The Metabolic Role of Retinol Binding Protein 4: An Update

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Key words

- insulin resistance
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- atherosclerosis

Abstract

Retinol binding protein 4 (RBP₄) is regarded as a novel cardiometabolic risk factor, which is secreted mainly by the hepatocytes and also by the adipose tissue. RBP₄ has been shown to induce insulin resistance, and plasma RBP₄ values are increased in type 2 diabetes mellitus, obesity, metabolic syndrome, and cardiovascular disease. Moreover, it has been found that circulating RBP₄ 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 RBP₄-related studies have investigated whether RBP₄ constitutes a causal factor of the above-mentioned metabolic conditions. Importantly, circulating RBP₄ is influenced by some nonmetabolic conditions, such as renal failure, acute illness, injury, and liver failure. Thus, further studies investigating the metabolic roles of RBP₄ should be carefully planned, taking into account the effects of nonmetabolic conditions on circulating RBP₄.

Introduction

Retinol-binding protein 4 (RBP₄), 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 RBP₄ under normal conditions, adipose tissue has the second highest expression level [2]. The only known role of RBP₄ was that of retinol transport, until 2005, when Yang et al. [3] reported that RBP₄ is a novel adipocyte-secreted hormone that is upregulated in insulin resistant states associated with obesity, and also RBP₄ provokes insulin resistance. Since then, RBP₄ 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, RBP₄ 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 RBP₄, the results of these studies are in some cases conflicting resulting in a discrepancy regarding some of the possible metabolic roles of RBP₄. 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 RBP₄. In this context, this article reviews the major aspects of the possible metabolic actions of RBP₄ and attempts to elucidate any resting confusion on this matter. The literature search was based on PubMed listings up to 1 August 2011.

Type 2 Diabetes and Insulin Resistance

Relationship between RBP₄ and clinical and laboratory parameters of insulin resistance

Circulating RBP₄ and expression of RBP₄mRNA 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 RBP₄ and synthesis rates of RBP₄ appear to be lower in type 1 diabetes mellitus (T1DM) compared to normal, nondiabetic individuals [8–11]. Furthermore, circulating RBP₄ 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 RBP₄ levels correlate inversely with the glucose disposal rate (GDR), which is a

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Bibliography

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strong predictor of future development of diabetes in such persons, indicating that circulating RBP₄ could serve as an early prognostic marker of the future development of T2DM in such individuals [6].

Additionally, a relationship between circulating RBP₄ and biochemical markers of carbohydrate metabolism has been reported. Specifically, circulating RBP₄ 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 RBP₄ 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 RBP₄ 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 RBP₄ 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, RBP₄ appears to be associated not only with insulin resistance but with β -cell function as well.

Mechanisms of RBP₄-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 RBP₄ causes insulin resistance in these mice [3]. From this point of view, RBP₄ appears to constitute a factor, which is secreted by the GLUT4^{-/-} adipocytes to induce insulin resistance in skeletal muscles [3].

The mechanism by which RBP₄ induces insulin resistance in mice was investigated by Yang et al. [3]. Specifically, it was found that RBP₄ 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 RBP₄ 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 RBP₄, using subcutaneous abdominal adipose tissue from nondiabetic subjects [19]. In that study, incubation of the adipocytes with RBP₄ 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 RBP₄ was not transmitted further downstream to reduce insulin-sensitivity for protein kinase B phosphorylation. The fact that RBP₄ 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, RBP₄ 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 RBP₄ 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 RBP₄ 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 RBP₄mRNA 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 RBP₄, which may cause the insulin resistance. However, a positive relationship between RBP₄mRNA 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 RBP₄ levels and thus the status of insulin resistance. Furthermore, circulating RBP₄ possibly causes a compensatory upregulation of the expression of GLUT4mRNA in subcutaneous adipose tissue, as indicated by one study [19]. Therefore, RBP₄ appears to induce insulin resistance in skeletal muscle, in liver and in adipose tissue, as well.

