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 and 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. 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. 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. 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. Similarly, this proportion increased from 2.6% in 1995–1999 to 19.5% in 2010–2014 in France. Therefore, NAFLD poses a substantial burden on global health resources and the economy. However, unlike other highly prevalent diseases, NAFLD has received little attention. 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. RBP4 is the specific carrier of retinol, which is responsible for delivering retinol from the storage sites to the target tissues. Since its first isolation from the human serum in 1968, RBP4 has been isolated from other species, such as fish and birds. RBP4 is predominantly expressed in the liver, followed by the adipose tissue. During adipogenesis, the expression and secretion of RBP4 markedly increase; RBP4 is mainly expressed in mature adipocytes in the adipose tissue.

Retinol (vitamin A) can be obtained in the form of retinyl esters and carotenoids from plant-based and animal-based food products, respectively. This fat-soluble vitamin plays vital roles in vision, immunity, embryonic development, and other physiological processes. Due to the hydrophobicity of retinol, proteins that solubilize this vitamin in different compartments of the body have evolved. 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.

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. 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. 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. STRA6 is expressed in the retinal pigment of the epithelial cells of the eye, pancreas, adipose tissue, spleen, and brain, whereas the expression level of STRA6 in the liver is undetectable. Intriguingly, basic studies involving STRA6-knockout mice showed that STRA6 is not essential for retinol homeostasis in tissues, except for the eye.

Free RBP4 can be easily filtered in the glomeruli due to its small molecular weight. Transthyretin (TTR), a thyroid hormone carrier, binds RBP4 in a 1:1 ratio to prevent RBP4 from being filtered freely in the glomeruli. In addition, holo-RBP4 is kept from binding to STRA6 by TTR, and holo-RBP4 shows a similar binding affinity to STRA6 and TTR. 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. 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. Numerous observational studies have also explored the association of serum RBP4 with NAFLD risks but have reported contradictory results. Part of the underlying mechanisms responsible for this association has been revealed, and the possible clinical implications of treating NAFLD by targeting RBP4 have been demonstrated. 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. 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). 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). 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).

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). Two other Greek studies also reported that serum RBP4 level was not associated with the degree of hepatic steatosis or fibrosis. 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. 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). 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).

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. 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. In addition, studies reporting that serum RBP4 was an independent risk factor of NAFLD were all conducted in Asia, whereas the other studies drawing negative conclusions were conducted in Western countries. The limited sampling size was also the source of the heterogeneity. Among the aforementioned studies, all but three included approximately 50 participants. 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.
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[34]   | Korea     | Cross-sectional | Group 1: 73 nondiabetic NAFLD; Group 2: 86 nondiabetic adults without NAFLD          | Ultrasoundography             | 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 was associated with the risk of NAFLD (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 | Ultrasoundography             | 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[36] | USA     | Cross-sectional | 49 biopsy-proven NAFLD, mean BMI 32.3 ± 5.0 kg/m² | 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[41] | 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 |
| China 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 (27.4–43.6) 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[40] | Turkey   | Case-control  | Group 1: 63 obese NAFLD; Group 2: 85 obese non-NAFLD                                 | Ultrasoundography             | ELISA      | Group 1 vs. Group 2: 33.2 ± 7.5 mg/L vs. 13.9 ± 5.0 mg/L (P < 0.05)                                                  | – |
| Huang and Yang[42] | China  | Cross-sectional | Group 1: 46 NAFLD; Group 2: 173 non-NAFLD                                            | Ultrasoundography             | 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[43] | Greece | Cross-sectional | Group 1: 14 biopsy-proven NASH; Group 2: 13 biopsy-proven nonalcoholic fatty liver; Group 3: 25 controls | Liver biopsy                | ELISA      | Group 1 vs. Group 2 vs. Group 3: 8.3 ± 1.9 mg/mL vs. 13.9 ± 2.7 mg/ml vs. 15.9 ± 2.2 mg/mL (all P < 0.05) | Serum RBP4 was not significantly correlated with NAS |
| Cai et al[44]   | 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 | Ultrasoundography             | 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) | – |
| Wang et al[45]  | China     | Prospective, 3-5-year follow-up | 2945 Chinese adults aged 40–75 years were divided into two groups according to baseline status of NAFLD | Ultrasoundography             | ELISA      | 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 immunosassay; 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.
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. Siruin-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,\(^{[53]}\) 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 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.\(^{[27]}\) 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. 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. 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 in and priming the nucleotide oligomerization domain-like receptor-domain-containing adapter-inducing interferon-

