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# Regulation of hepatic energy metabolism by the nuclear receptor PXR☆

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ABSTRACT

The pregnane X receptor (PXR) is a nuclear receptor that is traditionally thought to be specialized for sensing xenobiotic exposure. In concurrence with this feature PXR was originally identified to regulate drug-metabolizing enzymes and transporters. During the last ten years it has become clear that PXR harbors broader functions. Evidence obtained both in experimental animals and humans indicate that ligand-activated PXR regulates hepatic glucose and lipid metabolism and affects whole body metabolic homeostasis. Currently, the consequences of PXR activation on overall metabolic health are not yet fully understood and varying results on the effect of PXR activation or knockout on metabolic disorders and weight gain have been published in mouse models. Rifampicin and St. John's wort, the prototypical human PXR agonists, impair glucose tolerance in healthy volunteers. Chronic exposure to PXR agonists could potentially represent a risk factor for diabetes and metabolic syndrome. This article is part of a Special Issue entitled: Xenobiotic nuclear receptors: New Tricks for An Old Dog, edited by Dr. Wen Xie.



## 1. Introduction

Pregnane X receptor (PXR, systematic name NR1I2) was identified 1998 as a xenobiotic-sensing nuclear receptor that mediates induction of cytochrome P450 (CYP) genes [1]. Among the nuclear receptors PXR is exceptional, because it can accept wide variety of ligands with significant structural differences, although generally quite high concentrations of ligands are needed for activation. The large and flexible ligand binding pocket allowing this variety of ligands makes PXR a perfect tool for sensing changes in chemical environment. There are, however, also endogenous ligands for PXR including some steroids and bile acids. PXR functions as a ligand-activated transcription factor and together with another nuclear receptor retinoid X receptor (RXR) forms a heterodimer, which bounds to specific DNA elements regulating transcription of numerous genes. Furthermore, PXR may interact with and repress function of other transcription factors [2]. Details of the PXR function including binding cistrome, coregulatory protein preference and posttranslational modifications have been studied during the recent years, but many open questions remain concerning the regulatory hierarchy [3,4].

PXR senses changes in chemical environment and adjusts cellular functions accordingly including induction of xenobiotic biotransformation and transport. Consequently, PXR plays an important role in clinically important drug–drug interactions. While the role in protective detoxification network was easy to understand and predict,

*Abbreviations:* ACAA2, acetyl-CoA acyltransferase 2; ACC1, acetyl-CoA carboxylase 1; ACL, adenosine triphosphate citrate lyase; ACOX1, acyl-coenzyme A oxidase 1; AKR1B10, aldo–keto reductase 1B10; CAR, constitutive androstane receptor; CD36, cluster of differentiation 36; CPT1A, carnitine palmitoyltransferase 1A; CREB, cAMP response element-binding protein; CYP, cytochrome P450; ELOVL6, elongation of very-long-chain fatty acids 6; FAS, fatty acid synthase; FOX, forkhead box protein; G6Pase, glucose-6-phosphatase; GCK, glucokinase; GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide-1; GLUT, glucose transporter; HFD, high-fat diet; HMGCS2, 3-hydroxy-3-methylglutarate-CoA synthase 2; HNF4, hepatocyte nuclear factor 4; HOMA-IR, homeostatic model assessment of insulin resistance; INSIG1, insulin induced gene 1; IPGTT, intraperitoneal glucose tolerance test; IRS-1, insulin receptor substrate 1; ITT, insulin tolerance test; JNK, c-Jun. NH2-terminal kinase; MUFA, monounsaturated fatty acid; NALFD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; OGTT, oral glucose tolerance test; PCN, pregnenolone 16α-carbonitrile; PEPCK, phosphoenolpyruvate carboxykinase; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1α; PP2Cα, protein phosphatase 2Cα; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor; RXR, retinoid X receptor; SGK2, serum/glucocorticoid regulated kinase 2; SIRT1, sirtuin 1; SLC13A5, solute carrier family 13 member 5; SREBP1, sterol regulatory element-binding protein 1; THRSP, thyroid hormone-responsive spot 14 protein; WAT, white adipose tissue.

