REVIEW

Retinol Binding Protein 4 in Relation to Diet, Inflammation, Immunity, and Cardiovascular Diseases[1,2]

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ABSTRACT

Retinol binding protein 4 (RBP4), previously called retinol binding protein (RBP), is considered a specific carrier of retinol in the blood. It is also an adipokine that has been implicated in the pathophysiology of insulin resistance. RBP4 seems to be correlated with cardiometabolic markers in inflammatory chronic diseases, including obesity, type 2 diabetes, metabolic syndrome, and cardiovascular diseases (CVDs). It has recently been suggested that inflammation produced by RBP4 induces insulin resistance and CVD. The clinical relevance of this hypothesis is discussed in this review. Knowledge concerning the association of RBP4 with inflammation markers, oxidative stress, and CVDs as well as concerning the role of diet and antioxidants in decreasing RBP4 concentrations are discussed. Special attention is given to methodologies used in previously published studies and covariates that should be controlled when planning new studies on this adipokine. *Adv Nutr* 2015;6:748–62.

Keywords: retinol binding protein 4, cardiovascular diseases, inflammation, immunity, antioxidants, diet, adipokines

Introduction

Vitamin A is an essential nutrient that plays a key role in vision, cell growth, differentiation, and embryonic development (1, 2). The liver is the main storage site for retinol (3). Retinol-binding protein 4 (RBP4)[8], also known as retinol binding protein (RBP), is a plasma retinol transporter that carries retinol from the liver to the periphery, and very little plasma RBP4 originates from adipose tissue (4). The loss of RBP4 induces vitamin A deficiency in *Rbp4* knockout mice (5). Apo-RBP4 is defined as RBP4 that is not bound to retinol, whereas retinol-bound RBP4 (holo-RBP4) associates with transthyretin (TTR) in plasma to prevent the loss of RBP through kidney filtration (6). The cell surface receptor for RBP4 is known as stimulated by retinoic acid 6 (STRA6) (7, 8), which is not only a vitamin A transporter but also a surface signaling receptor (9).

RBP4 has been known as a negative acute phase inflammatory reactant. Yang et al. (10) indicated that RBP4 was a novel adipokine and that its concentrations are elevated in insulin-resistant states associated with obesity and type 2 diabetes (T2D). Several other studies observed high concentrations of RBP4 in obesity (11–15) as a chronic inflammatory state and in its complications including T2D (16–20), metabolic syndrome (21–27), and cardiovascular diseases (CVDs) (28-38).

RBP4 plays a role in progression of insulin resistance through immunity (39) and inflammatory mechanisms (40) in adipose and vascular tissues. Recently, RBP4 was implicated in cardiovascular incidents. However, there is controversy about the role of RBP4 as a marker of inflammation and CVD prediction. Therefore, a critical review of those studies is needed. To the extent of our knowledge, there are no RBP4 reviews investigating completely all of the inflammatory and immunity effects of RBP4, especially in CVD, and its association with diet and antioxidants. This article reviews recent studies of the role of RBP4 in chronic

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[8] Abbreviations used: CVD, cardiovascular disease; E-selectin, endothelial-leukocyte adhesion molecule 1; FABP, fatty acid binding protein 4; ICAM-1, intercellular adhesion molecule 1; MCP-1, monocyte chemoattractant protein1; RBP, retinol binding protein; RBP4, retinol binding protein 4; SOCS3, suppressor of cytokine signaling 3; STRA6, stimulated by retinoic acid 6; T2D, type 2 diabetes; TTR, transthyretin; VCAM-1, vascular cell adhesion molecule 1; 8-isoPGF2α, 8-iso-prostaglandin F2α.

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inflammation, immune response, and oxidative stress and of the relation between RBP4 and diet and antioxidants, with a special focus on CVD, to show areas of ambiguity related to this topic. The literature search was based on PubMed listings up to 10 September 2014.

RBP4 and CVD

Finding risk factors of CVD and using them as targets for drug or diet therapy has been an interesting area of research that has made it possible to prevent and treat CVD. RBP4 has recently been implicated in the pathogenesis of CVD. Higher circulating RBP4 concentrations have been observed in subjects with previous clinical arteriosclerosis (41, 42), high-grade carotid stenosis (43), inflammatory dilated cardiomyopathy (28), coronary artery disease (32), and advanced heart failure (36) compared with control subjects. However, other studies did not find significant differences in RBP4 concentrations between patients with coronary artery disease and healthy individuals (44, 45). These findings are mainly in agreement with the hypothesis that circulating RBP4 could be a possible marker of atherosclerosis.

Chronic vascular inflammation exerts a prominent role in the development of atherosclerosis (46–48). Vascular inflammation begins with endothelial secretion of proinflammatory cell surface adhesion molecules, including vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), and endothelial-leukocyte adhesion molecule 1 (E-selectin), as well as endothelial production of soluble proinflammatory factors, including monocyte chemoattractant protein 1 (MCP-1) and IL-6. MCP-1 promotes the recruitment and adherence of leukocytes to the endothelium (46).

The relation between RBP4 and oxidative stress markers is less controversial. Most of the studies reported a positive relation between RBP4 and oxidative stress markers [urinary 8-isoprostane (49), 8-iso-prostaglandin F2α (8-isoPGF2α) (50), 13-(S)-hydroxyoctadecadienoic acid (50), and malondialdehyde (51)] and a negative relation between RBP4 and antioxidant glutathione (49). Thus, RBP4 may have a role in oxidative stress, and its mechanisms need to be elucidated (**Table 1**).