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. Similar effects of the two forms of RBP4 on triggering hepatic de novo lipogenesis were observed in hepatocytes. Interestingly, STRA6 was expressed at lower than the detectable level in primary human and mouse macrophages. It is well established that STRA6 on the cell membrane is activated by the holo-RBP4 complex rather than apo-RBP4, which may explain why apo-RBP4 could also exert its impact in STRA6-null macrophages. Similarly, STRA6 was undetectable in the liver. Injection of RBP4 did not significantly block insulin signaling in the liver of C57BL/6 mice, and liver-specific RBP4-overexpressing mice did not show significant changes in whole-body insulin resistance. However, other studies observed that genetic or pharmacologic overexpression of RBP4 aggravated hepatic steatosis. On the basis of these findings, we speculate 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-β mutant mice showed decreased hepatic Rbp4 mRNA and RBP4 protein levels. 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. 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 systemic insulin resistance in different populations. However, other studies have drawn opposite conclusions. Remarkably, in the studies that did not establish this correlation, 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, livers, and skeletal muscles. The three predominant insulin-responsive tissues. Although experimental studies have demonstrated that pharmacological treatment with RBP4 and systemic 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 future studies.

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. Intriguingly, the variation in RBP4 levels was positively associated with the improvement of hepatic fat content, lipid profiles, adiposity, and insulin resistance. 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. Intraperitoneal injection of fenretinide ameliorated the development of NAFLD by promoting hepatic fatty acid oxidation and improved whole-body insulin resistance and glucose intolerance. Feeding mice with fenretinide for a long period of time also alleviated diet-induced obesity and hepatic steatosis. 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.

Fenretinide was effective in lowering circulating RBP4 but showed no effect on hepatic RBP4 expression. 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. In addition, overexpression of RBP4 in adipocytes induced hepatic steatosis, which was rescued by orally bioavailable RBP4 antagonists. 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.

References

1. Younossi ZM. Non-alcoholic fatty liver disease - A global public health perspective. J Hepatol 2018;70:351–354. doi: 10.1016/j.jhep.2018.10.063.

2. Targher G, Tilg H, Byrne CD. Non-alcoholic fatty liver disease: a multistep disease requiring a multidisciplinary and holistic approach. Lancet Gastroenterol Hepatol 2021;6:578–588. doi: 10.1016/S2468-1253(21)00020-0.

3. Cho EJ, Kwack MS, Jang ES, You SJ, Lee JH, Kim YJ, et al. Relative etiologico-temporal role of prior hepatitis B virus infection and nonalcoholic fatty liver disease in the development of non-B non-C hepatocellular carcinoma in a hepatitis B-endemic area. Digestion 2011;84 (Suppl 1):17–22. doi: 10.1159/0003333210.

4. Lazarus JV, Mark HE, Anstee QM, Wisse E, Arab JP, Batterham RL, Castera L, et al. Advancing the global public health agenda for NAFLD: a consensus statement. Nat Rev Gastroenterol Hepatol 2021;19:60–72. doi: 10.1038/s41575-020-0369-0.

5. Colantuoni V, Romano V, Bensi G, Santoro C, Costanzo F, Raugei G, et al. Cloning and sequencing of a full-length cDNA coding for human retinol-binding protein. Nucleic Acids Res 1983;11:7769–7776. doi: 10.1093/nar/11.22.7769.

6. O’Byrne SM, Blaner WS. Retinol and retinyl esters: biochemistry and physiology. J Lipid Res 2013;54:1731–1743. doi: 10.1194/jlr.R037648.

7. Oda Y, Kanazawa K, Fujita N, et al. Serum retinol-binding protein is more highly expressed in visceral adipose tissue than in subcutaneous adipose tissue and is a marker of intra-abdominal fat mass. Cell Metab 2007;6:79–87. doi: 10.1016/j.cmet.2007.06.002.