Relationship between RBP₄ 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, RBP₄ 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 RBP₄ and the markers of the macrophages CD68 and MCP1 in subcutaneous abdominal adipose tissue has been reported, indicating a relationship between RBP₄ and macrophage infiltration of adipose tissue [22]. Furthermore, serum RBP₄ levels are positively correlated with circulating inflammatory factors, such as high-sensitivity CRP (hsCRP) and IL-6 [24]. However, the positive relationship between circulating RBP₄ and markers of low grade inflammation should be discriminated from the negative correlation between circulating RBP₄ and factors of clinical inflammation. The latter negative correlation is attributed to the property of RBP₄ to be a negative acute phase reactant and thus circulating RBP₄ 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 RBP₄ (see Table 1)

Regarding the impact of insulin on plasma RBP₄ 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 RBP₄ 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; RBP₄: Retinol binding protein 4

Symbols: ↑: Increase; ↓: Decrease; ↔: No change

may explain the decrease in circulating RBP₄ in T1DM individuals compared to normal subjects [8–11]. Consistently, untreated streptozotocin-induced diabetic rats had decreased circulating RBP₄ compared to controls, whereas insulin treated diabetic rats had increased circulating RBP₄ compared to untreated diabetic rats and lower circulating RBP₄ than controls [26]. Moreover, when visceral adipose tissue explants were cultured with recombinant insulin, there was not any change in RBP₄ 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 RBP₄ levels, whereas the deprivation of the physiological actions of insulin can cause a decrease in circulating RBP₄.

Although there are some studies mentioning no change in circulating RBP₄ during thiazolidinedione treatment, circulating RBP₄ appears to decrease during thiazolidinedione treatment. Specifically, Yang et al. reported that rosiglitazone treatment of adipose GLUT4^{-/-} mice completely normalized the elevated serum RBP₄ levels and reduced the elevated RBP₄mRNA levels in adipose tissue [3]. Moreover, thiazolidinedione treatment of subjects with T2DM reduces circulating RBP₄ [28–30]. However, thiazolidinedione treatment of IGT or nondiabetic subjects does not cause any change in circulating RBP₄ [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 RBP₄ 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 RBP₄ [22,29]. Furthermore, sulfonylurea treatment appears to increase plasma RBP₄ levels [30,33]. Moreover, circulating RBP₄ 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 RBP₄ metabolism cannot be concluded accurately.

Obesity

Relationship between RBP₄ and adipose tissue

Circulating RBP₄ is elevated in obese subjects compared to lean ones [3,6,24] and in morbidly obese patients compared to lean individuals [35]. Consistently, circulating RBP₄ 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 RBP₄ is related with abdominal obesity. Additionally, RBP₄mRNA in visceral and subcutaneous abdominal adipose tissue has been shown to increase in obese patients compared to lean ones [7].

RBP₄mRNA is elevated in visceral compared to subcutaneous adipose tissue and circulating RBP₄ is correlated more strongly with RBP₄mRNA in visceral adipose tissue than RBP₄mRNA in subcutaneous adipose tissue [7]. Furthermore, circulating RBP₄ is positively correlated with visceral fat area, but not with abdominal subcutaneous fat area [28], and even more the change in circulating RBP₄ 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 RBP₄ and RBP₄mRNA 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 RBP₄ 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 RBP₄ 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 RBP₄ levels are positively correlated with liver fat [14]. Consistently, circulating RBP₄ 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 RBP₄ 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 RBP₄ levels, it remains to be elucidated whether RBP₄ causes or simply reflects NAFLD. Furthermore, circulating RBP₄ 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 RBP₄ 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 RBP₄ 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 RBP₄ 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 RBP₄ 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 RBP₄ is compatible with an inverse relationship between circulating RBP₄ 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 RBP₄ and leptin [14,46,53], a positive association between the decrease in circulating RBP₄ 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 RBP₄mRNA 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 RBP₄ secretion [27]. Notably, the studies mentioning an absence of negative association between RBP₄ 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 RBP₄ 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 RBP₄ 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 RBP₄ 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.