Cellular oxidative stress leads to the activation of vascular inflammation (46, 47). Because RBP4 concentrations are positively related to oxidative stress markers (Table 1), RBP4 may have a role in the initiation of endothelial inflammation. Farjo et al. (40) indicated that RBP4 induces gene expression of factors that are implicated in the initiation of vascular inflammation. Despite no observed relation between RBP4 and VCAM-1 (49, 52) in human studies, a positive relation between RBP4 and ICAM-1 and between RBP4 and E-selectin was evident only in diabetic patients (54) (**Table 2**). Surprisingly, an inverse relation was observed between RBP4 and E-selectin and between RBP4 and ICAM-1 in obese patients with rheumatoid arthritis, with no known reason (52) (Table 2). Collectively, although RBP4 has been implicated in the initiation of vascular inflammation in vitro (40), controversial results were observed in human studies.

In vitro studies indicated that RBP4 has a major role in plaque rupture by increasing vascular smooth muscle cell proliferation (55) and the induction of E-selectin (40) as a plaque formation marker (56). RBP4 is also known as an independent determinant index of plaque severity (43). Therefore, it should be expected that RBP4 is positively related to carotid artery intima media thickness as a marker of subclinical atherosclerosis (57). The relation between RBP4 concentrations and carotid artery intima media thickness and between RBP4 and E-selectin is controversial in human studies (Table 2 and **Table 3**). The positive relation of RBP4 with coronary artery calcification was shown in a study by Huang et al. (29) (Table 3). Additional studies are needed to confirm this observation. RBP4 concentrations are positively related to the early endothelial dysfunction marker flow-mediated dilation (60) in patients with rheumatoid arthritis (52) but not in patients with endothelial dysfunction (51). The inverse relation between RBP4 and flow-mediated vasodilatation (flow-mediated dilation) are evident in normotensive individuals and in diabetic patients (20, 51, 54). The association of RBP4 with CVD markers, including fatty acid binding protein 4 (FABP4) (44), lipocalin 2 (44), and the Framingham risk score (38), is shown in Table 2. The controversial results on the relation between RBP4 and hypertension are shown in **Table 4**. Although RBP4 is correlated with some CVD markers, it is unlikely to have a role in vascular dysfunction.

RBP4 concentrations have been shown to be predictive of CVD in several studies. RBP4 reduction predicted ischemia events in patients with familial hypercholesterolemia (64). It was also shown that RBP4 acts as an independent predictor of macrovascular diseases in patients with T2D (65). RBP4 concentrations manifest a linear trend with visceral obesity and Framingham risk score as a coronary heart disease risk point scale independent of obesity (38). Two longitudinal studies with controversial findings show the relation of RBP4 with CVD risk factors, despite not detecting holo- or apo-RBP4 (37, 66). Using a longer follow-up time and MS assay, Sun et al. (37) showed that full-length and total RBP4 were associated with coronary heart disease risk markers. In contrast, Mallat et al. (66), studying a larger sample size, did not report the same results. Reasons behind these conflicting results may be the different methods of measuring of RBP4 concentrations and differences in sample size, sex, and ethnicity.

For many years, biomarkers have been seen as a promising tool for improvement in prevention, early diagnosis, and management of CVD. However, the relation between RBP4 and cardiovascular risk factors is still controversial. To our knowledge, few longitudinal studies using more accurate assays have investigated the relation between RBP4 and CVD risk factors. Well-designed longitudinal studies that use larger sample sizes and that consider potential confounders are needed to confirm previous observations.

RBP4 in Relation to Inflammation and Immune Responses

Obesity is identified as a low-grade chronic inflammation state and defined as an elevated expression of inflammatory

TABLE 1 Methodology of studies investigating the association between RBP4 and oxidative stress[1]

Markers	Study design	Study population	Sex	Age,[2] y	Study size, *n*	Cytokine measurement method	RBP4 measurement method	Analysis	Relation	RBP4 specimen	Authors, year (ref)
Uri-8-isoPGF2α	CC	101 Subjects with T2D; 22 controls	62 M; 39 F	61.6 ± 4.5	123	EIA kit	EIA	Correlation	None	Serum	Takebayashi et al., 2007 (20)
Urinary 8-isoprostane	CS	Healthy obese and overweight children	45 M; 38 F	8–15	83	ELISA	Immunononenephelometry	ANCOVA	+	Serum	Codoñer-Franch et al., 2013 (49)
Uri-8-isoPGF2α	CS	Chinese	910 M; 838 F	50–70	1748	ELISA	ELISA	Correlation	+	Plasma	Liu et al., 2014 (50)
13-HOD	CS	Chinese	910 M; 838 F	50–70	1748	Colorimetric EIA kit	ELISA	Correlation	+	Plasma	Liu et al., 2014 (50)
Glutathione	CS	Healthy obese and overweight children	45 M; 38 F	8–15	83	ELISA	Immunononenephelometry	Multivariate regression	−	Serum	Codoñer-Franch et al., 2013 (49)
MDA	CC	Normotensive subjects	111 M; 47 F	49	66	TBAR	ELISA validated by Western blot	Linear correlation	+	Plasma	Solini et al., 2012 (51)

[1] CC, case-control; CS, cross-sectional; EIA, enzyme immunoassay; F, female; M, male; MDA, malondialdehyde; RBP4, retinol binding protein 4; ref, reference; T2D, type 2 diabetes; Uri-8-isoPGF2α, urinary 8-iso-prostaglandin F2α; 13-HOD, 13-(S)-hydroxyoctadecadienoic acid; +, positive; −, negative.

[2] Values are ranges, means ± SDs, or the median.

cytokines and infiltration of immune cells into adipocytes (67). The development of inflammatory responses leads to the induction of insulin resistance and a higher incidence of cardiovascular events (68). Inflammation may be the crucial way through which RBP4 exerts its function in the pathogenesis of insulin resistance and CVDs (40, 69).

Holo-RBP4 binds to STRA6 and directly induces insulin resistance in adipocytes through c-Jun N-terminal kinases (9). Subsequently, RBP4 suppresses insulin signaling like other cytokines by inducing suppressor of cytokine signaling 3 (SOCS3) (70). Either holo- or apo-RBP4 indirectly inhibits insulin signaling by inducing cytokine secretion in macrophages cocultured with adipocytes independent of retinol and STRA6 through the c-Jun N-terminal kinases pathway and Toll-like receptor 4 (69, 70). RBP's role in inducing endothelial cell inflammation through NAD(P)H and NF-κB pathways occurs independently of STRA6 and retinol (40). It is unclear whether holo- or apo-RBP4 or both are involved in the induction of insulin resistance and endothelial inflammation.

