Chapter 17
Bilirubin: A Ligand of the PPARα Nuclear Receptor

Stephen Hong, Darren Gordon, David E. Stec, and Terry D. Hinds Jr

Abstract Bilirubin is the product from red blood cell lysis, which releases heme that is reduced to biliverdin by heme oxygenases (HO). Later, the biliverdin is converted to bilirubin by the biliverdin reductase (BVR) enzyme. Studies have revealed that bilirubin is significantly lower in obese patients with nonalcoholic fatty liver disease (NAFLD). While the mechanisms that reduce plasma bilirubin are unknown, it has been shown that increasing plasma bilirubin lowers body fat percentage and liver fat content in obese animal models. The bilirubin actions have been attributed to a newly revealed function that it is a hormone, which binds directly to the PPARα nuclear receptor transcription factor. PPARα regulates fatty acid oxidation (FAO) and peroxisomal function to maintain cellular homeostasis and catabolism of fatty acids. Here, we discuss the partnership of bilirubin-PPARα, along with the two other PPAR isoforms PPARβ/δ and PPARγ, and how they function to control peroxisomes and mitochondria that mediates fatty acid β-oxidation and adiposity. There may be clinical interest in bilirubin-PPARα functionality to rectify NAFLD and insulin resistance in the obese.

Keywords Mitochondria · Peroxisome · Fatty acid oxidation · Obesity · Fatty liver disease · NAFLD · Biliverdin reductase · BVR · BVRA · Heme oxygenase · HO-1 · HO-2 · Bilirubin

S. Hong · D. Gordon
Department of Neurosciences, University of Toledo College of Medicine and Life Sciences, Toledo, OH, USA

D. E. Stec
Center for Excellence in Cardiovascular-Renal Research, Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, MS, USA

T. D. Hinds Jr (✉)
Department of Pharmacology and Nutritional Sciences, University of Kentucky, Lexington, KY, USA
e-mail: Terry.Hinds@uky.edu

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17.1 Introduction

With the growing obesity epidemic, multiple diseases have manifested that affects tens of millions of people. Consequentially, nonalcoholic fatty liver disease (NAFLD) is on the rise, which plays a role in developing insulin-resistant diabetes and cardiovascular diseases. NAFLD progression without corrective measures can worsen to nonalcoholic steatohepatitis (NASH), a fatty degeneration that can culminate into cirrhosis or hepatocellular carcinoma [1]. When examining the pathogenesis of NAFLD, the "two-hit" model focuses on the steatosis of the liver (first "hit") and then inflammation that follows (second "hit") [2]. The liver's fatty buildup can be initiated by hepatic insulin resistance, which increases circulating insulin inducing peripheral tissue to develop glucose intolerance [3]. The circulating glucose is collected by the liver to be converted into fat for de novo lipogenesis, leading to fatty liver development [4]. This process is exacerbated in patients with hyperinsulinemia, as there is an even higher amount of glucose deposition into the liver [4]. Consuming high caloric intake causes hepatic fat accumulation by two primary methods: (1) hepatic de novo lipid synthesis or (2) peripheral fat content in the body is redirected to the liver [5]. Sustained elevated levels of fat in the liver can result in pathological sequelae, such as inflammation and oxidative stress, which are natural responders against the adverse environment. NAFLD treatments are currently limited to lifestyle changes and diet modifications, as weight loss is the most effective method to reverse NAFLD [6]. However, drugs are being developed to target the fat accumulation pathways and inflammation, but these are yet to be approved for clinical use.

To reduce fat, mitochondrial or peroxisomal oxidation can play a vital role and serve as primary therapeutic targets for NAFLD. Although most oxidation is mediated through mitochondrial β-oxidation, peroxisomes assist mitochondria in oxidizing fat [7]. Peroxisomes have the ability to begin the oxidative process of very-long-chain fatty acids (VLCFAs) that cannot be oxidized by mitochondria [8]. Increasing this activity could be crucial in correcting NAFLD, which can be enhanced by activating the peroxisome proliferator-activating receptors (PPARα) nuclear receptor transcription factors. The PPARs were initially discovered by ligands that were thought to increase cellular peroxisomes directly, hence, peroxisome proliferation [9]. However, peroxisomal proliferation is based on the cellular response of PPARα activation. PPARβ/δ and PPARγ are two isoforms that were classified as PPARs based on their homology to the PPARα gene; however, neither induces peroxisome proliferation [10]. PPARα has been mostly studied for its role in lipid-lowering effect and, more recently, anti-inflammatory role [11, 12]. All of the PPAR isoforms reduce inflammation, which can provide additional protection from NAFLD [13]. Recently, bilirubin was shown to be a ligand for PPARα [14–16], which also significantly reduces hepatic fat accumulation [13, 17, 18]. In patients, bilirubin levels are inversely correlated with body weight [19–22]. These finds suggest that antioxidants, at least in the case of bilirubin, may reduce adiposity and NAFLD via PPARs. Here, we aim to elucidate the protective pathway that

