Interplay of retinol binding protein 4 with obesity and associated chronic alterations (Review)

YACCIL ADILENE FLORES-CORTEZ[1], MARTHA I. BARRAGÁN-BONILLA[1], JUAN M. MENDOZA-BELLO[1], CECILIA GONZÁLEZ-CALIXTO[2], EUGENIA FLORES-ALFARO[3] and MÓNICA ESPINOZA-ROJO[1]

[1]Laboratory of Molecular Biology and Genomic, Faculty of Biological Chemical Sciences, Autonomous University of Guerrero, Chilpancingo, Guerrero 39087; [2]Faculty of Nursing, Autonomous University of Guerrero, Acapulco, Guerrero 39610; [3]Laboratory of Clinical and Molecular Epidemiology, Faculty of Biological and Chemical Sciences, Autonomous University of Guerrero, Chilpancingo, Guerrero 39087, Mexico

Received January 20, 2022; Accepted April 26, 2022

DOI: 10.3892/mmr.2022.12760

Abstract. Obesity is a multifactorial disease, defined as excessive fat deposition in adipose tissue. Adipose tissue is responsible for the production and secretion of numerous adipokines that induce metabolic disorders. Retinol-binding protein 4 (RBP4) is an adipokine that transports vitamin A or retinol in the blood. High levels of RBP4 are associated with development of metabolic disease, including obesity, insulin resistance (IR), metabolic syndrome, and type 2 diabetes (T2D). The present review summarizes the role of RBP4 in obesity and associated chronic alterations. Excessive synthesis of RBP4 contributes to inflammatory characteristic of obesity by activation of immune cells and release of proinflammatory cytokines, such as TNFα and ILs, via the Toll-like receptor/JNK pathway. The retinol-RBP4 complex inhibits insulin signaling directly in adipocytes by activating Janus kinase 2 (JAK2)/STAT5/suppressor of cytokine signaling 3 signaling. This mechanism is retinol-dependent and requires vitamin A receptor stimulation by retinoic acid 6 (STRA6). In muscle, RBP4 is associated with increased serine 307 phosphorylation of insulin receptor substrate-1, which decreases its affinity to PI3K and promotes IR. In the liver, RBP4 increases hepatic expression of phosphoenolpyruvate carboxykinase, which increases production of glucose. Elevated serum RBP4 levels are associated with β-cell dysfunction in T2D via the STRA6/JAK2/STAT1/insulin gene enhancer protein 1 pathway. By contrast, RBP4 induces endothelial inflammation via the NF-κB/nicotinamide adenine dinucleotide phosphate oxidase pathway independently of retinol and STRA6, which stimulates expression of proinflammatory molecules, such as vascular cell adhesion molecule 1, E-selectin, intercellular adhesion molecule 1, monocyte chemoattractant protein 1 and TNFα. RBP4 promotes oxidative stress by decreasing endothelial mitochondrial function; overall, it may serve as a useful biomarker in the diagnosis of obesity and prognosis of associated disease, as well as a potential therapeutic target for treatment of these diseases.

Correspondence to: Professor Mónica Espinoza-Rojo, Laboratory of Molecular Biology and Genomic, Faculty of Biological Chemical Sciences, Autonomous University of Guerrero, Av. Lázaro Cárdenas S/N, Ciudad Universitaria, Chilpancingo, Guerrero 39087, Mexico
E-mail: monicaespinozarojo2014@gmail.com

Key words: retinol-binding protein 4, obesity, type 2 diabetes, oxidative stress, insulin resistance

Contents

1. Introduction

Obesity is a complex multifactorial disease, defined as excessive fat deposition in adipose tissue, which may be harmful to health. The degree of obesity is measured by body mass index (BMI). BMI is calculated using weight in kilograms divided by height in meters squared. For adults, a BMI of 25.0-29.9 kg/m^2 is defined as overweight and ≥30 kg/m^2 as obese (1). The worldwide prevalence of obesity tripled between 1975 and 2016. The World Health Organization estimated obesity had a global prevalence rate of 13 and 39% of people aged ≥18 years was overweight in 2016 (2). In Mexico, an increase in the prevalence of overweight and obese subjects was reported in different age groups in 2018 compared with 2016, while in adults aged ≥20 years, the prevalence of overweight and obese subjects were 73.0 and 30.5%, respectively (3).

Obesity is considered to be an epidemic worldwide. This condition increases the risk of numerous types of disease, including insulin resistance (IR), type 2 diabetes (T2D) and cardiovascular disease (CVD). Obesity is associated with a low-grade inflammation state where increased production and secretion of inflammatory factors, such as TNFα, IL-1β and IL-6, result in alteration of key steps in the insulin signaling pathway, which leads to development of IR (4). In addition, the high fat content in adipose tissue, which is characterized by increased palmitic acid and lipopolysaccharides concentration, leads to mitochondrial dysfunction, increased reactive oxygen species (ROS) production, inflammation and therefore higher risk of IR (5,6).

Adipose tissue is responsible for production and secretion of numerous adipokines, including leptin, adiponectin and retinol-binding protein 4 (RBP4), which leads to development of metabolic disorder such as IR, T2D, CVD, dyslipidemia and liver steatosis (6,7). RBP4 is expressed in the liver and adipose tissue. In the circulation, RBP4 is responsible for transport of retinol from the liver to peripheral tissue where it is metabolized to retinoic acid (RA). In addition, RBP4 induces secretion of proinflammatory cytokines in macrophages and activates antigen-presenting cells in adipose tissue (6,7). RBP4 is an adipokine that is expressed and secreted in mature adipocytes, as well as in adipocyte and preadipocyte cultures (8). Epidemiological studies have reported an association between high levels of RBP4 and development of metabolic disease, including obesity, IR and T2D in humans (9-12) and animals (13,14), suggesting the role of RBP4 as a potential biomarker of inflammation and oxidative stress (OS). In this context, the current review article analyzed the role of RBP4 in obesity and associated chronic alterations.

2. RBP4

RBP4 is a serum polypeptide of 201 amino acids and a size of 21 kDa. It consists of an N-terminal loop, β-barrel core, α helix and C-terminal loop. The β-barrel core is a structural part that specifically hosts one molecule of retinol (15). By ensuring retention of this hydrophobic metabolite in an aqueous medium, it allows retinol transportation in blood (15). This protein is encoded by the *RBP4* gene located on chromosome 10q23-24 in humans. It is synthesized primarily in the liver and adipose tissue, and to a lesser extent in other organs, such as lungs, kidney, testes, brain and retina (7).

The primary function of RBP4 is transport of retinol, an active metabolite of vitamin A, from the liver to target tissue, such as adipose tissue, the retina, brain, epidermis, kidney, lung, sexual organs and inmune system cells (15). Vitamin A is a fat-soluble compound with key biological activity in maintenance of immune function, maintenance of epithelial cell integrity, physiology of vision, reproduction as well as lipid metabolism. The physiological actions of vitamin A are mediated by its bioactive metabolite, retinoic acid. The vitamin A is found as provitamin A in carotenoids; these include β-carotene obtained from fruit and vegetables. Vitamin A is also found as preformed retinoids in the form of retinyl esters, these compounds include retinol (alcohol), retinal (aldehyde), retinoic acid (irreversibly oxidized form of retinol), which are derived from animal sources (16). Following intestinal absorption, retinyl esters are transported in chylomicrons to the liver for storage. When the body is deficient in vitamin A, retinyl esters are hydrolyzed and released as retinol, which binds to RBP4 and is secreted into the circulation (17).

In the circulation, the retinol-RBP4 complex binds transthyretin (TTR) to form a tertiary retinol-RBP4-TTR complex. Binding to TTR stabilizes the retinol-RBP4 complex, decreases RBP4 loss by renal filtration and allows RBP4 to be recycled following retinol uptake into cells (7,18). Retinol spontaneously dissociates from RBP4 due to its hydrophobic nature. Retinol readily moves into cells by diffusion through the plasma membrane (19). Nevertheless, in certain tissue, such as retina, brain, spleen, kidney, testis and adipose, the retinol-RBP4 complex is recognized by vitamin A receptor stimulated by retinoic acid 6 (STRA6) that transports retinol from binding protein RBP4 into cells (19,20). Intracellularly, phosphorylated STRA6 associates directly with intracellular retinol-binding protein (CRBP1), transferring extracellular retinol molecule from RBP4 to intracellular CRBP1, which prevents retinol dissociation in the aqueous medium of the cytosol. Following binding of CRBP1 to retinol, the CRBP1 dissociates from STRA6 and delivers retinol to a retinol-metabolizing enzyme, such as lecithinretinol acyltransferase (19,21). This catalyzes transformation of retinol to retinol esters, which are stored in the endoplasmic reticulum (22). In the absence of retinol, RBP4 is freely filtered by glomeruli due to its small molecular size and partially reabsorbed in cells of the proximal tubule, allowing its reuse (10).

Due to their chemical stability and hydrophobicity, retinol esters serve an essential role in visual function, both for color vision and adaptation in darkness. In the retina, retinol esters are hydrolyzed, isomerized and oxidized by retinol isomerase and 11-cis-retinol dehydrogenases to form 11-cis-retinal, which binds to opsin protein to form the visual pigment rhodopsin (22,23). The activated rhodopsin breaks down in all-trans-retinal and opsin following exposure to a photon light source. When a photon light source enters the retina it is absorbed by rhodopsin; this energy produces the rupture of 11 cis-retinal, causing its isomerization to its trans form and releasing opsin. This isomerization induces generation of an electric signal in the optic nerve, which is interpreted as vision (24,25).

