REVIEW
Obesity, insulin resistance and diabetes: sex differences and role of oestrogen receptors

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Abstract
Obesity increases the risk of coronary artery disease through insulin resistance, diabetes, arterial hypertension and dyslipidemia. The prevalence of obesity has increased worldwide and is particularly high among middle-aged women and men. After menopause, women are at an increased risk to develop visceral obesity due to the loss of endogenous ovarian hormone production. Effects of oestrogens are classically mediated by the two nuclear oestrogen receptors (ERs) α and β. In addition, more recent research has shown that the intracellular transmembrane G-protein-coupled oestrogen receptor (GPER) originally designated as GPR30 also mediates some of the actions attributed to oestrogens. Oestrogen and its receptors are important regulators of body weight and insulin sensitivity not only in women but also in men as demonstrated by ER mutations in rodents and humans. This article reviews the role of sex hormones and ERs in the context of obesity, insulin sensitivity and diabetes as well as the related clinical issues in women and men.

Keywords adipocyte, aromatase, atherosclerosis, oestradiol, myocardial infarction, visceral fat.

Obesity: a cardiovascular risk factor with a high prevalence
An increase in food intake combined with reduced energy expenditure (as a result of and aggravated by physical inactivity) has led to a dramatic increase in the prevalence of obesity, which is now considered a global epidemic (WHO, 2000, French et al. 2001, Barton & Furrer 2003, James 2008). Obesity has been recognized as an independent cardiovascular risk factor (Yusuf et al. 2004), mostly because of the hypertension, diabetes and dyslipidemia associated with it (Mokdad et al. 2003, Ogden et al. 2007) (Fig. 1). Risk is particularly high in individuals with large amounts of abdominal (visceral) fat (Kannel et al. 1991), which is a source of bioactive mediators that not only directly contribute to insulin resistance (Xu et al. 2003) but also adversely affect lipid profiles, blood pressure and vascular inflammation (Van Gaal et al. 2006). As a consequence of increased activity and production of growth factors with pro-inflammatory activity including angiotensin II and endothelin-1 (Barton et al. 2003, Barton 2010), obese patients are at an increased risk for atherosclerotic vascular complications such as myocardial infarction and stroke (WHO, 2000, Yusuf et al. 2004, Ogden et al. 2007). Despite their high prevalence, obesity and associated diseases remain undertreated in primary care (Bramlage et al. 2004, Stewart et al. 2009).
in young males are associated with insulin resistance and abnormal IGF-1 levels (Smith et al. 1994) as well as with premature coronary artery disease (Sudhir et al. 1997).

**Cellular targets and functions of oestrogens**

Human oestrogens comprise a group of structurally related steroid molecules, namely 17β-oestradiol, oestrone and oestriol, which are the most important regulators of the female and male reproductive systems. Oestrogens also interact with a number of non-reproductive organs, such as bone tissue, cardiovascular, immune and central nervous systems (Gruber et al. 2002). Oestrogens activate nuclear ERs in target cells, acting as transcription factors to regulate the expression of target genes, ultimately controlling cell growth, differentiation and homeostasis (Meyer et al. 2009). Two nuclear ERs located on distinct chromosomes have been identified (Walter et al. 1985, Green et al. 1986, Greene et al. 1986, Kuiper et al. 1996) and termed ERα and ERβ. A subpopulation of ERα and ERβ is localized to the plasma membrane, where their activation induces a variety of intracellular signalling cascades thereby mediating the ‘rapid effects’ of oestrogen (Hammes & Levin 2007, Meyer et al. 2009). Some of these ‘rapid effects’ are now known to be also mediated by the novel G-protein-coupled oestrogen receptor (GPER), previously termed GPR30, which is predominantly located to the endoplasmic reticulum (Revankar et al. 2005). GPER is widely expressed in numerous human organs, including adipose tissue (Hugo et al. 2008, Nadal et al. 2009, Prossnitz & Barton 2009) and has been implicated in oestrogen-dependent physiology of immune function as well as the central nervous and cardiovascular systems (Meyer et al. 2009, Prossnitz & Barton 2009). GPER has been associated with diseases such as obesity, insulin resistance and hormone-sensitive cancers (Martensson et al. 2009, Nadal et al. 2009, Prossnitz & Barton 2009). There also appears to be complex interplay between ERα and GPER, which has not yet been fully defined (Albanito et al. 2007, Prossnitz & Barton 2009, Vivacqua et al. 2009).

