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Roles of G protein-coupled estrogen receptor GPER in metabolic regulation

Geetanjali Sharma[a], Franck Mauvais-Jarvis[b], Eric R. Prossnitz[a,c,*]

[a] Division of Molecular Medicine, Department of Internal Medicine, University of New Mexico Health Sciences Center, Albuquerque, NM, 87131, United States
[b] Diabetes Discovery and Gender Medicine Laboratory, Section of Endocrinology and Metabolism, Department of Medicine,Tulane University Health Sciences Center, School of Medicine, New Orleans, LA, 70112, United States
[c] University of New Mexico Comprehensive Cancer Center, University of New Mexico Health Sciences Center, Albuquerque, NM 87131, United States

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ABSTRACT

Metabolic homeostasis is differentially regulated in males and females. The lower incidence of obesity and associated diseases in pre-menopausal females points towards the beneficial role of the predominant estrogen, 17β-estradiol (E2). The actions of E2 are elicited by nuclear and extra-nuclear estrogen receptor (ER) α and ERβ, as well as the G protein-coupled estrogen receptor (GPER, previously termed GPR30). The roles of GPER in the regulation of metabolism are only beginning to emerge and much remains unclear. The present review highlights recent advances implicating the importance of GPER in metabolic regulation. Assessment of the specific metabolic roles of GPER employing GPER-deficient mice and highly selective GPER-targeted pharmacological agents, agonist G-1 and antagonists G-15 and G36, is also presented. Evidence from *in vitro* and *in vivo* studies involving either GPER deficiency or selective activation suggests that GPER is involved in body weight regulation, glucose and lipid homeostasis as well as inflammation. The therapeutic potential of activating GPER signaling through selective ligands for the treatment of obesity and diabetes is also discussed.

1. Introduction: obesity, metabolic syndrome and sexual dimorphism

Obesity is a global health crisis affecting millions of people and represents a serious health and economic burden [1,2]. It is a heterogeneous disease that leads to insulin resistance and glucose intolerance, elevated lipid levels, hypertension and inflammation, all risk factors contributing to type 2 diabetes and cardiovascular disease [3,4]. Metabolic syndrome is a term that has been coined to refer to the cluster of metabolic disorders that frequently result from obesity [5]. Current major causes of obesity include calorie (sugar and fat) –rich diets and a sedentary lifestyle, resulting in higher levels of circulating lipids and excessive lipid deposition in metabolically important tissues such as skeletal muscle, liver, adipose and pancreas [6–9]. Increased lipid deposition leads to insulin resistance, which in turn results in further increases in triglyceride and non-esterified fatty acid release from adipose tissue, decreases in insulin-stimulated glucose uptake in skeletal muscle and increased hepatic glucose production [10,11]. The ensuing lipotoxicity and glucotoxicity in the islets of the pancreas reduces insulin production from pancreatic β-cells, eventually leading to diabetes [12,13]. Thus, obesity and its associated comorbidities pose severe economic threats to our modern society and it is therefore critical to understand the underlying mechanisms and develop therapeutic strategies to fight obesity.

The prevalence of obesity and its associated risk factors (termed metabolic syndrome) exhibit a sexual dichotomy. Whereas pre-menopausal women are largely protected against weight gain, inflammation and the ensuing metabolic and cardiovascular dysfunction compared to age-matched men, this protection is lost after menopause [14,15]. Postmenopausal women exhibit a decrease in energy expenditure, increased visceral fat deposition, insulin resistance and impaired glucose and lipid metabolism, with estrogen/hormone therapy normalizing these abnormalities to some extent [16–22]. Furthermore, in addition to increased total fat deposition, differences exist between pre- and post-menopausal women and men in terms of fat distribution [14]. Although premenopausal women exhibit more overall fat compared to age matched men, the fat in premenopausal women is stored to a

* Corresponding author at: Division of Molecular Medicine, Department of Internal Medicine, University of New Mexico Health Sciences Center, Albuquerque, NM, 87131, United States.
E-mail address: eprossnitz@salud.unm.edu (E.R. Prossnitz).

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greater extent in the lower body, exhibiting a gynoid distribution that is more resistant to lipolysis. On the other hand, men and postmenopausal women exhibit an upper body/visceral or android distribution of fat, which is more effective in mobilizing lipids [23]. The expansion of android fat depots is usually associated with a greater risk of developing diseases associated with metabolic syndrome. As increased central/android fat deposition alters the secretion of adipose-derived hormones and cytokines, increases circulating levels of lipids and glucose and induces systemic inflammation, obesity produces multiple negative effects on organs throughout the body [24–26]. A sexual dimorphism is also evident in rodents as male mice exhibit increased weight gain on a high-fat diet compared with females [27] but surprisingly are also responsive to estrogen and estrogen mimetics [28]. Hence, the increase in the incidence of obesity-related metabolic disorders is observed in men and postmenopausal women compared to premenopausal women.