In addition, retinol induces expression of multiple genes involved in energy homeostasis, insulin response, fatty acid metabolism and the gene that codes for RBP4. To accomplish this, it must be converted to retinaldehyde by the enzyme retinol dehydrogenase 10. Subsequently, retinaldehyde is oxidized to RA by retinaldehyde dehydrogenases 1-3 (26-28). RA activates or suppresses gene transcription via nuclear receptors, namely retinoic acid receptor (RAR)α, RARβ and RARγ (28,29). In the absence of RA, RBP4 secretion is inhibited, which leads to its accumulation in the endoplasmic reticulum until the RA is available (30,31).

3. RBP4 in obesity

In obesity, white adipose tissue secretes large amounts of adipokines, including RBP4, which have local and systemic effects (retinol homeostasis and transport) and contribute to IR (15,32). The association between elevated serum levels of

RBP4 (analyzed by ELISA) and obesity/T2D was reported by Yang *et al* (26) in the Chinese population in obese patients with and without diabetes. However, no significant association was noted between non-diabetic and diabetic groups, suggesting that hyperglycemia was not associated with elevated serum RBP4 levels (33). The association between RBP4 and obesity and associated disease has been investigated in humans and animal and cell culture models (7,9,11,13,18,32-39). In a 10-year follow-up study of 3,445 Chinese school-age children (11-12 years), an association between serum RBP4 levels and adverse cardiovascular risk, metabolic syndrome (MetS), IR, hyperglycemia, hypertension and hyperlipidemia was reported, suggesting that RBP4 may be an early biomarker of MetS (11). Moreover, elevated serum RBP4 concentrations, analyzed by ELISA, were positively associated with BMI, waist-hip index, elevated blood pressure (BP), triglycerides (TG), total cholesterol (TC) (11) low-density lipoprotein (LDL) (11,35), homeostatic model assessment of IR (HOMA-IR) and elevated leptin levels. Furthermore, elevated RBP4 levels were associated with lower levels of high-density lipoprotein (HDL) cholesterol, regardless of sex, age, puberty and time of follow-up (11). Elevated levels of RBP4 in serum are significantly associated with incidence of MetS and levels of very low-density lipoprotein and LDL (35).

Conversely, Korek *et al* (36) published a study of a Polish population, which did not identify significant differences in serum RBP4 concentration levels between obese (BMI: 32.05-39.51 kg/m^2) and lean subjects (BMI: 21.14-24.21 kg/m^2). However, a positive correlation was noted between concentration of RBP4 in serum (analyzed by ELISA) and levels of TG in obese and control groups (36). A study of obese Romanian children reported that following adjustment for age, sex and BMI, blood levels of RBP4 (analyzed by ELISA) were not associated with characteristics of MetS (obesity, IR and hypercholesterolemia) body composition (fat and muscle mass) or anthropometric profile (height, BMI, abdominal circumference, tricipital skin-fold thickness, waist circumference/height ratio) (32). Although controversial results have been published, a correlation between elevated levels of RBP4 in the blood and the incidence of IR, serum lipid levels, and anthropometric parameters has been reported (9,11,32,35-39). Therefore, population studies implementing different dietary plans have been performed to evaluate the association between weight loss and concentration of adipokines, including RBP4 (37,38,40). A study of Spanish women (age, 18-80 years) with obesity who consumed a calorie-restricted diet with 20, 27 or 35% protein for 3 months aimed to evaluate weight loss in these subjects; serum levels of RBP4 (analyzed by ELISA) were decreased by 12.50, 3.56 and 17.50%, respectively. Women following the 35% protein diet exhibited a 30% greater decrease in RBP4 levels than those following the 20% protein diet (following adjustment for weight loss). In addition, RBP4 levels were directly associated with TG concentration. However, the association between serum RBP4 and levels of other adipokines, such as leptin, was not significant (37). Similar results were demonstrated in an intervention study of obese Spanish individuals (BMI $\geq$35 kg/m^2) who were subjected to a very low-calorie diet for 6 months (40). Dietary intervention induced a significant decrease in serum levels of RBP4 and inflammatory markers, such as TNFα and C-reactive protein (CRP), which suggested attenuation of inflammation is associated with obesity (40).

Previous studies have yielded similar results (38-40). A study of American women (age, 18-62 years) with obesity and MetS who followed a diet and physical activity plan for two months to lose weight reported that serum levels of RBP4, analyzed by ELISA, were significantly associated with concentration of TC and TG (38). However, serum RBP4 levels were not associated with changes in body composition (weight, BMI, waist, trunk fat), suggesting that serum RBP4 levels were stable in this study population (38). However, a study of Japanese men (age, 48±2 years) who participated in a physical training program for 3 months demonstrated that obese individuals with physical training without calorie restriction exhibited significantly decreased levels of cardiovascular risk factors, such as TC, TG, LDL cholesterol, RBP4, leptin and IL-6 (39). Regression analysis revealed that change in serum RBP4 levels were associated with change in TG levels. This suggested that physical training contributes to decrease in RBP4 levels, regardless of body mass and caloric intake (39).

Other studies have shown a significant association between elevated serum levels of RBP4 and hypertriglyceridemia (11,36,41). Therefore, the association between RBP4 and TG may be representative of the association between RBP4 and obesity. RBP4 increases expression levels of genes that promote fatty acid synthesis in adipose tissue. This suggests that RBP4 expression is associated with visceral fat accumulation (42). Physiologically, high concentrations of RBP4 increase transport of retinol in the blood, which is catabolized to RA isomers in the liver. These isomers serve as ligands for nuclear receptors, such as RAR and retinoid X receptor (RXR), to regulate transcription of numerous genes, including those associated with glucose and lipid metabolism (37).

Furthermore, RXR forms heterodimers with nuclear receptors, including liver X receptor (LXR), RAR and peroxisome proliferator-activated receptors (PPARs), which serve key roles in signal transduction. LXR is a key modulator of lipid metabolism and inflammatory cell signaling and regulates cholesterol efflux in macrophages. Cholesterol and its metabolites are natural ligands that activate LXR. In addition, activation of stearoyl-coenzyme A desaturase and elongase 6, which are expressed in adipogenic tissue, including hepatic and adipose tissue, has been shown to serve a key role in lipid metabolism. Both enzymes are regulated by sterol regulatory element-binding protein 1 (SREBP1) (43). SREBP1 is a key transcription factor involved in expression of lipogenic genes that is induced by activation of LXR and RXR (43). Increased lipid synthesis is associated with increased RBP4 secretion and synthesis. The latter process occurs in brown adipose tissue, primarily via the RXR/PPAR heterodimer. Specifically, PPARγ and PPARα are involved in this process; these are both activated by cyclic AMP (cAMP) (44).

In addition, excessive synthesis of RBP4 in adipose tissue contributes to the inflammatory process, which is characteristic of obesity, via activation of Toll-like receptor (TLR) 2 and the TLR4/myeloid differentiation factor 2 receptor complex in macrophages (45). TLRs possess an extracellular domain that recognizes pathogen-associated molecular patterns, a transmembrane region and a cytosolic toll/interleukin-1 receptor domain that interacts with adaptor molecules, such as myeloid

differentiation primary response 88 (MyD88) and TNFα receptor. These proteins induce activation of JNK-dependent pathways and NF-κB by phosphorylating inhibitor of NF-κB (IκB) kinase, which phosphorylates IκB for proteasomal degradation (46). Following degradation of IκB, NF-κB translocates to the nucleus and induces expression levels of inflammatory mediators, such as TNFα, monocyte chemotactic protein-1, INF-γ and IL-6, -1β, -2 and -12, that are required for activation of macrophages and dendritic cells (45). In adipocytes, TNFα stimulates lipolysis (47). This is associated with inflammation and serves a causal role in IR as release of free fatty acids increases endogenous glucose production and decreases muscle glucose uptake (48,49). The JNK pathway induces activation of antigen-presenting cells, such as macrophages and dendritic cells, resulting in proliferation of pro-inflammatory cluster of differentiation 4 T cells, which promotes inflammation and systemic IR (Fig. 1A and B) (6).

In addition, activation of TLR causes activation of the IL-1 receptor-associated kinase (IRAK) family (46). The IRAK1-IRAK2-IRAK4 complex binds MyD88, thenIRAK-4phosphorylates to IRAK1 at serine and threonine residues, this event activates TNF-receptor-associated factor 6 (TRAF6), which subsequently activates TGF-β-activated protein kinase 1 (TAK1). TAK1 forms a complex with TGF-β-activated kinase 1/MAP3K7 binding protein 1 (TAB1), TAB2 and TAB3 (46). Moreover, TAK1 activates MAPK3 and MAPK6 to activate an alternative pathway that phosphorylates JNK and p38 (46). In addition, RBP4 induces endothelial inflammation, which is dependent on NF-κB and NADPH oxidase and is independent of retinol and STRA6. This suggests that RBP4 exerts retinol-dependent and -independent effects via activation of STRA6 and different receptors and signaling pathways in different types of cell (50) (Fig. 1C).

4. RBP4 in IR

IR involves excessive secretion of adipokines, including RBP4. RBP4 is considered to be involved in the pathogenesis of obesity and development of IR. The retinol-RBP4 complex directly inhibit insulin signaling in adipocytes by activating Janus kinase 2 (JAK2)/STAT5 signaling, which induces suppressor of cytokine signaling (SOCS) 3 expression (an inhibitor of insulin signaling); this mechanism is retinol-dependent and requires the membrane receptor STRA6 (Fig. 1A) (42,51). In addition, the retinol-RBP4-TTR complex is associated with development of IR in obese patients or subjects with T2D (7,41). In a study of Indian adults of similar age (36-46 years), ELISA was used to analyze serum levels of RBP4 and TTR (52). The subjects were stratified into three groups: Normal glucose tolerance (NGT; n=90), impaired glucose tolerance (IGT; n=70) and T2D (n=90). The mean serum RBP4 and TTR levels were higher in subjects with T2D (RBP4, 13; TTR, 832 μg/ml), followed by subjects with IGT (RBP4, 10.5; TTR, 720.0 μg/ml) and NGT (RBP4, 8.7; TTR, 551.5 μg/ml). RBP4 and TTR exhibited a significant association with T2D regardless of other factors associated with IR, such as age, sex, TG and HDL cholesterol. Serum RBP4 levels exhibited a significant association with IR in all subjects, even in the NGT group. However, TTR was not associated with IR (52).

