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Retinol-binding protein-4 and nonalcoholic fatty liver disease

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is becoming increasingly common as the global economy grows and living standards improve. Timely and effective preventions and treatments for NAFLD are urgently needed. Retinol-binding protein-4 (RBP4), the protein that transports retinol through the circulation, was found to be positively related to diabetes, obesity, cardiovascular disease, and other metabolic diseases. Observational studies on the association between serum RBP4 level and the prevalence of NAFLD found contradictory results. Some of the underlying mechanisms responsible for this association have been revealed, and the possible clinical implications of treating NAFLD by targeting RBP4 have been demonstrated. Future studies should focus on the predictive value of RBP4 on NAFLD development and its potential as a therapeutic target in NAFLD.

Keywords: Nonalcoholic fatty liver disease; Retinol-binding protein-4; Metabolic disease

Introduction

Nonalcoholic fatty liver disease (NAFLD) is a potentially serious chronic liver disease that affects nearly 25% of adults worldwide.[1] NAFLD is closely related to a series of intra- and extra-hepatic diseases, including hepatocellular carcinoma, colorectal carcinoma, cardiovascular disease, diabetes, obesity, and other diseases.[2] Compared with healthy controls, NAFLD patients were 1.33 times more likely to have coronary heart disease (CHD), and the CHD prevalence increased in parallel with the severity of hepatic steatosis.[3] In recent years, the prevalence of NAFLD-associated hepatocellular carcinoma has shown an increasing trend in many countries. The proportion of hepatocellular carcinoma attributed to NAFLD tripled from 3.8% in 2001–2005 to 12.2% in 2006–2010 in Korea.[4] Similarly, this proportion increased from 2.6% in 1995–1999 to 19.5% in 2010–2014 in France.[5] Therefore, NAFLD poses a substantial burden on global health resources and the economy. However, unlike other highly prevalent diseases, NAFLD has received little attention.[6] The pathogenesis of NAFLD remains to be elucidated, and curative treatment remains to be explored.

Retinol-binding protein-4 (RBP4) is a member of the lipocalin family, with a molecular weight of ~21 kDa.[7] The ligands of members of this family are small and include hydrophobic molecules such as retinol. In the circulation, RBP4 is the specific carrier of retinol, which is responsible for delivering retinol from the storage sites to the target tissues.[8] Since its first isolation from the human serum in 1968, RBP4 has been isolated from other species, such as fish and birds.[9] RBP4 is predominantly expressed in the liver, followed by the adipose tissue.[10] During adipogenesis, the expression and secretion of RBP4 markedly increase; RBP4 is mainly expressed in mature adipocytes in the adipose tissue.[11]

Retinol (vitamin A) can be obtained in the form of retinyl esters and carotenoids from plant-based and animal-based food products, respectively.[12] This fat-soluble vitamin plays vital roles in vision, immunity, embryonic development, and other physiological processes.[13-15] Due to the hydrophobicity of retinol, proteins that solubilize this vitamin in different compartments of the body have evolved.[16] In the circulation, RBP4 is the specific transporter of retinol and hence can be divided into retinol-bound RBP4 (holo-RBP4) and retinol-free RBP4 (apo-RBP4). Retinol is mainly stored in the liver and transported to extrahepatic organs by binding to RBP4.[8]

In the target tissues, retinol can be taken up by the binding of RBP4 to cell membrane receptors. Stimulated-by-retinoic acid-6 (STRA6) is the specific receptor of RBP4, and it mediates the influx of retinol from the circulation to the target cells.[16] Holo-RBP4 triggers the phosphorylation of STRA6 and then activates Janus kinase-2 (JAK2) and signal transducer-and-activator of transcription

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(STAT)-3/5.[17] STRA6 does not directly deliver retinol from extracellular RBP4 to the cytosol but to the cellular retinol-binding protein-1, which then transports retinol to the related metabolic enzymes.[18] STRA6 is expressed in the retinal pigment of the epithelial cells of the eye, pancreas, adipose tissue, spleen, and brain,[19,20] whereas the expression level of STRA6 in the liver is undetectable.[17] Intriguingly, basic studies involving STRA6-knockout mice showed that STRA6 is not essential for retinol homeostasis in tissues, except for the eye.[21]

