# Liver heparan sulfate proteoglycans: Old molecules provide new insights on lipoprotein metabolism – Commentary

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# Liver Heparan Sulfate Proteoglycans: Old Molecules Provide New Insights on Lipoprotein Metabolism

*MacArthur JM, Bishop JR, Stanford KI, Wang L, Bensadoun A, Witztum JL, et al.* Liver heparan sulfate proteoglycans mediate clearance of triglyceride-rich lipoproteins independently of LDL receptor family members. J Clin Invest 2007;117:153-164. (Reprinted with permission.)

## Abstract

**We examined the role of hepatic heparan sulfate in triglyceride-rich lipoprotein metabolism by inactivating the biosynthetic gene GlcNAc N-deacetylase/N-sulfotransferase 1 (Ndst1) in hepatocytes using the Cre-loxP system, which resulted in an approximately 50% reduction in sulfation of liver heparan sulfate. Mice were viable and healthy, but they accumulated triglyceride-rich lipoprotein particles containing apoB-100, apoB-48, apoE, and apoCI-IV. Compounding the mutation with LDL receptor deficiency caused enhanced accumulation of both cholesterol- and triglyceride-rich particles compared with mice lacking only LDL receptors, suggesting that heparan sulfate participates in the clearance of cholesterol-rich lipoproteins as well. Mutant mice synthesized VLDL normally but showed reduced plasma clearance of human VLDL and a corresponding reduction in hepatic VLDL uptake. Retinyl ester excursion studies revealed that clearance of intestinally derived lipoproteins also depended on hepatocyte heparan sulfate. These findings show that under normal physiological conditions, hepatic heparan sulfate proteoglycans play a crucial role in the clearance of both intestinally derived and hepatic lipoprotein particles.**

## Comment

Experimental and clinical evidence indicates that the proteoglycan protein superfamily plays a crucial role in homeostasis at the cellular and tissue level. Indeed, modulation of cell/extracellular matrix interactions by these glycoconjugates influences cell growth, embryonic development, and postnatal tissue remodeling and seems to be directly implicated in various disease conditions. A recent report highlighting the influence of heparan sulfate proteoglycans (HSPGs) on plasma lipoprotein metabolism[1] brings these macromolecules back to the arena of liver biology. By reducing the liver content of mature heparan sulfate (HS) through liver-specific gene targeting, MacArthur et al. elegantly showed that hepatic HSPGs have a physiologically relevant role in the clearance of different classes of plasma lipoproteins.[1] The contribution of these macromolecules to lipoprotein metabolism was previously suggested by *in vitro* and *in vivo* studies.[2-4] However, confirmatory *in vivo* studies have been extremely elusive, mainly because of the intrinsic complexity of proteoglycan biology.

Proteoglycan structure consists of a core protein with covalent substitutions of one or several glycosaminoglycan (GAG) chains. Even though the protein component itself may display binding sites for specific ligands, the refined chemical structure of GAGs defines most of the PG binding properties, which are very diverse, leading to exquisitely specific ligand recognition. The structural features of GAGs are cell type–specific and tissue-specific. For instance, liver HSPGs hold almost 2-fold more sulfate content than those derived from other tissues. Furthermore, multiple GAGs within a single PG molecule determine a large number of low-affinity binding sites, turning PGs into virtual reservoirs of their ligands. In particular, HSPGs bind several growth factors, morphogens, and extracellular proteases as well as some key modulators of lipoprotein metabolism such as lipases and apolipoproteins. Interestingly, the particular distribution of sulfate groups along the liver HSPGs confers increased affinity for apolipoprotein E (apoE) compared to HSPGs expressed in the vascular bed.

Among the PG superfamily, HSPGs are the predominant proteoglycan class found in the liver, accounting for more than two-thirds of total tissue GAG content, followed by chondroitin/dermatan sulfate PGs; no hepatic keratan sulfate PGs have been reported thus far. The relative GAG content in the liver is altered (significantly increased in chondroitin sulfate PGs) under different physiological and pathological conditions, such as hepatic regeneration,[5] cholestasis,[6] liver cirrhosis,[7] and HCC.[8] These changes in hepatic PG composition may have substantial pathogenic implications for liver diseases, because it can affect the normal interaction between several growth factors (e.g., hepatocyte growth factor, transforming growth factor-$\beta$) with their respective liver cell surface receptors, thus modulating hepatocyte proliferation and fibrogenesis.