By contrast, a study of 167 Thai participants (age, 35-66 years) without T2D or any other chronic disease, assessed certain anthropometric parameters such as fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c), fasting insulin and serum lipid levels, considered risk factors for the development of T2D (41). The participants of the aforementioned study were divided into normal (FBG <100 mg/dl) and high T2D risk group (FBG ≥100 mg/dl). The high T2D risk group exhibited increased TTR levels and HOMA-IR values, as well as decreased serum RBP4 levels as determined by ELISA compared with the normal T2D risk group. Significant positive correlations were noted between elevated serum TG levels and RBP4, TTR and HOMA-IR (41). Patients with primary hypertriglyceridemia exhibit increased rates of non-esterified fatty acid turnover and secretion into adipose tissue, supplying excess fatty acids to the liver for TG synthesis (53). This increase suggests that hypertriglyceridemic patients develop IR in adipose tissue. Furthermore, RBP4 and TTR have been hypothesized to play a role in the development of IR (52).

The role of RBP4 in development of IR was analyzed by Yang *et al* (33) in mice with adipose-specific deletion of glucose transporter-4 (adipose-*GLUT4*$^{-/-}$), transgenic mice selectively overexpressing GLUT4 in adipocytes (adipose-GLUT4-Tg), RBP4-overexpressing mice (RBP4-Tg) and mice with deletion of RBP4 (*RBP4*$^{-/-}$). The aforementioned study demonstrated that adipose-*GLUT4*$^{-/-}$ mice developed IR in muscle and liver tissue and exhibited increased levels of RBP4 mRNA and protein in serum, as determined by western blot analysis. By contrast, adipose-GLUT4-Tg mice exhibited decreased RBP4 levels in adipose tissue. Moreover, *RBP4*$^{-/-}$ mice exhibited improved insulin sensitivity, while RBP4-Tg mice developed IR. Considering that RBP4 serves an essential role in the development of IR, insulin signaling was analyzed in muscle and liver tissue of *RBP4*$^{-/-}$ and RBP4-Tg mice; administration of insulin increased PI3K activity in the muscle of control mice, whereas its effect was decreased by 30% in RBP4-Tg mice compared with wild type. By contrast, PI3K activity increased by 80% in muscle tissue from *RBP4*$^{-/-}$ compared with control mice. However, PI3K activity was not altered in the liver of RBP4-Tg mice (33).

In the skeletal muscle of Wistar rats, RBP4 levels decrease insulin-dependent glucose uptake (54). RBP4 is associated with impaired insulin signaling, decreased affinity of insulin receptor substrate (IRS) 1 for PI3K and decreased GLUT4 translocation to the cell membrane, which decreases glucose uptake and increases IR in the muscle (Fig. 1E) (33,55). In adipose tissue, the retinol-RBP4 complex interacts with its membrane receptor STRA6, which activates the JAK2/STAT3/5 pathway, stimulating expression of SOCS3, an inhibitor of insulin signaling. SOCS3 specifically inhibits binding of PI3K to IRS in the PI3K/AKT pathway, leading to IR in adipose tissue (Fig. 1A) (19). In addition, RBP4 increases hepatic expression of phosphoenolpyruvate carboxykinase (PEPCK), a molecule that participates in gluconeogenesis by catalyzing conversion of oxaloacetate to phosphoenolpyruvate, which increases production of glucose in the liver and decreases insulin activity to promote development of hyperglycemia (Fig. 1D) (33,42). Biological processes involving RBP4, such as inflammation in obesity, dyslipidemia, IR and hyperglycemia, are key risk factors for development of T2D and associated disease.

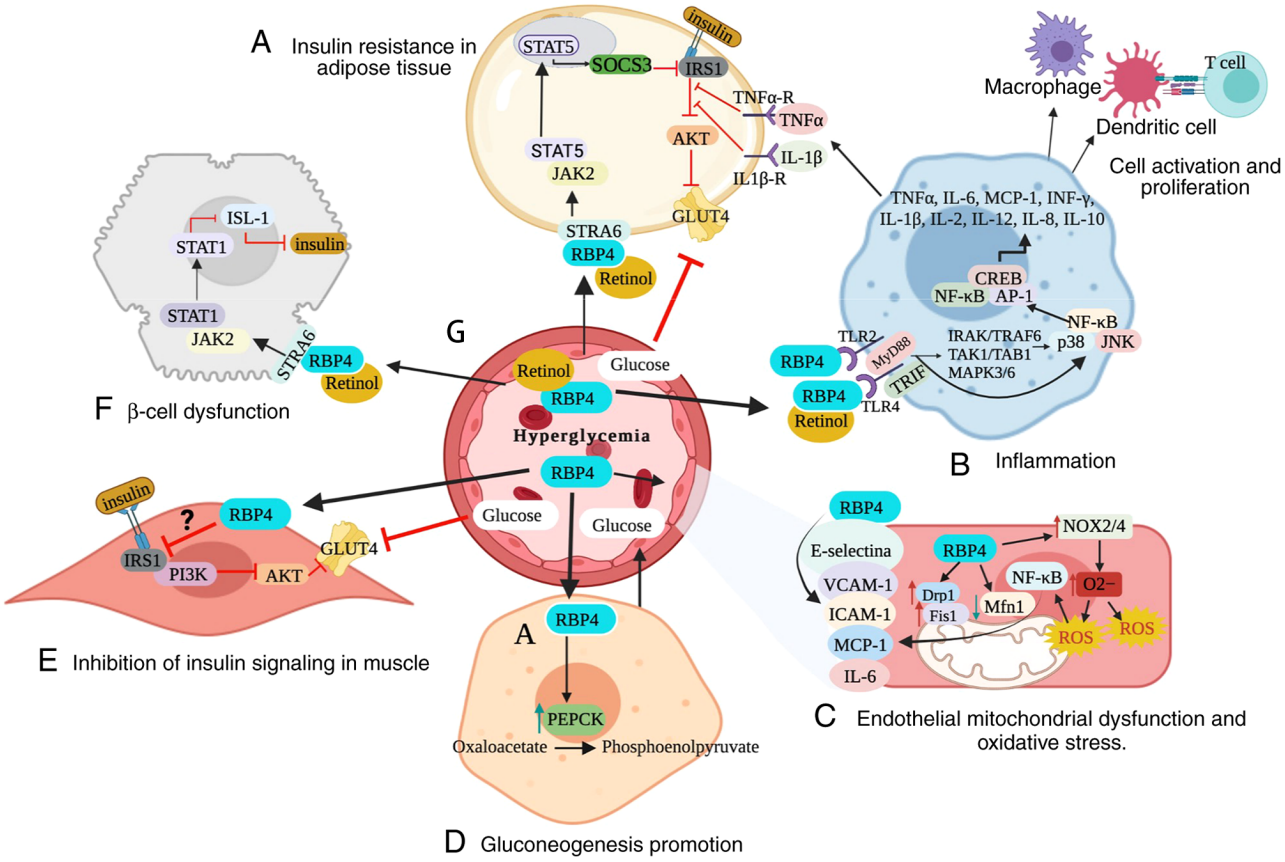

Figure 1. Molecular mechanisms of RBP4 in obesity and associated disease. (A) In adipocytes, the retinol-RBP4 complex directly inhibits insulin signaling by activating the JAK2/STAT5/SOCS3 pathway, leading to IR. (B) In adipose tissue, macrophages, retinol-RBP4 and RBP4 interact with TLR2 and TLR4/MD2 and downstream pathways of MyD88 and TRIF, releasing TNFα, IL-6, MCP-1, INF-γ, IL-1β, IL-2, IL-12, IL-8 and IL-10. This results in activation of the immune system and promotes an inflammatory state as well as inhibition of insulin signaling. (C) RBP4 induces NF-κB and NADPH oxidase-dependent endothelial inflammation, leading to development of OS by mitochondrial dysfunction. (D) RBP4 increases hepatic expression of PEPCK, thus increasing glucose production in the liver. (E) In skeletal muscle, RBP4 is associated with high serine 307 phosphorylation of IRS-1, which decreases its affinity for PI3K and inhibits insulin signaling. (F) Retinol-RBP4 promotes β-cell dysfunction via the JAK2/STAT1/ISL-1 pathway. (G) Aforementioned mechanisms promote development of hyperglycemia. PKB, Protein kinase B (PKB); AP-1, Activator protein 1; Drp1, Dynamin-related protein 1; Fis1, Mitochondrial fission 1 protein; GLUT 4, Glucose transporter type 4; ICAM-1, Intercellular Adhesion Molecule 1; IRS1, Insulin receptor substrate-1; ISL-1, Insulin gene enhancer protein; JAK2, Janus Kinase 2; MCP-1, Monocyte chemotactic protein-1; MCP-1, Monocyte chemotactic protein-1; Mfn1, Mitofusin-1; MyD88, Myeloid differentiation primary response 88; NOX2, NADPH oxidase; PEPCK, Phosphoenolpyruvate carboxykinase; RBP4, Retinol Binding Protein 4; ROS, reactive oxygen species; SOCS3, Suppressor of cytokine signaling 3; TLR, Toll-like receptor; TNFα-R, tumor necrosis factor α receptor; TRIF, TIR-domain-containing adapter-inducing interferon-β; VCAM-1, Vascular cell adhesion protein 1.

