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Potential Role of the Vitamin D Receptor in Control of Cholesterol Levels

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CYP7A1, a cholesterol 7 α -hydroxylase, is the rate-limiting enzyme in bile acid synthesis and its expression is thought to control levels of serum cholesterol.^{1,2} Humans deficient in CYP7A1 have increased serum cholesterol,³ and mice having constitutive expression of a human CYP7A1 transgene are protected from a high-fat-diet-induced hypercholesterolemia.⁴ The *Cyp7a1* gene is primarily regulated through activation of the ligand-dependent nuclear receptor farnesoid X receptor (FXR) by endogenous bile acids.^{5–7} Bile acids are able to suppress their synthesis and to increase their secretion from the liver into the bile ducts. Suppression of *Cyp7a1* by hepatic FXR is due in part to an induction of the small heterodimer protein (SHP), a member of the nuclear receptor superfamily that lacks a DNA binding domain but has the domain required for dimerization with other nuclear receptors.⁸ SHP is able to form a complex with the liver receptor homolog-1 (LRH-1), a positive regulator of *Cyp7a1*, rendering it unable to activate the *Cyp7a1* gene.⁹ Intriguingly, the importance of the intestine in the regulation of hepatic *Cyp7a1* has been highlighted by the discovery of the FXR-mediated induction in the ileum of fibroblast growth factor (FGF) 19/15, a hormone that is secreted in the portal blood and signals to the liver to repress *Cyp7A1* expression synergistically with SHP.¹⁰ Thus, hepatic and intestinal FXR cooperate to down-regulate *Cyp7A1* expression (Figure 1). The use of tissue-specific *Fxr*-null or transgenic mice demonstrated a much more prominent role for the intestinal FXR-FGF15 pathway, with respect to the hepatic FXR-SHP pathway, in repressing *Cyp7a1* expression.^{11,12}

Vitamin D receptor (VDR) is also reported to have effects on bile acid synthesis and cholesterol levels in hepatocytes and serum. VDR is activated by 1 α , 25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), an active form of vitamin D₃, and mediates vitamin D signaling in numerous physiologic and pharmacologic processes.¹³ VDR is expressed in kidney, intestine, and bone, and at low levels in most other tissues.¹⁴ It is not expressed to a significant extent in liver and thus its physiologic role in this tissue is questionable. Deficiency of VDR leads to hypocalcemia, hyperparathyroidism, rickets, osteomalacia,

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Conflicts of interest

The authors disclose no conflicts.

alopecia, uterine hypoplasia, and growth retardation, which might result from repression of calcium absorption owing to the down-regulation of duodenal epithelial calcium channels.^{15–19} VDR could affect the levels of cholesterol in liver and serum, possibly through controlling expression of genes involved in bile acid synthesis from cholesterol. However, earlier studies in mouse and human hepatoma cells revealed that activation of the VDR actually blocked the expression of FXR, which would result in suppression of *Cyp7a1* expression leading to decreased cholesterol levels.^{20,21}

To clarify the role of VDR in control of *Cyp7a1* and serum cholesterol levels, the current study by Chow et al²² was carried out using in vivo experimentation and various gene knockout mouse models to investigate the mechanism by which VDR affects cholesterol levels. They found that VDR is expressed at low but measurable levels in the livers of mice and is able to activate its main target gene *Cyp24a1* upon injection of 1,25(OH)₂D₃. In contrast with earlier studies in cultured tumor cells, they show that activation of VDR by 1,25(OH)₂D₃ represses SHP expression through suppression of *Shp* gene transcription. They also convincingly demonstrate that 1,25(OH)₂D₃ reduces both liver and serum cholesterol that they ascribe to the activation of *Cyp7a1*; this change was not observed in mice lacking expression of SHP in the liver (Figure 1). This study illustrates the importance of in vivo experimentation to determine the effects of drugs and other compounds on hepatic gene expression. Tumor cells frequently lack all of the transcription machinery found in the liver and even liver cells in culture differ from intact liver in response to stimulation because hepatocyte function depends on liver architecture and the presence of other nonhepatocytes, such as stromal cells and Kupffer cells.