PPARs and peroxisomes play against NAFLD with a discussion of the role of bilirubin.

17.2 PPARα Protein Structure

The three PPAR isoforms, α, β/δ, and γ, have different tissue expression [9]. They all work similarly in that ligand activation induces binding to DNA and heterodimerization with the retinoid X receptor (RXR) on PPAR response elements (PPREs), eliciting a gene response (Fig. 17.1) [23]. PPARα is a nuclear protein produced in response to reduced serum nutrients, such as in the case of fasting. It should be noted that after discovering that PPARs bind fatty acids, the mainstream thinking was that increased fatty acids during fasting must be inducing PPARα in the liver during this time. However, this is no longer the mainstream thinking as there was a published paper showing that PPARβ/δ is increased in the liver by free fatty acids

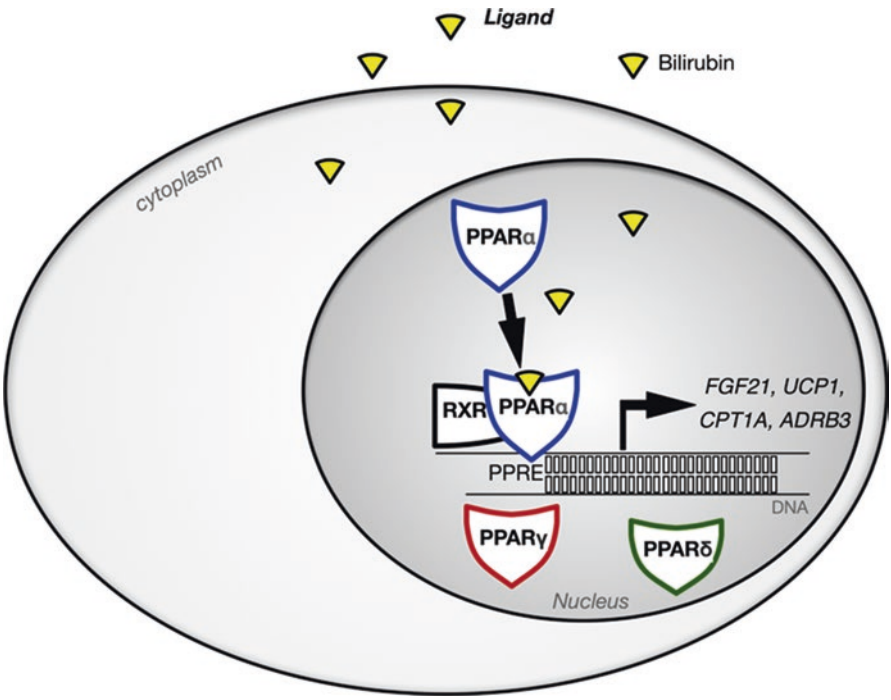

Fig. 17.1 PPAR Transcriptional Signaling Pathway. The PPARs are a superfamily of nuclear receptors, and three isoforms exist: α, β/δ, and γ. All isoforms perform through ligand activation that induces binding to DNA on PPAR response elements (PPREs) and heterodimerization with the retinoid X receptor (RXR), which controls gene transcription. PPARα binding with its ligands (e.g., bilirubin) upregulates transcription of genes that increase metabolism, such as *FGF21, UCP1, CPT1A*, and *ADRB3*

and that it serves as the "plasma free fatty acid sensor in liver" [24]. Since this paper, others have shown data supporting these findings and have rerouted the thinking of free fatty acids for increasing PPARα in the liver during fasting. PPARα being increased during fasting most likely occurs by lower circulating insulin levels, but this is still inclusive.