A number of studies have investigated the association between RBP4 and inflammatory markers, including C-reactive protein (CRP) (**Table 5**), IL-6 (**Table 6**), other cytokines (**Table 7**), and TNF (**Table 8**). Biological studies have shown that holo- or apo-RBP4 induces the secretion of certain inflammatory and/or cardiovascular risk markers, including TNF-α, IL-6, MCP-1, IFN-γ, IL-1β, IL-2, IL-12, IL-10, and IL-8 in macrophages (69), as well as ICAM-1, VCAM-1, E-selectin, IL-6, and MCP-1 in endothelial cells (40). The many conflicting results in human studies make it difficult to reach a definitive conclusion on the relation between cytokines and either circulating concentrations or adipose tissue gene expression of *Rbp4*.

With no known molecular mechanism, few human studies have shown a positive relation between RBP4 and CRP (50, 71, 72). Most studies observed no association between RBP4 and CRP (12, 20, 24, 31, 49, 52, 72–75) (Table 5). When macrophages were incubated with IL-6, the secretion and expression of *Rbp4* were not affected by IL-6 (80). Although RBP4 induces IL-6 secretion in macrophages (69) and endothelial cells (40), few human studies observed a positive relation between RBP4 and IL-6 (50, 71), whereas other studies found a negative (77) or no (49, 52, 75, 77, 78) relation (Table 6).

Studies that investigated RBP4 in relation to other inflammatory markers are shown in Table 7. Plasma RBP4 concentrations are positively correlated with plasma IL-8 as a neutrophil chemoattractant factor in patients with inflammatory dilated cardiomyopathy (28) (Table 7). The in vitro part of that same study revealed that IL-8 intensifies the expression of *Rbp4* by adipocytes dose-dependently (28). Furthermore, a positive correlation was observed between the adipose tissue expression of *Rbp4* and the inflammatory marker CD68 (53) (Table 7). Although these results implicated the role of RBP4 in adipose tissue inflammation, additional studies are needed to confirm these findings.

In vitro RBP4 induces TNF-α secretion in T cells (39) and macrophages (69), whereas TNF-α inhibits *Rbp4* gene

TABLE 2 Methodology of studies investigating the association between RBP4 and other CVD risk markers[1]

Marker	Study design	Study population	Sex	Age,[2] y	Study size, *n*	Cytokine measurement method	RBP4 measurement method	Analysis	Relation	RBP4 specimen	Authors, year (ref)
MCP-1	CS	Chinese	910 M; 838 F	50–70	1748	Milliplex (Millipore) human cytokine/chemokine panel	Sandwich ELISA	Correlation	+	Plasma	Liu et al., 2014 (50)
MCP-1	Basement analysis of an intervention	Healthy obese subjects	NM	24–62	16	Gene expression	Gene expression	Correlation	+	Adipose biopsy	Yao-Borengasser et al., 2007 (53)
MCP-1	CS	Obese patients with rheumatoid arthritis	NM	NM	217	Solid-phase HS and RD system	Solid-phase sandwich ELISA	Mixed regression method	None	Serum	Dessein et al., 2014 (52)
E-selectin	CC	Hypertensive patients with endothelial dysfunction	111 M; 47 F	49	92	ELISA	ELISA validated by Western blot	Linear correlation	None	Plasma	Solini et al., 2012 (51)
E-selectin	CS	Obese patients with rheumatoid arthritis	NM	NM	217	Solid-phase HS and RD system	Solid-phase sandwich ELISA	Mixed regression method	−	Serum	Dessein et al., 2014 (52)
E-selectin	CS	Patients with T2D	32 M; 18 F	20–80	50	ELISA	ELISA	Correlation	+	Serum	Park et al., 2009 (54)
VCAM-1	CS	Obese patients with rheumatoid arthritis	NM	NM	217	Solid-phase HS and RD system	Solid-phase sandwich ELISA	Mixed regression method	None	Serum	Dessein et al., 2014 (52)
VCAM-1	CS	Healthy obese and overweight children	45 M; 38 F	8–15	83	ELISA	Immunononenephelometry	Multivariate regression	None	Serum	Codoñer-Franch et al., 2013 (49)
ICAM-1	CS	Obese patients with rheumatoid arthritis	NM	NM	217	Solid-phase HS and RD system	Solid-phase sandwich ELISA	Mixed regression method	None	Serum	Dessein et al., 2014 (52)
ICAM-1	CS	Patients with T2D	32 M; 18 F	20–80	50	ELISA	ELISA	Correlation	+	Serum	Park et al., 2009 (54)
FABP4	CS	OD, nOD, OnD, and CVD patients and controls	139 M; 145 F	51–64	248	Sandwich immunoassay	Monoclonal antibody–based immunoassay	Correlation	+	Serum	Alkharfy et al., 2012 (44)
Lipocalin 2	CS	OD, nOD, OnD, and CVD patients and controls	140 M; 145 F	51–65	249	Monoclonal antibody–based rapid immunoassay	Monoclonal antibody–based rapid immunoassay	Correlation	+	Serum	Alkharfy et al., 2012 (44)

[1] CC, case-control; CS, cross-sectional; CVD, cardiovascular disease; E-selectin, endothelial-leukocyte adhesion molecule 1; F, female; FABP4, fatty acid binding protein 4; ICAM-1, intercellular adhesion molecule 1; M, male; MCP-1, monocyte chemoattractant protein 1; NM, not mentioned; nOD, nonobese diabetic; OD, obese diabetic; OnD, obese nondiabetic; RBP4, retinol binding protein 4; ref, reference; T2D, type 2 diabetes; VCAM-1, vascular cell adhesion molecule 1; positive; −, negative.

