The Metabolic Role of Retinol Binding Protein 4: An Update

Authors
G. A. Christou[1], A. D. Tselepis[2], D. N. Kiortsis[1]

Affiliations
[1] Laboratory of Physiology, Medical School, University of Ioannina, Ioannina, Greece
[2] Laboratory of Biochemistry, Department of Chemistry, University of Ioannina, Ioannina, Greece

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- insulin resistance
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Abstract

Retinol binding protein 4 (RBP_4) is regarded as a novel cardiometabolic risk factor, which is secreted mainly by the hepatocytes and also by the adipose tissue. RBP_4 has been shown to induce insulin resistance, and plasma RBP_4 values are increased in type 2 diabetes mellitus, obesity, metabolic syndrome, and cardiovascular disease. Moreover, it has been found that circulating RBP_4 decreases during medical interventions that result in amelioration of the metabolic profile, such as diet, exercise, oral antidiabetic drugs, and hypolipidemic agents. However, only few of the RBP_4-related studies have investigated whether RBP_4 constitutes a causal factor of the above-mentioned metabolic conditions. Importantly, circulating RBP_4 is influenced by some nonmetabolic conditions, such as renal failure, acute illness, injury, and liver failure. Thus, further studies investigating the metabolic roles of RBP_4 should be carefully planned, taking into account the effects of nonmetabolic conditions on circulating RBP_4.

Introduction

Retinol-binding protein 4 (RBP_4), a transport protein for vitamin A, is synthesized mainly by the hepatocyte and secreted into the circulation bound to vitamin A and transthyretin [1]. Although hepatocytes are regarded as the principal source of circulating RBP_4 under normal conditions, adipose tissue has the second highest expression level [2]. The only known role of RBP_4 was that of retinol transport, until 2005, when Yang et al. [3] reported that RBP_4 is a novel adipocyte-secreted hormone that is upregulated in insulin resistant states associated with obesity, and also RBP_4 provokes insulin resistance. Since then, RBP_4 has been regarded as an adipokine, which constitutes a hormone that signals changes in fatty-tissue mass and energy status in order to control fuel usage [3]. Even more, RBP_4 has been recently proposed as an emerging cardiometabolic risk factor [4].

Although, there is a considerable number of studies focusing on the various metabolic roles of RBP_4, the results of these studies are in some cases conflicting resulting in a discrepancy regarding some of the possible metabolic roles of RBP_4. From this point of view, there is a need for a critical review of these studies. To the best of our knowledge, there are no reviews investigating thoroughly all the metabolic effects of RBP_4. In this context, this article reviews the major aspects of the possible metabolic actions of RBP_4 and attempts to elucidate any resting confusion on this matter. The literature search was based on PubMed listings up to 1 August 2011.

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Correspondence
A. D. Tselepis, MD, PhD
Professor of Biochemistry – Clinical Chemistry
Department of Chemistry
University of Ioannina
45110 Ioannina
Greece
Tel.: +30/265/1008 365
Fax: +30/265/1008 785
atselep@uoi.gr

Type 2 Diabetes and Insulin Resistance

Relationship between RBP_4 and clinical and laboratory parameters of insulin resistance

Circulating RBP_4 and expression of RBP_4mRNA in abdominal adipose tissue are increased in subjects with type 2 diabetes mellitus (T2DM) or impaired glucose tolerance (IGT), compared to subjects with normal glucose tolerance (NGT) [5–7]. However, circulating RBP_4 and synthesis rates of RBP_4 appear to be lower in type 1 diabetes mellitus (T1DM) compared to normal, nondiabetic individuals [8–11]. Furthermore, circulating RBP_4 is higher in women with gestational diabetes mellitus compared to healthy pregnant women [12, 13]. Moreover, in nonobese, normoglycemic subjects with at least one first-degree relative with T2DM, serum RBP_4 levels correlate inversely with the glucose disposal rate (GDR), which is a

strong predictor of future development of diabetes in such persons, indicating that circulating RBP_4 could serve as an early prognostic marker of the future development of T2DM in such individuals [6].