Free RBP4 can be easily filtered in the glomeruli due to its small molecular weight.[22] Transthyretin (TTR), a thyroid hormone carrier, binds RBP4 in a 1:1 ratio to prevent RBP4 from being filtered freely in the glomeruli.[23] In addition, holo-RBP4 is kept from binding to STRA6 by TTR, and holo-RBP4 shows a similar binding affinity to STRA6 and TTR.[24,25] It has been reported that serum RBP4 is increased in patients and animal models with insulin resistance, in which the serum RBP4 level exceeds TTR and has the possibility of binding and activating STRA6.[26] Interest has increased regarding the role of RBP4-induced STRA6 activation in the pathogenesis of insulin resistance.

The role of RBP4 in the development of insulin resistance and obesity has received much attention.[27-34] Numerous observational studies have also explored the association of serum RBP4 with NAFLD risks but have reported contradictory results.[35-43] Part of the underlying mechanisms responsible for this association has been revealed,[26,44-47] and the possible clinical implications of treating NAFLD by targeting RBP4 have been demonstrated.[47-51] In this review, we summarize the relationship between RBP4 and NAFLD, aiming to provide new strategies for the early prevention of and interventions in NAFLD.

Observational Studies Exploring the Relationship Between RBP4 and NAFLD

In 2005, Yang *et al*[26] first reported that elevated serum RBP4 levels were associated with insulin resistance, and genetic overexpression or pharmacological injection of RBP4 significantly induced insulin resistance and hepatic gluconeogenesis. Given that insulin resistance plays a critical role in the pathogenesis of NAFLD, many subsequent studies have explored the clinical association of serum RBP4 levels with NAFLD but have reached inconsistent conclusions [Table 1].

It was first reported in 2008 by a Chinese study that in diabetic patients, serum RBP4 levels in the third tertile were associated with an increased risk of NAFLD compared with those with RBP4 levels in the first tertile (OR: 9.897, 95% CI: 2.281–42.936; $P < 0.001$).[35] Another study conducted in Korea also revealed that in nondiabetic adults, the serum RBP4 level was robustly higher in NAFLD patients than that in controls, and serum RBP4 level had a significant association with NAFLD risks (OR: 1.065, 95% CI: 1.020–1.113; $P = 0.004$).[36] A prospective study conducted in China further supported a causal relationship of RBP4 with NAFLD. The researchers found that baseline levels of serum RBP4 were independent predictors of incident NAFLD (OR: 2.01, 95% CI: 1.33–3.04; $P = 0.003$) and NAFLD regression (OR: 0.52, 95% CI: 0.34–0.80; $P < 0.001$).[37]

However, conflicting results were observed when investigating whether serum RBP4 level was associated with histological changes in NAFLD. A study conducted in 49 NAFLD patients who were diagnosed by liver biopsy reported that there was no significant difference in serum RBP4 levels between patients with simple steatosis and steatohepatitis, and no significant correlation was found between RBP4 and NAFLD activity score (NAS).[38] Two other Greek studies also reported that serum RBP4 level was not associated with the degree of hepatic steatosis or fibrosis.[39,40]

The conflicting relationship between serum RBP4 level and NAFLD was also observed in children and adolescents. In a Turkish study, obese children with NAFLD showed more than two-fold-higher serum levels of RBP4 than obese children without NAFLD.[41] Another study conducted in China reported that children with elevated serum RBP4 levels exhibited higher risks of developing NAFLD than controls (OR: 1.116, 95% CI: 1.001–1.245; $P = 0.048$).[42] Converse conclusions were drawn from an Italian study, which observed that serum RBP4 level was negatively correlated with NAS in pediatric NAFLD patients ($r = -0.86$, $P < 0.001$).[43]

These contradictory findings may result from the heterogeneity of fatty liver detection methods and race, as well as the limited sampling size. The studies observing no significant association or inverse association between RBP4 level and NAFLD all diagnosed fatty liver by biopsy.[38-40] Liver biopsy is the gold standard for the diagnosis of NAFLD. It is worth noting that none of these studies found a significant correlation between serum RBP4 level and body mass index, waist circumference, and fasting plasma glucose or insulin levels, although the correlation has been confirmed in a large body of studies.[52-57] In addition, studies reporting that serum RBP4 was an independent risk factor of NAFLD were all conducted in Asia[35-37,42], whereas the other studies drawing negative conclusions were conducted in Western countries.[38-40,43] The limited sampling size was also the source of the heterogeneity. Among the aforementioned studies, all but three included approximately 50 participants.[38,40,43] Therefore, further large-scale and well-designed clinical studies are needed to clarify the association of serum RBP4 level with the presence and severity of NAFLD.