[2] Values are ranges or the median.

TABLE 3 Methodology of studies investigating the association between RBP4 and CVD risk markers[1]

Marker	Study design	Study population	Sex	Age,[2] y	Study size, *n*	RBP4 measurement method	Analysis	Relation	RBP4 specimen	Authors, year (ref)
CIMT	CS	Postmenopausal women	F	42–58	709	Western blot	ANOVA or KW based on RBP4 quartile	None	Serum	Huang et al., 2012 (29)
CIMT	CS	NM	50% F	70	1008	ELISA	Multivariable-adjusted analyses	−	NM	Ingelsson and Lind, 2009 (30)
CIMT	CS	35 hypertensive and 35 normotensive healthy lean subjects	F	<65	70	ELISA	Overall linear regression	+	Serum	Solini et al., 2009 (42)
CIMT	CS	T2D	32 M; 18 F	20–80	50	ELISA	Multiple linear regression	None	Serum	Park et al., 2009 (54)
CIMT	CS	T2D	144 M; 140 F	35–70	284	ELISA	Correlation	+	Serum	Xiao et al., 2013 (58)
CIMT	CS	34 Patients with T2D and 8 smokers	44 M; 52 F	55 ± 1.3	96	ELISA	Correlation	+	Serum	Bobbert et al., 2010 (59)
CIMT	CC	101 Patients with T2D and 22 controls	62 M; 39 F	61.6 ± 4.5	123	EIA	Linear regression analysis	None	Serum	Takebayashi et al., 2007 (20)
CIMT	CS	Obese patients with rheumatoid arthritis	NM	NM	217	Solid-phase sandwich ELISA	Mixed regression method	+	Serum	Dessein et al., 2014 (52)
FMD	CS	Obese patients with rheumatoid arthritis	NM	NM	218	Solid-phase sandwich ELISA	Mixed regression method	+	Serum	Dessein et al., 2014 (52)
FMD	CS	Hypertensive patients with endothelial dysfunction	111 M; 47 F	49	92	ELISA validated by Western blot	Correlation	None	Plasma	Solini et al., 2012 (51)
FMD	CS	Patients with T2D	32 M; 18 F	20–80	50	ELISA	Correlation	−	Serum	Park et al., 2009 (54)
FMD	CC	101 Patients with T2D and 22 controls	62 M; 39 F	61.6 ± 4.5	123	EIA	Linear regression analysis	−	Serum	Takebayashi et al., 2007 (20)
FMD	CC	Normotensive subjects	112 M; 47 F	47	66	ELISA	Correlation	−	Plasma	Solini et al., 2012 (51)
CAC	CS	Postmenopausal women	F	42–58	709	Western blot	Curvilinear association	+	Serum	Huang et al., 2012 (29)
Framingham score	CS	Healthy adults	116 M; 175 F	19–70	291	EIA	Multiple linear regression	+	Serum	Won et al., 2012 (38)

[1] CAC, coronary artery calcification; CC, case-control; CIMT, carotid intima media thickness; CS, cross-sectional; CVD, cardiovascular disease; EIA, enzyme immunoassay; F, female; FMD, flow-mediated dilation; KW, Kruskal-Wallis test; M, male; NM, not mentioned; RBP4, retinol binding protein 4; ref, reference; T2D, type 2 diabetes; +, positive; −, negative.

[2] Values are ranges, means ± SDs, or medians.

TABLE 4 Methodology of studies investigating the association between RBP4 and blood pressure[1]

Study design	Study population	Sex	Age,[2] y	Study size, *n*	RBP4 measurement method	Analysis	Relation	RBP4 specimen	Authors, year (ref)
CS	Chinese	910 M; 838 F	50–70	1748	Sandwich ELISA	Multivariable logistic regression	None	Plasma	Liu et al., 2014 (50)
CS	30 Patients with MetS + HT vs. 30 NT patients with MetS	11 M; 34 F	38 ± 11	60	NM	Unpaired Student's *t* test	+	NM	Gil et al., 2013 (61)
CS	35 HT and 35 NT patients	F	<65	70	ELISA	Univariate correlation	+	Plasma	Solini et al., 2009 (42)
CS	Obese and nonobese HT and control subjects	225 M; 106 F	<20	331	ELISA	Linear regression	+	Serum	Deng et al., 2014 (62)
CS	Healthy subjects	M	59 ± 14	153	ELISA	Correlation	None	Serum	Chiba et al., 2009 (63)
CS	Healthy subjects	F	57 ± 14	224	ELISA	Correlation	+	Serum	Chiba et al., 2009 (63)
CS	HT vs. NT patients	111 M; 47 F	49	92	ELISA validated by Western blot	Student's *t* test or Mann-Whitney	None	Plasma	Chiba et al., 2009 (63)

[1] CS, cross-sectional; F, female; HT, hypertensive; M, male; MetS, metabolic syndrome; NM, not mentioned; NT, normotensive; RBP4, retinol binding protein 4; ref, reference; +, positive; −, negative.
[2] Values are ranges, means ± SDs, or the median.

expression in adipocytes (81) and macrophages (80). These results indicate that RBP4 might be a positive regulator of TNF-α and that RBP4 may be inhibited by TNF-α. However, conflicting results were observed in clinical studies of the relation between RBP4 and TNF-α (Table 8). Several in vivo studies showed a positive relation between RBP4 and TNF-α (35, 50, 72, 77) but not between RBP4 and soluble TNF-α receptor 1 and soluble TNF-α receptor 2 (75). Other studies did not show any relation between RBP4 and TNF-α (51, 74, 75, 77) (Table 8). These discrepancies may be due to variations in study design and methodologies.

