158** 1161–1170. (doi:10.1093/aje/kwg271)

- Karas RH, Hodgin JB, Kwoun M, Kregge JH, Aronovitz M, Mackey W, Gustafsson JA, Korach KS, Smithies O & Mendelsohn ME 1999 Estrogen inhibits the vascular injury response in estrogen receptor beta-deficient female mice. *PNAS* **96** 15133–15136. (doi:10.1073/pnas.96.26.15133)
- Keller H, Givel F, Perroud M & Wahli W 1995 Signaling cross-talk between peroxisome proliferator-activated receptor/retinoid X receptor and estrogen receptor through estrogen response elements. *Molecular Endocrinology* **9** 794–804. (doi:10.1210/me.9.7.794)
- Krom YD, Pires NM, Jukema JW, de Vries MR, Frants RR, Havekes LM, van Dijk KW & Quax PH 2007 Inhibition of neointima formation by local delivery of estrogen receptor alpha and beta specific agonists. *Cardiovascular Research* **73** 217–226. (doi:10.1016/j.cardiores.2006.10.024)
- Kuhl J, Hilding A, Ostenson CG, Grill V, Efendic S & Bavenholm P 2005 Characterisation of subjects with early abnormalities of glucose tolerance in the Stockholm Diabetes Prevention Programme: the impact of sex and type 2 diabetes heredity. *Diabetologia* **48** 35–40. (doi:10.1007/s00125-004-1614-1)
- Lamon-Fava S, Asztalos BF, Howard TD, Reboussin DM, Horvath KV, Schaefer EJ & Herrington DM 2010 Association of polymorphisms in genes involved in lipoprotein metabolism with plasma concentrations of remnant lipoproteins and HDL subpopulations before and after hormone therapy in postmenopausal women. *Clinical Endocrinology* **72** 169–175. (doi:10.1111/j.1365-2265.2009.03644.x)
- Laudenslager ML, Wilkinson CW, Carlisle HJ & Hammel HT 1980 Energy balance in ovariectomized rats with and without estrogen replacement. *American Journal of Physiology* **238** R400–R405.
- Liang YQ, Akishita M, Kim S, Ako J, Hashimoto M, Iijima K, Ohike Y, Watanabe T, Sudoh N, Toba K *et al.* 2002 Estrogen receptor beta is involved in the anorectic action of estrogen. *International Journal of Obesity and Related Metabolic Disorders* **26** 1103–1109. (doi:10.1038/sj.ijo.0802054)
- Lindell K, Bennett PA, Itoh Y, Robinson IC, Carlsson LM & Carlsson B 2001 Leptin receptor 5' untranslated regions in the rat: relative abundance, genomic organization and relation to putative response elements. *Molecular and Cellular Endocrinology* **172** 37–45. (doi:10.1016/S0303-7207(00)00382-8)
- de Luca C & Olefsky JM 2008 Inflammation and insulin resistance. *FEBS Letters* **582** 97–105. (doi:10.1016/j.febslet.2007.11.057)
- Lundholm L, Bryzgalova G, Gao H, Portwood N, Falt S, Berndt KD, Dicker A, Galuska D, Zierath JR, Gustafsson JA *et al.* 2008 The estrogen receptor {alpha}-selective agonist propyl pyrazole triol improves glucose tolerance in ob/ob mice; potential molecular mechanisms. *Journal of Endocrinology* **199** 275–286. (doi:10.1677/joe-08-0192)
- Machinal F, Dieudonne MN, Leneuve MC, Pecquery R & Giudicelli Y 1999 *In vivo* and *in vitro* ob gene expression and leptin secretion in rat adipocytes: evidence for a regional specific regulation by sex steroid hormones. *Endocrinology* **140** 1567–1574. (doi:10.1210/en.140.4.1567)