Possible Mechanisms Linking RBP4 with NAFLD

The precise mechanisms for the association between serum RBP4 level and NAFLD remain unclear. However, several hypotheses have been proposed as follows [Figure 1].

First, RBP4 may induce hepatic steatosis by enhancing lipogenesis. RBP4 could upregulate the expression of peroxisome proliferator-activated receptor-c coactivator-1β in a cyclic adenosine monophosphate-response element binding protein-dependent pathway, leading to the activation of sterol-regulatory element binding

Table 1: Studies investigating the relationship of circulating RBP4 with NAFLD.

Reference	Country	Study design	Subjects and number	Fatty liver detection methods	RBP4 assay	Comparison of circulating RBP4 levels between groups	Effect size for health outcome
Seo *et al*[36]	Korea	Cross-sectional	Group 1: 73 nondiabetic NAFLD; Group 2: 86 nondiabetic adults without NAFLD	Ultrasonography	ELISA	Group 1 *vs*. Group 2: 62.8 ± 16.0 mg/L *vs*. 51.7 ± 14.6 mg/L ($P < 0.05$)	An increase of serum RBP4 per unit was related to the risk of NAFLD ascertained by ultrasonography (OR: 1.065, 95% CI: 1.020–1.113)
Wu *et al*[35]	China	Case–control	Group 1: 52 T2DM with NAFLD; Group 2: 50 age- and gender-matched T2DM without NAFLD	Ultrasonography	RIA	Group 1 *vs*. Group 2: 41.3 ± 9.8 μg/mL *vs*. 32.0 ± 8.9 μg/mL ($P < 0.05$)	Subjects with serum RBP4 in the third tertial had a 9.897-fold risk of NAFLD compared with those having values in the first tertial (OR: 9.897, 95% CI: 2.281–42.936)
Alkhouri *et al*[38]	USA	Cross-sectional	49 biopsy-proven NAFLD, mean BMI 32.3 ± 5.0 kg/m^2	Liver biopsy	ELISA	NAFL *vs*. NASH: 26.8 ± 3.6 mg/L *vs*. 21.3 ± 2.1 mg/L ($P > 0.05$); Cirrhosis *vs*. noncirrhosis: 14.1 ± 11.1 mg/L *vs*. 27.9 ± 13.6 mg/L ($P < 0.05$)	Correlation of serum RBP4 with NAS was nonsignificant
Nobili *et al*[43]	Italy	Cross-sectional	59 biopsy-proven pediatric NAFLD	Liver biopsy	ELISA	NAFL *vs*. NASH: 3.8 mg/dL *vs*. 1.9 mg/dL ($P < 0.05$)	Correlation coefficient of serum RBP4 with NAS was −0.86
Schina *et al*[39]	Greece	Case–control	Group 1: 30 biopsy-proven NAFLD without T2DM; Group 2: 30 age- and gender-matched controls	Liver biopsy	ELISA	Group 1 *vs*. Group 2: 25.2 (20.7–27.4) μg/mL *vs*. 34.7 (27–43.6) μg/mL ($P < 0.05$)	Correlation of grade of hepatic steatosis and fibrosis and NAS with liver immunohistochemical RBP4 score was significant but was nonsignificant with serum RBP4