Additionally, a relationship between circulating RBP_4 and biochemical markers of carbohydrate metabolism has been reported. Specifically, circulating RBP_4 has been found to be positively correlated with fasting serum glucose levels (Glc), fasting serum insulin levels (Ins), glycated hemoglobin (HbA1c) [6], and homeostasis model assessment of insulin resistance (HOMA)-index [14], and negatively correlated with GDR [6,14,15]. Furthermore, circulating RBP_4 has been reported to be positively associated with plasma glucose levels at 2 h during oral glucose tolerance test (OGTT) and negatively correlated with insulin sensitivity, as estimated by the formula of Matsuda and DeFronzo during OGTT [14,16]. Plasma RBP_4 levels are negatively correlated with peripheral insulin sensitivity, as estimated by the insulin-stimulated rates of glucose and fat oxidation, and with hepatic insulin sensitivity, as assessed by the difference in hepatic glucose production between the basal state and upon insulin infusion [16]. Notably, circulating RBP_4 has been reported to be negatively correlated with β-cell function, as estimated by the first-phase disposition index (D_{i1}) during an intravenous glucose tolerance test [16]. Thus, RBP_4 appears to be associated not only with insulin resistance but with β-cell function as well.

Mechanisms of RBP_4-induced insulin resistance

It is known that skeletal muscle is the principal site of insulin-stimulated glucose uptake, whereas adipose tissue takes up much less glucose under normal conditions [17]. Moreover, mice with markedly reduced GLUT4 expression in adipose tissue, but normal GLUT4 expression in muscle, are insulin resistant [18]. Adipose-specific deletion of GLUT4 leads to secondary defects in insulin action in muscle and liver [18]. Yang et al. showed that RBP_4 causes insulin resistance in these mice [3]. From this point of view, RBP_4 appears to constitute a factor, which is secreted by the $GLUT4^{-/-}$ adipocytes to induce insulin resistance in skeletal muscles [3].

The mechanism by which RBP_4 induces insulin resistance in mice was investigated by Yang et al. [3]. Specifically, it was found that RBP_4 causes a reduction in insulin-stimulated phosphoinositide 3-kinase [PI(3)K] activity in muscle and in insulin-stimulated tyrosine phosphorylation of insulin receptor substrate-1 (IRS1) at tyrosine residue 612, an important docking site for the p85 subunit of PI(3)K. In the same study, it was shown that RBP_4 can act directly to increase the expression of the gluconeogenic enzyme phosphoenolpyruvate kinase (Pepck) in hepatocytes and the basal glucose production and can impair the suppression of hepatic glucose production in response to insulin.

Ost and Danielsson et al. gave a new insight in the mechanism of inducement of insulin resistance by RBP_4, using subcutaneous abdominal adipose tissue from nondiabetic subjects [19]. In that study, incubation of the adipocytes with RBP_4 for 24 h reduced the insulin-induced phosphorylation of IRS1 on tyrosine and on serine 307 (phosphorylation of serine 307 by insulin results in an increased steady-state level of tyrosine phosphorylation of IRS1 in response to physiological concentrations of insulin). In adipocytes, IRS1 transmits the insulin signal further downstream via a number of signaling mediators. These mediators include protein kinase B, eventually regulating glucose uptake and other metabolic effects, and MAP kinase regulating the mitogenic signaling of insulin [20]. Ost and Danielsson et al. reported that the insulin-desensitizing effects of RBP_4 was not transmitted further downstream to reduce insulin-sensitivity for protein kinase B phosphorylation. The fact that RBP_4 treatment did not influence the metabolic signaling of insulin via protein kinase B phosphorylation was attributed to the relatively short incubation period of 24 h, which may have not been long enough to elicit full-blown insulin resistance. Interestingly, RBP_4 treatment reduced the insulin sensitivity for downstream signaling to MAP kinases ERK1/2 phosphorylation. Furthermore, in this study, the incubation of isolated adipocytes from patients with T2DM with antibodies against RBP_4 restored the ability of insulin to elicit the phosphorylation of IRS1 at serine 307 and enhanced the insulin-induced phosphorylation of the MAP kinases ERK1/2. This finding is indicative of the ability of RBP_4 to induce insulin resistance in an autocrine or paracrine fashion, in the adipose tissue of patients with T2DM.

It is noteworthy that the studies, which were performed until today, have found an inverse relationship between the expression of RBP_4mRNA and GLUT4mRNA in visceral adipose tissue [3,7]. A possible explanation is that, in states of insulin resistance, where exists a downregulation of GLUT4 expression in visceral adipose tissue, there is an increased expression of RBP_4, which may cause the insulin resistance. However, a positive relationship between RBP_4mRNA and GLUT4mRNA in subcutaneous adipose tissue has been reported [16,21,22] or no association between them [7]. A plausible explanation for these findings is that subcutaneous adipose tissue may be less important than visceral adipose tissue in determining the plasma RBP_4 levels and thus the status of insulin resistance. Furthermore, circulating RBP_4 possibly causes a compensatory upregulation of the expression of GLUT4mRNA in subcutaneous adipose tissue, as indicated by one study [19]. Therefore, RBP_4 appears to induce insulin resistance in skeletal muscle, in liver and in adipose tissue, as well.