- Macotela Y, Boucher J, Tran TT & Kahn CR 2009 Sex and depot differences in adipocyte insulin sensitivity and glucose metabolism. *Diabetes* **58** 803–812. (doi:10.2337/db08-1054)
- Maffei L, Murata Y, Rochira V, Tubert G, Aranda C, Vazquez M, Clyne CD, Davis S, Simpson ER & Carani C 2004 Dysmetabolic syndrome in a man with a novel mutation of the aromatase gene: effects of testosterone, alendronate, and estradiol treatment. *Journal of Clinical Endocrinology and Metabolism* **89** 61–70. (doi:10.1210/jc.2003-030313)
- Martensson UE, Salehi SA, Windahl S, Gomez MF, Sward K, Daszkiewicz-Nilsson J, Wendt A, Andersson N, Hellstrand P, Grande PO *et al.* 2009 Deletion of the G protein-coupled receptor 30 impairs glucose tolerance, reduces bone growth, increases blood pressure, and eliminates estradiol-stimulated insulin release in female mice. *Endocrinology* **150** 687–698. (doi:10.1210/en.2008-0623)
- Matthews J & Gustafsson JA 2003 Estrogen signaling: a subtle balance between ER alpha and ER beta. *Molecular Interventions* **3** 281–292. (doi:10.1124/mi.3.5.281)
- Miller DS 1982 Factors affecting energy expenditure. *Proceedings of the Nutrition Society* **41** 193–202. (doi:10.1079/PNS19820030)
- Morishima A, Grumbach MM, Simpson ER, Fisher C & Qin K 1995 Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. *Journal of Clinical Endocrinology and Metabolism* **80** 3689–3698. (doi:10.1210/jc.80.12.3689)
- Morton GJ, Cummings DE, Baskin DG, Barsh GS & Schwartz MW 2006 Central nervous system control of food intake and body weight. *Nature* **443** 289–295. (doi:10.1038/nature05026)
- Musatov S, Chen W, Pfaff DW, Mobbs CV, Yang XJ, Clegg DJ, Kaplitt MG & Ogawa S 2007 Silencing of estrogen receptor alpha in the ventromedial nucleus of hypothalamus leads to metabolic syndrome. *PNAS* **104** 2501–2506. (doi:10.1073/pnas.0610787104)
- Nabulsi AA, Folsom AR, White A, Patsch W, Heiss G, Wu KK & Szklo M 1993 Association of hormone-replacement therapy with various cardiovascular risk factors in postmenopausal women. The Atherosclerosis Risk in Communities Study Investigators. *New England Journal of Medicine* **328** 1069–1075. (doi:10.1056/NEJM199304153281501)
- Nilsson S & Gustafsson JA 2010 Estrogen receptors: therapies targeted to receptor subtypes. *Clinical Pharmacology and Therapeutics* **89** 44–55. (doi:10.1038/clpt.2010.226)
- Nuutila P, Knuuti MJ, Maki M, Laine H, Ruotsalainen U, Teras M, Haaparanta M, Solin O & Yki-Jarvinen H 1995 Gender and insulin sensitivity in the heart and in skeletal muscles. Studies using positron emission tomography. *Diabetes* **44** 31–36. (doi:10.2337/diabetes.44.1.31)
- Oger E, Alhenc-Gelas M, Plu-Bureau G, Mennen L, Cambillau M, Guize L, Pujol Y & Scarabin P 2001 Association of circulating cellular adhesion molecules with menopausal status and hormone replacement therapy. Time-dependent change in transdermal, but not oral estrogen users. *Thrombosis Research* **101** 35–43. (doi:10.1016/S0049-3848(00)00382-0)
- Okura T, Koda M, Ando F, Niino N, Ohta S & Shimokata H 2003 Association of polymorphisms in the estrogen receptor alpha gene with body fat distribution. *International Journal of Obesity and Related Metabolic Disorders* **27** 1020–1027. (doi:10.1038/sj.ijo.0802378)