The PPARα gene is located on Chromosome 22 in humans and 15 in mice and is studied primarily for its role in lipid and glucose regulation in the liver, adipose, and other tissues [25, 26]. The PPAR proteins are arranged in similar domains: the N-terminal domain that includes the amino-terminal transactivation region (AF-1), DNA-binding domain (DBD), the hinge region, and the ligand-binding domain or LBD (also known as activation factor-2, AF2) [27]. Investigating the role of these domains can enhance our understanding of their significance in cellular metabolic pathways and potential impact on metabolic disease. While there are structural similarities, the PPARs have different LBDs that lead to diverse functionalities when bound to their cognate ligand. PPARα activation can lead to specific coregulator (coactivators and corepressors) recruitment that might affect the other PPAR isoforms differently. The AF-1 region is critical in recruiting coregulators that lead to the control of gene activity by the PPARs [28]. Our lab has previously identified an inhibitory PPARα phosphorylation amino acid at serine 73, which is in the AF-1 region. Serine 73 phosphorylation is lower in mice with elevated bilirubin levels [13]. The DBD plays an essential role in the heterodimerization of PPARα with RXR, enhancing PPARα functionality [29], and this cooperativity enhances the expression of metabolic genes [30, 31]. The DBD also contains phosphorylation sites, including the threonine 129 site, which is based on PKC activation and enhances PPARα activity [32]. The hinge region of PPARα serves several roles, which include coregulator binding [33] and is a target of both phosphorylation (serine 179) as well as SUMOylation (lysine 185) [32, 34]. Lastly, while there are some pan-PPAR agonists [31, 35, 36], structural differences in the LBD/AF2 cause preferential activation of specific PPAR isoforms to regulate gene-specific responses [15, 37]. Understanding the structural layout of PPARα is crucial as we explore the effects of several ligands and their ability to alter the coregulator recruitment to PPARα, which controls gene-pathway specific actions. We have previously shown by in silico analysis that bilirubin docks in the LBD of PPARα [15], which we later showed by competitive ligand-binding assays that bilirubin and fenofibrate compete for the same binding regions in the LBD [16]. We also showed that bilirubin induced a specific set of coregulators to PPARα protein (mouse and human) [16]. These indicate that bilirubin has a hormonal function by activating PPARα by direct binding that induces a physiological change.

17.3 Bilirubin Generation and Excretion

One enzyme that may have a key role in attenuating the detrimental impacts of liver pathology is biliverdin reductase (BVR) [13, 38–40]. During red blood cell destruction, hemoglobin is released into the plasma and is converted into biliverdin via heme oxygenase (HO) [41]. Then, through the conversion of a double bond to a single bond by BVR, biliverdin is converted to bilirubin [42–45]. The excretory process for bilirubin is mediated by the UDP-glucuronosyltransferase 1-1 (UGT1A1) enzyme that conjugates bilirubin [46], allowing for excretion into the biliary canaliculi and deposition in the intestine [5, 47]. The gut microbiome transforms conjugated bilirubin into other forms, such as urobilinogen and stercobilin, which are mostly excreted, although some can be reabsorbed [5]. The role of bilirubin metabolism and hepatic function has yet to be elucidated. However, recently, bilirubin nanoparticles were shown to improve fatty liver and reduce hepatic biomarkers AST and ALT enzymes [17].

The two known isozymes of BVR (BVRA and BVRB) have different structural and functional properties, which is further implicated as the genes are located on two different chromosomes [20]. BVRB compared to BVRA in zebra fish larvae has increased expression in states of oxidative stress [48], and global BVRA knockout mouse was subjected to greater oxidative stress [49]. Hepatic BVRA has also been implicated in metabolism as it was shown to protect PPARα for GSK3β inhibition by phosphorylated PPARα at serine 73 to increase turnover and decrease transactivity [13, 40]. BVRA may protect against Alzheimer's disease, which is known to be associated with insulin resistance [50–56]. There remain many scientific questions on BVRA and BVRB and their involvement with the PPAR isoforms, such as regulating all PPAR isoforms, or do they play an integral role in the use of bilirubin by PPARα. Future work on the BVR isozymes and how they signal to the PPAR isoforms is needed.