Relationship between RBP_4 and low-grade inflammation

It is well known that obesity is associated with low-grade inflammation, which is causally involved in the development of insulin resistance [23]. Although there are some conflicting data, RBP_4 appears to be related with some markers of low-grade inflammation and by this mechanism may cause, at least in part, insulin resistance. Specifically, a positive correlation between the expression of RBP_4 and the markers of the macrophages CD68 and MCP1 in subcutaneous abdominal adipose tissue has been reported, indicating a relationship between RBP_4 and macrophage infiltration of adipose tissue [22]. Furthermore, serum RBP_4 levels are positively correlated with circulating inflammatory factors, such as high-sensitivity CRP (hsCRP) and IL-6 [24]. However, the positive relationship between circulating RBP_4 and markers of low grade inflammation should be discriminated from the negative correlation between circulating RBP_4 and factors of clinical inflammation. The latter negative correlation is attributed to the property of RBP_4 to be a negative acute phase reactant and thus circulating RBP_4 is downregulated in illness- or injury-related inflammation [25]. These conditions are fundamentally different from obesity-related low-grade inflammation.

The effects of antidiabetic drugs on RBP_4 (○ Table 1)

Regarding the impact of insulin on plasma RBP_4 levels, it can be claimed that insulin is important for protein synthesis and in this aspect the insulin deficit, which occurs in T1DM patients

Table 1 The impact of medical interventions on plasma RBP_4 levels.

Medical interventions	Plasma RBP_4 levels	Comments
Insulin	↑	Insufficient data
Thiazolidinediones	↓	Mainly in patients with T2DM
Metformin	↔	Insufficient data
Sulfonylureas	↑	Insufficient data
Exenatide	↓	Insufficient data
Acarbose	↓	Insufficient data
Diet	↓	Well established. Negative energy balance is possibly more important than body weight per se
Exercise	↓	Resistance exercise is possibly more effective in reducing circulating RBP_4, than aerobic exercise
Orlistat	↓	Unknown if there is an impact of orlistat on circulating RBP_4 independently from the applied diet
Sibutramine	↓	Unknown if there is an impact of sibutramine on circulating RBP_4 independently from the applied diet
Rimonabant	↓	The decrease in circulating RBP_4 is possibly due to the rimonabant-induced reduction in production of RBP_4 by adipose tissue
Bariatric Surgery	↓	Well established. Negative energy balance is possibly more important than body weight per se
Statins	↓ or ↔	Conflicting data
Fibrates	Early ↑ and late ↓	The metabolic action of fibrates reduces circulating RBP_4. The fenofibrate-induced change in renal function increases circulating RBP_4
Cholestyramine	↓	Insufficient data
Ezetimibe	↔	Insufficient data

T1DM: Type 1 Diabetes Mellitus; T2DM: Type 2 Diabetes Mellitus; RBP_4: Retinol binding protein 4
Symbols: ↑: Increase; ↓: Decrease; ↔: No change

may explain the decrease in circulating RBP_4 in T1DM individuals compared to normal subjects [8–11]. Consistently, untreated streptozotocin-induced diabetic rats had decreased circulating RBP_4 compared to controls, whereas insulin treated diabetic rats had increased circulating RBP_4 compared to untreated diabetic rats and lower circulating RBP_4 than controls [26]. Moreover, when visceral adipose tissue explants were cultured with recombinant insulin, there was not any change in RBP_4 secretion [27]. A plausible explanation for the above mentioned data is that, when there is not insulin deficit, insulin treatment may not have any significant impact on plasma RBP_4 levels, whereas the deprivation of the physiological actions of insulin can cause a decrease in circulating RBP_4.

Although there are some studies mentioning no change in circulating RBP_4 during thiazolidinedione treatment, circulating RBP_4 appears to decrease during thiazolidinedione treatment. Specifically, Yang et al. reported that rosiglitazone treatment of adipose $GLUT4^{-/-}$ mice completely normalized the elevated serum RBP_4 levels and reduced the elevated RBP_4mRNA levels in adipose tissue [3]. Moreover, thiazolidinedione treatment of subjects with T2DM reduces circulating RBP_4 [28–30]. However, thiazolidinedione treatment of IGT or nondiabetic subjects does not cause any change in circulating RBP_4 [22,31]. These results may be attributed to the fact that the study subjects did not have overt diabetes and thus the insulin sensitizing effects of thiazolidinediones did not fully appear. Similarly, in another study, when in the oral antidiabetic medication of subjects with T2DM 15 mg pioglitazone was added per day for 8 months, no change was found in circulating RBP_4 compared to baseline values [32]. Given the relatively low pioglitazone dose used and the fact that the patients had already been receiving antidiabetic medication, the results of this study should be interpreted with the appropriate caution.