Among the unresolved issues in this study is the translatability to humans. This is of great importance because oral vitamin D₃ and its derivatives are used as dietary supplements and are frequently prescribed to patients with low vitamin D₃ levels. However, a randomized, placebo-controlled trial revealed that short-term treatment with 50,000 IU of vitamin D₃ weekly for 8 weeks had no effect on serum cholesterol levels²³; similar results were obtained with a long-term treatment.²⁴ It should be noted in this context, that these studies were done with oral dosing, whereas Chow et al²² treated mice by injecting 1,25(OH)₂D₃. Injection of humans with vitamin D₃ is not practical as a means for therapeutic delivery for lowering cholesterol, even if it is shown to be efficacious by this route of administration. However, the present work does give a hint that endogenously produced vitamin D₃ and hepatic VDR could affect cholesterol levels in humans.

The *Cyp7a1* gene is positively regulated by several transcription factors including LRH-1, liver X receptor (LXR), and hepatocyte (HNF4 α). SHP represses *Cyp7a1* by binding to and inactivating the transcription potential of LRH-1 and this pathway is conserved in humans and mice.²⁵ SHP could also affect signaling by HNF4 α .^{26,27} There exists a species difference in control of the mouse *Cyp7a1* and human *CYP7A1* genes. LXR activates the mouse *Cyp7a1* gene, but does not activate the human *CYP7A1* gene owing to the lack of an LXR binding site (direct repeat 4 element) in the *CYP7A1* promoter.^{1,26} HNF4 α activates human *CYP7A1* but not mouse *Cyp7a1*²⁸ or rat²⁶ *CYP7A1* transcription. Does the lack of an LXR binding site in the human *CYP7A1* promoter affect the suppression of this gene by SHP? The effect of SHP on HNF4 α and the *CYP7A1* gene could also impact the signaling

in humans. Chow et al²² did show that 1,25(OH)₂D₃ induced *CYP7A1* mRNA in human hepatocytes, although they did not see an effect on *SHP* expression which was attributed to the short half-life on *SHP* mRNA in these cells. Promoter studies revealed a conserved VDR binding site (direct repeat 3 element) in both the mouse *Cyp7a1* and human *CYP7A1* promoters, suggesting that this pathway is also active in humans.

Others found that mice lacking VDR had a decrease in *Shp* RNA and an increase in *Cyp7a1* mRNA and that injection of 1,25(OH)₂D₃ increased expression of *Fgf15* mRNA in the intestine with subsequent inhibition of hepatic *Cyp7a1* mRNA.²⁹ In contrast with these data, administration of 1,25(OH)₂D₃ in the present study induced *Cyp7a1* mRNA. However, as the authors' noted, the dose used by Schmidt et al²⁹ was an order of magnitude higher than that employed in the present study although they used a single IP injection and the studies were short term. Thus, it is possible that a dose-dependent effect could characterize the apparent discrepancy in enterohepatic VDR driven regulation of *Cyp7a1* expression.

Among the most interesting findings in this paper is that activated VDR repressed expression of the *Shp* gene. The authors provide evidence using reporter assays in cultured tumor cells and chromatin immunoprecipitation studies in 1,25(OH)₂D₃-treated mice that VDR binds to an upstream element in the *Shp* gene. Gene repression by nuclear receptors was found in earlier studies, but the molecular mechanism of repression of transcription had not been flushed out. VDR binds to upstream sites of *Cyp24a1* as a heterodimer with the retinoid X receptor (RXR) and activates transcription.^{30,31} In the current study, VDR/RXR bound to an upstream element of the *Shp* gene but instead of activating transcription, the heterodimer represses transcription. What element of the *Shp* promoter mediates this differential response? For nuclear receptors to activate transcription, co-activators must be recruited along with other transcription machinery that modifies chromatin (methylation and acetylation) and attracts RNA polymerase 2. Does binding of VDR/RXR to the *Cyp7a1* element recruit a co-repressor instead of a co-activator? Is chromatin modified to a form that promotes transcription or that suppresses transcription after receptor binding? Finally, regulation of the *Cyp7a1* gene involves many transcription factors (Figure 1). How does VDR influence the FXR-mediated activation of the *Shp* gene? In the presence of increased hepatic bile acids, does FXR signaling negate the effects of VDR on *Shp*? These are questions that can be addressed experimentally.

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Figure 1. Transcriptional regulation of hepatic *Cyp7a1* by bile acids and 1α, 25-dihydroxyvitamin D₃ through nuclear receptors farnesoid X receptor (FXR), vitamin D receptor (VDR), and small hetero-dimer protein (SHP), and intestinal fibroblast growth factor (FGF)15.