RBP₄ during weight loss treatment

Dietary treatment

With regard to dietary interventions, the majority of the relevant studies showed a decrease in circulating RBP₄ during hypocaloric diets [14,24,59,64–70], whereas few studies did not find any change in circulating RBP₄ 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 RBP₄ levels decreased during VLCD and subsequently gradually increased during LCD and WM phases [71]. Thus, at the end of the whole dietary intervention plasma RBP₄ 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 RBP₄ 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 RBP₄ 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 RBP₄ 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 RBP₄ levels compared to low-fat diet [59]. Moreover, during an application of a hypocaloric Mediterranean diet, the reduction in RBP₄ 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 RBP₄, most of the relevant studies have shown that exercise reduces circulating RBP₄ [6,56,73,74], whereas some of them did not find any change in RBP₄ [75,76] (● **Table 1**). There have been some reports of the influence of RBP₄ 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 RBP₄, but moderate-intensity activity, low-intensity activity, or walking does not have any significant impact on RBP₄ [73]. Furthermore, circulating RBP₄ 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 RBP₄ during resistance exercise. Additionally, the exercise-induced change in RBP₄ 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 RBP₄ 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 RBP₄ may have 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 RBP₄ apart from the caloric restriction. As for the impact of CB1 blockers on RBP₄, 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 RBP₄ and circulating RBP₄ was positively correlated with the percentage change of HOMA-index [64]. A possible mechanism for the rimonabant-induced reduction in circulating RBP₄ is the decrease in excretion of RBP₄ from adipose tissue, as indicated by a study showing that rimonabant treatment reduced RBP₄mRNA 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 RBP₄ reported a decrease in circulating RBP₄ (○ Table 1), which was associated with concomitant improvements in various metabolic parameters [35,69,81–84]. Furthermore, the reduction in circulating RBP₄ after bariatric surgery occurs mainly during periods of active weight loss, whereas the decrease in circulating RBP₄ 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 RBP₄ 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 RBP₄ between patients undergoing gastric banding and gastric bypass [84].

Metabolic Syndrome

All the previous studies have shown that circulating RBP₄ is higher in patients with metabolic syndrome (MS) than in subjects without MS [36,37,48,49,85]. Moreover, circulating RBP₄ 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 RBP₄ 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 RBP₄ has been noticed for hypertriglyceridemia [36,37], whereas the weakest and the least frequent association with RBP₄ has been found for hyperglycemia [37,85].

Lipoprotein Metabolism

Relationship between RBP₄ and lipid parameters

Circulating RBP₄ 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 RBP₄ with TRG. Furthermore, a study by our group investigated the relationship between the changes in circulating RBP₄ 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 RBP₄ 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 RBP₄ was the best predictor of the diet-induced percentage change of ApoB. As the possible mechanism of the relationship between RBP₄ 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, RBP₄ 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 RBP₄ 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 RBP₄ was the best predictor of the diet-induced percentage change of sdLDL-C. The same study proposed that the mechanism linking RBP₄ 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 RBP₄ and hepatic lipase activity has been previously reported [88].

Importantly, the majority of the RBP₄-related studies have reported strong and persistent associations with the ApoB-containing lipoproteins, whereas there are much fewer studies mentioning an association of RBP₄ with the ApoAI-containing HDL-C. Possibly, the inverse relationship between RBP₄ and HDL-C can be attributed to the positive association between RBP₄ and TRG and the well known inverse relationship between TRG and HDL-C [89]. From this point of view, the relationship between RBP₄ and HDL-C may reflect the previously reported strong association between RBP₄ and TRG. Notably, most of the studies investigating the relationship between RBP₄ 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 RBP₄ and LDL-C [52,64,86]. Thus, the absence or the weakness of the association between RBP₄ 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 RBP₄ with sdLDL-C, in a steady metabolic state [52]. This study found a positive relationship between circulating RBP₄ 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 RBP₄ and sdLDL-C needs to be confirmed in hypertriglyceridemic subjects, as well.