17.4 Bilirubin as a PPARα Ligand

The beneficial effects of elevated bilirubin levels have been observed in humans with the Gilbert's polymorphism. They contain a polymorphism in the *UGT1A1* promoter that lowers its expression, increasing plasma bilirubin. People with Gilbert's have lower rates of ischemic heart disease and higher rates of high-density lipoproteins (HDL), also known as "good cholesterol" [57]. A humanized Gilbert's syndrome mouse model has been generated using the human UGT1A1*28 polymorphism and has been found to have significantly less fat mass, body fat percentage, cholesterol in the liver, liver stenosis, fasted blood glucose levels, and plasma insulin levels on a high-fat diet (HFD) compared to control on HFD [58]. In addition, there was a significant increase in the CYP4A subfamily of enzymes that are also activated by PPARα [59]. This same model was later shown to

hyperphosphorylate PPARα in white adipose tissue (WAT) and enhanced coactivator recruitment [16].

Previous research has shown that PPARα ligands, such as WY 14,643 and fenofibrate, act as selective PPAR modulators (SPPARM) based on different binding affinity or the recruitment of different coregulators [60–62]. Ultimately, the transcriptional differences provide different protective factors as fibrates are better for anti-inflammatory processes, while WY 14,643 is more suited for lowering blood glucose levels [63]. Based on nonpathogenic increased levels of bilirubin, it may influence the positive effects of both fibrates and WY 14,643.

These beneficial outcomes can be seen in experiments with the direct application of bilirubin to diseased tissues. Colitis-induced rats were given intragastric gavages of unconjugated bilirubin and were shown to have decreased inflammation and a faster recovery rate than controls [64]. These anti-inflammatory effects were further supported using intravenous administration of polyethylene glycol (PEG)-bound bilirubin (bilirubin nanoparticles) to colitis-induced rats. Bilirubin nanoparticles preferentially localized to areas of inflamed colon and significantly halted the process of inflammation in these areas [65]. However, neither of these studies could elucidate the mechanism of how bilirubin was able to reduce inflammation.

To further stress the point of bilirubin activation of PPARα, bilirubin has been shown to attenuate inflammatory processes similarly to PPARα [66, 67]. Two different studies examined the effect of fenofibrate and bilirubin on the proliferation of Th17 differentiation, a T-helper cell associated with autoimmune diseases [68]. Chang et al. found that the activation of PPARα via fenofibrate decreased Th17 cell differentiation by inhibiting the IL-6/STAT3/RORγt pathway [69]. Congruently, Longhi et al. showed that the introduction of bilirubin to mononuclear cells downregulated Th17 cells and IFNγ production via ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1 or CD39) [70]. CD39 is essential for autoimmunity as it hydrolyzes extracellular ATP down to AMP that is converted to immunosuppressive adenosine [71]. In both cases, the downstream effect leads to decreased IL-17, a pro-inflammatory cytokine of Th17 cells, and increased Foxp3 expression, the master regulator of Treg cells. In addition, multiple sources have found that PPARα can upregulate the activity of CD39, showing a more significant correlation between bilirubin and its ability to activate PPARα pathways [72, 73]. The similarities of these results continue to support the interaction between bilirubin and PPARα.

Recent studies have shown that bilirubin activates the PPARα pathway by directly activating the receptor [13, 15]. It had been previously established that activation of PPARα and its other two isoforms leads to increased heme oxygenase-1 (HO-1), the rate-limiting enzyme responsible for synthesizing the bilirubin precursor biliverdin [74–80]. This indicates that there is a positive feedback mechanism between PPARα and bilirubin. There are also limits on this positive feedback mechanism as PPARα has been shown to activate UDP-glucuronosyltransferase 1 family, polypeptide A1 (UGT1A1), the enzyme that conjugates bilirubin, its primary clearance mechanism [79, 81]. This correlation between bilirubin and PPARα may be

able to explain bilirubin's protective ability against NAFLD by a peroxisomal mechanism.

By establishing the relationship between bilirubin and PPARα, it is possible to formulate a mechanism the body naturally forms to protect against the increased inflammation and hepatic damage in NAFLD. The process begins with an increase in bilirubin levels, a general indicator of liver damage or disease. The activation of PPARα ensues, which activates multiple pathways, including the positive and negative feedback loops, indicated previously. The major pathways important for NAFLD protection are the increase in fatty acid oxidation (FAO) to lower the overall fat content within the body and the attenuation of inflammation. Although PPARs were discovered to increase peroxisome levels [9], it is essential where these cells are activated as it can be assumed that more FAO indicates greater protection from NAFLD. Fenofibrate increases PPARα transcription and induces general downstream gene expression of enzymes in skeletal muscle [82], including FAO gene production in lean and obese patients [83]. This is highly warranted as the sheer amount of muscle mass outweighs any other organ that contains high levels of PPARα. It can be assumed that bilirubin can have a similar effect in skeletal muscle as it can be transported ubiquitously in the body. In addition to just increasing peroxisomal content, there is evidence that bilirubin through PPARα activation can bolster peroxisomal and mitochondrial FAO through other supportive mechanisms.