Boyraz *et al*[41]	Turkey	Case–control	Group 1: 63 obese NAFLD; Group 2: 85 obese non-NAFLD	Ultrasonography	ELISA	Group 1 *vs*. Group 2: 33.2 ± 7.5 μg/mL *vs*. 13.9 ± 7.0 μg/mL ($P < 0.05$)	–
Huang and Yang[42]	China	Cross-sectional	Group 1: 46 NAFLD; Group 2: 173 non-NAFLD	Ultrasonography	ELISA	Group 1 *vs*. Group 2: 26.6 ± 5.9 mg/L *vs*. 22.6 ± 5.3 mg/L ($P < 0.05$)	Serum RBP4 was positively associated with the risks of NAFLD (OR: 1.116, 95% CI: 1.001–1.245)
Polyzos *et al*[40]	Greece	Cross-sectional	Group 1: 14 biopsy-proven NASH; Group 2: 15 biopsy-proven nonalcoholic fatty liver; Group 3: 25 controls	Liver biopsy	ELISA	Group 1 *vs*. Group 2 *vs*. Group 3: 8.3 ± 1.9 ng/mL *vs*. 13.9 ± 2.7 ng/mL *vs*. 15.9 ± 2.2 ng/mL (all $P > 0.05$)	Serum RBP4 was not significantly correlated with NAS
Cai *et al*[81]	China	Cross-sectional	Group 1: 51 postmenopausal women with NAFLD; Group 2: 19 postmenopausal women without NAFLD; Group 3: 41 premenopausal women with NAFLD; Group 4: 42 premenopausal women without NAFLD	Ultrasonography	ELISA	Group 1 *vs*. Group 2 *vs*. Group 3 *vs*. Group 4: 30.99 (23.40–40.32) *vs*. 21.30 (17.62–25.08) *vs*. 26.32 (21.30–36.85) *vs*. 18.18 (14.61–22.43) (all $P < 0.05$, except for Group 2 *vs*. Group 3 and Group 3 *vs*. Group 4	–
Wang *et al*[37]	China	Prospective, 3.09-year follow-up	2945 Chinese adults aged 40–75 years were divided into two groups according to baseline status of NAFLD Group 1: 1318 non-NAFLD at baseline, of whom, 410 developed incident NAFLD; Group 2: 1382 NAFLD at baseline, of whom, 339 regressed to non-NAFLD	Ultrasonography	ELISA	In Group 1: incident NAFLD *vs*. non-NAFLD: 36.50 ± 5.96 μg/mL *vs*. 34.73 ± 6.67 μg/mL ($P < 0.05$); in Group 2: sustained NAFLD *vs*. regressed NAFLD: 38.33 ± 6.45 μg/mL *vs*. 35.58 ± 6.47 μg/mL ($P < 0.05$)	In Group 1, subjects with basal serum RBP4 in the fourth quartile had a 2.01-fold risk of incident NAFLD compared with those having values in the first quartile (OR: 2.01, 95% CI: 1.33–3.04); in Group 2, basal RBP4 was inversely related to NAFLD regression (Q4 *vs*. Q1, OR: 0.52, 95% CI: 0.34–0.80)