Although, there are some clinical studies mentioning no association between circulating RBP₄ and serum free fatty acids (FFAs) levels [14,45], RBP₄^{+/-} and RBP₄^{-/-} mice have decreased circulating FFAs compared to wild type mice [3]. A possible explanation is that RBP₄ may be involved in the regulation of FFA metabolism, but RBP₄ alone does not appear to be significant enough to determine serum FFA levels, at least in normal states.

RBP₄ levels and hypolipidemic agents (see Table 1)

Statins cause a significant reduction of circulating RBP₄ [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 RBP₄ [94] or to the relative low statin dose used [95]. The mechanism underlying the potential statin-induced reduction of circulating RBP₄ may involve the statin-LDL lowering effect, given the previously reported relationship between RBP₄ and LDL metabolism [64,67].

Fibrates decrease RBP₄ levels [64,93,96], possibly due to the fenofibrate-induced suppression of RBP₄ mRNA levels, in adipose tissue [96]. Furthermore, a study by our group [64] investigated the impact of 3-month fenofibrate treatment on plasma RBP₄ 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, RBP₄ belongs to low molecular weight proteins that are traced in urine samples [97], the early rise in circulating RBP₄ during the first month of fenofibrate treatment was attributed to the fenofibrate-induced decrease in renal clearance of RBP₄. Subsequently, the fall in circulating RBP₄ during the following 2 months resulted from the metabolic action of fenofibrate.

Circulating RBP₄ 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 RBP₄, whereas the cholestyramine per se did not have any significant impact on circulating RBP₄. It has been reported that ezetimibe treatment does not influence plasma RBP₄ levels [98].

Cardiovascular Disease

Circulating RBP₄ has been found to be associated with some measures of subclinical cardiovascular disease (CVD). Specifically, plasma RBP₄ 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]. Consist-

ently, 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 RBP₄ and this is also observed when every vascular disease category is considered separately [4]. Similarly, circulating RBP₄ has been associated with any prior cerebrovascular disease and with any prior hospitalization for CVD [36]. Moreover, the circulating RBP₄ 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 RBP₄ [50]. Although a well-documented relationship exists between RBP₄ and CVD, it remains to be elucidated whether RBP₄ is causally involved in the development of CVD.

Regarding the possible mechanism underlying the association of RBP₄ with CVD, in subjects with T2DM, circulating RBP₄ is positively associated with the soluble adhesion molecules sICAM-1 and sE-selectin, indicating that circulating RBP₄ may be responsible for the development of vascular complications in T2DM [53]. Moreover, given the well known strong association of RBP₄ with the atherogenic ApoB-containing lipoproteins and especially with triglycerides, this relationship appears to be a plausible mechanism linking at least in part RBP₄ with CVD. Indeed, the association between circulating RBP₄ and CVD becomes insignificant after adjustment for TRG [36,87]. Furthermore, the negative association between mean IMT and retinol/RBP₄ ratio persists even after adjustment for established cardiovascular risk factors [101]. Thus, given that the retinol/RBP₄ ratio indicates the saturation of RBP₄ with retinol, retinol-free RBP₄ (apo-RBP₄) may have a specific role in the development of atherosclerosis.

The Impact of Renal Function on Circulating RBP₄

It is well known that RBP₄ is filtered through the glomerulus and subsequently is reabsorbed into the proximal tubular cells [102,103]. Moreover, RBP₄ belongs to low molecular weight proteins that are traced in urine samples [97]. From this point of view, plasma RBP₄ 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 RBP₄

Regarding the procedure of measurement circulating RBP₄, 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 RBP₄ in diabetic subjects, due to assay saturation, and quantitative Western blotting has been proposed as the most reliable method for assaying circulating RBP₄ [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 RBP₄. Another aspect, which should be taken into account in the measurement of circulating RBP₄, 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