Additionally, metformin treatment has been found to cause no significant change in circulating RBP_4 [22,29]. Furthermore, sulfonylurea treatment appears to increase plasma RBP_4 levels [30,33]. Moreover, circulating RBP_4 is reduced after treatment with exenatide [33] or acarbose [34]. It should be noticed that the main drawback of the majority of the above-mentioned studies is that the addition of the new antidiabetic drug was performed on patients already receiving antidiabetic medication. Thus, by this way the impact of the studied antidiabetic drug per se on RBP_4 metabolism cannot be concluded accurately.

Obesity

Relationship between RBP_4 and adipose tissue

Circulating RBP_4 is elevated in obese subjects compared to lean ones [3,6,24] and in morbidly obese patients compared to lean individuals [35]. Consistently, circulating RBP_4 has been found to be positively correlated with body mass index (BMI) [6,7,36], waist circumference (WC) [7,36], and waist to hip ratio [37,38], indicating that RBP_4 is related with abdominal obesity. Additionally, RBP_4mRNA in visceral and subcutaneous abdominal adipose tissue has been shown to increase in obese patients compared to lean ones [7].

RBP_4mRNA is elevated in visceral compared to subcutaneous adipose tissue and circulating RBP_4 is correlated more strongly with RBP_4mRNA in visceral adipose tissue than RBP_4mRNA in subcutaneous adipose tissue [7]. Furthermore, circulating RBP_4 is positively correlated with visceral fat area, but not with abdominal subcutaneous fat area [28], and even more the change in circulating RBP_4 after weight loss is significantly associated with the change in visceral fat area, but not with the change in subcutaneous fat area [39]. Consistently, circulating RBP_4 and RBP_4mRNA in both visceral and subcutaneous adipose depots have been found to be increased in subjects with visceral obesity compared to subjects with nonvisceral obesity [7,28,40]. The majority of the relevant studies have found that circulating RBP_4 is positively correlated with percent trunk fat (trunk fat divided by the total body fat), rather than the absolute amount of trunk fat, the total body fat or the percent body fat [14,15,41]. From all the above-mentioned data, it can be concluded that visceral adipose tissue is possibly more important than the subcutaneous one in determining plasma RBP_4 levels and even more the ratio of the visceral to the subcutaneous adipose mass appears to be more crucial than the absolute amount of these stores.

Additionally, plasma RBP_4 levels are positively correlated with liver fat [14]. Consistently, circulating RBP_4 has been found to be increased in patients with nonalcoholic fatty liver disease (NAFLD) compared to subjects who do not have NAFLD and are matched for age and gender with the patients with NAFLD [42–44]. It should be underlined that, until today, a causal link between elevated serum RBP_4 and NAFLD beyond simple association could not be convincingly established. Thus, given the fact that the presence of NAFLD is an indicator of insulin resistance, which is associated with elevated plasma RBP_4 levels, it remains to be elucidated whether RBP_4 causes or simply reflects NAFLD. Furthermore, circulating RBP_4 appears not to be associated with ectopic fat deposition in muscles [14,22,45].

Although many studies have shown an inverse relationship between the circulating RBP_4 and adiponectin [24,28,37,46–51], some other studies failed to find any association between these adipokines [14,27,52–55]. Notably, the major relevant studies, which included above 1000 Asians, reported a weak negative association [28,37,49]. Apart from the above-mentioned studies, which were performed in a steady metabolic state, an inverse relationship between the induced changes of the circulating RBP_4 and adiponectin during aerobic exercise has been shown [56]. Moreover, one study reported that when isolated adipocytes from mammary adipose tissue were incubated with adiponectin, there was no significant change in RBP_4 production, which may be attributed to the fact that mammary adipose tissue is more similar to subcutaneous adipose tissue than the visceral one [57]. Similarly, regarding high molecular weight (HMW) adiponectin, which is considered the most active form of the adiponectin and with the greatest clinical significance [58], an inverse relationship between the serum levels of RBP_4 and HMW adiponectin has been reported [50]. However, this relationship was not found in a population of nondiabetic subjects [55], indicating that this association exists mainly in diabetics. It should be noticed that, given the well known causal link between adiponectin and insulin sensitivity [58], the prevalent notion of the inducement of insulin resistance by RBP_4 is compatible with an inverse relationship between circulating RBP_4 and adiponectin. Further studies are needed to confirm the existence of this association and to investigate whether it is causal or not.