5. RBP4 in T2D and its comorbidities, such as retinopathy, nephropathy and CVD

T2D is a chronic metabolic disorder characterized by hyperglycemia, which results from defective insulin action. An imbalance between insulin and insulin sensitivity leads to IR, primarily affecting middle-aged and older adults who have prolonged hyperglycemia due to obesity (56). An association between serum RBP4 levels in obese subjects and IR has been previously demonstrated by factors (obesity, IR, OS, metabolic disorder) that contribute to the development of T2D (9-14). Involvement of RBP4 has been assessed in the pathogenesis of T2D. A prospective population-based study of 1,011 Chinese participants with prediabetes (age, 55.6±7.2 years) demonstrated that serum RBP4 levels (analyzed by ELISA) <31 (HR, 2.01; 95% CI, 1.31-3.09) and >55 μg/ml (HR, 1.94; 95% CI, 1.32-2.93) are associated with increased risk of incidence of T2D independent of adiposity and HOMA-IR, suggesting that serum RBP4 levels may increase the risk of T2D via pathways that do not involve IR (9). For example, higher serum RBP4 levels may be involved in pathogenesis of β cell dysfunction (9).

Huang *et al* (57) reported that increased circulating RBP4 levels are associated with progression of hyperglycemia in db/db mice and inversely associated with insulinogenic index (index that determinates the glucose stimulated insulin secretion). *In vitro*, RBP4 inhibits glucose-stimulated insulin secretion (GSIS) in a dose- and time-dependent manner in primary islets isolated from C57BL/J mice and in a β cell line (INS-1E). RBP4-transgenic overexpressing mice (RBP4-Tg; age, 8 weeks) exhibit a dynamic decrease in GSIS prior to impairment of insulin sensitivity and glucose tolerance. The aforementioned study also showed *in vivo* that islets isolated from RBP4-Tg mice exhibit significantly decreased levels of GSIS (57). In addition, STRA6 was expressed in pancreatic β cells, which mediates the inhibitory effect of RBP4 on insulin synthesis via activation of the JAK2/STAT1/insulin gene enhancer protein-1 (ISL-1) pathway (58). ISL-1 is a transcription factor involved in regulation of insulin synthesis

and maintenance of normal β cell function (57,58). Moreover, decreased circulating RBP4 levels effectively restores β cell function and ameliorate hyperglycemia in db/db mice. These findings indicated that β-cell dysfunction is associated with increased RBP4 circulation levels and hyperglycemia (Fig. 1F) (58).

RBP4 is associated with multiple T2D comorbidities. For example, plasma RBP4 levels analyzed by ELISA are significantly higher in patients with diabetic retinopathy (DR), as well as those with vision-threatening DR (VTDR) compared with those without DR or VTDR (59). A meta-analysis demonstrated an association between elevated RBP4 levels and development of DR in subjects with T2D (60). The mechanisms underlying the RBP4/DR axis may involve an association of plasma RBP4 levels with adverse profile of inflammatory markers and OS, which is associated with progression of DR (59) due to the presence of a high levels of lipids in retinal tissue (61); increased serum RBP4 levels, which induce inflammation in human retinal endothelial cells and RBP4-mediated progression of DR via a unique and independent proinflammatory mechanism that increases retinal levels of IL-18 RNA and protein (60).

By contrast, diabetic nephropathy (DN) is a common and serious microvascular complication of diabetes and one of the leading causes of end-stage renal disease worldwide (62). It has been reported that serum RBP4 levels (analyzed by ELISA) are significantly increased in diabetic patients with macro- and microalbuminuria compared with those in control and normoalbuminuria groups (63). Furthermore, serum RBP4 levels are positively associated with clinical parameters, such as systolic and diastolic blood pressure (S and DBP), glucose, HbA1c, HOMA-IR, albumin/creatinine ratio (ACR) and TG levels. Moreover, serum RBP4 levels are inversely associated with glomerular filtration rate (GFR) (63). A meta-analysis (62) indicated that levels of circulating RBP4 are significantly elevated in microalbuminuria and macroalbuminuria groups compared with normal albuminuria subjects with T2D. In addition, significantly increased RBP4 levels were identified in T2D subjects with chronic kidney disease (CKD) and low GFR and RBP4 levels were positively associated with ACR but negatively associated with GFR (62). The latter inverse association has been observed in subjects with prediabetes (9). Retinol homeostasis is mediated by GF and reabsorption of RBP4 in the proximal tubule. Therefore, decreased catabolism resulting from microvascular damage in the kidney leads to a gradual increase in plasma RBP4 concentration in subjects with DN compared with patients with T2D without DN (62). The alterations in RBP4 metabolism during CKD are important for development of T2D as patients with T2D are exposed to increased OS, which is associated with endothelial dysfunction (63). Patients with T2D often exhibit kidney dysfunction and therefore RBP4 may increase OS via its action on polymorphonuclear leukocytes by interacting with TLR4 in leucocytes and inducing inflammation (6,45,63).

The potential association between RBP4 and cardiovascular events has also been evaluated. Circulating adipokines and vascular function were assessed in 3,505 participants of the third-generation Framingham cohort, which were free of CVD (mean age, 40 years; 53% female, 47% male). In the aforementioned study, mean arterial pressure was positively associated with serum RBP4, as determined by ELISA (64). Furthermore, in a study of Chinese individuals with prehypertension (Pre-HT) (65), elevated serum levels of RBP4 were noted in subjects with Pre-HT compared with subjects with normal BP. Moreover, higher serum RBP4 levels were identified in obese patients with Pre-HT compared with non-obese patients with Pre-HT. Serum RBP4 levels, determined by ELISA, were positively correlated with BMI, SBP and DBP (65). However, a prospective study of 950 Chinese subjects with T2D (follow-up, 22 years) indicated that higher serum RBP4 levels were not significantly associated with decreased CVD mortality (66).

In addition, a prospective cohort study revealed that RBP4 gene overexpression in transgenic mice (RBP4-Tg) increases incidence of atherosclerotic events by inducing macrophage-derived foam cell formation (67). In another study of diabetic rats with atherosclerosis, serum RBP4 levels, analyzed by ELISA, were positively associated with HOMA-IR, SBP, atherogenic index (predictor of atherosclerosis) and fasting insulin, TG and CRP levels (42). RBP4 stimulates expression of proinflammatory molecules, such as vascular cell adhesion protein-1, E-selectin, intercellular adhesion molecule-1, monocyte chemotactic protein-1, IL-6, TNFα and CRP, which induce endothelial inflammation (Fig. 1C) (18). RBP4-mediated endothelial inflammation is independent of retinol and signaling receptor and transporter of retinol *STRA6* via the NADPH/NF-κB-pathway. In addition, RBP4 has a key role in increasing nitric oxide (NO) production due to stimulation of the PI3K/AKT/NO synthase pathway and inhibition of extracellular signal-regulated kinase 1/2 phosphorylation and insulin-induced endothelin-1 secretion, leading to vasodilatation (68).

RBP4 is involved in development of vascular and endothelial inflammation following oxidative damage (50,63). OS serves a key role in the pathophysiology of IR and diabetes; it decreases peripheral insulin sensitivity via at least five key molecular mechanisms, including β cell dysfunction, inflammatory response, GLUT-4 downregulation and/or membrane localization, mitochondrial dysfunction and impairment of normal insulin signaling pathways (69).

6. RBP4, hyperglycemia and OS

Elevated RBP4 levels promote hyperglycemia and IR by inhibiting PI3K activity in skeletal muscle and increasing PEPCK synthesis in the liver, which increases hepatic glucose production, inhibits insulin signaling and impairs glucose uptake in skeletal muscle cells (Fig. 1D and E) (42,62). Hyperglycemia induces free radical formation and impairs endogenous antioxidant defense systems via different mechanisms, including increased polyol (sorbitol) and hexosamine pathway signaling, increased advanced glycation end-product formation and activation of protein kinase C isoforms. OS is defined as a disturbance in prooxidant-antioxidant balance of the cell in favor of the former (69,70). Excess production of ROS, such as the superoxide anion (O_2^-) and hydroxyl free radical and hydrogen peroxide, are harmful to cell components, notably proteins, lipids and DNA, which damages the cell (70).

Adipokines are associated with numerous types of OS-associated disease and pathological conditions (obesity,

inflammation, IR, diabetes). RBP4 promotes OS by impairing endothelial mitochondrial function (Fig. 1C) (71). The association between RBP4 levels and OS markers has been demonstrated by multiple studies (34,71,72). Codoñer-Franch *et al* (34) demonstrated a positive association between RBP4 and the inflammatory state in obesity and OS; there was a positive association between RBP4 and urinary 8-isoprostane (a marker of lipid peroxidation damage) and a negative association between RBP4 and decreased glutathione and GFR, suggesting that OS was dependent on RBP4 levels.

Liu *et al* (72) investigated the association between RBP4 and OS in a Chinese population; their results indicated a positive association between RBP4 and 8-iso-prostaglandin F2α and 13-(S)-hydroxyoctadecadienoic acid (13-HODE), which are both products of peroxidation of unsaturated fatty acids. The aforementioned study established an association between RBP4 and OS markers in humans (72). In a study of human aortic endothelial cells (HAECs), the treatment of 40 μg/ml RBP4 increased mitochondrial O_2^- production, which promoted mitochondrial dysfunction and increased membrane potential. RBP4 suppresses mitofusin-1 protein expression and enhances dynamin-related protein-1 and fission-1 protein expression levels in HAECs (Fig. 1C), suggesting an impairment of mitochondrial fusion and fission dynamics (73). Moreover, mitochondrial damage induces endothelial apoptosis, whereas RBP4 stimulation suppresses PI3K/AKT signaling in HAECs (73).