In addition, the potential role of hepatic HSPGs in lipoprotein metabolism *in vivo* was postulated, based on the pioneering work led by Mahley.[2-4] After establishing that the interaction between apoE and HSPG was important for the uptake of triglyceride-rich lipoproteins by cultured cells,[2,3] a classic study showed that direct infusion of HS-degrading enzymes into the portal circulation of mice dramatically reduced plasma clearance and hepatic uptake apoE-enriched lipoproteins,[4] suggesting a functional role for cell surface–associated HSPGs, either directly as lipoprotein receptors or indirectly by a bridging interaction with the low-density lipoprotein receptor (LDLR)-related protein. From a clinical perspective, supporting evidence was also provided by the analysis of HSPG binding activities of dominant variants of apoE that cause human type III hyperlipoproteinemia.[9]

By targeting genes encoding different HS-processing enzymes, it has been possible to gain significant insights

about the biological functions of HSPGs *in vivo*.[10] Germline disruption of the Ndst1 (GlcNAc *N*-deacetylase/*N*-sulfotransferase) gene, which encodes an enzyme isoform that plays a key role in the maturation of HS, leads to a dramatic decrease in *N*-sulfated HSPGs in all tissues, resulting in embryonic and perinatal death. To overcome lethality, liver-specific Ndst1 null mice generated by MacArthur et al.[1] are an extraordinary tool to establish more definitively the importance of hepatic HSPGs in lipoprotein physiology. These mice exhibited a 2.5-fold increase in plasma total triglyceride levels accumulated in remnant lipoproteins under chow diet as well as significantly higher plasma cholesterol levels transported in LDL particles when crossbred to LDLR-deficient mice. This work indicates that hepatic HSPGs are indeed critical for LDLR-independent chylomicron and very low density lipoprotein (VLDL) remnant clearance *in vivo*. This study also reveals that HSPGs may also play a significant role in hepatic LDL uptake when the classic LDL receptor pathway is absent or down-regulated.

Additional studies have begun to address the molecular identity of specific hepatic HSPGs that may account for their effects on lipid metabolism. As a sinusoidal transmembrane HSPG facing the plasma compartment, syndecan-1 is a very attractive candidate to be involved in lipoprotein physiology. Indeed, syndecan-1 mediates clathrin-independent endocytic uptake of lipoprotein lipase-enriched LDL[11] and apoE-rich and triglyceride-rich VLDL[12] in cultured cells. In addition, inhibition of syndecan-1 expression decreases binding of remnant lipoproteins in HepG2 cells.[13] To further assess the role of liver syndecan-1 *in vivo*, we overexpressed this HSPG by adenoviral gene transfer in mice,[14] which exhibited a significant hyperlipidemic response due to accumulation of all plasma lipoprotein classes that were also enriched in apoE. We postulated that excessive hepatic overexpression of syndecan-1 may lead to abnormal sequestration of lipoproteins within the space of Disse, precluding their physical interaction and clearance through sinusoidal receptors of the LDLR family.[14] Whether other HSPGs (e.g., perlecan) and additional PG classes (e.g., decorin) are also critical for hepatic lipoprotein uptake and plasma lipid level regulation *in vivo* remains an open question.

The study by MacArthur et al.[1] may have important implications for pathophysiology and therapy of human primary and secondary dyslipidemias. Interestingly, animal models of insulin resistance and diabetes exhibit altered expression of hepatic PGs (lower HSPG/chondroitin sulfate PG ratio) as well as reduced HSPG levels associated with decreased sulfation of GAG chains.[15,16] Whether these findings are also present and pathogenically relevant in patients with diabetes and metabolic syndrome remains to be established. If HSPGs indeed are critical players in controlling lipoprotein metabolism, polymorphism analysis of genes encoding HSPG protein cores and/or GAG biosynthetic enzymes may define novel markers of predisposition to dyslipidemias or response to lipid-lowering therapy. On the other hand, nuclear farnesoid X receptor agonism transcriptionally stimulates the expression of syndecan-1 in cultured cells, including cultured hepatocytes.[17] It would be interesting to evaluate if the hypotriglyceridemic effect induced by these farnesoid X receptor compounds in rodents is indeed dependent on increased expression of hepatic HSPGs. If so, these glycoconjugated protein macromolecules may represent novel targets for development of lipid-lowering drugs.