- Payette C, Blackburn P, Lamarche B, Tremblay A, Bergeron J, Lemieux I, Despres JP & Couillard C 2009 Sex differences in postprandial plasma tumor necrosis factor-alpha, interleukin-6, and C-reactive protein concentrations. *Metabolism* **58** 1593–1601. (doi:10.1016/j.metabol.2009.05.011)
- Peter I, Shearman AM, Vasan RS, Zucker DR, Schmid CH, Demissie S, Cupples LA, Kuvini JT, Karas RH, Mendelsohn ME *et al.* 2005 Association of estrogen receptor beta gene polymorphisms with left ventricular mass and wall thickness in women. *American Journal of Hypertension* **18** 1388–1395. (doi:10.1016/j.amjhyper.2005.05.023)
- Regitz-Zagrosek V, Lehmkuhl E & Weickert MO 2006 Gender differences in the metabolic syndrome and their role for cardiovascular disease. *Clinical Research in Cardiology* **95** 136–147. (doi:10.1007/s00392-006-0351-5)
- Revankar CM, Cimino DE, Sklar LA, Arterburn JB & Prossnitz ER 2005 A transmembrane intracellular estrogen receptor mediates rapid cell signaling. *Science* **307** 1625–1630. (doi:10.1126/science.1106943)
- Schuit SC, Oei HH, Witteman JC, Geurts van Kessel CH, van Meurs JB, Nijhuis RL, van Leeuwen JP, de Jong FH, Zillikens MC, Hofman A *et al.* 2004 Estrogen receptor alpha gene polymorphisms and risk of myocardial infarction. *Journal of the American Medical Association* **291** 2969–2977. (doi:10.1001/jama.291.24.2969)
- Shearman AM, Karasik D, Gruenthal KM, Demissie S, Cupples LA, Housman DE & Kiel DP 2004 Estrogen receptor beta polymorphisms are associated with bone mass in women and men: the Framingham Study. *Journal of Bone and Mineral Research* **19** 773–781. (doi:10.1359/jbmr.0301258)
- Shi H, Kokoeva MV, Inouye K, Tzamelis I, Yin H & Flier JS 2006 TLR4 links innate immunity and fatty acid-induced insulin resistance. *Journal of Clinical Investigation* **116** 3015–3025. (doi:10.1172/JCI28898)
- Shimizu H, Shimomura Y, Nakanishi Y, Futawatari T, Ohtani K, Sato N & Mori M 1997 Estrogen increases *in vivo* leptin production in rats and human subjects. *Journal of Endocrinology* **154** 285–292. (doi:10.1677/joe.0.1540285)
- Shoelson SE, Herrero L & Naaz A 2007 Obesity, inflammation, and insulin resistance. *Gastroenterology* **132** 2169–2180. (doi:10.1053/j.gastro.2007.03.059)

- Simerly RB, Chang C, Muramatsu M & Swanson LW 1990 Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an *in situ* hybridization study. *Journal of Comparative Neurology* **294** 76–95. (doi:10.1002/cne.902940107)
- Simonian SX & Herbison AE 1997 Differential expression of estrogen receptor alpha and beta immunoreactivity by oxytocin neurons of rat paraventricular nucleus. *Journal of Neuroendocrinology* **9** 803–806. (doi:10.1046/j.1365-2826.1997.00659.x)
- Simpson KA, Martin NM & Bloom SR 2009 Hypothalamic regulation of food intake and clinical therapeutic applications. *Arquivos Brasileiros de Endocrinologia e Metabologia* **53** 120–128. (doi:10.1590/S0004-27302009000200002)
- Song Q, Wang SS & Zafari AM 2006 Genetics of the metabolic syndrome. *Hospital Physician* **42** 51–61.
- Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS & Lazar MA 2001 The hormone resistin links obesity to diabetes. *Nature* **409** 307–312. (doi:10.1038/35053000)
- Thomas P, Pang Y, Filardo EJ & Dong J 2005 Identity of an estrogen membrane receptor coupled to a G protein in human breast cancer cells. *Endocrinology* **146** 624–632. (doi:10.1210/en.2004-1064)
- Turgeon JL, Carr MC, Maki PM, Mendelsohn ME & Wise PM 2006 Complex actions of sex steroids in adipose tissue, the cardiovascular system, and brain: insights from basic science and clinical studies. *Endocrine Reviews* **27** 575–605. (doi:10.1210/er.2005-0020)
- Uhler ML, Marks JW, Voigt BJ & Judd HL 1998 Comparison of the impact of transdermal versus oral estrogens on biliary markers of gallstone formation in postmenopausal women. *Journal of Clinical Endocrinology and Metabolism* **83** 410–414. (doi:10.1210/jc.83.2.410)
- Voisin DL, Simonian SX & Herbison AE 1997 Identification of estrogen receptor-containing neurons projecting to the rat supraoptic nucleus. *Neuroscience* **78** 215–228. (doi:10.1016/S0306-4522(96)00551-9)
- Wang X & Kilgore MW 2002 Signal cross-talk between estrogen receptor alpha and beta and the peroxisome proliferator-activated receptor gamma in MDA-MB-231 and MCF-7 breast cancer cells. *Molecular and Cellular Endocrinology* **194** 123–133. (doi:10.1016/S0303-7207(02)00154-5)
- Wang D, Oparil S, Chen YF, McCrory MA, Skibinski GA, Feng W & Szalai AJ 2005 Estrogen treatment abrogates neointima formation in human C-reactive protein transgenic mice. *Arteriosclerosis, Thrombosis, and Vascular Biology* **25** 2094–2099. (doi:10.1161/01.ATV.0000179602.85797.3f)
- Wang HH, Afdhal NH & Wang DQ 2006a Overexpression of estrogen receptor alpha increases hepatic cholesterogenesis, leading to biliary hypersecretion in mice. *Journal of Lipid Research* **47** 778–786. (doi:10.1194/jlr.M500454-JLR200)
- Wang W, Lee ET, Fabsitz RR, Devereux R, Best L, Welty TK & Howard BV 2006b A longitudinal study of hypertension risk factors and their relation to cardiovascular disease: the Strong Heart Study. *Hypertension* **47** 403–409. (doi:10.1161/01.HYP.0000200710.29498.80)
- Wang HH, Liu M, Clegg DJ, Portincasa P & Wang DQ 2009 New insights into the molecular mechanisms underlying effects of estrogen on cholesterol gallstone formation. *Biochimica et Biophysica Acta* **1791** 1037–1047. (doi:10.1016/j.bbap.2009.06.006)
- Wellen KE & Hotamisligil GS 2003 Obesity-induced inflammatory changes in adipose tissue. *Journal of Clinical Investigation* **112** 1785–1788. (doi:10.1172/JCI20514)
- Wellen KE & Hotamisligil GS 2005 Inflammation, stress, and diabetes. *Journal of Clinical Investigation* **115** 1111–1119. (doi:10.1172/JCI25102)
- Yamada Y, Ando F, Niino N, Ohta S & Shimokata H 2002 Association of polymorphisms of the estrogen receptor alpha gene with bone mineral density of the femoral neck in elderly Japanese women. *Journal of Molecular Medicine* **80** 452–460. (doi:10.1007/s00109-002-0348-0)
- Yepuru M, Eswaraka J, Kearbey JD, Barrett CM, Raghov S, Veverka KA, Miller DD, Dalton JT & Narayanan R 2010 Estrogen receptor- β -selective ligands alleviate high-fat diet- and ovariectomy-induced obesity in mice. *Journal of Biological Chemistry* **285** 31292–31303. (doi:10.1074/jbc.M110.147850)
- Yoshihara R, Utsunomiya K, Gojo A, Ishizawa S, Kanazawa Y, Matoba K, Taniguchi K, Yokota T, Kurata H, Yokoyama J *et al.* 2009 Association of polymorphism of estrogen receptor-alpha gene with circulating levels of adiponectin in postmenopausal women with type 2 diabetes. *Journal of Atherosclerosis and Thrombosis* **16** 250–255.
- Zhao C, Dahlman-Wright K & Gustafsson JA 2008 Estrogen receptor beta: an overview and update. *Nuclear Receptor Signaling* **6** e003.
- Zhou L, Chen H, Xu P, Cong LN, Sciacchitano S, Li Y, Graham D, Jacobs AR, Taylor SI & Quon MJ 1999 Action of insulin receptor substrate-3 (IRS-3) and IRS-4 to stimulate translocation of GLUT4 in rat adipose cells. *Molecular Endocrinology* **13** 505–514. (doi:10.1210/me.13.3.505)
- Zirilli L, Rochira V, Diazzi C, Caffagni G & Carani C 2008 Human models of aromatase deficiency. *Journal of Steroid Biochemistry and Molecular Biology* **109** 212–218. (doi:10.1016/j.jsbmb.2008.03.026)

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