The binding of RBP4 to retinol and TTR is affected by several factors, including serum retinol, vitamin A intake, the acute phase response, protein-energy malnutrition, and liver and renal diseases (82–85). In addition, age, sex, BMI, body fat percentage, lipid variables, fasting blood glucose, physical activity, TTR, waist circumference, waist-to-hip ratio, ethnicity, insulin concentration, intakes of SFAs and TGs, and blood pressure are potential covariates that should be adjusted to find the precise correlation between RBP4 and cytokines (86-89). For studies to succeed, all of these covariates must be considered and adjustments made.

Circulating RBP4 concentration depends on vitamin A status (90).Therefore, serum retinol concentration and vitamin A intake should be included as covariates in association analysis involving RBP. To avoid false associations, apo- and holo-RBP4 should be analyzed separately in relation to cytokines (12, 91). Several studies found a positive correlation between CRP and RBP4 (50, 63, 71, 72) when serum retinol was not considered as a covariate. Furthermore, a few studies considered vitamin A status (retinol/RBP) (12, 91) and dietary vitamin A intake (12) as confounders. By considering these covariates, no significant correlations were observed between RBP and CRP or IL-6 (12, 91).

The use of ELISA kits, with limited dynamic range, could result in conflicting results. Commercial ELISA kits do not differentiate between holo- and apo-RBP4 and evaluate the whole RBP4 concentration. Therefore, it is not clear whether holo- or apo-RBP4 is related to inflammation or CVD. According to in vitro studies, apo-RBP4 induces inflammatory cascades (40, 69). Clinical studies have consistently shown that apo-RBP4 is secreted by adipose tissue (91) and is elevated in obese individuals (91) and patients with T2D (77). Apo-RBP4 should be incorporated in association analysis. (69). Thus, apo-RBP4 should be involved in association analysis with inflammatory and cardiovascular risk markers.

Variability in findings may emerge due to a lack of high-affinity and reliable methods of RBP4 measurement (92). Previous studies used commercial ELISA kits, which underestimate RBP4 concentrations due to assay saturation (93). They are unable to distinguish between the full-length and the truncated forms of RBP4 that may affect metabolic risk factors (92). Western blot measures the full length of RBP4 and is considered the gold-standard method (93). MS immunoassay quantitates the full-length and truncated isoforms of RBP4 (92). Although these 2 assays may analyze RBP4 more accurately and precisely than ELISA, only a few studies used Western blot (31, 75) or MS (37, 92, 94) to measure RBP4 concentrations. Another reason behind the conflicting results may be the type of specimen used to evaluate RBP4 concentrations because the use of plasma anticoagulants may cause spurious results (93).

Different populations, sample sizes, geographical regions, health status, BMI, and ethnicity may justify the conflicting results observed in studies. In one study, the role of single nucleotide polymorphisms in *Rbp4* was not considered during the evaluation of the relation between RBP4 and

TABLE 5 Methodology of studies investigating the association between RBP4 and CRP[1]

Study design	Study population	Sex	Age,[2] y	Study size, *n*	Cytokine measurement method	RBP4 measurement method	Analysis	Relation	RBP4 specimen	Authors, year (ref)
CS	Normal, obese, and overweight children	42 M; 37 F	14–6	79	Chemiluminescent immunometric assay	ELISA	Correlation	None	Serum	Aeberli et al., 2007 (12)
In	Obese adolescents	NM	14–18	15	Particle-enhanced immunonephlometry assay	Particle-enhanced immunonephlometry assay	Multivariant regression	+	Serum	Balagopal et al., 2007 (71)
CC	102 Patients with T2D and 22 controls	63 M; 39 F	61.6 ± 4.5	123	High-Sensitivity CRP assay	EIA	Correlation	None	Serum	Takebayashi et al., 2007 (20)
CS	Postmenopausal women	F	42–58	709	DPC Immulite 2000 (Diagnostic Products Corporation)	Western blot	ANOVA or KW based on RBP4 quartile	None	Serum	Huang et al., 2012 (24)
CS	Healthy Chinese	910 M; 838 F	50–70	1748	Particle-enhanced immunonephlometry assay	ELISA	Correlation	+	Plasma	Liu et al., 2014 (50)
CS	Healthy Chinese	1458 M; 1831 F	50–70	3289	Particle-enhanced immunoturbidimetric assay	Sandwich ELISA	Correlation	None	Plasma	Qi et al., 2007 (24)
CS	Nonobese, nondiabetic patients with CKD	28 M; 18 F	44–80	51	ELISA	Adipose expression	Correlation	None	Adipose biopsy	Barazzoni et al., 2011 (72)
CS	Nonobese, nondiabetic patients with CKD	29 M; 18 F	44–80	51	ELISA	ELISA	Linear and multiple regression	+	Plasma	Barazzoni et al., 2011 (72)
CC	Children with T1D or controls	51 M; 35 F	14.2	40 T1D,41 controls	ELISA	ELISA	Mann-Whitney	None	Serum	Espe et al., 2007 (73)
CC	Children with T1D or controls	51 M; 35 F	14.2	40 T1D,41 controls	ELISA	HPLC	Mann-Whitney	None	Retinol	Espe et al., 200 (73)
CC	Children with T1D or controls	51 M; 35 F	14.2	40 T1D,41 controls	ELISA	HPLC	Mann-Whitney	None	Retinol/RBP4	Espe et al., 200 (73)
CS	Healthy obese subjects	F	45 ± 9.3	63	Nephelometry	Nephelometry	NM	None	Serum	Broch et al., 2010 (74)
CS	Healthy Swedish men	M	58	100	ELISA	Western blot	Correlation	None	Serum	Wallenius et al., 2011 (75)
CS	Healthy obese and overweight children	45 M; 38 F	15–8	83	Immunonephelometry	Immunononenephelometry	ANCOVA	None	Adipose biopsy	Codoner-Franch et al., 2013 (49)
CS	Obese patients with rheumatoid arthritis	NM	NM	217	Immunoturbidimetric method	Solid-phase sandwich ELISA	Mixed regression method	None	Serum	Dessein et al., 2014 (52)
CS	Patients with ovarian cancer	F	59.2	41	NM	Latex agglutination immunoassay	Correlation	−	Serum	Watanabe et al., 2014 (76)
CC	1036 CAD patients and 1889 controls	1846 M; 1079 F	45–79	2925	Sandwich ELISA	Particle-enhanced immunonephlometry assay	Correlation	−	Serum	Mallat et al., 2009 (66)
CS	Patients with T2D	32 M; 18 F	20–80	50	NM	ELISA	Correlation	None	Serum	Park et al., 2009 (54)
CS	Healthy subjects	M	59 ± 14	153	Nepherometry	ELISA	Correlation	None	Serum	Chiba et al., 2009 (63)
CS	Healthy subjects	F	57 ± 14	224	Nepherometry	ELISA	Correlation	+	Serum	Chiba et al., 2009 (63)
CC	Healthy subjects	F	59.5 ± 6.6	472	NM	MS immunoassay	Correlation	None	Plasma	Sun et al., 2013 (37)