2. Estrogen and metabolism

In premenopausal females, the protective effects on metabolism are largely attributed to estrogens, an important class of female sex hormones. The most potent of estrogens is 17β-estradiol (E2), which modulates food intake and energy expenditure through the central nervous system [29] and also exerts direct effects on the physiology of important metabolism-regulating tissues such as adipose, skeletal muscle, liver, pancreas as well as immune cells [30–32]. Besides regulating amount of fat, E2 also regulates the site of fat deposition as well as inflammation and glucose and lipid homeostasis [30,32–35]. E2 specifically promotes accumulation of subcutaneous fat and inhibits visceral fat deposition and suppresses lipogenesis in adipose tissue, skeletal muscle, liver and pancreas [36–39]. In addition, E2 maintains glucose homeostasis by enhancing glucose-induced insulin secretion from pancreatic β-cells [19,40]. Ovariectomized mice, where surgical removal of the ovaries eliminates the endogenous production of E2, is a frequently used model to study the metabolic effects of E2 deprivation [41]. Ovariectomized mice and postmenopausal women share similar metabolic traits resulting from E2 reduction, such as increased adiposity, changes in body fat distribution (specifically central fat), lower energy expenditure, glucose intolerance and reduced insulin sensitivity [16,41]. Administration of E2 or conjugated equine estrogens (CE) to postmenopausal women or ovariectomized female rodents alleviates multiple aspects of metabolic syndrome. Specifically, treatment with estrogens reduces fat mass, decreases fasting glucose and insulin levels, improves dyslipidemia, and ameliorates insulin resistance and glucose intolerance [32,42–44]. Ovariectomized mice, supplemented with E2 or CE, exhibit increased lipid oxidation with a concomitant decrease in lipogenesis in metabolically active peripheral tissues such as adipose, liver and skeletal muscle, thereby preventing lipid accumulation and improving metabolic output of these tissues [30,44]. Weight gain in ovariectomized mice is associated with increases in adipocyte size and visceral fat pads resulting in the altered production of hormones and other bio-active substances such as leptin, resistin, pro-inflammatory cytokines and reactive oxygen species, which further mediate many pathological consequences of obesity on metabolism [45,46]. Treatment of ovariectomized mice with E2 or CE reduces the size of adipocytes and visceral fat pads [41,44,47]. Furthermore, E2 and CE confer protection against oxidative stress and inflammation both in postmenopausal women as well as ovariectomized rodent models [44,48,49]. Another important mouse model that reinforces the importance of E2 in regulation of metabolism is the aromatase knockout (ArKO) mice. Aromatase is the key enzyme involved in the final step of E2 biosynthesis from testosterone. Male and female ArKO mice with E2 insufficiency develop adiposity due to an accumulation of intra-abdominal fat and displayed increases in adipocyte volume, circulating levels of leptin, insulin and cholesterol as well as a decrease in lean mass and physical activity [50]. Thus, E2 supplementation exerts pleiotropic effects via multiple pathways in metabolic tissues to mitigate the effects of adiposity and associated metabolic dysfunction arising from E2 insufficiency.

3. Estrogen receptors

Estrogen, via its receptors, regulates a myriad of physiological effects in multiple metabolic tissues. Effects of E2 are mediated by nuclear and extranuclear estrogen receptors (ERs), ERα and ERβ, which mediate genomic responses through transcriptional activation and rapid actions [51–53]. In the last decade evidence has emerged towards the role of the G protein-coupled estrogen receptor (GPER) in metabolic regulation [54,55], as well as cardiovascular physiology [56–59], immune regulation [60–62], the nervous system [63,64], reproduction [65,66] and cancer [67–73]. GPER is known to exert its effects through non-genomic rapid signaling as well as transcriptional activation [74]. The receptors and mechanisms involved in the actions of E2 are dependent on the type of tissue, the abundance of receptor types present and cross talk between different receptor types. Non-genomic rapid signaling in response to E2 via GPER can activate multiple signaling pathways including MAPK, PI3 K, PKC, Ca^{2+} mobilization and adenylyl cyclase (to produce cAMP) [54,75]. As described above, loss of E2 leads to metabolic imbalance in humans as well as mice. Similarly, mice lacking either ERα or GPER recapitulate multiple aspects of metabolic syndrome, such as obesity, dyslipidemia, insulin resistance, glucose intolerance as well as inflammation [76–78]. With advances in the medical sciences and an increase in life expectancy, women live a large part (up to half) of their life after menopause with an elevated risk of obesity, diabetes and cardiovascular disease. Although menopausal hormone therapy (containing estrogens) in postmenopausal women has shown promise in alleviating these metabolic disturbances, this benefit is not without side effects such as an increased risk of some cancers. Thus, it is imperative to identify new targets for therapeutic intervention of obesity and diabetes to improve the quality of life in post-menopausal women. Numerous studies have elucidated the importance of ERα in energy homeostasis, either through ERα KO mice or by activating ERα through semi-selective ligands (Reviewed in [32,70,79,80]). In the present review, we focus on the recent advances in understanding the effects of GPER deletion or selective activation in the regulation of obesity and metabolic function.

4. GPER expression and selectivity

GPER is a 7-transmembrane G protein-coupled receptor (GPCR), first described as an orphan GPCR in late 1990s [81]. It has since been shown to bind E2 and activate multiple signaling pathways [82–84]. Antibodies raised against GPER have demonstrated its expression and distribution in a wide variety of cells and tissues. Although GPER, as a 7-transmembrane GPCR, is presumed to be located on the plasma membrane as most GPCRs are, many reports observed that in diverse cells and tissues, GPER is predominantly, though not exclusively, localized to intracellular membranes, particularly those of the endoplasmic reticulum and Golgi apparatus [82,85,86]. GPER is widely expressed in reproductive tissues, heart, intestines, ovary, CNS, pancreatic islets, adipose tissue, skeletal muscle, liver, neurons and inflammatory cells [87]. In some tissues, GPER expression is developmentally regulated, such as in developing female reproductive tissues and during the

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