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Molecular effects of ER alpha- and beta-selective agonists on regulation of energy homeostasis in obese female Wistar rats

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ABSTRACT

The molecular mechanisms underlying the effects of selective ER subtype activation on lipogenesis, adipogenesis, lipid utilization and storage as well as glucose metabolism are currently largely unknown and were analyzed in female OVX Wistar rats on a high-fat diet. Rats received estradiol (E2), ER subtype-selective agonists (Alpha and Beta), and genistein (Gen) for 10 weeks. In adipose tissue, treatment with E2, Alpha, and Beta significantly decreased lipogenic (SREBP-1c, FAS) and adipogenic genes (LPL, PPAR gamma). In liver and skeletal muscle of E2-, Alpha-, Beta-, and Gen-treated animals, lipogenesis and triglyceride accumulation were significantly reduced. Increased hepatic and muscular PPAR gamma mRNA expression was observed in untreated, Beta- and Gen-treated animals, which correlates with increased hepatic glucose uptake. However, only untreated animals showed impaired insulin sensitivity compared to all other groups. Therefore, PPAR gamma up-regulation in untreated animals suggests a compensatory mechanism, probably due to increased triglyceride accumulation in non-adipose tissues. Beta- and Gen-treated animals may benefit from the anabolic potency of ER beta that ameliorates lipid and glucose utilization in muscle. Activation of either ER subtype reduces fat enrichment and improves insulin sensitivity. Depending on the investigated tissue, different molecular pathways seem to be involved.

1. Introduction

Obesity, defined as excessive fat accumulation, is one of the greatest public health challenges of our time. Between 1980 and 2008, its prevalence has nearly doubled worldwide, with tripling rates being observed in many European countries. In 2008, 1.5 billion adults were overweight (BMI greater than 25 kg/m^2) worldwide. Of those affected, more than 200 million men and around 300 million women can be classified as obese (BMI greater than 30 kg/m^2) (WHO, 2012). In addition, the number of overweight children has steadily increased since 1990. In 2010, nearly 43 million children under five were overweight worldwide (WHO, 2010). These numbers are expected to continue to rise, particularly among children. Excessive body weight drastically increases the risk of developing a number of chronic diseases. These include cardiovascular diseases (mainly heart disease and stroke), T2DM, musculoskeletal disorders, dyslipidemia, hypertension, and certain forms of cancer. Consequently, obesity and co-morbidities are responsible for constantly rising health costs and premature death. Obesity is most likely caused by combination of genetic, behavioral, and environmental factors (Chen et al., 2009; Comuzzie et al., 2001; Goulart et al., 2009). However, the most common reason for the epidemic growth of obesity over the last several decades is thought to be a chronic imbalance of energy homeostasis due to increased caloric intake combined with a sedentary lifestyle (Chen et al., 2009; Low et al., 2009; Newbold et al., 2009; Wasan and Looije, 2005).

Abbreviations: Alpha, ER alpha-selective agonist 16α-LE2; Beta, ER beta-selective agonist 8β-VE2; BMI, body mass index; E2, 17β-estradiol; ER, estrogen receptor; FAS, fatty acid synthase; Gen, genistein; Glut4, glucose transporter 4; HF, high fat; HOMA, Homeostasis Assessment Model; IRMS, Isotope-ratio mass spectrometry; LDL, low-density lipoprotein cholesterol; LF, low fat; LME, linear mixed effects; OVX, ovariectomized; PPAR, peroxisome proliferator-activated receptor; PPRE, PPAR responsive element; SREBP-1c, sterol regulatory element binding protein 1c; T2DM, type 2 diabetes mellitus; TC, total cholesterol; TG, triglyceride; VLDL, very-low-density lipoprotein cholesterol; VPDB, Vienna Pee Dee Belemnite.

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Estrogens are well known to regulate energy homeostasis through the modulation of glucose and fat metabolism (Ropero et al., 2008). For example, systemic loss of endogenous estrogens in women following menopause is associated with body weight gain combined with the development of visceral obesity, insulin resistance and T2DM (Curtis and Wilson, 2005; Ley et al., 1992; Louet et al., 2004; Toth et al., 2000). Numerous clinical studies investigating the effect of hormone replacement therapy on postmenopausal women have shown a decrease of central obesity, lower incidence of T2DM, increased sensitivity towards glucose and insulin, and an improvement in lipid metabolism (Andersson et al., 1997; Curtis and Wilson, 2005; Kanaya et al., 2003; Pentti et al., 2009; Samaras et al., 1999; Santen et al., 2010). Similar observations have been made in several animal models. Ovariectomy in rodents leads to body weight gain and the development of obesity, whereas E2 supplementation antagonized these effects (Hertrampf et al., 2006, 2008b; Naaz et al., 2002; Zoth et al., 2010).

Estrogens exert their physiological effects via two ER subtypes, ER alpha and ER beta. How these subtypes, especially ER alpha, affect metabolic pathways, has been intensively investigated in the last decade. The suppressive role of ER alpha on body weight gain has been convincingly shown in a number of studies (Bryzgalova et al., 2006; Heine et al., 2000; Hertrampf et al., 2007, 2008a,b). The influence of ER alpha and ER beta on energy intake is still a matter of debate. Both the activation of ER alpha (Naaz et al., 2002) and ER beta (Liang et al., 2002) have been described to lower food intake.