[1] CAD, coronary artery disease; CC, case-control; CKD, chronic kidney disease; CRP, C-reactive protein; CS, cross-sectional; F, female; In, intervention; M, male; NM, not mentioned; RBP4, retinol binding protein 4; ref, reference; T1D, type 1 diabetes; T2D, type 2 diabetes; +, positive; −, negative.

[2] Values are ranges, means ± SDs, or medians.

TABLE 6 Methodology of studies investigating the association between RBP4 and IL-6[1]

Study design	Study population	Sex	Age,[2] y	Study size, *n*	Cytokine measurement method	RBP4 measurement method	Analysis	Relation	RBP4 specimen	Authors, year (ref)
In	Obese adolescents	NM	14–18	15	ELISA	Particle-enhanced immunonephlometry assay	Correlation	+	Serum	Balagopal et al., 2007 (71)
CS	Normal and impaired glucose-tolerant subjects and obese, nonobese patients with and without T2D	164 M; 69 F	48.3 ± 1.8 to 63.2 ± 3.1	233	ELISA	Gene expression	Multivariate regression	−	Adipose biopsy	Erikstrup et al., 2009 (77)
CS	Normal and impaired glucose-tolerant subjects and obese, nonobese patients with and without T2D	164 M; 69 F	48.3 ± 1.8 to 63.2 ± 3.1	233	Muscle or adipose gene expression	Gene expression	Multivariate regression	None	Adipose biopsy	Erikstrup et al., 2009 (77)
CS	Chinese	910 M; 838 F	50–70	1748	Milliplex (Millipore) human cytokine/ chemokine panel	ELISA	Correlation	+	Plasma	Liu et al., 2014 (50)
CS	Healthy Swedish men	M	58	100	ELISA	Western blot	Correlation	None	Serum	Wallenius et al., 2011 (75)
Basement analysis of an intervention	Healthy men	M	19–29	65	Access immunoassay system	RIA kit	Partial correlation for age and BMI	None	Serum	Shea et al., 2007 (78)
CC	Patients with psoriasis	19 M; 11 F	48.6 ± 10.7	30	ELISA	ELISA	Correlation	−	Serum	Nakajima et al., 2013 (79)
CS	Healthy obese and overweight children	45 M; 38 F	15–8	83	ELISA	Immunonenephelometry	Multivariant regression	None	Serum	Codoñer-Franch et al., 2013 (49)
CS	Obese patients with rheumatoid arthritis	NM	NM	217	Solid-phase sandwich ELISA	Solid-phase sandwich ELISA	Mixed regression method	None	Serum	Dessein et al., 2014 (52)

[1] CC, case-control; CS, cross-sectional; In, intervention; F, female; M, male; NM, not mentioned; RBP4, retinol binding protein 4; ref, reference; T2D, type 2 diabetes; +, positive; −, negative.
[2] Values are ranges, means ± SDs, or medians.

TABLE 7 Association between RBP4 and other cytokines[1]

Cytokine	Study design	Study population	Sex	Age,[2] y	Study size, *n*	Cytokine measurement method	RBP4 measurement method	Analysis	Relation	RBP4 specimen	Authors, year (ref)
IL-8	CC	Patients with dilated inflammatory cardiomyopathy	48 M; 6 F	41.84 ± 9.77 to 49.54 ± 13.97	54	Multiplex ELISA (Biorad)	ELISA	Correlation	+	Plasma	Bobbert et al., 2009 (28)
IL-10	CS	Nonobese, nondiabetic CKD patients undergoing MHD treatment	29 M; 18 F	44–80	15	mRNA level	ELISA	Correlation	None	Plasma	Barazzoni et al., 2011 (72)
IL-18	CS	Healthy obese subjects	F	45 ± 9.3	63	Solid-phase enzyme immunoassay	Nephelometry	NM	None	Serum	Broch et al., 2010 (74)
CD68	Basement analysis of an intervention	Healthy obese subjects	NM	24–62	16	Gene expression	Gene expression	Correlation	+	Adipose biopsy	Yao-Borengasser et al., 2007 (53)

[1] CC, case-control; CKD, chronic kidney disease; CS, cross-sectional; F, female; M, male. MHD, maintenance hemodialysis; NM, not mentioned; RBP4, retinol binding protein 4; ref, reference; +, positive.
[2] Values are ranges or means ± SDs.

biomarkers (95). RBP4 concentrations differ according to acute inflammatory states, obesity, insulin resistance, glucose intolerance, CVDs, and the use of certain drugs (86, 87). Most of the case-control studies analyzed RBP4 in correlation with cytokines in whole study populations in which the effects of diseases and drugs may have been ignored. It is better to make correlations in patients and healthy subjects separately because inflammatory states and drugs directly affect RBP4 associations. To evaluate the impact of RBP4 on chronic inflammation in human subjects, there is an emerging need to design studies with large sample sizes in which all of the confounders can be controlled.