17.5 Peroxisomal Protection against NAFLD

Peroxisomes are single-membrane organelles that contain matrix proteins used for fatty acid metabolism, sequestration of reactive oxygen species (ROS), and biosynthesis of phospholipids [84]. Peroxisomes have often been characterized as the sidekick to mitochondria for their ability to break down VLCFAs and eliminate ROS generated by mitochondria (Fig. 17.2) [85]. Thus, a known partnership exists among the two, which is supported by both organelles' FAO, following the same process of dehydrogenation, hydration, dehydration, and thiolytic cleavage, albeit with different enzymes [86]. However, peroxisomal FAO is not limited to very-long-chain fatty acids. Recent research found that in mitochondrial fatty acid transport dysfunction or overload, peroxisomes can oxidize medium- and long-chain fatty acids [87], making them relevant in reducing NAFLD. The closeness in the relationship between peroxisomes and mitochondria is shown in the coordination of peroxisome-mitochondria FAO using shared enzymes and the mirroring between transport enzymes such as the peroxisome's ATP-binding cassette subfamily D (ABCD) transporters and the mitochondria's carnitine palmitoyl-transferases (CPT) [88, 89]. Furthermore, the main regulator of mitochondrial biosynthesis, peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC-1α), utilizes the PPAR pathway to increase the expression of peroxisomal enzymes required for FAO [11, 90].

