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Does bilirubin prevent hepatic steatosis through activation of the PPARα nuclear receptor?

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ABSTRACT

Several large population studies have demonstrated a negative correlation between serum bilirubin levels and the development of obesity, hepatic steatosis, and cardiovascular disease. Despite the strong correlative data demonstrating the protective role of bilirubin, the mechanism by which bilirubin can protect against these pathologies remains unknown. Bilirubin has long been known as a powerful antioxidant and also has anti-inflammatory actions, each of which may contribute to the protection afforded by increased levels. We have recently described a novel function of bilirubin as a ligand for the peroxisome proliferator-activated receptor-alpha (PPARα), which we show specifically binds to the nuclear receptor. Bilirubin may function as a selective PPAR modulator (SPPARM) to control lipid accumulation and blood glucose. However, it is not known to what degree bilirubin activation of PPARα is responsible for the protection afforded to reduce hepatic steatosis. We hypothesize that bilirubin, acting as a novel SPPARM, increases hepatic fatty acid metabolism through a PPARα-dependent mechanism which reduces hepatic lipid accumulation and protects against hepatic steatosis and non-alcoholic fatty liver disease (NAFLD).

Introduction

Hepatic steatosis is a serious pathologic condition in which the liver accumulates fatty acids to an excessive degree resulting in the development of non-alcoholic fatty liver disease (NAFLD) (Fig. 1). Hepatic steatosis is initiated by several distinct injurious pathways rather than a true "hit" to the liver [1]. When coupled with another 'hit', such as increased oxidative stress (reactive oxygen species), insulin resistance, or inflammation, NAFLD can lead to the development of non-alcoholic steatohepatitis (NASH). The initial 'two-hit' theory for explaining the progression from NAFLD to NASH is now being modified to a 'multiple parallel hits' hypothesis [2]. In this model, the build-up of lipids causes a reduction in insulin clearance in the liver, which in turn, promotes peripheral tissue insulin resistance. Peripheral insulin resistance causes alterations in adipose lipolysis [3], which increases the delivery of free fatty acids from the adipose to the liver resulting in the first "hit", thus, leading to hepatic steatosis. This "hit" increases the vulnerability of the liver to other factors that may follow such as increased oxidative stress and inflammation which then leads to hepatocyte injury and progression to NASH [4].

We and others have previously shown that the lower expression and activity of PPARα in the obese is directly linked to hepatic lipid accumulation and glucose intolerance [5–12]. PPARα is a transcription factor that upon activation promotes uptake, utilization and catabolism of fatty acids by the upregulation of genes involved in fatty acid transport and peroxisomal and mitochondrial fatty acid β-oxidation. We have demonstrated that increasing plasma bilirubin levels attenuated adiposity in obese mice by significantly increasing expression of PPARα [6]. Interestingly, bilirubin levels are also decreased in human obese patients as well as several rodent models of obesity (Fig. 1) [6,13–15]. Bilirubin is primarily known as a scavenger of free radicals and acts as an antioxidant [16–18]. Bilirubin is a product derived from the breakdown of heme released from red blood cells by heme oxygenase enzymes in the spleen. Heme oxygenase is found in two forms, the constitutively expressed form, HO-2, and the inducible form HO-1. Heme oxygenase enzymes metabolize heme to biliverdin which is rapidly converted to bilirubin by the ubiquitous enzyme biliverdin reductase. Bilirubin can also be produced intracellularly as a result of recycling of heme contained in cytochrome P450 proteins. Bilirubin also protects against other forms of cellular stress including endoplasmic reticulum (ER) stress in a model of type II diabetes [19]. In addition to being a potent antioxidant, bilirubin also has anti-inflammatory actions

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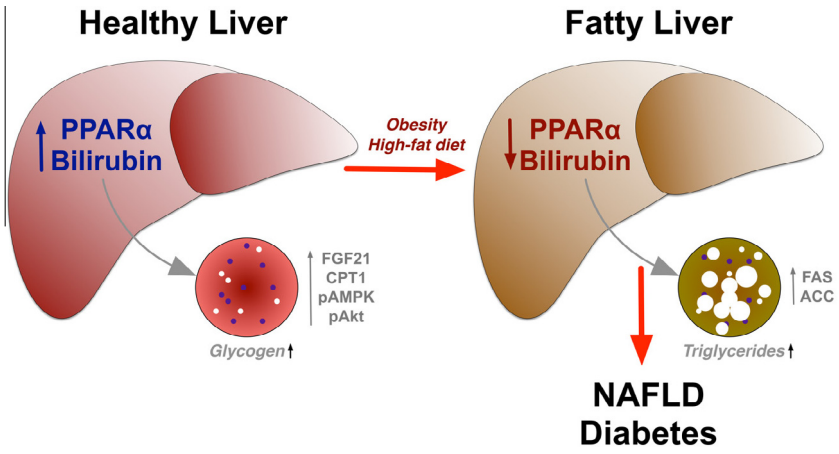