RBP4 in Relation to Diet and Antioxidants

Despite the conflicting results in studies on the relation between RBP4 and cytokines and CVD risk factors, lifestyle intervention studies (low-calorie diet and exercise) were less controversial in cases of lowering RBP4 concentrations. Previously published studies indicate that several drugs (20, 22, 53, 65, 96) and antioxidants (97–101) may decrease *Rbp4* gene expression. RBP4 concentrations were not influenced by the consumption of fruit and vegetable juice concentrates (100), but they were decreased by vitamin D– or vitamin D plus calcium–fortified yogurt beverages (102). However, none of the studies evaluated changes in serum retinol after a decrease in RBP4 concentrations. Despite having sufficient dietary vitamin A intake in *Rbp4* knockout mice, serum vitamin A concentrations decreased to levels similar to those seen in later stages of human vitamin A deficiency (103, 104). Because vitamin A circulates mainly in the form of holo-RBP4 in blood (3, 105), pharmacologic doses of antioxidants and anti-inflammatory drugs may possibly reduce retinol and RBP4 concentrations. However, because vitamin A is provided to peripheral tissues in the form of retinyl esters and β-carotene in chylomicrons (106), it is not clear whether a decrease in RBP4 affects human vitamin A status. The possibility of a decrease in the ratio of RBP to retinol by antioxidants, drugs, and lifestyle-induced weight loss should be investigated. Additional studies are needed to evaluate the effect of antioxidants and anti-inflammatory drugs on vitamin A utilization and alteration of liver reserves.

Conflicting results were found in studies of the relation between vitamin A intake and RBP4 concentrations. Hermsdorff et al. (88) found a positive association between vitamin A intake and RBP4 concentrations in healthy, nonobese Spanish women. Consistently, RBP deficiency in mice embryos increased the vulnerability of the embryos to alterations in maternal vitamin A intake (107). However, lower concentrations of RBP4 caused by dichlorodiphenyltrichloroethane (DDT) in patients with malaria were not associated with lower vitamin A intake (108). Similarly, *Rbp4* promoter polymorphism in mice with T2D was not associated with retinol intake (109). The relation between vitamin A intake and concentrations of RBP4 is not clear, and additional studies are needed.

Lifestyle intervention seems to be the best way of alleviating RBP4 concentrations in patients with obesity, T2D,

TABLE 8 Methodology of studies investigating the association between RBP4 and TNF-α[1]

Cytokine	Study design	Study population	Sex	Age,[2] y	Study size, *n*	Cytokine measurement method	RBP4 measurement method	Analysis	Relation	RBP4 specimen	Authors, year (ref)
TNF-α	CC	Patients with T2D	62 M; 75 F	30–60	137	ELISA	ELISA	Correlation	+	Serum	Al-Daghri et al., 2009 (35)
TNF-α	CS	Obese and nonobese T2D patients with NIGT	164 M; 69 F	48.3 ± 1.8 to 63.2 ± 3.1	233	ELISA	Gene expression	Multivariant regression	+	Adipose biopsy	Erikstrup et al., 2009 (71)
TNF-α	CS	Obese and nonobese T2D patients with NIGT	164 M; 69 F	48.3 ± 1.8 to 63.2 ± 3.1	233	Muscle gene expression	Gene expression	Multivariant regression	+	Adipose biopsy	Erikstrup et al., 2009 (71)
TNF-α	CS	Obese and nonobese T2D patients with NIGT	164 M; 69 F	48.3 ± 1.8 to 63.2 ± 3.1	233	Adipose gene expression	Gene expression	Multivariant regression	None	Adipose biopsy	Erikstrup et al., 2009 (71)
TNF-α	CS	Obese and nonobese T2D patients with NIGT	164 M; 69 F	48.3 ± 1.8 to 63.2 ± 3.1	233	Muscle gene expression	RBP or retinol or the RBP-to-retinol ratio (ELISA)	Multivariant regression	None	Plasma	Erikstrup et al., 2009 (71)
TNF-α	CS	Chinese	910 M; 838 F	50–70	1748	Milliplex (Millipore) human cytokine panel	ELISA	Correlation	+	Plasma	Liu et al., 2014 (50)
TNF-α	CS	Nonobese, nondiabetic patients with CKD	165 M; 69 F	44–80	15	mRNA level	ELISA	Correlation	+	Plasma	Barazzoni et al., 2011 (66)
TNF-α/IL-10	CS	Nonobese nondiabetic CKD patients undergoing MHD treatment	910 M; 838 F	44–80	15	mRNA level	ELISA	Correlation	+	Plasma	Barazzoni et al., 2011 (66)
sTNFR1,2	CS	Healthy obese subjects	29 M; 18 F	45 ± 9.3	63	Sandwich ELISA	Nephelometry	NM	None	Serum	Broch et al., 2010 (67)
TNF-α	CS	Healthy Swedish men	M	58	100	ELISA	Western blot	Correlation	−	Serum	Wallenius et al., 2011 (69)
sTNFR1,2	CS	Healthy Swedish men	M	58	100	ELISA	Western blot	Correlation	None	Serum	Wallenius et al., 2011 (69)
TNF-α	CC	66 NT or 92 HT patients	111 M; 47 F	49	66	ELISA	ELISA validated by Western blot	Correlation	None	Plasma	Solini et al., 2012 (51)

[1] CC, case-control; CKD, chronic kidney disease; CS, cross-sectional; F, female; HT, hypertensive; M, male; MHD, maintenance hemodialysis; NIGT, normal or impaired glucose tolerance; NM, not mentioned; NT, normotensive; RBP, retinol binding protein; RBP4, retinol binding protein 4; ref, reference; sTNFR1,2, soluble TNF-α receptors 1 and 2; T2D, type 2 diabetes; +, positive; −, negative.
[2] Values are ranges, means ± SDs, or medians.