**Production of oestrogens and its role in human obesity**

While in pre-menopausal women, 17β-oestradiol is primarily and variably synthesized in the ovaries during the menstrual cycle, depletion of ovarian follicles in the perimenopausal period leads to a steady decline in 17β-oestradiol production. Thus, oestrone becomes the predominant oestrogen in post-menopausal women (Gruber et al. 2002). Therefore, in post-menopausal women, the main source of oestrogens is the conversion of oestrone and oestriol in the peripheral tissues.
of the adrenal androgens testosterone and androstenedione into 17β-oestradiol and oestrone, respectively, which mainly takes place in adipose tissue (Sietti 1987). This conversion is catalysed by the enzyme aromatase, the activity of which increases with ageing (Cleland et al. 1985). Of note, the conversion rate, measured as the proportion of oestrogens and androgens as a surrogate of aromatization, is also accelerated in obese individuals (Sietti 1987), likely as a result of increased numbers of adipocytes (where aromatase is highly expressed) rather than increases in aromatase activity (Cleland et al. 1985). Indeed, changes in body fat mass are positively correlated with total serum 17β-oestradiol and oestrone concentrations in post-menopausal women (Haffner et al. 1991, Kaye et al. 1991, Baglietto et al. 2009). Interestingly, this association varies with time from the onset of menopause and the changes in hormonal status may take up to 6 years to develop (Baglietto et al. 2009). Moreover, physical activity lowers serum oestrone levels (Haffner et al. 1991). Thus, oestrogen synthesis in post-menopausal women is determined by age, body weight and physical fitness (Gruber et al. 2002). Conversely, plasma concentrations of sex hormone-binding globulin, which is the binding protein of sex steroids in plasma, decreases with increasing body weight and specifically, abdominal adiposity (Haffner et al. 1991, Kaye et al. 1991, Baglietto et al. 2009). This results in an increase of unbound, biologically active oestrogen which has been associated with an increased risk for hormone-sensitive tumours, such as breast cancer in obese women as well as in men (Rinaldi et al. 2006, Brinton et al. 2010).

Oestrogen serum levels are increased in hypogonadal men, which is caused by increased aromatization of androgens in the adipose tissue (Schneider et al. 1979, Cleland et al. 1985, Sietti 1987). As a result, although plasma oestrogen levels in men are low compared to women, local concentrations might be much higher and physiologically relevant at the site of production and/or action, where they may reach micromolar concentrations (Sugioka et al. 1987). Moreover, increased oestrogen levels confer a hypogonadal state in men, possibly mediated by inhibition of gonadotropin release via activation of hypothalamic ERs (Zitzmann 2009). Testosterone deficiency may aggravate the development of obesity and hyperinsulinemia, which, in turn, will suppress testicular androgen synthesis even further, resulting in a vicious cycle (Zitzmann 2009). Insulin resistance in a man with a homozygous inactivating mutation of the aromatase gene (Maffei et al. 2004, 2007) as well as in a patient with a mutation of ERα (Smith et al. 1994) have been reported, indicating that oestrogens and their cellular targets are also important for the maintenance of energy homeostasis in males. Taken together, disturbances and changes in the relationship between oestrogens and androgen metabolism seem to adversely affect fat metabolism and insulin sensitivity independent of sex.

**Role of oestrogens in regulation of body weight and insulin sensitivity**

Oestrogens are known as a regulator of body composition, energy balance and insulin sensitivity in both women and men, recently reviewed elsewhere (Geer & Shen 2009, Shi & Clegg 2009, Brown & Clegg 2010). Body weight increases in several conditions associated with oestrogen deficiency such as ovariectomy, polycystic ovary syndrome (PCOS), or the lack of a functional aromatase gene and all can be corrected by 17β-oestradiol treatment (Pedersen et al. 1992, Asarian & Geary 1999, Jones et al. 2000, Gambineri et al. 2002, Misso et al. 2003, Takeda et al. 2003, Maffei et al. 2007). Oestrogens not only decrease food intake through ‘direct’ (central nervous system) effects (Wade 2009) but also through interactions with other hormones that regulate food intake such as insulin, leptin, ghrelin and neuropeptide Y (Brown & Clegg 2010). Moreover, animals and humans lacking endogenous oestrogen synthesis exhibit insulin resistance, which can be treated by oestrogen supplementation (Bailey & Ahmed-Sorour 1980, Morishima et al. 1995, Jones et al. 2000, Takeda et al. 2003). In particular, oestrogens increase hepatic insulin sensitivity by decreasing gluconeogenesis and glycogenolysis (Ahmed-Sorour & Bailey 1981) and increasing insulin release in islets of Langerhans (Alonso-Magdalena et al. 2008). Oestrogens also prevent β-cell apoptosis (Le May et al. 2006), reduce pro-inflammatory signalling (Evans et al. 2001, 2002) and improve insulin action (Brussaard et al. 1997). Therefore, the greater amount of visceral adipose tissue in conjunction with lower endogenous oestrogen levels found in men may be related to the higher insulin resistance when compared with pre-menopausal women (Geer & Shen 2009) and could thus contribute to the sex differences seen with cardiovascular disease (Meyer et al. 2006).