RBP₄ 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 RBP₄ both with the atherogenic ApoB-containing lipoproteins and CVD has been reported. Notably, an important limitation of some of the RBP₄-related studies is that they included patients who received drugs, known to influence circulating RBP₄, such as oral antidiabetic drugs and hypolipidemic agents, or patients in conditions influencing circulating RBP₄, such as renal or hepatic damage. Furthermore, regarding the associations of RBP₄ 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 RBP₄ demands the demonstration that RBP₄ is causally linked with the above-mentioned metabolic parameters. Therefore, further carefully planned studies are needed, focusing on the investigation of whether RBP₄ constitutes a causal metabolic factor and on the molecular mechanism of action of RBP₄.

References

- Blaner WS. Retinol-binding protein: the serum transport protein for vitamin A. *Endocr Rev* 1989; 10: 308–316
- Tsutsumi C, Okuno M, Tannous L, Piantadosi R, Allan M, Goodman DS, Blaner WS. Retinoids and retinoid-binding protein expression in rat adipocytes. *J Biol Chem* 1992; 267: 1805–1810
- Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, Kotani K, Quadro L, Kahn BB. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature* 2005; 436: 356–362
- Cabre A, Lazaro I, Girona J, Manzanares AF, Plana N, Heras M, Masana L. Retinol-binding protein 4 as a plasma biomarker of renal dysfunction and cardiovascular disease. *J Intern Med* 2007; 262: 496–503
- Cho YM, Youn BS, Lee H, Lee N, Min SS, Kwak SH, Lee HK, Park KS. Plasma retinol-binding protein-4 concentrations are elevated in human subjects with impaired glucose tolerance and type 2 diabetes. *Diabetes Care* 2006; 29: 2457–2461
- Graham TE, Yang Q, Bluher M, Hammarstedt A, Ciaraldi TP, Henry RR, Wason CJ, Oberbach A, Jansson P, Smith U, Kahn BB. Retinol-Binding Protein 4 and Insulin Resistance in Lean, Obese, and Diabetic Subjects. *N Engl J Med* 2006; 354: 2552–2563
- Kloting N, Graham TE, Berndt J, Kralisch S, Kovacs P, Wason CJ, Fasshauer M, Schon MR, Stumvoll M, Bluher M, Kahn BB. Serum Retinol-Binding Protein is more highly expressed in visceral than in subcutaneous adipose tissue and is a marker of intra-abdominal fat mass. *Cell Metab* 2007; 6: 79–87
- Kemp SF, Frindik JP. Effect of metabolic control on serum protein concentrations in diabetes. *Acta Paediatr Scand* 1991; 80: 938–943
- Basu TK, Tze WJ, Leichter J. Serum vitamin A and retinol-binding protein in patients with insulin-dependent diabetes mellitus. *Am J Clin Nutr* 1989; 50: 329–331
- Jourdan M, Jaleel A, Karakelides H, Ford GC, Kahn BB, Nair KS. Impact of type 1 diabetes and insulin treatment on plasma levels and fractional synthesis rate of retinol-binding protein 4. *J Clin Endocrinol Metab* 2009; 94: 5125–5130
- Krill D, O'Leary L, Koehler AN, Kramer MK, Warty V, Wagner MA, Dorman JS. Association of retinol binding protein in multiple-case families with insulin-dependent diabetes. *Hum Biol* 1997; 69: 89–96
- Chan TF, Chen HS, Chen YC, Lee CH, Chou FH, Chen JJ, Chen SY, Jong SB, Tsai EM. Increased serum retinol-binding protein 4 concentrations in women with gestational diabetes mellitus. *Reprod Sci* 2007; 14: 169–174
- Su Y, Hong J, Yan Q, Xu C, Gu W, Zhang Y, Shen C, Chi Z, Dai M, Xu M, Zhang Y, Liu Q, Wang WQ, Li XY, Ning G. Increased serum retinol-binding protein-4 levels in pregnant women with and without gestational diabetes mellitus. *Diabetes Metab* 2010; 36: 470–475
- Stefan N, Hennige AM, Staiger H, Machann J, Shick F, Schleicher E, Fritsche A, Haring HU. High circulating retinol-binding protein 4 is associated with elevated liver fat but not with total, subcutaneous, visceral, or intramyocellular fat in humans. *Diabetes Care* 2007; 30: 1173–1178
- Gavi S, Stuart LM, Kelly P, Melendez MM, Mynarcik DC, Gelato MS, McNurlan MA. Retinol-binding protein 4 is associated with insulin resistance and body fat distribution in nonobese subjects without type 2 diabetes. *J Clin Endocrinol Metab* 2007; 92: 1886–1890