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unexpectedly PXR has been shown to know many more tricks. During the last ten years an increasing number of studies have associated PXR with several important diseases and physiological functions with no obvious connection to the original function as part of the detoxification machinery against foreign chemicals [2,5]. PXR activation may affect diseases such as inflammatory bowel disease, primary biliary cirrhosis, osteomalacia and multiple components of metabolic syndrome [2]. Metabolic diseases such as obesity, type 2 diabetes and lipid disorders are some of the most burning medical problems of our time. While sedentary lifestyle and excess caloric intake are probably the major etiological factors, additional elements including exposure to chemical endocrine disruptors may play a role in the escalating problem [6]. New therapeutic opportunities are urgently needed calling for better understanding of the molecular mechanisms behind the metabolic diseases. PXR has been shown to regulate glucose and lipid homeostasis and modify the risk of hyperglycemia, diabetes, obesity, dyslipidemia and hepatosteatosis [5]. Interestingly, PXR deficiency also decreases atherosclerosis, the major consequence of the glucose and lipid disorders, in apoE-deficient mice [7].

Liver is a central metabolic organ and plays an important role in maintenance of the whole body energy homeostasis and interacts with other tissues of metabolic importance including skeletal muscle and adipose tissue [8]. In the postprandial state glucose is taken up by the liver and condensed into glycogen or metabolized into fatty acids or amino acids. In fasted state or during exercise the liver releases glucose and ketone bodies to supply metabolic fuels for the needs of the extrahepatic tissues [8]. The liver also senses portal glucose level and signals to pancreatic beta cells to regulate glucose-stimulated insulin excretion [9]. Hepatic energy metabolism is tightly controlled by hormonal and neuronal signals. Especially insulin and glucagon play major roles by regulating functions of transcription factors, enzymes and transporters; however, there are obviously several other ways of fine-tuning the processes. PXR is an emerging new regulator of the hepatic energy metabolism that connects sensing of chemical environment and metabolic health with exciting implications for the etiology of the metabolic diseases, but perhaps also offers potential for new therapies. Further, the metabolic effects of PXR activation raise the question of putative long term, harmful effects of PXR-activating drug therapies.

## 2. Clinical and epidemiological evidence connecting PXR and glucose homeostasis

There is increasing evidence that PXR agonists cause hyperglycemia in humans. Most clinical studies indicate that PXR-activating drugs such as rifampicin, phenobarbital, phenytoin, cyclophosphamide as well as many cholesterol-lowering statins (HMG-CoA reductase inhibitors) and antiretroviral medications cause impaired glucose tolerance (for review see [10,11]). In our recent clinical study we reported that treatment with the prototypical PXR agonist rifampicin increased blood glucose levels during oral glucose tolerance test (OGTT) in healthy volunteers [12]. In this randomized, open, placebo-controlled crossover trial the 1-week administration of rifampicin led to elevated glucose and insulin levels during the OGTT without affecting fasting indexes of glucose metabolism such as the homeostatic model assessment of insulin resistance (HOMA-IR). These findings are supported by the earlier OGTT study of rifampicin dosing in patients with tuberculosis [13], although two other studies of rifampicin-treated tuberculosis patients have given contradictory results [14,15]. The subjects in our study had no interfering medications and no active infection such as tuberculosis.