**Importance of ERα and ERβ for insulin function in obesity and diabetes**

**Body weight, food intake and obesity**

Subcutaneous and intra-abdominal adipose tissues express both ERα and ERβ with a predominance of ERα being expressed in intra-abdominal adipose tissue (Dieudonne et al. 2004). The development of knockout animals has provided a powerful tool to examine the role of individual ERs in the function of adipose tissue (Fig. 2). Female and male mice lacking ERα develop
central obesity with increases in white adipose tissue and body weight, which is reflected by increased adipocyte number and size (Heine et al. 2000). Despite this increase in tissue and body weight, food consumption and energy intake do not differ between ERα-knockout animals and controls; but energy expenditure is reduced in the absence of ERα (Heine et al. 2000).

Similarly, silencing of ERα by RNA interference in the hypothalamus reduces energy expenditure and increases food intake in animals (Musatov et al. 2007). Work from Mauvais-Jarvis’ group has shown that oestrogens help to sustain insulin production in diabetic male and female mice and that this effect is at least in part ERα dependent (Le May et al. 2006). Recent work from the same investigators has extended these findings demonstrating that E2 – independent of ERα – can stimulate islet insulin synthesis through interactions between the extranuclear/membrane ERα and the tyrosine kinase Src, which activates ERK1/2 MAPK (Wong et al. 2010). An anti-diabetic role of ERα is also suggested by Ribas et al. indicating that ERα deficiency increases fasting insulin levels, impairs glucose tolerance and results in skeletal muscle insulin resistance (Ribas et al. 2010).

Gustafsson and co-workers showed that after sexual maturation, body fat increases in male mice lacking either ERα or both ERα and ERβ, an effect, which was not observed in ERβ-knockout animals (Ohlsson et al. 2000). Obese ERα-knockout animals also display increased serum cholesterol levels (Ohlsson et al. 2000). These findings support a substantial physiological role for ERα in mediating the effects of oestrogens in the control of body weight. Consistent with these results, insulin resistance developed in a 28-year-old man with a mutation in the ERα gene; this individual also had increased height as a result of insufficient epiphysial plate fusion (Smith et al. 1994). ERα gene expression in subcutaneous adipose tissue and isolated adipocytes is reduced in obese pre-menopausal women, but increases after weight reduction (Nilsson et al. 2007). Moreover, several ERα single nucleotide polymorphisms have been associated with obesity phenotypes in women and men (Deng et al. 2000, Okura et al. 2003, Fox et al. 2005).

The effect of oestrogens on adipose tissue development has also been investigated in ovariectomized ERα-knockout mice. Loss of oestrogen following ovariectomy in these animals resulted in decreased body weight through the regulation of body weight and maintenance of glucose homeostasis. In pre-menopausal women, 17β-oestradiol (E2) is the predominant oestrogen released by the ovaries. The main source of oestrogen in men and post-menopausal women is adipose tissue, where E2 is converted from androgen precursors by the aromatase enzyme. E2 not only has paracrine effects on adipocytes but also acts centrally in the brain as well as peripherally in organs regulating glucose homeostasis such as the endocrine pancreas, liver and skeletal muscle. Note that ERα and ERβ generally mediate opposing effects whereas the role of GPER has only been investigated in part. In addition, insulin released by pancreatic β-cells regulates hepatic glucose production via gluconeogenesis and glucose uptake in skeletal muscle, which is impaired by the action of inflammatory mediators released by adipose tissue. +, stimulatory effect; –, inhibitory effect; ?, effect unknown.
weight, fat-pad weight and adipocyte size, an effect that was reversed by 17β-oestradiol treatment (Naaz et al. 2002), suggesting that increases in body weight were mediated by an ER other than ERα, possibly by ERβ. In addition, only small effects on retroperitoneal fat-pad weight were observed in ERβ-knockout mice whereas animals lacking ERα demonstrated a markedly increased amount of total body fat, suggesting an adipo-genic role of ERβ (Ohlsson et al. 2000). In contrast, mice lacking both ERα and ERβ also develop obesity questioning a role for ERβ (Ohlsson et al. 2000). In addition, Ouchi and co-workers suggested that in rats, ERβ inhibits food intake and reduces body weight through effects in the central nervous system (Liang et al. 2002). In humans, polymorphisms in the ERβ gene have been associated with lower BMI although other investigators found no correlations (Goulart et al. 2002). In humans, polymorphisms in the ERβ gene have been associated with lower BMI although other investigators found no correlations (Goulart et al. 2002). Indeed, ERβ mediated gene expression in certain cell types and often opposes the action of ERα (Matthews & Gustafsson 2003), an interaction that might be also important for the regulation of body weight. Finally, it should be noted that compensatory developmental changes in both animal models and humans may alter hormone responsiveness in ways that are different from the inherent biology in healthy individuals or ‘wild type’ animals respectively.