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## Treatment for Cirrhosis-Associated Hyponatremia? Vaptans and Aquaresis

*Schrier RW, Gross P, Gheorghiade M, Berl T, Verbalis JG, Czerwiec FS, Orlandi C, and SALT Investigators.* Tolvaptan, a selective oral vasopressin V2-receptor antagonist, for hyponatremia. N Engl J Med. 2006;355:2099-2112. (Reprinted with permission.)

## Abstract

**BACKGROUND: Hyponatremia (serum sodium concentration, <135 mmol per liter) is a predictor of death among patients with chronic heart failure and cirrhosis. At present, therapy for acute and chronic hyponatremia is often ineffective and poorly tolerated. We investigated whether tolvaptan, an orally active vasopressin V(2)-receptor antagonist that promotes aquaresis—excretion of electrolyte-free water—might be of benefit in hyponatremia. METHODS: In two multicenter, randomized, double-blind, placebo-controlled trials, the efficacy of tolvaptan was evaluated in patients with euvolemic or hypervolemic hyponatremia. Patients were randomly assigned to oral placebo (223 patients) or oral tolvaptan (225) at a dose of 15 mg daily. The dose of tolvaptan was increased to 30 mg daily and then to 60 mg daily, if necessary, on the basis of serum sodium concentrations. The two primary end points for all patients were the change in the average daily area under the curve for the serum sodium concentration from baseline to day 4 and the change from baseline to day 30. RESULTS: Serum sodium concentrations increased more in the tolvaptan group than in the placebo group during the first 4 days (P<0.001) and after the full 30 days of therapy (P<0.001). The condition of patients with mild or marked hyponatremia improved (P<0.001 for all comparisons). During the week after discontinuation of tolvaptan on day 30, hyponatremia recurred. Side effects associated with tolvaptan included increased thirst, dry mouth, and increased urination. A planned analysis that combined the two trials showed significant improvement from baseline to day 30 in the tolvaptan group according to scores on the Mental Component of the Medical Outcomes Study 12-item Short-Form General Health Survey. CONCLUSIONS: In patients with euvolemic or hypervolemic hyponatremia, tolvaptan, an oral vasopressin V2-receptor antagonist, was effective in increasing serum sodium concentrations at day 4 and day 30. (ClinicalTrials.gov numbers, NCT00072683 [ClinicalTrials.gov] [SALT-1] and NCT00201994 [ClinicalTrials.gov] [SALT-2].).**

## Comment

Hyponatremia is the most frequent metabolic disorder encountered in hospitalized patients. In chronic liver disease, hyponatremia (serum sodium <135 mmol/l) is seen in 49% of hospitalized patients with cirrhosis and is associated with an increased rate of encephalopathy, development of spontaneous bacterial peritonitis, onset of the hepatorenal syndrome, as well as worsening of ascites.[1] Hyponatremia in cirrhosis adds predictive value to the MELD score and is thus a significant predictor of decreased patient survival and the need for liver transplant.[2] The pathogenesis of cirrhosis-associated hyponatremia is secondary to increase renal water retention due to a reduced aquaresis (solute-free water clearance) although it should be recognized that not all patients with decompensated cirrhosis have abnormal free water clearance. Thus, these patients have increased total body water as well as increased total body sodium despite the hyponatremia; this condition is called dilutional hyponatremia.[3]

Vasopressin release in cirrhosis is the most important factor mediating a reduction in aquaresis via $V_2$ receptors on the collecting ducts of the renal nephron.[4] Vasopressin binding to $V_2$ receptors results in insertion of aquaporin-2 water channels into the collecting ducts. The net result is retention of free water. Treatment of significant hyponatremia in cirrhosis has always been difficult. Traditional approaches include cessation of diuretics in combination with water restriction.[5] However, such approaches rarely work for any length of time. Additional approaches include the use of hypertonic saline if the serum sodium is extremely low and potentially life threatening.[5] It should also be noted that too rapid an increase in serum sodium in these situations is potentially hazardous, because of the complication central pontine myelinolysis.

Because there is a lack of specific and efficient treatment of hyponatremia, oral $V_2$ receptor vasopressin antagonists (vaptans) have been developed to induce an aquaresis.[4] A recent report by Schrier et al. describes 2 identically designed clinical trials (SALT 1 and SALT 2) in which a vasopressin $V_2$ receptor antagonist, tolvaptan, was used to treat hyponatremia in a randomized, double-blinded, placebo-controlled study of 448 patients.[6] The trials were performed predominantly on outpatients with serum sodium levels <130 mmol/l on enrollment. Fluid restriction was not mandatory, and patients were allowed to continue concurrent diuretic use. Patients with cirrho-