In addition to endothelial dysfunction, development of OS and elevated RBP4 levels may be due to certain OS products, such as 13-HODE, which serve as a ligand for PPARγ, causing its activation (74). In brown adipose tissue cells that PPARγ and cAMP-mediated PPARα act on the promoter of the RBP4 gene to positively regulate RBP4 expression (44). This reveals a direct association between certain OS markers and increased RBP4 levels.

Table I summarizes studies that have analyzed the association between RBP4 and obesity and associated chronic alterations in different populations worldwide and in animal models, along with sex, age, sample size and association between variables.

7. RBP4 in cancer and obesity-associated disease

RBP4 is associated with other types of obesity-associated disease, including liver, bone and joint pathology, sleep apnea, hepatocellular carcinoma, breast cancer, lung cancer and colorectal adenoma and ovarian cancer (75-90). Elevated levels of RBP4 have been reported in Japanese patients with severe and moderate obstructive sleep apnea (75). Despite the lack of association of RBP4 levels with HOMA-IR or BP, a positive association between visceral fat and TG has been noted (75). However, in Greek patients, no correlation has been noted between RBP4 levels and sleep apnea, anthropometric characteristics (BMI, neck circumference, waist circumference, hip circumference, waist-to-hip ratio), glycemic markers or lipid profile (76).

By contrast, high levels of RBP4 have been observed in obese patients with non-alcoholic fatty liver disease (NAFLD) with and without prediabetes (77). In a murine model, it has been observed, that RBP4 and retinol levels were significantly decreased in NAFLD (78). By contrast, elevated concentrations of RBP4 in Chinese subjects have been associated with development and progression of NAFLD (79). Elevated RBP4 levels are considered a risk factor for progression of CKD in patients with NAFLD (80).

Recent data have shown an association between RBP4 and bone and joint pathology (81-84). RBP4 levels are significantly elevated in patients with psoriatic arthritis and psoriasis (80). In addition, RBP4 is secreted in osteoarthritic joints and is positively associated with expression levels of other adipokines and extracellular matrix metalloproteinases (MMPs) 1 and 3 (82). However, a Mendelian randomization study found no association between RBP4 levels and osteoarthritis (83).

RBP4 is associated with the incidence of numerous types of cancer (84-89). RBP4 mRNA and protein expression is decreased in hepatocellular carcinoma (HCC) but high in normal tissue. Higher RBP4 expression is associated with improved overall survival time in patients with HCC (84). RBP4 is associated with complement and coagulation cascades, metabolic pathways, biosynthesis of antibiotics and peroxisome proliferator-activated receptor signaling and pyruvate metabolism pathways (84). Elevated levels of RBP4 in plasma, tumor tissue, liver and abdominal fat are associated with greater blood flow impairment and metastatic potential in patients with breast cancer (85); conversely, another study found that gene expression levels of RBP4 are significantly decreased and associated with unfavorable prognosis and pathogenesis of breast cancer (86). However, Tsakogiannis *et al* (87) published a study in 2021 that did not demonstrate a significant association between RBP4 and breast cancer. By contrast, high levels of serum RBP4 are associated with increased risk of non-small cell lung cancer (88). RBP4 has been proposed as a potential biomarker for early detection of high-risk colorectal cancer and adenoma (89). Overexpression of RBP4 in ovarian cancer cells induces expression levels of MMP2 and MMP9, as well as activation of the RhoA/Rock1 and cyclin D pathways, promoting migration and proliferation of cancer cells (90). A schematic representation of altered RBP4 levels in obesity and their association with other types of pathology is shown in Fig. 2.

8. Clinical applications of RBP4 and future perspectives

Currently, use of drugs that modulate RBP4 levels is proposed for clinical improvement of patients with numerous types of pathology (hepatic steatosis, obesity, IR, diabetes, atrophic macular degeneration) (15,33,91-95). One of the first RBP4 agonist drugs to be discovered was fenretinide. This synthetic retinoid increases urinary excretion of RBP4 (33). By binding to RBP4, it increases its hepatic secretion and sterically alters formation of complexes with TTR, which increases renal clearance of RBP4 and decreases its serum concentration (15). In an *in vivo* model of obesity induced by a high-fat diet, treatment with fenretinide increases renal clearance of RBP4, decreases its serum levels and improves IR and glucose intolerance in study mice (33). In addition, fenretinide has an inhibitory effect on development of hepatic steatosis in a murine model of obesity (91). However, fenretinide treatment inhibits vitamin A synthesis in

Table I. RBP4 is associated with obesity and associated clinical/metabolic disorder and disease.

First author/s, year	Study subjects	Sex	Age	Sample size, n	Clinical/metabolic disorder or disease	Association	(Refs.)
Fan, 2019	Chinese population	Male (n=295) and female (n=716)	56±7 years	1,011	HOMA-IR, TG, LDLc in DT2	+	(9)
					TG	-	
Li, 2018	Chinese population	Male (n=1754) and female (n=1591)	6-18 years	3,345	BMI, WC, BP, TG, TC, LDLc, HOMA-IR, leptin, IR	+	(11)
					HDLc	-	
Boaghi, 2020	Romanian population	Male and female (data not available)	5-17 years	213	TG, HDLc in obesity	-	(32)
Yang Q, 2005	Chinese population	NA	NA	NA	Obesity, DT2	+	(33)
Yang Q, 2005	Mouse model	Male	NA	36	IR, obesity, DT2	+	(33)
Wessel, 2019	Dutch population	Male (n=29) and female (n=49)	>18 years	78	VLDL and LDLc in MetS	+	(35)
Korek E, 2018	Polish population	Male (n=41) and female (n=12)	24-63 years	53	TG in obesity	+	(36)
Mateo-Gallego R, 2018	Spanish population	Female	18-80 years	76	TG in obesity	+	(37)
Comerford KB, 2014	US population	Female	NA	35	TC, TG in obesity	+	(38)
Numao S, 2012	Japanese population	Male	48±2 years	29	TG in obesity	+	(39)
Kwanbunjan K, 2018	Thai population	Male (n= 47) and female (n=120)	35-66 years	167	TTR, HOMA-IR and TG in healthy individuals	+	(41)
Zhou, 2018	Rat model	Male	4 weeks	46	DA, TG, HOMA-IR, SBP CRP in DT2	+	(42)
Pandey, 2015	Indian population	Male (n=123) and female (n=127)	20 years	250	Obesity, IR, DT2	+	(52)
Huang, 2021	Cell model INS-1E	NA	NA	NA	β cell dysfunction	+	(57)
Li JY, 2018	Chinese population	Male (n=155) and female (n=132)	62±7 years	287	DR, CVDR in DT2	+	(59)
Mahfouz MH, 2016	Saudi Arabia population	Male (n=74) and female (n=76)	NA	150	HT, Glucose, HbA1c, CRP in DN, DT2	+	(63)
					GFR	-	
Zachariah, 2016	US population	Male (n=1644) and female (n=1861)	40 years	3,505	Arterial pressure	+	(64)
Zhang, 2017	Chinese population	Male (n=188) and female (n=132)	NA	320	BMI, SBP, DBP in Pre-HT	+	(65)
Liu, 2016	Chinese population	Male	NA	950	TG	+	(66)
					GFR	-	
					Mortality in CVD in DT2	-	
Liu Y, 2017	Mouse model	NA	NA	NA	Atherosclerosis	+	(67)

NA, not available; AMI, Acute myocardial infarction; BMI, body mass index; CAD, coronary artery disease; CVD, cardiovascular disease; CRP, C-Reactive protein; CVDR, compromised vision due to diabetic retinopathy; DA, diabetic atherosclerosis; DBP, diastolic blood pressure; DN, diabetic nephropathy; DR, diabetic retinopathy; DT2, diabetes mellitus type 2; GFR, glomerular filtration rate; HbA1c, glycohemoglobin; HDLc, High-density lipoprotein; HOMA-IR, insulin resistance index; HT, arterial hypertension; IR, insulin resistance; LDLc, low density lipoprotein cholesterol; MetS, metabolic syndrome; Pre-HT, prehypertensive; RA, Rheumatoid Arthritis; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; TTR, transthyretin; VLDL, very-low-density lipoprotein; WC, waist-hip index; +, positive; -, negative.