8. Lee JW, Im JA, Lee HR, Shim JY, Youn BS, Lee DC. Visceral adiposity is associated with serum retinol binding protein-4 levels in healthy women. Obesity 2007;15:2225–2232. doi: 10.1038/oby.2007.264.

9. Kim YL, Kim TK, Cheong ES, Shin DG, Choi GS, Jung J, et al. Relation of absolute or relative adiposity to insulin resistance, retinol binding protein-4, leptin, and adiponectin in type 2 diabetes. Diabetes Metab J 2012;36:415–421. doi: 10.4093/dmj.2012.36.6.415.
32. Zemany I, Kraus BJ, Norsen J, Saito T, Peroni OD, Johnson RL, et al. Downregulation of STRA6 in adipocytes and adipose stromal-vascular fraction from obesity-associated effects of adipose-specific STRA6 knockout in vivo. Mol Cell Biol 2014;34:1170–1186. doi: 10.1128/mcb.01106-13.

33. Norsen J, Hosooka T, Hammarstedt A, Yore MM, Kant S, Aryal P, et al. Retinol-binding protein 4 inhibits insulin signaling in adipocytes by inducing proinflammatory cytokines in macrophages through a c-Jun N-terminal kinase- and toll-like receptor 4-dependent and retinol-independent mechanism. Mol Cell Biol 2012;32:2010–2019. doi: 10.1128/mcb.01913-11.

34. Moraes-Vieira PM, Yore MM, Sontheimer-Phelps A, Castoldi A, Norseen J, Aryal P, et al. Retinol binding protein 4 primes the NLRP3 inflammasome by signaling through Toll-like receptors 2 and 4. Proc Natl Acad Sci U S A 2020;117:31309–31314. doi: 10.1073/pnas.2003771117.

35. Wu H, Jia W, Bao Y, Lu J, Zhu J, Wang R, et al. Serum retinol binding protein 4 and nonalcoholic fatty liver disease in patients with metabolic syndrome children with nonalcoholic fatty liver disease. Chin Med Pract 2008;79:185–190. doi: 10.11690/diabetes.2007.08.016.

36. Seo JA, Kim NH, Park SY, Kim HY, Ryu OH, Lee KW, et al. Serum retinol-binding protein 4 levels are elevated in non-alcoholic fatty liver disease. Clin Endocrinol 2008;68:555–560. doi: 10.1111/j.1365-2265.2007.03072.x.

37. Wang X, Chen X, Zhang H, Pang J, Lin J, Xu X, et al. Circulating retinol-binding protein 4 is associated with the development and regulation of non-alcoholic fatty liver disease. Biomed Res Int 2019;2019:119–128. doi: 10.1186/s12896-019.04.009.

38. Alkhouri N, Lopez R, Berk M, Feldstein AE. Serum retinol-binding protein 4 levels in patients with nonalcoholic fatty liver disease. J Clin Gastroenterol 2009;43:985–989. doi: 10.1097/MCG.0b013e3181a998ad.

39. Schina M, Koskinas J, Timiakos D, Hadziyannis E, Savvas S, Karamanos B, et al. Circulating and liver tissue levels of retinol-binding protein-4 in non-alcoholic fatty liver disease. Hepatology Res 2009;39:972–978. doi: 10.1111/j.1740-0939.2009.00354.x.

40. Polyzos SA, Kountouras J, Polymerou V, Papadimitriou KG, Zavos C, Katsinelos P. Vaspin, resistin, retinol-binding protein-4, interleukin-1 and interleukin-6 in patients with nonalcoholic fatty liver disease. J Clin Endocrinol Metab 2016;101:705–714. doi: 10.1210/jc.2015-3486.

41. Boyraz M, Cekmez F, Karaoglu A, Cinaz P, Durak M, Bideci A. Retinol-binding protein 4 inhibits insulin signaling in adipocytes by inducing proinflammatory cytokines in macrophages through a c-Jun N-terminal kinase- and toll-like receptor 4-dependent and retinol-independent mechanism. Mol Cell Biol 2012;32:2010–2019. doi: 10.1128/mcb.01913-11.