Regarding glucose metabolism, the loss of ER alpha was shown to impair systemic glucose tolerance and insulin sensitivity in mice (Barros et al., 2009, 2006; Bryzgalova et al., 2006; Heine et al., 2000). By contrast, in ER beta-deficient mice glucose tolerance was similar to their wild type counterparts (Barros et al., 2009, 2006; Bryzgalova et al., 2006). Conversely, the activation of ER alpha using the ER alpha selective agonist PPT (propyl pyrazole triol) improved glucose tolerance and insulin sensitivity in mice compared to vehicle-treated animals (Lundholm et al., 2008). The role of ER beta was not investigated in this study. In skeletal muscle and white adipose tissue, ER alpha deficiency was shown to reduce Glut4 expression when compared to wild types, while the loss of ER beta revealed the opposite effect, suggesting a pro-diabetic effect of ER beta (Barros et al., 2009, 2006). Furthermore, ER alpha-deficient mice displayed also hepatic insulin resistance compared to wild types that was associated with increased expression of lipogenic genes in liver (Bryzgalova et al., 2006). The role of ER beta in hepatic lipogenesis was not investigated in this study. Moreover, in comparison to wild type mice, the loss of ER alpha in mice of both sexes was described to increase white adipose tissue mass (Heine et al., 2000). Another study using the stable ER alpha-transfected 3T3-L1 pre-/adipocyte cell line displayed reduced lipoprotein lipase mRNA and decreased TG accumulation (Homma et al., 2000). These results suggest an important role of ER alpha in adipose tissue biology, but again, the impact of ER beta was not investigated in these two studies. ER beta has been shown to reduce PPAR gamma expression in adipocytes (Foryst-Ludwig et al., 2008). Overexpression of ER beta, but not ER alpha, resulted in a decreased PPAR gamma transcriptional activity and inhibited adipocyte differentiation in 3T3-L1 preadipocytes. A subsequent *in vivo* experiment revealed that ER beta deficiency increased PPAR gamma activity in adipose tissue and led to increased body weight and fat mass in the presence of improved insulin sensitivity compared to wild type mice (Foryst-Ludwig et al., 2008). Taken together, activation of ER alpha has been shown to improve glucose and lipid metabolism, while the role of ER beta on energy homeostasis needs clarification.

Recently, we investigated the impact of two ER subtype-selective agonists, 16α-LE2 (Alpha) and 8β-VE2 (Beta), and the phytoestrogen genistein (Gen) on energy homeostasis in a rat model of nutrition-induced obesity. Our results clearly demonstrated that neither Alpha nor Beta affected food uptake significantly. Treatment with Alpha reduced body weight gain, visceral fat mass, adipocyte size, and serum levels of leptin and lipids compared to untreated rats. Beta treatment did not decrease blood lipids or body weight but reduced fat mass and adipocyte size. In addition, selective activation of ER beta increased skeletal muscle mass (Weigt et al., 2012) which could explain the higher body weight of these animals. By contrast, application of Gen (orally administered via Gen-enriched food in a dose that can be reached by dietary supplements in humans) had no significant influence on the investigated parameters in adipose tissue or blood (Weigt et al., 2012). However, Gen may affect skeletal muscle homeostasis similar to the ER beta subtype-selective agonist (Table 1).

The aim of the current study was to further investigate the underlying molecular mechanisms behind the effects of ER subtype-selective agonists and Gen on energy homeostasis. Therefore, the impact of Alpha-, Beta- and Gen-treatment on the expression of genes involved in the regulation of glucose and fatty acid metabolism in adipose tissue (SREBP-1c, FAS, LPL, and PPAR gamma), liver (SREBP-1c, FAS, PPAR alpha, and PPAR gamma), and soleus muscle (SREBP-1c, FAS, PPAR alpha, PPAR gamma, and PPAR delta) was investigated. TG content in soleus muscle and liver was determined. Furthermore, to investigate glucose tolerance in the animals, we measured the systemic insulin level and the hepatic glucose uptake by using isotope-ratio mass spectrometry.

2. Material and methods

2.1. Animals

Juvenile female Wistar rats (6-weeks old, 120–150 g) were obtained from Janvier (Janvier, Le Genest St Isle, France) and kept at constant room temperature (20 °C ± 1), relative humidity (50–80%) and illumination (12 h light/dark cycles). The animals were housed 3–4 in each cage, with food (SSniff GmbH, Soest, Germany) and water provided ad libitum. All animal procedures were approved by the Committee on Animal Care and compliant with accepted veterinary medical practice.

2.2. Animal diet

Two different diets were used: A phytoestrogen-free LF diet (ssniff® EF D12450B* (I) mod. *LS** containing a metabolizable energy of 15.0 MJ/kg) was used that contains soy oil as the sole source of fat (2.5%). Thus, this diet is rich in unsaturated fatty acids (approx. 25% C18:1 n9, >50% 18:2 n6) and served as control diet. As a second diet a phytoestrogen-free HF diet (ssniff® EF R/M acc. D12451 (I) mod.* containing a metabolizable energy of 19.2 MJ/kg) was used. The main source of fat (20.5%) in this diet originates from animals (lard) and only 5% from soy oil (to cover the needs of essential fatty acids), resulting in a high level of saturated fatty acids. This diet was designed to induce obesity and secondary diseases. The protein source in both diets was casein (detailed composition of diets was described before (Weigt et al., 2012)). A portion of the HF diet was enriched with genistein (700 mg/kg diet), enabling an oral route of exposure. Gen was obtained from LC Laboratories (Woburn, MA 01801 USA). Ssniff Spezialdiäten GmbH, Soest, Germany, provided the food enrichment with Gen.

2.3. Animal treatment

Prior to experimental exposure procedures, all animals were ovariectomized (OVX) or SHAM operated (SHAM) via the dorsal route. Following 2 weeks of regeneration and endogenous