BMI: Body mass index; CI: Confidence interval; ELISA: Enzyme-linked immunoassay; NAFL: Nonalcoholic fatty liver; NAFLD: Nonalcoholic fatty liver disease; NAS: NAFLD activity score; NASH: Nonalcoholic steatohepatitis; OR: Odds ratio; Q1: The first quartile; Q4: The fourth quartile; RIA: Radioimmunoassay; RBP4: Retinol-binding protein-4; T2DM: Type-2 diabetes mellitus; –: Not applicable.

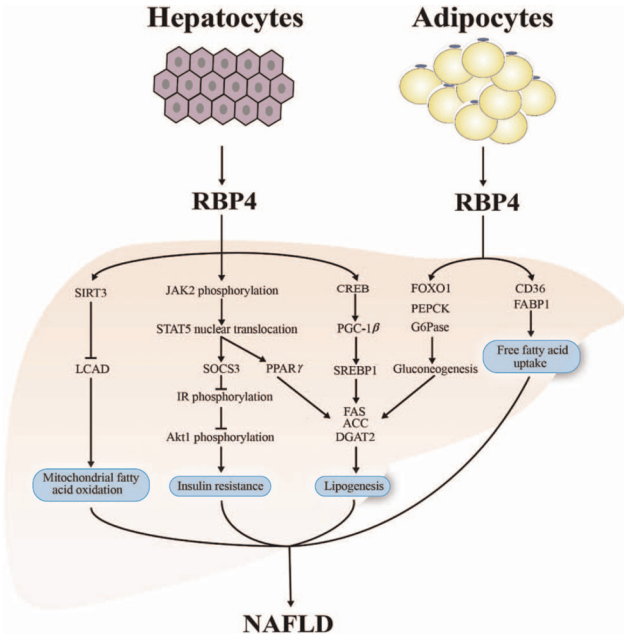

Figure 1: Potential mechanisms of RBP4 and NAFLD. ACC: Acetyl-CoA carboxylase; CD36: Cluster of differentiation-36; cAMP: Cyclic adenosine monophosphate; CREB: cAMP-response element binding protein; DGAT2: Diacylglycerol *O*-acyltransferase-2; FABP1: Fatty acid binding protein-1; FAS: Fatty acid synthase; FOXO1: Forkhead box protein-O1; G6Pase: Glucose-6-phosphatase; IR: Insulin receptor; JAK2: Janus kinase-2; LCAD: Long-chain acyl coenzyme A dehydrogenase; NAFLD: Nonalcoholic fatty liver disease; PEPCK: Phosphoenolpyruvate carboxykinase; PGC-1β: Peroxisome proliferator-activated receptor-c coactivator-1β; PPARγ: Peroxisome proliferator-activated receptor γ; RBP4: Retinol-binding protein-4; SIRT3: Sirtuin-3; SOCS3: Suppressor of cytokine signaling-3; SREBP1: Sterol-regulatory element binding protein-1; STAT5: Signal transducer and activator of transcription-5.

protein-1c (SREBP1c) and its downstream targets fatty acid synthase (FAS) and acetyl coenzyme A carboxylase-1 in hepatocytes.[44] Overexpression of RBP4 in adipocytes also caused hepatic steatosis. However, there were nonsignificant changes in hepatic mRNA levels of SREBP1c, FAS, ACC, stearoyl-coenzyme A desaturase-1, apolipoprotein B, and microsomal triglyceride transfer protein.[45] These results suggested that the underlying mechanism was neither an increase in hepatic *de novo* lipogenesis nor a decrease in very-low-density lipoprotein secretion. Intriguingly, the gene expression of cluster of differentiation-36 and fatty acid binding protein-1, which are involved in the hepatic uptake of fatty acids, was enhanced in RBP4-overexpressing adipocytes. In addition, overexpression of RBP4 in adipocytes and pharmacological injection of RBP4 upregulated the hepatic mRNA levels of forkhead transcription factor-O1 (FOXO1), phosphoenolpyruvate carboxykinase, and glucose-6-phosphatase, resulting in enhanced liver gluconeogenesis.[26,45]

Second, RBP4 may exaggerate hepatic steatosis by inhibiting mitochondrial fatty acid β-oxidation. Both hepatic *Rbp4* mRNA and protein levels were increased in NAFLD patients and mouse models.[46,47] Systematic overexpression of RBP4 caused obesity, impaired insulin sensitivity, and aggravated hepatic lipid deposition. Previous studies have validated the deleterious effects of mitochondrial protein hyperacetylation on mitochondrial function.[58] For instance, hyperacetylation of long-chain acyl coenzyme A dehydrogenase (LCAD), a key enzyme in mitochondrial fatty acid oxidation, led to a decrease in its activity. Sirtuin-3 (SIRT3) is a mitochondrial sirtuin responsible for modulating mitochondrial protein deacetylation.[59] RBP4 overexpression decreased hepatic mitochondrial content and destroyed its function, as evidenced by reduced adenosine triphosphate generation and downregulated expression of genes involved in mitochondrial β-oxidation.[46] These effects were initiated by RBP4-stimulated suppression of hepatic mitochondrial SIRT3, followed by impaired activity of LCAD. In addition, exposure to RBP4 caused mitochondrial dysfunction in endothelial cells, marked by reduced mitochondrial integrity and impaired mitochondrial fusion and fission dynamics.[60]