- Ribel-Madsen R, Friedrichsen M, Vaag A, Poulsen P. Retinol-binding protein 4 in twins: regulatory mechanisms and impact of circulating and tissue expression levels on insulin secretion and action. *Diabetes* 2009; 58: 54–60
- DeFronzo RA, Gunnarsson R, Björkman O, Olsson M, Wahren J. Effects of insulin on peripheral and splanchnic glucose metabolism in non-insulin-dependent (type II) diabetes mellitus. *J Clin Invest* 1985; 76: 149–155
- Abel ED, Peroni O, Kim JK, Kim YB, Boss O, Hadro E, Minnemann T, Shulman GI, Kahn BB. Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. *Nature* 2001; 409 (6821): 729–733
- Ost A, Danielsson A, Lidén M, Eriksson U, Nystrom FH, Strålfors P. Retinol-binding protein-4 attenuates insulin-induced phosphorylation of IRS1 and ERK1/2 in primary human adipocytes. *FASEB J* 2007; 21: 3696–3704
- Stenkula KG, Said L, Karlsson M, Thorn H, Kjølhed P, Gustavsson J, Söderström M, Strålfors P, Nystrom FH. Expression of a mutant IRS inhibits metabolic and mitogenic signalling of insulin in human adipocytes. *Mol Cell Endocrinol* 2004; 221: 1–8
- Janke J, Engeli S, Boschmann M, Adams F, Böhneke J, Luft FC, Sharma AM, Jordan J. Retinol-binding protein 4 in human obesity. *Diabetes* 2006; 55: 2805–2810
- Yao-Borengasser A, Varma V, Bodles AM, Rasouli N, Phanavanh B, Lee MJ, Starks T, Kern LM, Spencer HJ 3rd, Rashidi AA, McGehee RE Jr, Fried SK, Kern PA. Retinol binding protein 4 expression in humans: relationship to insulin resistance, inflammation, and response to pioglitazone. *J Clin Endocrinol Metab* 2007; 92: 2590–2597
- Zeyda M, Stulnig TM. Obesity inflammation and insulin resistance – A mini-review. *Gerontology* 2009; 55: 379–386
- Balogopal P, Graham TE, Kahn BB, Altomare A, Funanage V, George D. Reduction of elevated serum retinol binding protein in obese children by lifestyle intervention: association with subclinical inflammation. *J Clin Endocrinol Metab* 2007; 92: 1971–1974
- Fleck A. Clinical and nutritional aspects of changes in acute-phase proteins during inflammation. *Proc Nutr Soc* 1989; 48: 347–354
- Zobali F, Avci A, Canbolat O, Karasu C. Effects of vitamin A and insulin on the antioxidative state of diabetic rat heart: a comparison study with combination treatment. *Cell Biochem Funct* 2002; 20: 75–80
- Kos K, Wong S, Tan BK, Kerrigan D, Randeve HS, Pinkney JH, Wilding JP. Human RBP4 adipose tissue expression is gender specific and influenced by leptin. *Clin Endocrinol (Oxf)* 2011; 74: 197–205
- Jia W, Wu H, Bao Y, Wang C, Lu J, Zhu J, Xiang K. Association of serum retinol binding protein 4 and visceral adiposity in Chinese subjects with and without type 2 diabetes. *J Clin Endocrinol Metab* 2007; 92: 3224–3229
- Tetsuya T, Takeshi O, Kengo M, Masako Z, Kunichi K, Yushi H, Hideaki K, Masahiko F, Kazuro S, Masato K. Effects of pioglitazone and metformin on intracellular lipid content in liver and skeletal muscle of individuals with type 2 diabetes mellitus. *Metabolism* 2007; 56: 1418–1424
- Lin KD, Chang YH, Wang CL, Yang YH, Hsiao PJ, Li TH, Shin SJ. Thiazolidinedione addition reduces the serum retinol-binding protein 4 in type 2 diabetic patients treated with metformin and sulfonylurea. *Transl Res* 2008; 151: 309–314
- Pfützner A, Schöndorf T, Hanefeld M, Lübken G, Kann PH, Karagiannis E, Wilhelm B, Forst T. Changes in insulin resistance and cardiovascular risk induced by PPARgamma activation have no impact on RBP4 plasma concentrations in nondiabetic patients. *Horm Metab Res* 2009; 41: 202–206
- You SH, Kim BS, Hong SJ, Ahn CM, Lim DS. The effects of pioglitazone in reducing atherosclerosis progression and neointima volume in type 2 diabetic patients: prospective randomized study with volumetric intravascular ultrasonography analysis. *Korean Circ J* 2010; 40: 625–631
- Derosa G, Maffioli P, Salvadeo SA, Ferrari I, Ragonesi PD, Quersi F, Franzetti IG, Gadaleta G, Ciccarelli L, Piccinni MN, D'Angelo A, Cicero AF. Exenatide versus glibenclamide in patients with diabetes. *Diabetes Technol Ther* 2010; 12: 233–240