Figure 1. Schematic diagram of hypothesis. In a normal healthy liver, bilirubin and PPARα combine to reduced lipid storage through an increase of the β-oxidation pathway by an up-regulation of FGF21, CPT1, pAMPK, and pAkt. As a result of obesity or a high-fat diet, bilirubin and PPARα levels are decreased, which increases levels of FAS and ACC that enhances triglyceride accumulation. Fat accumulation in liver eventually leads to a non-alcoholic fatty liver disease (NAFLD) and type II diabetes mellitus. ACC, Acetyl-CoA carboxylase, pAMPK, phosphorylated AMP-activated protein kinase, pAkt, phosphorylated Akt, CPT1, Carnitine palmitoyltransferase I, FAS, fatty acid synthase, FGF21, fibroblast growth factor 21, NAFLD, non-alcoholic fatty liver disease.

through regulation of cytokine production as well as disruption of immune cell adhesion molecule-mediated migration [20,21]. These are well-known and established functions of bilirubin. The antioxidant properties of bilirubin protect against increases in ROS, which contributes to the increased oxidative stress that has been linked to the development of hepatocyte injury and progression to NASH.

Serum levels of bilirubin are regulated by the hepatic UDP-glucuronosyltransferase 1A1 (UGT1A1) which conjugates bilirubin for elimination in the bile [22,23]. Mutations in the UGT system results in elevated plasma levels of unconjugated bilirubin. Gilbert's syndrome (GS) is the most common hereditary cause of hyperbilirubinemia affecting approximately 5–10% of the population. GS is the result of reduced activity of the UGT enzyme, UGT1A1, resulting in higher plasma bilirubin levels. GS patients exhibiting mildly elevated levels of bilirubin were found to have reduced the risk of cardiovascular events and a lower risk for future heart disease [24]. Several large-scale population studies have demonstrated a negative relationship between serum bilirubin levels and the development of cardiovascular disease as well as obesity and diabetes [25–29]. Studies in several different patient populations have also demonstrated a negative relationship between serum bilirubin levels and hepatic steatosis [30–33]. Despite these correlative studies, the mechanism by which increases in serum bilirubin levels affords protection against hepatic steatosis is unknown. However, we have recently discovered a novel function for bilirubin as a selective modulator of the PPAR family of nuclear receptors (SPPARM), or more specifically its' direct binding to PPARα (34). The interaction of bilirubin with other PPARs is unknown. We hypothesize that bilirubin may protect against hepatic steatosis through activation of PPARα and it's associated pathways that promote β-oxidation of fatty acids and decrease fatty acid synthesis.

The hypothesis

Serum bilirubin and non-alcoholic fatty liver disease

NAFLD is an emerging liver pathology which is closely linked to the growing obesity epidemic [35]. It is estimated that the global incidence of NAFLD is 25% [36]. This percentage is likely to increase to parallel the global obesity epidemic which is estimated to affect well over a third of the world's population. The negative correlation between serum bilirubin levels and NAFLD has been observed in several different patient populations including children [30–33,37]. Both total, as well as unconjugated serum bilirubin levels, have been reported to be negatively correlated with the development of NAFLD [31,32]. Serum bilirubin levels have also been demonstrated to be *negatively* correlated with NASH, which is a pathological inflammatory condition of the liver that often results in the development of cirrhosis [37]. It is interesting that only a 1.5–2-fold difference in serum bilirubin levels affords protection against NAFLD and NASH in most patient populations observed. Moderate increases in serum bilirubin levels obtain the protective effects of bilirubin against NAFLD.

PPARα and non-alcoholic fatty liver disease

Given the profound effects that PPARα has on lipid metabolism, agonists have been developed as potential therapeutics for the treatment of NAFLD [38,39]. PPARα levels are decreased in animal models of NAFLD and patients with NASH (Fig. 1) [40,41]. Treatment with PPARα agonists has been demonstrated to protect against dietary-induced NAFLD in several different animal models [42–44]. PPARα agonists are believed to protect against hepatic steatosis through up-regulation of genes responsible for increasing β-oxidation of fatty acids. Despite the relative efficacy of PPARα agonists to protect against NAFLD they are not without their limitations. For example, the PPARα agonist, fenofibrate, was demonstrated to increase hepatomegaly despite decreasing steatosis, necro-inflammation, and collagen deposition in a dietary model of NAFLD in the rat [45]. The use of fibrates is also associated with gastrointestinal side effects such as nausea, stomach cramps, or diarrhea. Fibrates can also interact with other drugs such as blood thinners and statin medications to alter their effectiveness. These side effects observed with fibrate-based PPARα agonists have not been reported in bilirubin treated animals or patients with moderately increased levels.

Bilirubin and PPARα

We hypothesize that bilirubin acting through PPARα will attenuate and reverse NAFLD (Fig. 1). This hypothesis is based upon several lines of evidence. The first being the pyrrole-ring like structure of bilirubin which is very similar to known PPARα ligands such as WY-14643 and fenofibrate. Secondly, *in silico* modeling clearly