Third, RBP4 may aggravate insulin resistance, a pivotal component in the pathogenesis of NAFLD. Inverse correlation between serum RBP4 level and insulin resistance was observed in studies involving different populations, including subjects with newly diagnosed hypertension,[55] healthy but obese elderly individuals,[56] postmenopausal women with or without newly diagnosed type-2 diabetes mellitus (T2DM),[57] women with polycystic ovary syndrome,[61] nondiabetic women with or without obesity,[62] and nondiabetic and nonobese individuals.[63] In these studies, insulin resistance was assessed by homeostasis model assessment of insulin resistance (HOMA-IR) [56,64-66] or euglycemic–hyperinsulinemic clamp.[61-63] An elevated serum RBP4 level was also observed in patients and mouse models with insulin resistance.[26] Further, experiments demonstrated that both genetic overexpression and exogenous injection of RBP4 impaired insulin signaling by suppressing the insulin-induced phosphorylation of the insulin receptor (IR) and phosphatidylinositol-3-kinase.[26] Treatment of hepatocytes with RBP4 caused excessive triglyceride accumulation, mediated by escalating hepatic insulin sensitivity.[17] In cultured hepatocytes, holo-RBP4 bound to its receptor STRA6 and then phosphorylated JAK2, which in turn promoted STAT5 translocation to the nucleus. Activated STAT5 thereafter upregulated the expression of its targets, namely, suppressor of cytokine signaling-3 and peroxisome proliferator-activated receptor γ. RBP4 impaired insulin sensitivity in hepatocytes, marked by decreased insulin-induced phosphorylation of IR and its downstream effector protein kinase B (Akt1). Both baseline and insulin-induced transportations of glucose transporter-4 to the plasma membrane were also inhibited. However, these findings were not observed when injecting mice with RBP4 due to undetectable levels of hepatic STRA6 *in vivo*.

Fourth, RBP4 may cause inflammation in different cell types. Hepatic exposure to inflammation is a critical driving force for the progression of NAFLD, fueling the transition from simple steatosis to steatohepatitis.[67] In both microvascular and macrovascular endothelial cells, holo-RBP4 induced the expression of the proinflammatory factors interleukin (IL)-6 and monocyte chemoattractant protein-1 (MCP-1) and the adherent molecules E-selectin, intercellular adhesion molecule-1, and vascular cell

adhesion molecule-1 through nuclear factor-κB-dependent mechanisms.[68] In macrophages, RBP4 increased the expression and secretion of MCP-1, IL-6, and tumor necrosis factor-α (TNF-α) in a toll-like receptor (TLR)-4- and c-Jun N-terminal kinase-dependent pathway.[33] Similar effects were observed in adipocytes in which RBP4 triggered TNF-α and IL-1β expression and release by activating the TLR4/myeloid differentiation factor (MD2) complex and TLR2 and the downstream toll/IL-1 receptor-domain-containing adapter-inducing interferon-β (TRIF) and myeloid differentiation primary response-88 and priming the nucleotide oligomerization domain-like receptor family pyrin domain containing-3 inflammasome.[34] Moreover, systematic overexpression of RBP4 exacerbated high-fat-diet-induced increases in hepatic mRNA levels of TNF-α, MCP-1, IL-1β, and IL-6.[46]

Despite intensive studies, four major unresolved issues need to be clarified. First, is RBP4 essential for binding retinol and activating STRA6 to induce hepatic steatosis? Holo-RBP4 and apo-RBP4 were as potent as stimulating proinflammatory cytokine release in macrophages and adherent molecule secretion in endothelial cells.[33,34,68] Similar effects of the two forms of RBP4 on triggering hepatic *de novo* lipogenesis were observed in hepatocytes.[45] Interestingly, STRA6 was expressed at lower than the detectable level in primary human and mouse macrophages.[33] It is well established that STRA6 on the cell membrane is activated by the holo-RBP4 complex rather than apo-RBP4,[69] which may explain why apo-RBP4 could also exert its impact in STRA6-null macrophages.[33] Similarly, STRA6 was undetectable in the liver.[17] Injection of RBP4 did not significantly block insulin signaling in the liver of C57BL/6 mice,[17] and liver-specific RBP4-overexpressing mice did not show significant changes in whole-body insulin resistance.[70] However, other studies observed that genetic or pharmacologic overexpression of RBP4 aggravated hepatic steatosis.[17,45,46] On the basis of these findings, we speculated that RBP4 may exacerbate liver insulin resistance and steatosis by different molecular mechanisms, dependent and independent of retinol and STRA6, respectively.