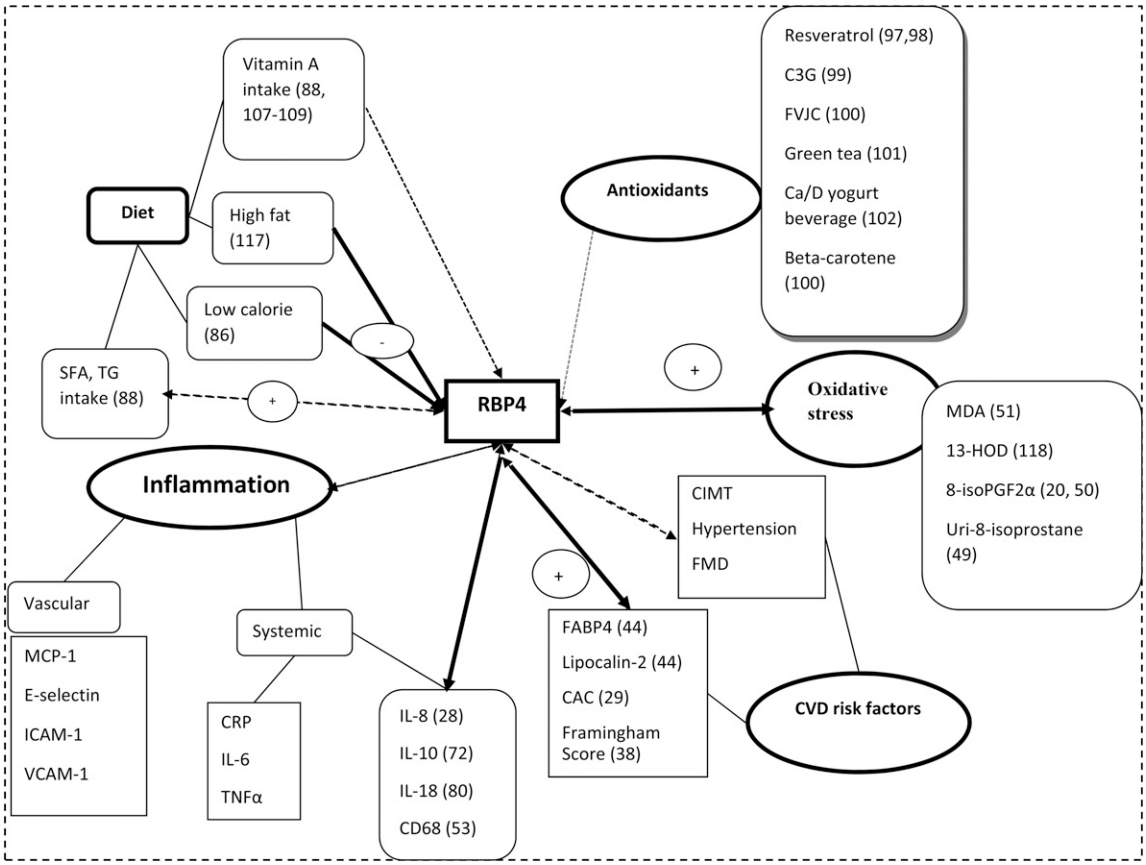

FIGURE 1 RBP4 in relation to diet, antioxidants, oxidative stress, inflammation, and CVD risk factors. C3G, cyanidin 3-glucoside; CAC, coronary artery calcification; Ca/D, calcium and vitamin D; CIMT, carotid intima media thickness; CRP, C-reactive protein; CVD, cardiovascular disease; E-selectin, endothelial-leukocyte adhesion molecule 1; FABP4, fatty acid binding protein 4; FMD, flow-mediated dilation; FVJC, fruit and vegetable juice concentrate; ICAM-1, intercellular adhesion molecule 1; MCP-1, monocyte chemoattractant protein 1; MDA, malondialdehyde; RBP4, retinol binding protein 4; Uri-8-isoprostane, urinary 8-isoprostane; VCAM-1, vascular cell adhesion molecule 1; 8-isoPGF2α, 8-iso-prostaglandin F2α; 13-HOD, 13-(S)-hydroxyoctadecadienoic acid.

and CVD. Studies have shown that a low-calorie diet considerably decreased RBP4 concentrations (13, 71, 110–116). However, the effect was dependent on the amount of weight loss as well as the quality and diversity of the diets (111, 116). In a few studies, lifestyle intervention caused a decrease in RBP4 concentrations independent of weight loss (71, 116). Because RBP4 is related to coronary risk factors (37, 38, 65) and can induce insulin resistance in lean subjects (69), weight loss is not enough to decrease RBP4 concentrations. To design a CVD prevention diet for healthy individuals, more studies are needed that will show the relations of dietary patterns, diet diversity, and different food groups with RBP4 concentrations.

Conclusions

The relation of RBP4 to diet, antioxidants, oxidative stress, inflammation, and CVDs is summarized in **Figure 1**. Although in vitro studies indicated that RBP4 directly induces insulin resistance and CVDs through inflammatory pathways, the clinical relevance of this claim is unclear. A number of human studies confirmed that higher RBP4 concentrations are positively related to inflammatory factors and CVD risks, whereas other studies showed negative or null associations. These discordances emerge from different methodologies in the measurement of RBP4 and cytokine concentrations as well as differences in characteristics of study participants. RBP4 concentrations are affected by many covariates, which need to be controlled for in studies. More longitudinal studies with larger sample sizes are needed to investigate the relation of RBP4 to inflammation, diet, and CVD risks and its role in CVD risk prediction. It is not clear in clinical studies whether apo- or holo-RBP4 is related to inflammatory markers and/or CVDs. There is an emerging need for biological research to evaluate the possible mechanisms that RBP4 exerts in oxidative stress, inflammation, and insulin resistance. Further studies are needed to show the effect of RBP4 on indexes of CVD progression and the molecular mechanisms of RBP4 on the initiation and progression of CVD.

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