Published in final edited form as:

Hepatology. 2013 August ; 58(2): 477–479. doi:10.1002/hep.26507.

RBP4 and fatty liver: a direct link?

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Retinol-binding protein 4 (RBP4) is a 21-kDa protein that facilitates the transport of retinol through the circulation to peripheral tissues. The principal source of RBP4 in the body is the liver. Hepatocytes are the primary producers of RBP4; they use the protein to contribute to total-body retinol homeostasis by complexing it with dietary retinol and secreting the retinol-RBP4 holoprotein into the circulation.¹ Although hepatocytes are clearly a dominant source of RBP4, cells from other tissues also synthesize the protein. An important secondary source is the adipocyte. Under lean conditions, adipocytes express about one-fifth as much RBP4 mRNA as a hepatocyte;² in obesity, this expression increases substantially.³

In 2005, a unique role was proposed for RBP4 in the pathogenesis of insulin resistance. This came about as a result of genetic manipulations of the glucose transporter GLUT4 in adipocytes.⁴ When GLUT4 was deleted in adipocytes, RBP4 was markedly up-regulated coincident with the development of insulin resistance; conversely, when GLUT4 was overexpressed in adipocytes, RBP4 was suppressed and insulin sensitivity was improved. A specific connection between RBP4 and insulin resistance was demonstrated by generating RBP4 transgenic mice or infusing wild-type mice with recombinant RBP4. Both manipulations provoked insulin resistance, which prompted the designation of RBP4 as an “adipokine” contributing to the metabolic syndrome.

Subsequent studies in humans revealed that RBP4 levels are elevated in the plasma of subjects with insulin resistance and that RBP4 correlates positively with disease severity.^{3, 5} In normal individuals, plasma RBP4 averages about 20 µg/mL; in patients with insulin resistance, RBP4 ranges from 40–90 µg/mL. Importantly, in some studies elevated plasma RBP4 has been linked to the presence of hepatic steatosis.^{6–8} Efforts to use RBP4 as a biomarker of fatty liver disease, have been less successful.^{9–12}

Whether RBP4 can actually stimulate hepatic steatosis, and if so, whether the effect is liver-autonomous, has not been clearly established. Initial studies in mice showed that exogenous RBP4 could induce hepatic expression of gluconeogenic enzymes,⁴ but it was not determined whether this represented a direct effect of the protein on hepatocytes. In the current issue of *HEPATOLOGY*, Xia and colleagues¹³ shed new light on the impact of RBP4 on hepatocyte metabolism. They demonstrate that RBP4 directly stimulates lipogenesis in hepatocytes, by stimulating a series of intracellular events that begins with up-regulation of peroxisome proliferator activated receptor-γ coactivator 1-β (PGC1β) (Figure 1).

Xia et al. began their experiments by treating HepG2 cells with RBP4 in a dose range spanning the plasma levels observed in normal and insulin-resistant humans (20–80 µg/mL). RBP4 stimulated lipogenesis in HepG2 cells in a dose-dependent fashion; this was accompanied by a comparable dose-dependent increase in cellular triglyceride. Similar

results were obtained in primary hepatocytes, although the magnitude of the response to RBP4 was less robust in primary cells than HepG2 cells. Interestingly, RBP4 stimulated lipogenesis in liver cells whether or not the protein was bound to retinol; thus, the apoprotein is the active moiety. Delving further into the mechanism by which RBP4 stimulates hepatic lipogenesis, Xia and colleagues demonstrated that RBP4 stimulated the expression and nuclear translocation of the lipogenic transcription factor sterol regulatory element binding protein-1 (SREBP1). RBP4 also induced the expression of genes downstream of SREBP1, including fatty acid synthase (FAS), acetyl CoA carboxylase-1 (ACC1) and diacylglycerol transferase-2 (DGAT2). The effect of RBP4 was seemingly specific to SREBP1, as it had no effect on the expression of genes regulated by SREBP2.