Although the studies examining nonobese subjects in a steady metabolic state did not find any relationship between circulating RBP_4 and leptin [14,46,53], a positive association between the decrease in circulating RBP_4 and the increase in circulating leptin during a carbohydrate-restricted diet has been reported [59]. Furthermore, leptin administration to ob/ob mice reduces the expression of RBP_4mRNA in adipose tissue [60]. On the contrary, when visceral adipose tissue explants from 10 nonobese women were cultured with recombinant leptin, there was an increase in RBP_4 secretion [27]. Notably, the studies mentioning an absence of negative association between RBP_4 and leptin included mostly nonobese subjects [14,27,46,53], indicating that this relationship may exist mainly in obese subjects.

Moreover, any significant association between RBP_4 and resistin does not appear to exist, because the relevant studies found a very weak association between these adipokines [61] or no significant association [46]. As for visfatin, a positive relationship between circulating RBP_4 and visfatin has been found in women with polycystic ovary syndrome (PCOS) [62].

Given the fact that the expression of the above-mentioned adipokines by the adipose tissue is known to be influenced by the presence of obesity and insulin resistance [63], the above-mentioned relationships between RBP_4 and the rest of adipokines are possible to reflect an indirect association between these adipokines, and not a causal link between them. Therefore, further studies are needed to investigate this topic.

RBP_4 during weight loss treatment

Dietary treatment

With regard to dietary interventions, the majority of the relevant studies showed a decrease in circulating RBP_4 during hypocaloric diets [14,24,59,64–70], whereas few studies did not find any change in circulating RBP_4 during such interventions [21] (**Table 1**). Importantly, when obese women followed a dietary intervention consisted of a 4 week very low-calorie diet (VLCD), a 2 month low-calorie diet and 3–4 months of a weight maintenance (WM) phase, plasma RBP_4 levels decreased during VLCD and subsequently gradually increased during LCD and WM phases [71]. Thus, at the end of the whole dietary intervention plasma RBP_4 levels were higher than the ones at the end of the VLCD, but lower than baseline values. A possible explanation for these results is that circulating RBP_4 is mainly influenced by the energy balance at a given time point, and not by the body weight per se.

It is well known that weight loss treatment causes an improvement of various metabolic parameters [72]. Therefore, the changes in circulating RBP_4 during dietary interventions that result in weight loss have been associated with the improvement of various metabolic parameters, such as BMI [64,68], liver fat [14], Ins [24,68], HOMA-index [68], hsCRP, IL-6 [24], quantitative insulin sensitivity check index (QUICKI) [68], insulin sensitivity index of Matsuda and DeFronzo during OGTT [14], and fractional catabolic rate (FCR) of LDL ApoB-100 [67].

The magnitude of the diet-induced decrease in circulating RBP_4 depended not only on the amount of weight loss, but also on the qualitative characteristics of the applied diet [59,70]. Specifically, carbohydrate-restricted diet results in greater reduction in serum RBP_4 levels compared to low-fat diet [59]. Moreover, during an application of a hypocaloric Mediterranean diet, the reduction in RBP_4 is significantly greater in individuals with a higher adherence to Mediterranean dietary pattern than individuals with lower adherence, independently of the magnitude of caloric restriction or weight loss [70].

Exercise

Regarding the exercise-induced changes in RBP_4, most of the relevant studies have shown that exercise reduces circulating RBP_4 [6,56,73,74], whereas some of them did not find any change in RBP_4 [75,76] (**Table 1**). There have been some reports of the influence of RBP_4 by the quantitative, as well as the qualitative characteristics of the physical activity [73,74]. Specifically, the vigorous-intensity activity is associated with lower circulating RBP_4, but moderate-intensity activity, low-intensity activity, or walking does not have any significant impact on RBP_4 [73]. Furthermore, circulating RBP_4 has been shown to decrease only during a resistance exercise program, but not during aerobic exercise [74]. Although the existing studies have shown that the improvement in insulin sensitivity after resistance exercise is similar with that after aerobic exercise [77], resistance exercise possibly has more favorable effects in the insulin sensitivity of skeletal muscles, as indicated by the above-mentioned more pronounced decrease in circulating RBP_4 during resistance exercise. Additionally, the exercise-induced change in RBP_4 is associ-

ated with the improvement in various metabolic parameters, such as GDR [6], WC, TRG, Glc, Ins, HOMA-index and the area under the curve of glucose during OGTT ($AUC_{glucose}$) [56].