42. Huang SC, Yang YJ. Serum retinol-binding protein 4 is elevated and positively associated with insulin resistance in polycystic ovary syndrome. J Endocrinol Invest 2008;31:950–955. doi: 10.1007/s12020-007-0429-7.

43. Nobili V, Alkhouri N, Altisi A, Ottino S, Lopez R, Manco M, et al. Retinol-binding protein 4: a promising circulating marker of liver damage in lean and obese women with normal glucose metabolism in lean and obese women with normal glucose metabolism. J Clin Endocrinol Metab 2009;94:231–244. doi: 10.1210/jc.2008-0077.

44. Xia M, Liu Y, Guo H, Wang D, Wang Y, Ling W. Retinol binding protein 4 primes the NLRP3 inflammasome by signaling through Toll-like receptors 2 and 4. Proc Natl Acad Sci U S A 2020;117:31309–31314. doi: 10.1073/pnas.2003771117.

45. Schina M, Koskinas J, Timiakos D, Hadziyannis E, Savvas S, Karamanos B, et al. Circulating and liver tissue levels of retinol-binding protein-4 in non-alcoholic fatty liver disease. Hepatology Res 2009;39:972–978. doi: 10.1111/j.1740-0939.2009.00354.x.

46. Polyzos SA, Kountouras J, Polymerou V, Papadimitriou KG, Zavos C, Katsinelos P. Vaspin, resistin, retinol-binding protein-4, interleukin-1 and interleukin-6 in patients with nonalcoholic fatty liver disease. J Clin Endocrinol Metab 2016;101:705–714. doi: 10.1210/jc.2015-3486.

47. Boyraz M, Cekmez F, Karaoglu A, Cinaz P, Durak M, Bideci A. Retinol-binding protein 4 inhibits insulin signaling in adipocytes by inducing proinflammatory cytokines in macrophages through a c-Jun N-terminal kinase- and toll-like receptor 4-dependent and retinol-independent mechanism. Mol Cell Biol 2012;32:2010–2019. doi: 10.1128/mcb.01913-11.

48. Huang SC, Yang YJ. Serum retinol-binding protein 4 is independently associated with pediatric NAFLD and fasting triglyceride levels. J Pediatr Gastroenterol Nutr 2015;61:145–150. doi: 10.1097/MPG.0b013e31827222ae.

49. Nobili V, Alkhouri N, Altisi A, Ottino S, Lopez R, Manco M, et al. Retinol-binding protein 4: a promising circulating marker of liver damage in lean and obese women with normal glucose metabolism in lean and obese women with normal glucose metabolism. J Clin Endocrinol Metab 2009;94:231–244. doi: 10.1210/jc.2008-0077. 66. Al-Daghri NM, Al-Attas OS, Alokail M, Draz HM, Bamakhramah A, Sabico S. Retinol binding protein-4 is associated with TNF-alpha and IL-6 levels in patients with type 2 diabetes mellitus. Diabetes Care 2006;29:2457–2461. doi: 10.2337/dcj06-0360.

50. Cho YM, Youn BS, Lee H, Lee N, Min SS, Kwak SH, et al. Plasma retinol-binding protein-4 concentration is elevated in human subjects with impaired glucose tolerance and type 2 diabetes. Diabetes Care 2006;29:2457–2461. doi: 10.2337/dcj06-0360.

51. Ram J, Snehalatha C, Selvam S, Nandiratha A, Shetty AS, Godland IB, et al. Retinol-binding protein-4 inhibits insulin signaling in adipocytes by inducing proinflammatory cytokines in macrophages through a c-Jun N-terminal kinase- and toll-like receptor 4-dependent and retinol-independent mechanism. Mol Cell Biol 2012;32:2010–2019. doi: 10.1128/mcb.01913-11.
67. Buzzetti E, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). Metabolism 2016;65:1038–1048. doi: 10.1016/j.metabol.2015.12.012.

68. Farjo KM, Farjo RA, Halsey S, Moiseyev G, Ma JX. Retinol-binding protein 4 induces inflammation in human endothelial cells by an NADPH oxidase- and nuclear factor kappa B-dependent and retinol-independent mechanism. Mol Cell Biol 2012;32:5103–5115. doi: 10.1128/mcb.00820-12.