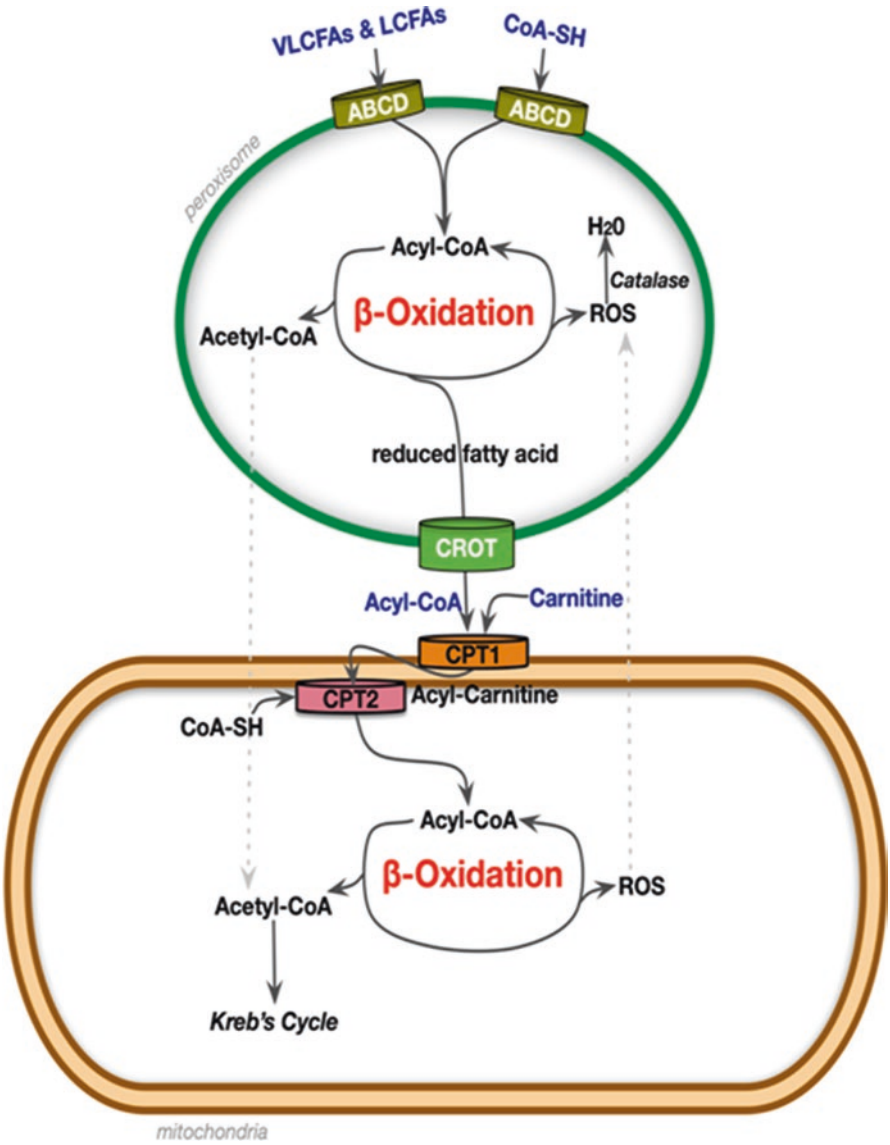

Fig. 17.2 Cross talk of mitochondrial and peroxisomal pathways. Peroxisomes communicate with mitochondria by preparing very-long-chain fatty acids (VLCFAs) into long- and medium-chain fatty acids (LCFAs and MCFAs) for β-oxidation. The VLCFAs and LCFAs enter the peroxisome via ABCD transporters, and, after catabolism, shorter-chain fatty acids are exported via CROT, a peroxisomal carnitine O-octanoyltransferase. The oxidizing of reactive oxygen species (ROS) created from mitochondria and peroxisomes protects the cell. The mitochondria import medium-chain fatty acids via the carnitine palmitoyl-transferase (CPT1) and communicate to CPT2 inside the mitochondria to signal for β-oxidation. Upregulation of the peroxisome allows for improved metabolic activity

In addition to FAO, peroxisomes contain both catalase (CAT) and superoxide dismutase 1 (SOD1), which are vital in the reduction of ROS generated through FAO and other cellular processes [91]. These enzymes represent the main mechanism of ROS removal by converting it into water. It is vital to eliminate these ROS as they damage DNA, including mitochondrial DNA [92]. This becomes a central issue in NAFLD as Nassir et al. have established the link between mitochondrial dysfunction and NAFLD [93], indicating that higher oxidative stress and ROS damage are the root cause of this dysfunction. Peroxisomes act as a significant defensive line against ROS-mediated mitochondrial DNA damage to maintain proper transcription and FAO. With the peroxisome's FAO and ROS sequestration abilities, these intracellular organelles' proliferation and activation may be a key in preventing and correcting NAFLD.

The biogenesis of peroxisomes is regulated by the transcription of peroxin (PEX) genes, which produces a family of proteins necessary for the formation and activity of peroxisomes [94]. Knockouts of essential PEX genes, such as Pex11a, showed significant increases in fat mass, body weight, blood glucose, hemoglobin A1C, insulin, hepatic triacylglycerol (TG), and many other factors [95–99]. This is mirrored in PPARα knockout mice, as similar results were found [11, 100, 101]. Removal of these integral proteins cause increased blood glucose and lipid deposition into the liver, which exacerbate NAFLD. Although it is unclear if most peroxisomes are formed de novo from the endoplasmic reticulum or the fission of preexisting peroxisomes [102, 103], the activation of PPARs directly expands the number of peroxisomes in cells [9].

17.6 Peroxisomes, Oxidative Stress, and Antioxidants

Peroxisomes can be both sources and traps for reactive oxygen species (ROS) due to the oxidative metabolism of fatty acids and the degradation of H_2O_2 by catalases. Peroxisomes contain a subset of enzymes, including the flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)-dependent oxidase, which generate H_2O_2 [104]. Peroxisomes also contain several H_2O_2-eliminating enzyme systems, including catalase and peroxiredoxins (PRDXs). Catalase is abundant in peroxisomes; however, during increased ROS production conditions, the importation of catalase into peroxisomes can be reduced to increase the amount of catalase in the cytosol to protect against H_2O_2 damage [105]. PRDX5 reduces peroxynitrite ($ONOO^-$) and a variety of lipid peroxides (LOOH) via NADPH-dependent thioredoxin (TXN)/TXN reductase (TXNTR) system. They can also transfer oxidizing equivalents from H_sO_s to target proteins through thiol-disulfide reshuffling [106]. PRDX5 and CAT play nonoverlapping roles in H2O2 clearance, supported by the distinct kinetic characteristics of both antioxidant enzymes. Catalase scavenges H_2O_2 in the low millimolar range, while PRDXs work in the low micromolar range [107]. Glutathione S-transferases and epoxide hydrolases can also contribute to ROS balance in peroxisomes [108].