Pharmacotherapy (○ Table 1)

Plasma RBP_4 levels decrease after the application of a weight loss program including caloric restriction with the concomitant administration of orlistat [78]. Similar results have been found concerning sibutramine [39,79]. However, in the studies with orlistat and sibutramine, the noticed reduction in circulating RBP_4 may has been caused due to the caloric restriction per se. Thus, it cannot be concluded whether the administration of orlistat or sibutramine has any additive impact on the reduction of circulating RBP_4 apart from the caloric restriction. As for the impact of CB1 blockers on RBP_4, there is only one study in humans, which was performed by our group [64]. In this study, it was found that rimonabant treatment along with a dietary intervention of obese subjects with hypertriglyceridemia for 3 months resulted in reduction of circulating RBP_4 and circulating RBP_4 was positively correlated with the percentage change of HOMA-index [64]. A possible mechanism for the rimonabant-induced reduction in circulating RBP_4 is the decrease in excretion of RBP_4 from adipose tissue, as indicated by a study showing that rimonabant treatment reduced RBP_4mRNA expression in visceral adipose tissue of ob/ob mice [80].

Surgical management

Most of the studies investigating the impact of bariatric surgery (gastric bypass or gastric banding) on circulating RBP_4 reported a decrease in circulating RBP_4 (○ **Table 1**), which was associated with concomitant improvements in various metabolic parameters [35,69,81–84]. Furthermore, the reduction in circulating RBP_4 after bariatric surgery occurs mainly during periods of active weight loss, whereas the decrease in circulating RBP_4 is minimal during periods of stabilized weight loss [81,83]. In this aspect, these results are in agreement with the above-mentioned results in dietary intervention studies. Therefore, these results imply that RBP_4 may be considered as a dynamic marker of negative energy balance, being reduced during weight loss when a negative energy balance threshold is reached, independently of the BMI of the individuals at a given time point. Moreover, there was no difference in the induced changes in circulating RBP_4 between patients undergoing gastric banding and gastric bypass [84].

Metabolic Syndrome

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All the previous studies have shown that circulating RBP_4 is higher in patients with metabolic syndrome (MS) than in subjects without MS [36,37,48,49,85]. Moreover, circulating RBP_4 has been associated with the number of the factors of MS [36,37] and also with the value of each individual constituent of MS. Specifically, circulating RBP_4 has been found to increase in the following states: hypertriglyceridemia, low HDL-C, hypertension, increased WC, and hyperglycemia. Among these factors, the strongest and more steady association with RBP_4 has been noticed for hypertriglyceridemia [36,37], whereas the weakest and the least frequent association with RBP_4 has been found for hyperglycemia [37,85].

Lipoprotein Metabolism

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Relationship between RBP_4 and lipid parameters

Circulating RBP_4 is positively correlated with total cholesterol (TC) [36,52], low density lipoprotein cholesterol (LDL-C) [7,52,67,86], triglycerides (TRG) [6,7,28,36,86], apolipoprotein B (ApoB) [87], small dense LDL cholesterol (sdLDL-C) [52] and negatively correlated with high density lipoprotein cholesterol (HDL-C) [7,86]. It should be noticed that the above-mentioned associations were found in a steady metabolic state (without any significant recent change in nutrient intake and body weight). Importantly, almost all of the relevant studies reported an association of RBP_4 with TRG. Furthermore, a study by our group investigated the relationship between the changes in circulating RBP_4 and the changes in parameters of lipoprotein metabolism, during medical interventions that alter the lipoprotein profile [64]. Specifically, in this study obese, hypertriglyceridemic patients followed dietary or fenofibrate treatment for 3 months. It was found that the percentage change of plasma RBP_4 levels during diet was positively correlated with the percentage change of TRG, very low density lipoprotein-cholesterol (VLDL-C), LDL-C, non-HDL-cholesterol (non-HDL-C), ApoB, and sdLDL-C. Similar associations were also reported during fenofibrate treatment. Multiple regression analysis revealed that the percentage change of circulating RBP_4 was the best predictor of the diet-induced percentage change of ApoB. As the possible mechanism of the relationship between RBP_4 and the ApoB-containing lipoproteins was suggested the regulation of the fractional catabolic rate (FCR) of LDL ApoB100, as indicated by another study [67]. In this aspect, RBP_4 appears to be linked with the metabolic pathway, which is responsible for the diet-induced changes in ApoB-containing lipoproteins. This link may be causal or not. To our knowledge, this matter has not been elucidated yet. Moreover, among all the associations of RBP_4 with the ApoB-containing lipoprotein subspecies, the strongest of them was the one referring to sdLDL-C. Furthermore, it was found that the percentage change of circulating RBP_4 was the best predictor of the diet-induced percentage change of sdLDL-C. The same study proposed that the mechanism linking RBP_4 with sdLDL may include not only the overproduction of sdLDL due to the increased VLDL-C, but also the regulation of delipidation cascade of triglyceride-rich LDL particles. Specifically, this regulation may be attributed to hepatic lipase activity, since a positive association between circulating RBP_4 and hepatic lipase activity has been previously reported [88].

