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Role of human pregnane X receptor in high fat diet-induced obesity in pre-menopausal female mice

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ABSTRACT

Obesity is a complex metabolic disorder that is more prevalent among women. Until now, the only relevant rodent models of diet-induced obesity were *via* the use of ovariectomized ("postmenopausal") females. However, recent reports suggest that the xenobiotic nuclear receptor pregnane X receptor (PXR) may contribute to obesity. Therefore, we compared the roles of mouse and human PXRs in diet-induced obesity between wild type (WT) and PXR-humanized (hPXR) transgenic female mice fed either control or high-fat diets (HFD) for 16 weeks. HFD-fed hPXR mice gained weight more rapidly than controls, exhibited hyperinsulinemia, and impaired glucose tolerance. Fundamental differences were observed between control-fed hPXR and WT females: hPXR mice possessed reduced estrogen receptor α (ERα) but enhanced uncoupling protein 1 (UCP1) protein expression in white adipose tissue (WAT); increased protein expression of the hepatic cytochrome P450 3A11 (CYP3A11) and key gluconeogenic enzymes phosphoenolpyruvate carboxykinase and glucose 6-phosphatase, and increased total cholesterol. Interestingly, HFD ingestion induced both UCP1 and glucokinase protein expression in WT mice, but inhibited these enzymes in hPXR females. Unlike WT mice, CYP3A11 protein, serum 17β-estradiol levels, and WAT ERα expression were unaffected by HFD in hPXR females. Together, these studies indicate that the hPXR gene promotes obesity and metabolic syndrome by dysregulating lipid and glucose homeostasis while inhibiting UCP1 expression. Furthermore, our studies indicate that the human PXR suppresses the protective role of estrogen in metabolic disorders. Finally, these data identify PXR-humanized mice as a promising *in vivo* research model for studying obesity and diabetes in women.
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1. Introduction

The prevalence of obesity has increased worldwide at an alarming rate and is now recognized as a serious global health problem [1]. In the US, obesity affects every age-group of the population, with 1 in 3 adults considered to be clinically obese [2]. In the context of this global obesity pandemic, a clear gender disparity exists, with women at greater risk of developing obesity than men [2]. Further, obesity is also a disease of racial disparity, in which African–American women are more frequently afflicted than Caucasian women [3]. The biological mechanisms that contribute to these gender and racial disparities remain known.

Similar to humans, several studies have reported differential susceptibility to obesity upon high-fat diet (HFD) ingestion by some strains of rats and mice [4–7]. Reports indicate that C57BL/6

Abbreviations: HFD, high-fat diet; PXR, pregnane X receptor; mPXR, mouse PXR; hPXR, PXR-humanized or human PXR; WT, wild type; WAT, white adipose tissue; BAT, brown adipose tissue; ALT, alanine transaminase; PPARs, peroxisome proliferator-activated receptors; LBD, ligand binding domain; H&E, hematoxylin and eosin; GTT, glucose tolerance test; IPGTT, intraperitoneal glucose tolerance test; CYP2E1, cytochrome P450 2E1; CYP3A11, cytochrome P450 3A11; AUC, area under the curve; PEPCK1, phosphoenolpyruvate carboxykinase 1; PCN, pregnenolone 16α-carbonitrile; UCP1, uncoupling protein 1; ERα, estrogen receptor α; ERβ, estrogen receptor β; CAR, constitutive androstane receptor; NEFA, nonesterified free fatty acid; G6Pase, glucose 6-phosphatase.

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(B6) mice will develop severe obesity, hyperglycemia, and insulin resistance if weaned unto a HFD compared to other strains, such as C3H/He, 129/Sv, and A/J mice, which are resistant to obesity and diabetes [7–12]. Interestingly, similar to the B6 mice, AKR/J mice are also sensitive to the development of obesity by a HFD, but are less hyperglycemic [7]. Several factors influence the development of obesity including genetics, sedentary lifestyles, and dietary fat amount and type [13–15]. Interestingly, the specific genes that determine sensitivity to dietary obesity remain unknown [13,14].

With respect to gender differences in obesity, both clinical data and animal studies indicate that sex hormones (estrogens in females and androgens in males) are the chief drivers of sexual dimorphisms; gonadal steroids contribute to the regulation of food intake, body weight, and lipid metabolism [16,17]. In females, the loss of estrogen production, either following menopause in humans or ovariectomy in rodents, frequently leads to obesity, suggesting that estrogens appear to play a role in lipid homeostasis to reduce overall body adiposity [18,19].

Consumption of a high caloric diet, rather than genetic makeup, is the most common cause of obesity in humans [20]. Therefore, a clinically relevant animal model of HFD-induced obesity would provide a valuable tool for investigating the molecular basis of obesity in women. Unfortunately, unlike humans, female rodents are more resistant to HFD-induced obesity than their male counterparts, and among female rodents, only ovariectomized animals are capable of significant HFD-induced weight gain [21]. Therefore, female mice are less prone to develop diabetes than humans [22]. Thus, data from both human and animal studies suggest that estrogen may not be the only factor that influences sexual dimorphism in obesity.