**Insulin sensitivity and inflammation**

Impaired insulin sensitivity/glucose intolerance and hyperinsulinaemia were noted in a man lacking functional ERα (Smith et al. 1994). A metabolic function of ERα is also supported by animal studies, which suggest oestrogen-dependent effects on glucose homeostasis through both ERα and ERβ (Fig. 2), whereas glucose tolerance is normal in ERβ-knockout mice (Heine et al. 2000, Naaz et al. 2002, Bryzgalova et al. 2006, Ribas et al., 2010). Impaired insulin sensitivity as determined by the hyperinsulineemic clamp technique in ERα-deficient animals was attributed to either inadequate suppression of hepatic glucose production by insulin or impaired insulin action in skeletal muscle (Bryzgalova et al. 2006, Ribas et al., 2010). In addition, adiponectin, an adipokine associated with suppression of insulin resistance and inflammation is decreased in the absence of ERα whereas PAI-1, a surrogate marker of systemic inflammation (Ridker et al. 2004) is increased (Ribas et al., 2009). Increased inflammation-associated changes following streptotoxin-induced injury of pancreatic islets have been described in ERα-deficient mice (Le May et al. 2006); moreover, enhanced inflammatory signalling and impaired fatty acid oxidation were also found in the skeletal muscle of ERα-knockout mice (Ribas et al., 2010), further indicating an ERα-dependent insulin sensitivity phenotype (Bandyopadhyay et al. 2006). Indeed, insulin-stimulated glucose uptake in skeletal muscle, mediated by the glucose transporter isoform GLUT4 (Ryder et al. 2001), is suppressed in the absence of ERα (Bryzgalova et al. 2006). GLUT4 expression was not affected in mice lacking ERβ arguing in favour of an oestrogen-dependent regulation of GLUT4 expression by ERα (Barros et al. 2006). In addition, insulin sensitivity is preserved in mice lacking ERβ although these animals, like wild-type C57BL/6J mice (Barton et al. 2000, Mundy et al. 2007), become obese following a high-fat diet (Foryst-Ludwig et al. 2008). In addition, ERβ acts as an inhibitor of peroxisome proliferators-activated receptor gamma activity, a major inhibitory regulator of glucose and lipid metabolism (Foryst-Ludwig et al. 2008).

Glucose- and arginine-stimulated insulin release in pancreatic islets is similar in mice lacking either ERα or ERβ when compared with control animals (Bryzgalova et al. 2006). ERα-knockout mice have an obese phenotype and develop insulin resistance (Heine et al. 2000), yet 17β-oestradiol is without effect in increasing insulin levels in isolated islets from ERα-knockout animals compared to controls or to ERα-knockout mice (Alonso-Magdalena et al. 2008). Moreover, in the absence of ERα, 17β-oestradiol only partially protects pancreatic β-cells from apoptosis (Le May et al. 2006). A recent study investigated the role of ERs in vascular inflammation associated with diabetes. In both healthy and diabetic mice lacking ERβ, 17β-oestradiol reduced inflammatory nitric oxide synthase expression in the aorta. This inhibitory effect was absent in ERα-knockout animals (Cignarella et al. 2009) indicating that the protective effects of oestrogens on inflammatory responses in the vessel wall are mediated by ERα (Cignarella et al. 2009). In summary, these studies not only indicate an important role of ERα in the regulation of insulin sensitivity but also point to an inhibitory effect of ERβ on ERα-dependent actions (Matthews & Gustafsson 2003).