Importantly, the majority of the RBP_4-related studies have reported strong and persistent associations with the ApoB-containing lipoproteins, whereas there are much fewer studies mentioning an association of RBP_4 with the ApoAI-containing HDL-C. Possibly, the inverse relationship between RBP_4 and HDL-C can be attributed to the positive association between RBP_4 and TRG and the well known inverse relationship between TRG and HDL-C [89]. From this point of view, the relationship between RBP_4 and HDL-C may reflect the previously reported strong association between RBP_4 and TRG. Notably, most of the studies investigating the relationship between RBP_4 and LDL-C assessed LDL-C by its indirect calculation using the Friedewald equation [LDL-C=TC−(HDL-C+TRG/5)], which is more inaccurate than the direct measurement of LDL-C, especially in subjects with considerable hypertriglyceridemia [90,91]. Moreover, studies assessing LDL-C by its direct measurement with lipoprotein electrophoresis found quite steady and strong associations

between RBP_4 and LDL-C [52,64,86]. Thus, the absence or the weakness of the association between RBP_4 and LDL-C, which has been reported in some studies, may not be true. Furthermore, to the best of our knowledge, there is only one study investigating the association of circulating RBP_4 with sdLDL-C, in a steady metabolic state [52]. This study found a positive relationship between circulating RBP_4 and sdLDL-C, in a population of young women. Notably, all these women had normal TRG. Given the fact that small dense LDL particles predominate in states of hypertriglyceridemia [92], the positive relationship between circulating RBP_4 and sdLDL-C needs to be confirmed in hypertriglyceridemic subjects, as well.

Although, there are some clinical studies mentioning no association between circulating RBP_4 and serum free fatty acids (FFAs) levels [14,45], $RBP_4^{+/-}$ and $RBP_4^{-/-}$ mice have decreased circulating FFAs compared to wild type mice [3]. A possible explanation is that RBP_4 may be involved in the regulation of FFA metabolism, but RBP_4 alone does not appear to be significant enough to determine serum FFA levels, at least in normal states.

RBP_4 levels and hypolipidemic agents (○ Table 1)

Statins cause a significant reduction of circulating RBP_4 [93] or not change [31] or a nonsignificant trend for decrease [94,95], which may be attributed either to the not long enough period of statin administration to induce changes in circulating RBP_4 [94] or to the relative low statin dose used [95]. The mechanism underlying the potential statin-induced reduction of circulating RBP_4 may involve the statin-LDL lowering effect, given the previously reported relationship between RBP_4 and LDL metabolism [64,67].

Fibrates decrease RBP_4 levels [64,93,96], possibly due to the fenofibrate-induced suppression of RBP_4mRNA levels, in adipose tissue [96]. Furthermore, a study by our group [64] investigated the impact of 3-month fenofibrate treatment on plasma RBP_4 levels, in obese hypertriglyceridemic patients. Specifically, this study elucidated that fenofibrate caused an increase in serum creatinine and a decrease in renal excretion of proteins. Given the fact that, RBP_4 belongs to low molecular weight proteins that are traced in urine samples [97], the early rise in circulating RBP_4 during the first month of fenofibrate treatment was attributed to the fenofibrate-induced decrease in renal clearance of RBP_4. Subsequently, the fall in circulating RBP_4 during the following 2 months resulted from the metabolic action of fenofibrate.

Circulating RBP_4 decreases after combination treatment of diet and cholestyramine [93]. However, in this case, it can not be excluded that the diet caused the decrease in circulating RBP_4, whereas the cholestyramine per se did not have any significant impact on circulating RBP_4. It has been reported that ezetimibe treatment does not influence plasma RBP_4 levels [98].