Peroxisomes play an essential role in a healthy redox balance demonstrated in conditions that interfere with normal peroxisomal function and biogenesis. Long-term exposure to peroxisomal proliferators, such as dibutyl phthalate and gemfibrozil, in rodents has been reported to cause oxidative liver damage, and ROS imbalances the induction of ROS-generating peroxisomal enzymes [109]. However, this effect of long-term exposure of peroxisomal proliferators on liver function may be species dependent. Humans appear to be relatively insensitive or nonresponsive at dose levels that produce a marked response in rodents.

17.7 Peroxisomes and Inflammation in NAFLD

The ABCD family of genes, found on the peroxisomal surface, helps import fatty acids or fatty acyl-CoAs [110]. Activation of PPARα significantly increases the transcription of both *ABCD2* and *ABCD3* transporters, increasing peroxisomal FAO [111–114]. In support of this greater import of fat into peroxisomes, bilirubin decreases the levels of the ATP-binding cassette subfamily A member 1 (ABCA1) transporters on macrophages [115]. This may seem counterintuitive as ABCA1, also known as cholesterol efflux regulatory protein (CERP), pumps cholesterol and phospholipids to an extracellular acceptor, apolipoprotein A1, a vital process in the formation of HDL [116]. However, this decrease in the ABCA1 export of lipids out of macrophages may reduce fat in the body. One of HDL's major functions is to return peripheral fat in the body to the liver to be excreted as bile [117], which is unwarranted in NAFLD. To correlate this decreased ABCA1 expression, HO-1 and PPARα agonists have been shown to promote M2 macrophage over M1 macrophage polarization {Stec, 2019 #25713} [118, 119]. M2 macrophages have anti-inflammatory properties and are considered the "repair" macrophages, following injury by promoting growth factors such as PDGF and VEGF [120, 121]. Unlike its M1 counterpart that uses glycolysis, these macrophages are powered by FAO through AMP-activated protein kinase (AMPK). Therefore, it is favorable that macrophages would want to lower the efflux of cholesterol and other fatty acids as they are actively degrading them for the activation of PPARα.

To highlight the importance of peroxisomes in glucose regulation, mice that lacked the *Cpt1b* gene, a key enzyme in mitochondrial FAO, exhibited enhanced glucose regulation by increasing peroxisomal activity [122]. Furthermore, mice with double knockouts of muscle-specific *Cpt1b* and *Pex5* exhibited impaired glucose tolerance due to the lack of peroxisomal compensatory activity [123]. To support this relationship in an endogenous system, our lab has shown that mice with the human *UGT1A1* locus with the Gilbert's polymorphism, a mutation in the gene that reduces bilirubin clearance increasing plasma levels, were protected against hepatic steatosis alongside improved glucose regulation as compared to control [58]. We revealed this is due to a decrease in PPARα phosphorylation in the S73 site, which downregulates PPARα activity [58], resulting in an enhanced gene activity.

Activation of PPARα by WY 14,643 and fenofibrate, both known PPARα ligands, increases catalase and SOD1 activity within cells [124, 125]. Whether this is due to increasing peroxisomal number or individual peroxisomal ROS removal efficiency is unknown. Regardless, this shows that PPARα activation protects the cell from ROS damage at least partially by peroxisomal activity [11]. In addition, upregulation of HO-1 and therefore bilirubin's peroxisomal activation via PPARα have been shown to increase catalase and SOD activity [126], which are further supplemented with bilirubin acting directly as an antioxidant by reducing ROS [127]. In relation to obesity and insulin resistance, why adiposity lowers bilirubin levels and, as a result, progresses to NAFLD will benefit millions of patients.