In the liver, SREBP1 expression is positively influenced by the transcriptional co-activator PGC1 β .¹⁴ Xia and colleagues tested the effect of RBP4 on PGC1 β expression, and found it up-regulated PGC1 β in a dose-dependent fashion. RBP4-mediated induction of PGC1 β was rapid; it occurred as early as 3 h, well before the increases in SREBP1 and lipogenic genes at 24 h. RBP4-mediated induction of PGC1 β was dependent upon CREB (cAMP response element-binding protein), a phosphoprotein involved in the regulation of many metabolic genes.¹⁵ How RBP4 promotes CREB phosphorylation remains unresolved.

To confirm that a link between RBP4 and lipogenesis also exists in the liver in vivo, Xia et al. treated mice with purified RBP4 for 14 days. They achieved circulating levels of RBP4 that were 2.3 times that of a normal mouse, which presumably corresponded to the levels observed in insulin-resistant humans. RBP4 treatment induced the expression of PGC1 β and SREBP1 in the liver; it also promoted the nuclear translocation of SREBP1 and stimulated lipogenic gene expression. The end result of these events was a doubling of hepatic triglyceride content. To determine whether PGC1 β played a pivotal role in RBP4-mediated lipogenesis in vivo, the authors repeated their RBP4 infusion experiment in PGC1 β -deficient mice. In the absence of PGC1 β , RBP4 had virtually no effect on SREBP1 or any of its downstream targets and failed to stimulate hepatic lipid accumulation.

Although other groups have shown that diet-induced hepatic steatosis can be prevented¹⁶ or improved¹⁷ by reducing circulating RBP4, the work of Xia and colleagues is unique in that it demonstrates a direct lipogenic effect of RBP4 that is completely independent of diet. Still, the experiments reported by Xia et al leave several questions unanswered. For example, how does RBP4 interact with hepatocytes to signal lipogenesis? What is the link between RBP4 and CREB phosphorylation? Also, if retinol is not required for RBP4 to stimulate hepatocyte lipogenesis, is the effect mediated simply by RBP4 binding to a hepatocyte surface protein? It was previously noted that RBP4 synthesized outside the liver cannot be internalized by hepatocytes.¹⁸ Is it possible that “exogenous” RBP4, such as that synthesized by adipocytes in insulin-resistant states, binds hepatocytes in a fashion different from that meant for retinol transport?¹⁹ If RBP4 molecules from different sources are ultimately found to exert unique effects on hepatocytes, it may solve the mystery why hepatocyte-derived RBP4 does not stimulate lipogenesis in an autocrine fashion.

If excess RBP4 promotes hepatic lipogenesis in culture and in vivo, as shown experimentally by Xia and colleagues, the data must be reconciled with clinical observations that do not consistently show a positive correlation between plasma RBP4 and hepatic steatosis.^{6–12} One explanation is that in at-risk patients, RBP4 is but one of many stimuli that influence hepatic lipogenesis. Another possibility is that hepatocyte injury from fatty liver disease impairs hepatic synthesis of RBP4;^{12, 20} this could hamper the distinction of patients with steatohepatitis from normals. Full insight into the association between RBP4 and fatty liver will require further study in both animals and humans. In regard to the latter, a clinical trial is currently underway to determine the effect of RBP4 suppression on insulin

resistance (ClinicalTrials.gov; NCT00546455). If this study yields a hepatic outcome, it should help establish the specific role of RBP4 among the many factors that control fat metabolism in the hepatocyte.

Acknowledgments

Grant support: R01 DK068450 (JJM)

P30 DK026743 (UCSF Liver Center)

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Figure 1.

Proposed scheme of RBP4-mediated hepatic lipogenesis, based on data from Xia et al.¹³ The information in the shaded box illustrates the classical role of RBP4 in vitamin A homeostasis. In this scheme, dietary vitamin A complexes with hepatocyte-derived RBP4, and the complex is released into the circulation for delivery to tissues. Apo-RBP4 then undergoes reuptake by hepatocytes. Below the shaded box is a second pathway illustrating a novel function for RBP4 as proposed by Xia et al. In this scenario, “exogenous” RBP4 (produced by extrahepatic tissues such as fat) induces CREB phosphorylation in hepatocytes by an as-yet-undetermined mechanism. CREB induces PGC1 β , which in turn up-regulates SREBP1 and leads to induction of several lipogenic genes. The end result of these events is an increase in triglyceride within hepatocytes.