Statins are known to induce hyperglycemia and increase the risk of type 2 diabetes [16–19]. The highest risk of incident diabetes has been reported for atorvastatin, simvastatin, and rosuvastatin whereas with pravastatin, lovastatin, and fluvastatin the risk is low or nonexistent [20,21]. Since no difference between various statins and risk of diabetes has also been reported [22], further studies are needed to determine whether all the statins have the same diabetogenic effect. Based on *in vitro* studies, atorvastatin, simvastatin, lovastatin and fluvastatin are PXR activators, whereas pravastatin and rosuvastatin are not PXR agonists, rosuvastatin having a weak constitutive androstane receptor (CAR) affinity [23–25]. The mechanism explaining the higher incidence of type 2 diabetes with statin use is not yet known, and PXR activation as a mediator of diabetogenicity in humans is being intensely investigated [26,27]. In a recent study atorvastatin 20 mg/day for 6 months increased fasting levels and area under the curve of insulin and decreased insulin sensitivity (insulinogenic and Matsuda indexes) in women with polycystic ovary syndrome [28]. In human hepatocyte cultures, atorvastatin had the highest relative efficacy of statins for CYP induction [29]. However, atorvastatin treatment (4 weeks, 10 mg/day) was reported not to affect urinary 6β-hydroxycortisol excretion, an index of CYP3A4 induction [30]. In another study 20 mg/day atorvastatin for 6 months inhibited CYP3A4 activity as measured by the 4β-hydroxycholesterol to cholesterol ratio [31], whereas in the study of Björkhem-Bergman et al. treatment with 80 mg/day atorvastatin for four weeks increased 4β-hydroxycholesterol to cholesterol ratio perhaps indicating PXR-mediated CYP3A4 induction [32]. In addition, simvastatin (5 mg/day for four weeks) was able to reduce the AUC of diltiazem, a CYP3A4 substrate, presumably *via* PXR-mediated CYP3A4 induction [33]. Altogether, atorvastatin and simvastatin clearly have a relatively high risk of incident diabetes, but more studies are needed to investigate whether PXR is involved in the atorvastatin and simvastatin-impaired glucose metabolism or diabetogenic effects of the other statins.

Many of the PXR activating drugs (phenobarbital, phenytoin, as well as ampenavir, lopinavir, tipranavir and efavirenz of antiretrotiviral drugs) are also activators of CAR, making it difficult to evaluate the effect of PXR agonism on glucose homeostasis [34–37]. In an elegant, placebo-controlled, double-blind, crossover study phenobarbital treatment did not have any effect on indices of glucose metabolism, including OGTT in type 2 diabetics [38]. In the earlier, to some extent less rigorous studies with the euglycemic clamp technique, another group published that phenobarbital lowered fasting insulin and increased glucose disposal rate and metabolic clearance rate of glucose in healthy volunteers [39,40], and increased insulin sensitivity in type 2 diabetic patients [41,42]. Phenobarbital affects glucose metabolism also by directly inhibiting glucose transporters (GLUT) GLUT1, GLUT2, and GLUT3 in addition to dual PXR/CAR agonism [43,44]. Phenytoin is another dual PXR/CAR activator associated with hyperglycemia [45–48]. The effect of phenytoin on glucose metabolism is mediated by interfering glucose-stimulated $Ca^{2+}$ uptake in pancreas which leads to an impaired release of insulin [10,49].

Many herbal remedies have PXR-activating properties [10,11,50]. Herbal PXR activators associated with glucose metabolism include *Ginkgo biloba* and St. John's wort. Ingestion of *G. biloba* have been shown to increase the insulin and C-peptide levels during OGTT and impair fasting insulin excretion in healthy volunteers and type 2 diabetics without affecting OGTT glucose levels [51,52]. Later it was shown by the same research group that *G. biloba* increased OGTT glucose AUC in type 2 diabetics and increased fasting insulin and C-peptide as well as OGTT C-peptide AUC in healthy volunteers [53]. In addition, *G. biloba* did not cause insulin resistance in the study with the euglycemic clamp technique [54]. The study of the association between *G. biloba* and diabetes is further complicated by the recent findings showing that 18 month treatment with *G. biloba* leaf extract lowered HbA1c levels in patients with type 2 diabetes [55]. It was recently shown that treatment with St. John's wort alone for 21 days impaired glucose tolerance by reducing insulin secretion in healthy young men [56]. St. John's wort contains hyperforin, a very potent PXR agonist [57]. It is noteworthy that most clear-cut results on the impaired glucose metabolism elicited by PXR agonists were obtained in studies with two of the most efficient prototypical PXR activators, *i.e.* rifampicin and hyperforin [12,56].

In addition to medicines and herbal remedies, PXR activators include various environmental and occupational chemicals including