17.8 Extra-Peroxisomal PPARα Pathways Against NAFLD

PPARα produces several other responses that combat NAFLD through extra-peroxisomal mechanisms via the upregulation of fatty acid transport proteins (FATPs) and solute carrier family 27 members 1, 2, and 4 (Slc27a1, Slc27a2, and Slc27a4) [128–130]. Complementary to this, PPARα has also been shown to increase adipose differentiation-related protein (ADRP), which stimulates fatty acid storage in cytosolic lipid droplets rather than the formation of very-low-density lipoproteins (VLDLs) that are released into the bloodstream {Stec, 2019 #25713} [131]. The increases in fatty acid transport into the cell and the decrease in VLDLs lower plasma TG levels [132]. PPARα is also a mediator for the activation of cytochrome P450 enzymes of 4A subfamily (CYP4A) [11, 133], which is a class of enzymes capable of hydroxylating the terminal ω-carbon of saturated and unsaturated fatty acids [134]. Not surprisingly, mice with hyperbilirubinemia have higher CYP4A expression [58]. This provides an additional possible mechanism of how PPARα lowers the levels of fatty acids.

NAFLD progression increases with inflammation, and PPARα and bilirubin have been shown to function in an anti-inflammatory capacity. PPARα activation reduced the initiation and progression of several inflammatory diseases, such as Parkinson's disease and autoimmune disorders [69, 135]. The protection against autoimmune disorders may be based on the reduced amount of reactive immune cells needed to handle infections. Peroxisomes are required for proper phagocytosis and clearance of bacteria through oxidative burst [136]. The activation of PPARα increases peroxisome concentration in immune cells and their ability to kill bacteria, indicating a need for less immune cells for infection clearance and a lowered chance for autoimmune disorders to develop. However, this hypothesis has yet to be proven. Nevertheless, the activation of PPARα is paramount in attenuating NAFLD as it reduces fat content and inflammation seen in the disease progression.

17.9 PPARγ and PPARβ/δ Effects on Peroxisomes and NAFLD

In the case of the other two isoforms of PPARs, not all things were created equally in protecting the body against NAFLD. Bilirubin was not shown to interact or activate the other PPAR isoforms PPARγ or PPARβ/δ [16]. In particular, PPARγ is critical in lipogenesis and adipocyte differentiation [137–140]. It is important to note that peroxisomes are also vital in producing phospholipids and other phospholipid derivatives critical for cellular functions, such as neuronal myelin sheaths and the formation of the pro-inflammatory precursor, arachidonic acid [89]. This lipid synthesis is typically associated more with PPARγ activation rather than the two other isoforms. Thiazolidinediones (TZDs), a well-known PPARγ activator, are used as an antidiabetic medication to increase insulin sensitivity without increasing hepatic glucose production [140, 141]. This may seem a practical pathway to nullify lipid accumulation as insulin sensitivity allows for greater efficiency of carbohydrate uptake and less lipid production. However, TZDs have been implicated in weight gain as a common side effect, significantly increasing subcutaneous fat compared to visceral fat due to a higher concentration of PPARγ receptors in this tissue [142].

PPARβ/δ is the least studied isoform of the three. This isoform may play a role similar to PPARα's protective nature as it has been shown to work with AMP-activated protein kinase (AMPK) [143, 144]. AMPK is a master regulator of energy metabolism and homeostasis at the cellular and full-body levels by controlling food intake [145, 146]. PPARβ/δ activation via exercise with AMPK synergy has been shown to increase β-oxidation in skeletal muscle cells [147]. However, with PPARs generally associated with anti-inflammatory properties, PPARβ/δ's role is complicated by conflicting reports [148]. One study has found that PPARβ/δ knockouts could not access the anti-inflammatory properties of exercise on vascular inflammation [149]. Counter to this, patients with psoriasis, an autoimmune condition of the skin, had higher PPARβ/δ, and activation of these receptors sustained inflammation [150]. Further studies will be required to understand a possible selective transcription and modulation of PPARβ/δ.

17.10 Conclusion

The recent discovery that bilirubin is a hormone that interacts with PPARα potentially explains the bilirubin-mediated improvement of several metabolic diseases, including obesity and diabetes. Potential mechanisms of how bilirubin is reduced during metabolic disease have implications for improving therapeutics. The bilirubin-PPARα axis appears to be essential in regulating peroxisomes and mitochondria that control fat-burning mechanisms to improve adiposity. Mounting experimental evidence has demonstrated a vital role for peroxisomes in protecting hepatic lipid accumulation and inflammation. Future studies are needed to precisely

determine if bilirubin acting through PPARα can directly stimulate peroxisomal fatty acid metabolism as well as peroxisomal proliferation. Further investigation into the role of PPARα in peroxisomes is needed to completely understand how peroxisomes contribute to the regulation of hepatic function so that novel therapies could be developed in the future to treat conditions such as NAFLD.

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