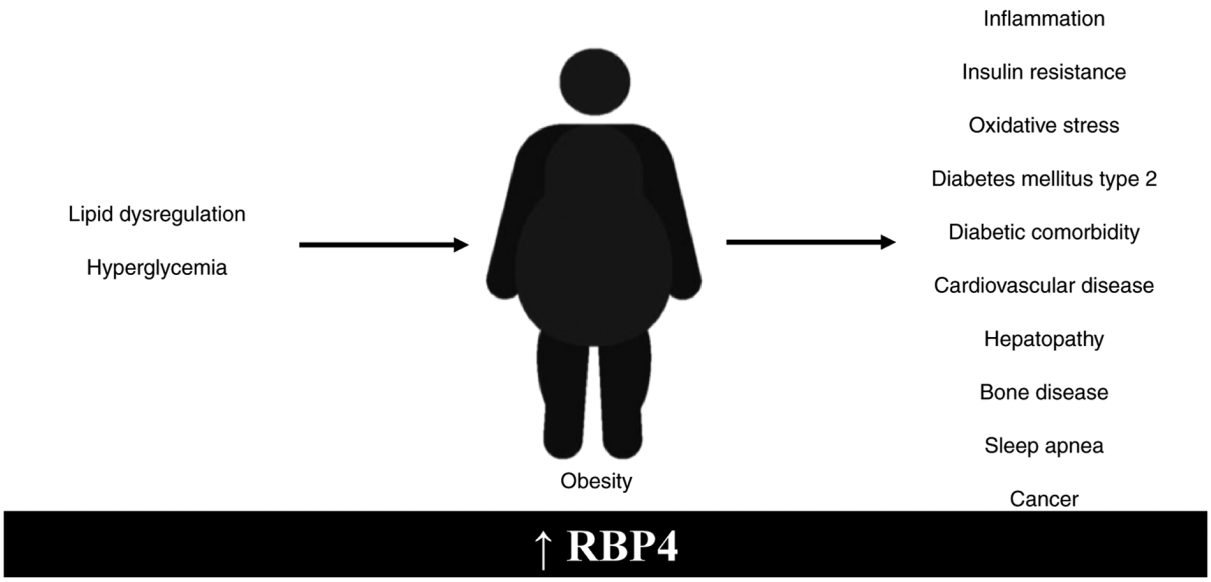

Figure 2. Schematic representation of alterations in RBP4 levels in obesity and its association with other types of pathology. Hyperglycemia and lipid dysregulation promote obesity, which induces the development of other pathology. Increased levels of RBP4 is a common factor.

β-carotene-fed mice, which is detrimental to visual function; therefore, further understanding of the effect of this drug on vitamin A homeostasis is required (96).

A1120 is a non-retinoid ligand for RBP4 that disrupts the RBP4-TTR complex by inducing a conformational change at the interaction interface, thereby decreasing serum RBP4 concentration and retinol levels (15). However, A1120 has low hepatic stability and no effect on glucose levels has been observed in animal models (93,97). As A1120 is not a retinoid and does not interfere with vision, it may be a more favorable drug compared with fenretinide (15).

BPN-14136 is another non-retinoid compound used to treat age-associated atrophic macular degeneration and Stargardt's disease. This drug disrupts the RBP4-TTR complex (15), decreases serum RBP4 levels and prevents high-fat diet-induced obesity and hepatic steatosis in mice overexpressing RBP4 in adipose tissue. It also decreases bisretinoid synthesis without affecting vision of mice and induces decreased CRP and levels of components involved in the complement cascade in the retina (98). Non-retinoid antagonists of RBP4 may represent a promising class of compounds as potential therapeutic agents.

Use of thiazolidinediones (synthetic PPAR ligands) has been proposed to inhibit expression of RBP4. For example, rosiglitazone decreases RBP4 levels in the adipose tissue of *Glut4*$^{-/-}$ mice (33). In addition, pioglitazone significantly decreases levels of RBP4 in an obese rat model, decreases body weight and improves insulin sensitivity (94). Finally, sitagliptin, a dipeptidyl peptidase-4 inhibitor, decreases RBP4 expression and improves insulin sensitivity in diabetic rats (95). However, thiazolidinediones are associated with adverse events, such as weight gain and edema.

9. Conclusion

RBP4 is an adipokine associated with obesity (32). RBP4 participates in lipid metabolism by activating RXR and LXR receptors (37). In addition, RBP4 promotes development of inflammation via release of proinflammatory cytokines and activation of macrophages and dendritic cells via the TLR4 and JNK pathways (45-47). Moreover, RBP4 serves a key role in IR-mediated activation of the JAK2/STAT5 signaling pathway (42,51). RBP4 also participates in development of hyperglycemia in muscle via inhibition of PI3K activity (19,33,54,55) and in liver via increased PEPCK activity (33,42). It also regulates OS via mitochondrial dysfunction (72,73). These processes participate in development of obesity, RI, diabetes and associated complications, such as retinopathy, nephropathy and CVD. RBP4 and its downstream signaling contribute to development of obesity and associated pathology, the regulation of this adipokine may be important for clinical improvement of patients. Further research is needed to develop an effective treatment to regulate levels of this adipokine without generating adverse effects.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

Not applicable.

Authors' contributions

YAFC, MER and EFA designed the review and wrote the manuscript. CGC, JMMB and MIBB edited the manuscript. Data authentication is not applicable. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Piché ME, Tchernof A and Després JP: Obesity phenotypes, diabetes, and cardiovascular diseases. Circ Res 126: 1477-1500, 2020.
2. WHO: World Health Organization. Obesity and overweight, 2021. Retrieved July 20. Available from https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight.
3. Shamah-Levy T, Vielma-Orozco E, Heredia-Hernández O, Romero-Martínez M, Mojica-Cuevas J, Cuevas-Nasu L and Rivera-Dommarco J: Encuesta Nacional de Salud y Nutrición 2018-19: Resultados Nacionales. Pública IN (ed). Instituto Nacional de Salud Pública, 2020.
4. Wu H and Ballantyne CM: Metabolic inflammation and insulin resistance in obesity. Circ Res 126: 1549-1564, 2020.
5. Wang L, Hu J and Zhou H: Macrophage and adipocyte mitochondrial dysfunction in obesity-induced metabolic diseases. World J Mens Health 39: 606-614, 2021.
6. Moraes-Vieira PM, Yore MM, Dwyer PM, Syed I, Aryal P and Kahn BB: RBP4 activates antigen-presenting cells, leading to adipose tissue inflammation and systemic insulin resistance. Cell Metab 19: 512-526, 2014.
7. Nono Nankam PA and Blüher M: Retinol-binding protein 4 in obesity and metabolic dysfunctions. Mol Cell Endocrinol 531: 111312, 2021.
8. Tsutsumi C, Okuno M, Tannous L, Piantedosi R, Allan M, Goodman DS and Blaner WS: Retinoids and retinoid-binding protein expression in rat adipocytes. J Biol Chem 267: 1805-1810, 1992.
9. Fan J, Yin S, Lin D, Liu Y, Chen N, Bai X, Ke Q, Shen J, You L, Lin X, *et al*: Association of serum retinol-binding protein 4 Levels and the risk of incident type 2 diabetes in subjects with prediabetes. Diabetes Care 42: 1574-1581, 2019.
10. Grosjean F, Esposito P, Maccarrone R, Libetta C, Dal Canton A and Rampino T: RBP4: A culprit for insulin resistance in end stage renal disease that can be cleared by hemodiafiltration. BioMed Res Int: Nov 23, 2017 (Epub ahead of print).
11. Li G, Esangbedo IC, Xu L, Fu J, Li L, Feng D, Han L, Xiao X, Li M, Mi J, *et al*: Childhood retinol-binding protein 4 (RBP4) levels predicting the 10-year risk of insulin resistance and metabolic syndrome: The BCAMS study. Cardiovasc Diabetol 17: 69, 2018.
12. Grzegorczyk EA, Harasim-Symbor E, Lukaszuk B, Harasiuk D, Choromanska B, Mysliwiec P, Zendzian-Piotrowska M and Chabowski A: Lack of pronounced changes in the expression of fatty acid handling proteins in adipose tissue and plasma of morbidly obese humans. Nutr Diabetes 8: 3, 2018.
13. Chen Y, Lv P, Du M, Liang Z, Zhou M and Chen D: Increased retinol-free RBP4 contributes to insulin resistance in gestational diabetes mellitus. Arch Gynecol Obstet 296: 53-61, 2017.
14. Abbas NAT and El Salem A: Metformin, sitagliptin, and liraglutide modulate serum retinol-binding protein-4 level and adipocytokine production in type 2 diabetes mellitus rat model. Can J Physiol Pharmacol 96: 1226-1231, 2018.
15. Steinhoff JS, Lass A and Schupp M: Biological functions of RBP4 and its relevance for human diseases. Front Physiol 12: 659977, 2021.
16. Fairulnizal Md Noh M, Devi Nair Gunasegavan R and Mustar S: Vitamin A in health and disease. Vitamin A, 2019.
17. Olsen T and Blomhoff R: Retinol, retinoic acid, and retinol-binding protein 4 are differentially associated with cardiovascular disease, type 2 diabetes, and obesity: An overview of human studies. Adv Nutr 11: 644-666, 2020.
18. Zabetian-Targhi F, Mahmoudi MJ, Rezaei N and Mahmoudi M: Retinol binding protein 4 in relation to diet, inflammation, immunity, and cardiovascular diseases. Adv Nutr 6: 748-762, 2015.
19. Noy N: Vitamin A in regulation of insulin responsiveness: Mini review. Proc Nutr Soc 75: 212-215, 2016.
20. Kawaguchi R, Yu J, Honda J, Hu J, Whitelegge J, Ping P, Wiita P, Bok D and Sun H: A membrane receptor for retinol binding protein mediates cellular uptake of vitamin A. Science 315: 820-825, 2007.
21. Napoli JL: Functions of intracellular retinoid binding-proteins. Subcell Biochem 81: 21-76, 2016.
22. Saari JC: Vitamin A metabolism in rod and cone visual cycles. Annu Rev Nutr 32: 125-145, 2012.
23. Dowling JE: Vitamin A: Its many roles-from vision and synaptic plasticity to infant mortality. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 206: 389-399, 2020.
24. Chelstowska S, Widjaja-Adhi MA, Silvaroli JA and Golczak M: Molecular basis for vitamin A uptake and storage in vertebrates. Nutrients 8: 676, 2016.
25. Wright CB, Redmond TM and Nickerson JM: A history of the classical visual cycle. Prog Mol Biol Transl Sci 134: 433-448, 2015.
26. Yang FC, Xu F, Wang TN and Chen GX: Roles of vitamin A in the regulation of fatty acid synthesis. World J Clin Cases 9: 4506-4519, 2021.
27. Iskakova M, Karbyshev M, Piskunov A and Rochette-Egly C: Nuclear and extranuclear effects of vitamin A. Can J Physiol Pharmacol 93: 1065-1075, 2015.
28. Ghyselinck NB and Duester G: Retinoic acid signaling pathways. Development 146: dev167502, 2019.
29. Balmer JE and Blomhoff R: Gene expression regulation by retinoic acid. J Lipid Res 43: 1773-1808, 2002.
30. Smith JE, Borek C, Gawinowicz MA and Goodman DS: Structure-function relationships of retinoids in their effects on retinol-binding protein metabolism in cultured H4II EC3 liver cells. Arch Biochem Biophys 238: 1-9, 1985.
31. Bellovino D, Lanyau Y, Garaguso I, Amicone L, Cavallari C, Tripodi M and Gaetani S: MMH cells: An in vitro model for the study of retinol-binding protein secretion regulated by retinol. J Cell Physiol 181: 24-32, 1999.
32. Boaghi A, Pop RM, Vasilache SL, Banescu C, Hutanu A, Marginean OC and Pascanu IM: Plasma RBP4 level in association with body composition, metabolic profile, STRA6 and RBP4 gene polymorphisms in obese Romanian children. Diabetes Metab Syndr Obes 13: 4643-4650, 2020.
33. Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, Kotani K, Quadro L and Kahn BB: Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature 436: 356-362, 2005.
34. Codoñer-Franch P, Mora-Herranz A, Simó-Jordá R, Pérez-Rambla C, Boix-García L and Faus-Pérez A: Retinol-binding protein 4 levels are associated with measures of liver and renal function and oxidant/antioxidant status in obese children. J Pediatr 163: 593-595, 2013.
35. Wessel H, Saeed A, Heegsma J, Connelly MA, Faber KN and Dullaart RPF: Plasma levels of retinol binding protein 4 relate to large VLDL and small LDL particles in subjects with and without type 2 diabetes. J Clin Med 8: 1792, 2019.
36. Korek E, Gibas-Dorna M, Chęcińska-Maciejewska Z, Krauss H, Łagiedo-Żelazowska M, Kołodziejczak B and Bogdański P: Serum RBP4 positively correlates with triglyceride level but not with BMI, fat mass and insulin resistance in healthy obese and non-obese individuals. Biomarkers 23: 683-688, 2018.
37. Mateo-Gallego R, Lamiquiz-Moneo I, Perez-Calahorra S, Marco-Benedí V, Bea AM, Baila-Rueda L, Laclaustra M, Peñalvo JL, Civeira F and Cenarro A: Different protein composition of low-calorie diet differently impacts adipokine profile irrespective of weight loss in overweight and obese women. Nutr Metab Cardiovasc Dis 28: 133-142, 2018.
38. Comerford KB, Buchan W and Karakas SE: The effects of weight loss on FABP4 and RBP4 in obese women with metabolic syndrome. Horm Metab Res 46: 224-231, 2014.
39. Numao S, Sasai H, Nomata Y, Matsuo T, Eto M, Tsujimoto T and Tanaka K: Effects of exercise training on circulating retinol-binding protein 4 and cardiovascular disease risk factors in obese men. Obes Facts 5: 845-855, 2012.
40. López-Domènech S, Abad-Jiménez Z, Iannantuoni F, de Marañón AM, Rovira-Llopis S, Morillas C, Bañuls C, Víctor VM and Rocha M: Moderate weight loss attenuates chronic endoplasmic reticulum stress and mitochondrial dysfunction in human obesity. Mol Metab 19: 24-33, 2019.