Numerous studies have utilized ovariectomy with or without repletion of estrogen and/or compared male and female mice provided a HFD as models to explore the contribution of estrogen to the sexual dimorphisms in obesity [18,19]. Obesity is the product of a chronic positive energy balance, and the health risks associated with obesity vary depending on the amount and location of adipose tissue [23–25]. While the ovariectomized rodent model shares similar characteristics with humans, this model requires a high level of surgical skill and does not completely match the physiology of postmenopausal women, most of whom retain their ovaries. For example, both ovariectomy in female rodents and menopause result in weight gain and increased adipose tissue mass, however, ovariectomy preferentially increases subcutaneous fat whereas menopause increases the metabolically more deleterious intraabdominal visceral fat [24–27]. Furthermore, ovariectomy leads to an immediate cessation of estrogen secretion and gain in lean body mass, whereas menopause occurs after a gradual cessation of estrogen secretion resulting in loss of lean body mass [26,27]. These dissimilar effects of ovariectomy and menopause suggest that the metabolic consequences of ovariectomy differ from that caused by menopause-induced obesity and that ovariectomy may not fully represent a rodent model for female obesity.

Nevertheless, estrogens and estrogen receptors (ERs) have been the primary focus of studies attempting to unravel the molecular basis of sexual dimorphisms in obesity. Estrogen activity occurs *via* the ER, a ligand-dependent transcription factor that consists of distinct modular domains, each with unique biological functions [28,29]. Importantly, ER-mediated transcriptional activity modulates other nuclear receptors, including the peroxisome proliferator-activated receptor α (PPARα), the constitutive androstane receptor (CAR), and the pregnane X receptor [PXR; NR1I2, its human homologue, steroid and xenobiotic receptor (SXR)] [30–32]. While the role of nuclear receptors in obesity is now well established, information is lacking about the contribution of nuclear receptors to the gender disparity of obesity incidence in humans and the functional differences between mouse and human nuclear receptors in diet-induced obesity.

PXR expressed primarily in the liver, intestine and kidney is a xenobiotic sensing nuclear receptor, involved in detoxification of drugs and other foreign chemicals [33]. While previous studies have established functional links between glucose homeostasis and PXR, the *in vivo* significance of PXR function in obesity and metabolic syndrome has not been fully explored [34–36]. The sequences of human and mouse PXRs share nearly 77% amino acid identity across the C-terminal ligand binding domain (LBD), 96% amino acid identity in the N-terminal DNA-binding domain, and the two PXRs display similar tissue-specific expression patterns [33]. However, there are major differences in responses to ligand activation by human and mouse PXR that are likely due to differences in LBD sequence [37]. As a result, some chemicals that activate human PXR usually have little effect on the mouse PXR and vice versa [38,39]. For example, rifampicin, phytoestrogens, and corticosterone potently activate human PXR, but not mouse PXR. In contrast, pregnenolone-16α-carbonitrile (PCN) and dexamethasone strongly activate mouse, but not human PXR [38].

To overcome the species differences in ligand specificity, PXR-humanized (hPXR) transgenic mice have provided a relevant *in vivo* model of the human xenobiotic response [40]. The hPXR mice were generated by bacterial artificial chromosome (BAC) transgenesis, in which the transgene contains the complete human *PXR* gene and the 5′- and 3′-flanking sequences and then bred with PXR-KO mice to produce hPXR mice carrying a C57BL/6 genetic background [40]. hPXR mice selectively express PXR in the liver and intestine in parallel with cytochrome P450 3A4 (CYP3A4), used to detoxify a wide range of substances [40]. Treatment with PXR ligands revealed a clear species difference between WT and hPXR mice in their response to xenobiotics, suggesting that this BAC transgenic hPXR mouse model is useful for investigating human PXR function *in vivo* [40]. Indeed, recent reports have demonstrated the utility of this whole body hPXR mouse model in exploring the xenobiotic and endobiotic role of PXR [40–45]. Our group and others have shown that the male mouse PXR (mPXR) promotes obesity, however, whether PXR regulates obesity with sexual dimorphism remains unknown [45,46]. Moreover, the effect of the human PXR gene on ER- and diet-induced obesity in a mouse model requires investigation. At the same time, gender-based obesity research has lacked a valid animal model that fully recapitulates the human disease.

To resolve discrepancies in the gender-specific molecular pathology of obesity between rodents and humans, female WT and hPXR transgenic mice were investigated with HFD treatment. Our results indicate that the human PXR gene when expressed transgenically in female mice produces an animal model that becomes rapidly obese, glucose intolerant, diabetic, and hyperinsulinemic when exposed to a HFD. These results suggest that human PXR promotes obesity in female mice and that hPXR transgenic mice will serve as a valuable *in vivo* model for obesity research in humans.

2. Materials and methods

2.1. Animals

Breeding-pairs of adult male and female C57BL/6 (WT) mice were purchased from Jackson Laboratories (Bar Harbor, ME). All mice were housed in polycarbonate cages on racks directly vented *via* the facility's exhaust system at 22 °C with a 12/12-h light/dark cycle at the Animal Resources Complex at North Carolina Central University. Breeding pairs of hPXR mice on a C57BL/6 background were transferred from the colony housed at the National Cancer