Second, the changes in hepatic RBP4 expression levels in NAFLD models remain controversial. C57BL/6J mice fed high-fat and high-cholesterol diets for 12 weeks or 20 weeks and Leptin^{ob} mutant mice showed decreased hepatic *Rbp4* mRNA and RBP4 protein levels.[71] However, other researchers observed that biopsy-proven NAFLD patients and apolipoprotein E-knockout mice fed high-fat and high-cholesterol diets for 16 weeks displayed vastly increased expression levels of hepatic RBP4.[46] Third, the relationship between RBP4 and insulin resistance is under debate. As mentioned above, many studies have confirmed the positive correlation of serum RBP4 levels with systematic insulin resistance in different populations.[55-57,61-63] However, other studies have drawn opposite conclusions.[64-66,72-74] Remarkably, in the studies that did not establish this correlation,[64-66,72-74] insulin resistance was assessed by HOMA-IR rather than using euglycemic–hyperinsulinemic clamp, which is the gold standard to evaluate insulin resistance. Moreover, the role of RBP4 in impairing insulin signaling has been verified in adipose tissues,[34] livers, and skeletal muscles,[17,26] the three predominant insulin-responsive tissues. Although experimental studies have demonstrated that pharmacological treatment with RBP4[44] and systematic or adipocyte-specific overexpression of RBP4 promoted the development of hepatic steatosis, the role of specifically overexpressed RBP4 in hepatocytes needs to be explored in the future.[45,46]

Clinical Implications of RBP4 in the Treatment of NAFLD

Several clinical trials have found that diet management, exercise, and bariatric surgery-induced weight loss could decrease serum RBP4 levels and ameliorate NAFLD, adiposity, and T2DM.[75-80] Intriguingly, the variation in RBP4 levels was positively associated with the improvement of hepatic fat content, lipid profiles,[75,76] adiposity, and insulin resistance.[77-80] These results provided a rationale for the role of serum RBP4 in predicting the treatment response to weight loss and insulin sensitization interventions.

In addition, the role of RBP4-targeting agents in the treatment of NAFLD has been explored in recent years. The combination of RBP4 with TTR prevents the free filtration of RBP4 in the glomeruli. Fenretinide, however, dissociates RBP4 from TTR, promoting the renal excretion of RBP4 and lowering the circulating levels of RBP4.[26] Intraperitoneal injection of fenretinide ameliorated the development of NAFLD by promoting hepatic fatty acid oxidation and improved whole-body insulin resistance and glucose intolerance.[48] Feeding mice with fenretinide for a long period of time also alleviated diet-induced obesity and hepatic steatosis.[49] Furthermore, fenretinide was used in clinical trials to explore its effects on insulin resistance in premenopausal women. Women taking fenretinide 200 mg/day for 2 years were seven times more likely to have improved insulin resistance than those who took a placebo.[50]

Fenretinide was effective in lowering circulating RBP4 but showed no effect on hepatic RBP4 expression.[48] Inhibiting *RBP4* gene expression in adipocytes with RNA oligonucleotides not only reduced serum RBP4 levels but also downregulated the levels of RBP4 protein and *Rbp4* mRNA in the liver. In addition, an anti-RBP4 RNA oligonucleotide attenuated hepatic steatosis and improved liver function, hyperglycemia, and hyperinsulinemia.[51] In addition, overexpression of RBP4 in adipocytes induced hepatic steatosis, which was rescued by orally bioavailable RBP4 antagonists.[47] For future studies, the effects of RBP-targeting agents on ameliorating NAFLD need to be assessed in clinical trials.

Conclusions

In this review, we discussed the association between RBP4 and NAFLD from three aspects. First, clinical observations found that circulating RBP4 levels were closely associated with NAFLD risk, but discrepancies still exist. Second, basic studies have verified that RBP4 is involved in the pathogenesis of NAFLD by inducing hepatic *de novo* lipogenesis, impairing fatty acid oxidation, exaggerating

insulin resistance, and promoting inflammation. Third, agents aimed at lowering circulating RBP4 levels and downregulating hepatic RBP4 expressions exerted protective effects against NAFLD. These findings raise the possibility of targeting RBP4 as a novel marker and a potential therapeutic target for NAFLD. A series of further studies exploring the role of RBP4 will provide more evidence for the prevention and treatment of NAFLD.

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Conflicts of interest

None.

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