Cardiovascular Disease

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Circulating RBP_4 has been found to be associated with some measures of subclinical cardiovascular disease (CVD). Specifically, plasma RBP_4 levels have been shown to be positively correlated with the echocardiographically measured left ventricular wall thickness and carotid intima-media thickness (IMT) and negatively correlated with the flow-mediated dilatation (FMD), as a measure of endothelial function, and with the gray scale median in IMT (GSM-IMT) (a lower value of GSM-IMT corresponds to a higher fat content of the carotid vessel wall) [53,99–101]. Consistently, the presence of clinical arteriosclerosis (defined as the presence of at least one of the following: coronary heart disease, stroke, or peripheral vascular disease) is associated with higher circulating RBP_4 and this is also observed when every vascular disease category is considered separately [4]. Similarly, circulating RBP_4 has been associated with any prior cerebrovascular disease and with any prior hospitalization for CVD [36]. Moreover, the circulating RBP_4 of patients who had fatal or nonfatal coronary artery disease during follow-up is higher compared to that of the individuals who remained free of cardiovascular disease during follow-up [87]. Furthermore, acute or subacute cerebral infarction has been associated with elevated circulating RBP_4 [50]. Although a well-documented relationship exists between RBP_4 and CVD, it remains to be elucidated whether RBP_4 is causally involved in the development of CVD.

Regarding the possible mechanism underlying the association of RBP_4 with CVD, in subjects with T2DM, circulating RBP_4 is positively associated with the soluble adhesion molecules sICAM-1 and sE-selectin, indicating that circulating RBP_4 may be responsible for the development of vascular complications in T2DM [53]. Moreover, given the well known strong association of RBP_4 with the atherogenic ApoB-containing lipoproteins and especially with triglycerides, this relationship appears to be a plausible mechanism linking at least in part RBP_4 with CVD. Indeed, the association between circulating RBP_4 and CVD becomes insignificant after adjustment for TRG [36,87]. Furthermore, the negative association between mean IMT and retinol/RBP_4 ratio persists even after adjustment for established cardiovascular risk factors [101]. Thus, given that the retinol/RBP_4 ratio indicates the saturation of RBP_4 with retinol, retinol-free RBP_4 (apo-RBP_4) may have a specific role in the development of atherosclerosis.

The Impact of Renal Function on Circulating RBP_4

It is well known that RBP_4 is filtered through the glomerulus and subsequently is reabsorbed into the proximal tubular cells [102,103]. Moreover, RBP_4 belongs to low molecular weight proteins that are traced in urine samples [97]. From this point of view, plasma RBP_4 levels are positively correlated with serum creatinine and degree of albuminuria, negatively correlated with Glomerular Filtration Rate (GFR) and they generally increase in renal dysfunction [104,105].

Measurement of RBP_4

Regarding the procedure of measurement circulating RBP_4, the majority of the relevant studies have used the ELISA method and the rest have implied quantitative Western blotting or nephelometry. ELISA method has been reported to underestimate circulating RBP_4 in diabetic subjects, due to assay saturation, and quantitative Western blotting has been proposed as the most reliable method for assaying circulating RBP_4 [106]. However, the procedure of quantitative Western blotting is too laborious, time-consuming and it needs the appropriate experience. Thus, it is not practical for studies using a big number of samples. Furthermore, ELISA results are similar and are strongly associated with Western blotting results [22,83,107,108] and ELISA method is much easier and more quicker than Western blotting. From this point of view, ELISA method appears to be a practical and respectable enough choice in measuring circulating RBP_4. Another aspect, which should be taken into account in the measurement of circulating RBP_4, is whether there have been used plasma or serum samples, because plasma anticoagulants may

cause spurious results [106]. However, manufacturers usually advocate the use of either serum or plasma samples.

Conclusions

RBP_4 appears to be an adipokine, which induces insulin resistance and is possibly involved in the pathogenesis of the metabolic complications of obesity. Importantly, the existence of a close association of the RBP_4 both with the atherogenic ApoB-containing lipoproteins and CVD has been reported. Notably, an important limitation of some of the RBP_4-related studies is that they included patients who received drugs, known to influence circulating RBP_4, such as oral antidiabetic drugs and hypolipidemic agents, or patients in conditions influencing circulating RBP_4, such as renal or hepatic damage. Furthermore, regarding the associations of RBP_4 with some metabolic parameters, there are no studies investigating the aspect of causality. In other words, the establishment of the various potential metabolic roles of RBP_4 demands the demonstration that RBP_4 is causally linked with the above-mentioned metabolic parameters. Therefore, further carefully planned studies are needed, focusing on the investigation of whether RBP_4 constitutes a causal metabolic factor and on the molecular mechanism of action of RBP_4.

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