41. Kwanbunjan K, Panprathip P, Phosat C, Chumpathat N, Wechjakwen N, Puduang S, Auyyuenyong R, Henkel I and Schweigert FJ: Association of retinol binding protein 4 and transthyretin with triglyceride levels and insulin resistance in rural thais with high type 2 diabetes risk. BMC Endocr Disord 18: 26, 2018.
42. Zhou W, Ye SD, Chen C and Wang W: Involvement of RBP4 in diabetic atherosclerosis and the role of vitamin D intervention. J Diabetes Res 2018: 7329861, 2018.
43. Weiss K, Mihály J, Liebisch G, Marosvölgyi T, Schmitz G, Decsi T and Rühl R: Effect of synthetic ligands of PPAR α, β/δ, γ, RAR, RXR and LXR on the fatty acid composition of phospholipids in mice. Lipids 46: 1013-1020, 2011.
44. Rosell M, Hondares E, Iwamoto S, Gonzalez FJ, Wabitsch M, Staels B, Olmos Y, Monsalve M, Giralt M, Iglesias R and Villarroya F: Peroxisome proliferator-activated receptors-α and -γ, and cAMP-mediated pathways, control retinol-binding protein-4 gene expression in brown adipose tissue. Endocrinology 153: 1162-1173, 2012.
45. Moraes-Vieira PM, Yore MM, Sontheimer-Phelps A, Castoldi A, Norseen J, Aryal P, Simonyté Sjödin K and Kahn BB: Retinol binding protein 4 primes the NLRP3 inflammasome by signaling through Toll-like receptors 2 and 4. Proc Natl Acad Sci USA 117: 31309-31318, 2020.
46. Yesudhas D, Gosu V, Anwar MA and Choi S: Multiple roles of toll-like receptor 4 in colorectal cancer. Front Immunol 5: 334, 2014.
47. Kilicarslan M, de Weijer BA, Simonyté Sjödin K, Aryal P, Ter Horst KW, Cakir H, Romijn JA, Ackermans MT, Janssen IM, Berends FJ, *et al*: RBP4 increases lipolysis in human adipocytes and is associated with increased lipolysis and hepatic insulin resistance in obese women. FASEB J 34: 6099-6110, 2020.
48. Grant RW and Stephens JM: Fat in flames: Influence of cytokines and pattern recognition receptors on adipocyte lipolysis. Am J Physiol Endocrinol Metab 309: E205-E213, 2015.
49. Morigny P, Houssier M, Mouisel E and Langin D: Adipocyte lipolysis and insulin resistance. Biochimie 125: 259-266, 2016.
50. Farjo KM, Farjo RA, Halsey S, Moiseyev G and 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 32: 5103-5115, 2012.
51. Norseen J, Hosooka T, Hammarstedt A, Yore MM, Kant S, Aryal P, Kiernan UA, Phillips DA, Maruyama H, Kraus BJ, *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 32: 2010-2019, 2012.
52. Pandey GK, Balasubramanyam J, Balakumar M, Deepa M, Anjana RM, Abhijit S, Kaviya A, Velmurugan K, Miranda P, Balasubramanyam M, *et al*: Altered circulating levels of retinol binding protein 4 and transthyretin in relation to insulin resistance, obesity, and glucose intolerance in Asian Indians. Endocr Pract 21: 861-869, 2015.
53. Grundy SM: Hypertriglyceridemia, insulin resistance, and the metabolic syndrome. Am J Cardiol 83: 25F-29F, 1999.
54. Yousefi MR and TaheriChadorneshin H: The effect of moderate endurance training on gastrocnemius retinol-binding protein 4 and insulin resistance in streptozotocin-induced diabetic rats. Interv Med Appl Sci 10: 59-63, 2018.
55. Marschner RA, Pinto G, Borges J, Markoski MM, Schaan BD and Lehnen AM: Short-term detraining does not change insulin sensitivity and RBP4 in rodents previously submitted to aerobic exercise. Horm Metab Res 49: 58-63, 2017.
56. Sapra A and Bhandari P: Diabetes mellitus. In: StatPearls. StatPearls Publishing, Treasure Island, FL, 2022.
57. Huang R, Bai X, Li X, Wang X and Zhao L: Retinol-binding protein 4 activates STRA6, provoking pancreatic β-cell dysfunction in type 2 diabetes. Diabetes 70: 449-463, 2021.
58. Ediger BN, Du A, Liu J, Hunter CS, Walp ER, Schug J, Kaestner KH, Stein R, Stoffers DA and May CL: Islet-1 Is essential for pancreatic β-cell function. Diabetes 63: 4206-4217, 2014.
59. Li JY, Chen XX, Lu XH, Zhang CB, Shi QP and Feng L: Elevated RBP4 plasma levels were associated with diabetic retinopathy in type 2 diabetes. Biosci Rep 38: BSR20181100, 2018.
60. Han W, Wei H, Kong W, Wang J, Yang L and Wu H: Association between retinol binding protein 4 and diabetic retinopathy among type 2 diabetic patients: A meta-analysis. Acta Diabetol 57: 1203-1218, 2020.
61. Behl T, Kaur I and Kotwani A: Implication of oxidative stress in progression of diabetic retinopathy. Surv Ophthalmol 61: 187-196, 2016.
62. Zhang L, Cheng YL, Xue S and Xu ZG: The role of circulating RBP4 in the type 2 diabetes patients with kidney diseases: A systematic review and meta-analysis. Dis Markers 2020: 8830471, 2020.
63. Mahfouz MH, Assiri AM and Mukhtar MH: Assessment of neutrophil gelatinase-associated lipocalin (NGAL) and retinol-binding protein 4 (RBP4) in type 2 diabetic patients with nephropathy. Biomark Insights 11: 31-40, 2016.
64. Zachariah JP, Hwang S, Hamburg NM, Benjamin EJ, Larson MG, Levy D, Vita JA, Sullivan LM, Mitchell GF and Vasan RS: Circulating adipokines and vascular function: Cross-sectional associations in a community-based cohort. Hypertension 67: 294-300, 2016.
65. Zhang JX, Zhu GP, Zhang BL and Cheng YY: Elevated serum retinol-binding protein 4 levels are correlated with blood pressure in prehypertensive Chinese. J Hum Hypertens 31: 611-615, 2017.
66. Liu G, Ding M, Chiuve SE, Rimm EB, Franks PW, Meigs JB, Hu FB and Sun Q: Plasma levels of fatty acid-binding protein 4, retinol-binding protein 4, high-molecular-weight adiponectin, and cardiovascular mortality among men with type 2 diabetes: A 22-year prospective study. Arterioscler Thromb Vasc Biol 36: 2259-2267, 2016.
67. Liu Y, Zhong Y, Chen H, Wang D, Wang M, Ou JS and Xia M: Retinol-binding protein-dependent cholesterol uptake regulates macrophage foam cell formation and promotes atherosclerosis. Circulation 135: 1339-1354, 2017.
68. Rychter AM, Skrzypczak-Zielińska M, Zielińska A, Eder P, Souto EB, Zawada A, Ratajczak AE, Dobrowolska A and Krela-Kaźmierczak I: Is the retinol-binding protein 4 a possible risk factor for cardiovascular diseases in obesity? Int J Mol Sci 21: 5229, 2020.
69. Yaribeygi H, Sathyapalan T, Atkin SL and Sahebkar A: Molecular mechanisms linking oxidative stress and diabetes mellitus. Oxid Med Cell Longev 2020: 8609213, 2020.
70. Papachristoforou E, Lambadiari V, Maratou E and Makrilakis K: Association of glycemic indices (hyperglycemia, glucose variability, and hypoglycemia) with oxidative stress and diabetic complications. J Diabetes Res 2020: 7489795, 2020.
71. Daneshzad E, Farsad-Naeimi A, Heshmati J, Mirzaei K, Maghbooli Z and Keshavarz SA: The association between dietary antioxidants and adipokines level among obese women. Diabetes Metab Syndr 13: 1369-1373, 2019.
72. Liu Y, Wang D, Li D, Sun R and Xia M: Associations of retinol-binding protein 4 with oxidative stress, inflammatory markers, and metabolic syndrome in a middle-aged and elderly Chinese population. Diabetol Metab Syndr 6: 25, 2014.
73. Wang J, Chen H, Liu Y, Zhou W, Sun R and Xia M: Retinol binding protein 4 induces mitochondrial dysfunction and vascular oxidative damage. Atherosclerosis 240: 335-344, 2015.
74. Umeno A, Sakashita M, Sugino S, Murotomi K, Okuzawa T, Morita N, Tomii K, Tsuchiya Y, Yamasaki K, Horie M, *et al*: Comprehensive analysis of PPARγ agonist activities of stereo-, regio-, and enantio-isomers of hydroxyoctadecadienoic acids. Biosci Rep 40: BSR20193767, 2020.
75. Makino S, Fujiwara M, Suzukawa K, Handa H, Fujie T, Ohtaka Y, Komatsu Y, Aoki Y, Maruyama H, Terada Y, *et al*: Visceral obesity is associated with the metabolic syndrome and elevated plasma retinol binding protein-4 level in obstructive sleep apnea syndrome. Horm Metab Res 41: 221-226, 2009.
76. Nena E, Steiropoulos P, Tzouvelekis A, Tsara V, Hatzizisi O, Kyriazis G, Froudarakis M, Trakada G, Papanas N and Bouros D: Reduction of serum retinol-binding protein-4 levels in nondiabetic obstructive sleep apnea patients under continuous positive airway pressure treatment. Respiration 80: 517-523, 2010.
77. Karamfilova V, Gateva A, Alexiev A, Zheleva N, Velikova T, Ivanova-Boyanova R, Ivanova R, Cherkezov N, Kamenov Z and Mateva L: The association between retinol-binding protein 4 and prediabetes in obese patients with nonalcoholic fatty liver disease. Arch Physiol Biochem 128: 217-222, 2022.
78. Saeed A, Bartuzi P, Heegsma J, Dekker D, Kloosterhuis N, de Bruin A, Jonker JW, van de Sluis B and Faber KN: Impaired hepatic vitamin A metabolism in NAFLD mice leading to vitamin A accumulation in hepatocytes. Cell Mol Gastroenterol Hepatol 11: 309-325.e3, 2021.