69. Kawaguchi R, Yu J, Honda J, Hu J, Whitelegge J, Ping P, et al. A membrane receptor for retinol binding protein mediates cellular uptake of vitamin A. Science 2007;315:820–825. doi: 10.1126/science.1136244.

70. Fedders R, Muenzner M, Weber P, Sommerfeld M, Knauer M, Kedziora S, et al. Liver-secreted RBP4 does not impair glucose homeostasis in mice. J Biol Chem 2018;293:15269–15276. doi: 10.1074/jbc.RA118.004294.

71. Saeed A, Bartuzi P, Heegsma J, Dekker D, Kloosterhuis N, de Bruin A, et al. Impaired hepatic vitamin A metabolism in NAFLD mice leading to vitamin A accumulation in hepatocytes. Cell Mol Gastroenterol Hepatol 2021;11:309–325. doi: 10.1016/j.ccm.2020.07.006.

72. Raila J, Henze A, Spranger J, Möhlig M, Pfeiffer AFH, Schweigert FJ. Microalbuminuria is a major determinant of elevated plasma retinol-binding protein 4 in type 2 diabetic patients. Kidney Int 2007;72:505–511. doi: 10.1038/sj.ki.5002372.

73. Ulgen F, Herder C, Kühn MC, Willenberg HS, Schott M, Scherbaum WA, et al. Association of serum levels of retinol-binding protein 4 with male sex but not with insulin resistance in obese patients. J Physiol Biochem 2010;116:57–62. doi: 10.3109/13813451003631421.

74. Wang YS, Ye J, Yang X, Zhang GP, Cao YH, Zhang R, et al. Association of retinol binding protein-4, cystatin C, homocysteine and high-sensitivity C-reactive protein levels in patients with newly diagnosed type 2 diabetes mellitus. Arch Med Sci 2018;15:1203–1216. doi: 10.5114/ams.2018.79565.

75. Numao S, Sasai H, Nomata Y, Matsu T, Tsujimoto T, et al. Effects of exercise training on circulating retinol-binding protein 4 and cardiovascular disease risk factors in obese men. Obes Facts 2012;5:845–855. doi: 10.1159/000346205.

76. Broch M, Gómez JM, August MT, Vilarroca N, Pastor R, Elio I, et al. Association of retinol-binding protein-4 (RBP4) with lipid parameters in obese women. Obes Surg 2010;20:1258–1264. doi: 10.1007/s11695-010-0200-5.

77. Haider DG, Schindler K, Prager G, Bohdjalian A, Luger A, Wolzt M, et al. Serum retinol-binding protein 4 is reduced after weight loss in morbidly obese subjects. J Clin Endocrinol Metab 2006;92:1168–1171. doi: 10.1210/jc.2006-1839.

78. Gómez-Ambrosi J, Rodríguez A, Catalán V, Ramírez B, Silva C, Rotellar F, et al. Serum retinol-binding protein 4 is not increased in obesity or obesity-associated type 2 diabetes mellitus, but is reduced after relevant reductions in body fat following gastric bypass. Clin Endocrinol 2007;69:208–215. doi: 10.1111/j.1365-2265.2007.03156.x.

79. Tscheroner A, Sturm W, Engl J, Kaser S, Laimer M, Laimer F, et al. Retinol-binding protein-4, visceral fat, and the metabolic syndrome: effects of weight loss. Obesity 2008;16:2439–2944. doi: 10.1038/oby.2008.391.

80. Lim S, Choi SH, Jeong IK, Kim JH, Moon MK, Park KS, et al. Insulin-sensitizing effects of exercise on adiponectin and retinol-binding protein-4 concentrations in young and middle-aged women. J Clin Endocrinol Metab 2008;93:2263–2268. doi: 10.1210/jc.2007-2028.

81. Cai H, Lu S, Chen Y, MrccogSDM, Niu Z, Zhuo G, et al. Serum retinol binding protein 4 and galectin-3 binding protein as novel markers for postmenopausal nonalcoholic fatty liver disease. Clin Biochem 2018;56:95–101. doi: 10.1016/j.clinbiochem.2018.04.017.

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