**Novel metabolic functions of G-protein-coupled oestrogen receptor**

**Body weight, food intake and obesity**

G-protein-coupled oestrogen receptor or GPER (originally cloned and designated as GPR30) is a transmembrane G-protein-coupled receptor located predominantly in the endoplasmic reticulum (Prossnitz et al. 2007, 2008). GPER binds 17β-oestradiol, an agonist activating three major ERs, with subsequent

Interestingly, sexual dimorphisms for GPER expression and/or function have been described not only in the brain (Canonaco et al. 2008) but also in the pancreatic islets, where it is expressed at a much higher level in women than in men (Balhuizen et al. 2010). Accordingly, a role for GPER, in addition to ERα and ERβ, in the regulation of obesity-associated metabolic functions has recently been proposed. Deficiency of GPER was found to be associated with increased visceral adiposity (Ford et al. 2010, Haas et al. 2009) whereas Martensson et al. (2009), using a different GPER knockout strategy, found changes in body weight that were limited to female GPER-deficient animals. The same investigators found no effect of GPER deficiency on the anti-obesity effects of 17β-oestradiol (Windahl et al. 2009). By contrast, Isensee et al. found no effect of GPER deficiency on body weight in animals on either a normal or high-fat diet in another model, LacZ-GPER reporter mice from Deltagen®, which represent a partial GPR30 deletion (Isensee et al. 2009, Olde & Leeb-Lundberg 2009, Langer et al. 2010). Experimental evidence from studies with tamoxifen and raloxifene – SERMs and SERDs that are also GPER agonists (Revankar et al. 2005, Lin et al. 2009, Chow et al. 2010, Meyer et al. 2010 and E.R. Prossnitz unpublished observation 2010) – supports the concept that GPER activation has inhibitory effects on food intake, body weight and fat mass (Baptista et al. 1997, Meli et al. 2004).

Insulin sensitivity

In normal animals and healthy humans, the expression of GPER is high in the pancreatic islets and in the liver, two important organs controlling insulin function (Liu et al. 2009, Samuel et al. 2010). Recent work from Mauvais-Jarvis’ group found that GPER has a critical role in islet survival (Liu & Mauvais-Jarvis 2009, Liu et al. 2009) although glucose tolerance of the normal-diet fed GPER-deficient mice was normal despite increased central obesity (Liu et al. 2009, Haas et al. 2009, Ford et al. 2010). In contrast, Martensson et al. (2009) reported that glucose tolerance is impaired only in female mice lacking GPER. The subsequent hyperglycaemia in these animals is caused by a loss of oestrogen-stimulated pancreatic insulin secretion (Martensson et al. 2009). In addition, deficiency of GPER predisposes to a loss of β-cells and a decrease in pancreatic insulin production after acute exposure to oxidative stress in females (Liu et al. 2009). Selective GPER activation, in turn, prevents apoptosis in islets as efficiently as non-selective ER activation by 17β-oestradiol (Liu et al. 2009). Together, these studies imply that GPER is a novel and important oestrogen-dependent regulator of glucose metabolism and body weight (Fig. 2), although to date, little is known about the individual anti-adipogenic actions of ERα, ERβ and GPER.

Conclusions

Oestrogens are important, sex-independent regulators of body weight, body fat distribution and insulin resistance. Although conventional oestrogen therapy might beneficially affect adiposity and diabetes risk, its previous use in women was associated with adverse effects including an increased risk for breast cancer and thromboembolism. This increased risk may partly result from non-selective activation of ERs, which are ubiquitously expressed in the human body. Future basic science investigations should therefore lead to a better understanding of the molecular mechanisms whereby different ERs regulate body weight and insulin sensitivity in both females and males. In particular, potential interactions and cross-talk between ERα and GPER, which seem to mediate most beneficial effects and ERβ that often opposes these functions, might be identified. This identification may help to define novel pharmacological targets selectively associated with fat metabolism and glucose homeostasis. Of note, such an approach would also imply a therapeutic potential in men bypassing the unwanted effects of oestrrogens.

From a clinical perspective, it should be noted that obesity is associated with increased cardiovascular risk regardless of the accompanying metabolic status (Arnlov et al. 2010). In addition, prevention of weight gain or loss and maintenance of body weight may also reduce the risk of several other obesity-associated diseases such as breast cancer in women (Harvie et al. 2010).
2005). In view of the number of obese children increasingly diagnosed with type 2 diabetes (Sorof & Daniels 2002, Ludwig 2007), arterial hypertension (Andrade et al. 2010), fatty liver disease (Denzel et al. 2009, Alisi et al. 2010), often in combination with a lack of exercise (Belcher et al. 2010, Chen et al. 2005), appropriate steps need to be taken to avoid the projected decline in life expectancy related to the long-term clinical complications of obesity (Olshansky et al. 2005, Stewart et al. 2009).

Conflict of interest

None

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