79. Wang X, Chen X, Zhang H, Pang J, Lin J, Xu X, Yang L, Ma J, Ling W and Chen Y: Circulating retinol-binding protein 4 is associated with the development and regression of non-alcoholic fatty liver disease. Diabetes Metab 46: 119-128, 2020.
80. Tsai YL, Liu CW, Huang SF, Yang YY, Lin MW, Huang CC, Li TH, Huang YH, Hou MC and Lin HC: Urinary fatty acid and retinol binding protein-4 predict CKD progression in severe NAFLD patients with hypertension: 4-Year study with clinical and experimental approaches. Medicine (Baltimore) 99: e18626, 2020.
81. Toussirot E, Aubin F, Desmarets M, Wendling D, Augé B, Gillard J, Messica O, Guillot X, Laheurte C, Monnet E and Dumoulin G: Visceral adiposity in patients with psoriatic arthritis and psoriasis alone and its relationship with metabolic and cardiovascular risk. Rheumatology (Oxford) 60: 2816-2825, 2021.
82. Scotece M, Koskinen-Kolasa A, Pemmari A, Leppänen T, Hämäläinen M, Moilanen T, Moilanen E and Vuolteenaho K: Novel adipokine associated with OA: Retinol binding protein 4 (RBP4) is produced by cartilage and is correlated with MMPs in osteoarthritis patients. Inflamm Res 69: 415-421, 2020.
83. Fan J, Zhu J, Sun L, Li Y, Wang T and Li Y: Causal association of adipokines with osteoarthritis: A Mendelian randomization study. Rheumatology (Oxford) 60: 2808-2815, 2021.
84. Li M, Wang Z, Zhu L, Shui Y, Zhang S and Guo W: Down-regulation of RBP4 indicates a poor prognosis and correlates with immune cell infiltration in hepatocellular carcinoma. Biosci Rep 41: BSR20210328, 2021.
85. Papiernik D, Urbaniak A, Kłopotowska D, Nasulewicz-Goldeman A, Ekiert M, Nowak M, Jarosz J, Cuprych M, Strzykalska A, Ugorski M, *et al*: Retinol-binding protein 4 accelerates metastatic spread and increases impairment of blood flow in mouse mammary gland tumors. Cancers (Basel) 12: 623, 2020.
86. Wu M, Li Q and Wang H: Identification of novel biomarkers associated with the prognosis and potential pathogenesis of breast cancer via integrated bioinformatics analysis. Technol Cancer Res Treat 20: 1533033821992081, 2021.
87. Tsakogiannis D, Kalogera E, Zagouri F, Zografos E, Balalis D and Bletsa G: Determination of FABP4, RBP4 and the MMP-9/NGAL complex in the serum of women with breast cancer. Oncol Lett 21: 85, 2021.
88. Hu X, Huang W, Wang F, Dai Y, Hu X, Yue D and Wang S: Serum levels of retinol-binding protein 4 and the risk of non-small cell lung cancer: A case-control study. Medicine (Baltimore) 99: e21254, 2020.
89. Komor MA, Bosch LJ, Coupé VM, Rausch C, Pham TV, Piersma SR, Mongera S, Mulder CJ, Dekker E, Kuipers EJ, *et al*: Proteins in stool as biomarkers for non-invasive detection of colorectal adenomas with high risk of progression. J Pathol 250: 288-298, 2020.
90. Wang Y, Wang Y and Zhang Z: Adipokine RBP4 drives ovarian cancer cell migration. J Ovarian Res 11: 29, 2018.
91. Preitner F, Mody N, Graham TE, Peroni OD and Kahn BB: Long-term Fenretinide treatment prevents high-fat diet-induced obesity, insulin resistance, and hepatic steatosis. Am J Physiol Endocrinol Metab 297: E1420-E1429, 2009.
92. Cioffi CL, Racz B, Varadi A, Freeman EE, Conlon MP, Chen P, Zhu L, Kitchen DB, Barnes KD, Martin WH, *et al*: Design, synthesis, and preclinical efficacy of novel nonretinoid antagonists of retinol-binding protein 4 in the mouse model of hepatic steatosis. J Med Chem 62: 5470-5500, 2019.
93. Kim N and Priefer R: Retinol binding protein 4 antagonists and protein synthesis inhibitors: Potential for therapeutic development. Eur J Med Chem 226: 113856, 2021.
94. Zhu C, Xiao Y, Liu X, Han J, Zhang J, Wei L and Jia W: Pioglitazone lowers serum retinol binding protein 4 by suppressing its expression in adipose tissue of obese rats. Cell Physiol Biochem 35: 778-788, 2015.
95. Hu H, Xu M, Qi R, Wang Y, Wang C, Liu J, Luo L, Xia L and Fang Z: Sitagliptin downregulates retinol-binding protein 4 and upregulates glucose transporter type 4 expression in a type 2 diabetes mellitus rat model. Int J Clin Exp Med 8: 17902-17911, 2015.
96. Miller AP, Black M and Amengual J: Fenretinide inhibits vitamin A formation from β-carotene and regulates carotenoid levels in mice. Biochim Biophys Acta Mol Cell Biol Lipids 1867: 159070, 2022.
97. Blaner WS: Vitamin A signaling and homeostasis in obesity, diabetes, and metabolic disorders. Pharmacol Ther 197: 153-178. 2019.
98. Racz B, Varadi A, Kong J, Allikmets R, Pearson PG, Johnson G, Cioffi CL and Petrukhin K: A non-retinoid antagonist of retinol-binding protein 4 rescues phenotype in a model of Stargardt disease without inhibiting the visual cycle. J Biol Chem 293: 29. 11574-11588, 2018.