- 34 Derosa G, Maffioli P, D'Angelo A, Fogari E, Bianchi L, Cicero AF. Acarbose on insulin resistance after an oral fat load: a double-blind, placebo controlled study. *J Diabetes Complications* 2011; 25: 258–266
- 35 Haider DG, Schindler K, Prager G, Bohdjalian A, Luger A, Wolzt M, Ludvik B. Serum retinol-binding protein 4 is reduced after weight loss in morbidly obese subjects. *J Clin Endocrinol Metab* 2007; 92: 1168–1171
- 36 Ingelsson E, Sundstrom J, Melhus H, Michaelsson K, Berne C, Vasan RS, Riserus U, Blomhoff R, Lind L, Arnlov J. Circulating retinol-binding protein 4, cardiovascular risk factors and prevalent cardiovascular disease in elderly. *Atherosclerosis* 2009; 206: 38–39
- 37 Qi Q, Yu Z, Ye X, Zhao F, Huang P, Hu FB, Franco OH, Wang J, Li H, Liu Y, Lin X. Elevated Retinol-binding protein 4 levels are associated with Metabolic Syndrome in Chinese people. *J Clin Endocrinol Metab* 2007; 92: 4827–4834
- 38 Lee DC, Lee JW, Im JA. Association of serum retinol binding protein 4 and insulin resistance in apparently healthy adolescents. *Metabolism* 2007; 56: 327–331
- 39 Lee JW, Lee HR, Shim JY, Im JA, Lee DC. Abdominal visceral fat reduction is associated with favorable changes of serum retinol binding protein-4 in nondiabetic subjects. *Endocr J* 2008; 55: 811–818
- 40 Lee JW, Im JA, Lee HR, Shim JY, Youn BS, Lee DC. Visceral adiposity is associated with serum retinol binding protein-4 levels in healthy women. *Obesity (Silver Spring)* 2007; 15: 2225–2232
- 41 Silha JV, Nyommba BLG, Leslie WD, Murphy LJ. Ethnicity, insulin resistance and inflammatory adipokines in women at high and low risk for vascular disease. *Diabetes Care* 2007; 30: 286–291
- 42 Wu H, Jia W, Bao Y, Lu J, Zhu J, Wang R, Chen Y, Xiang K. Serum retinol binding protein4 and nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus. *Diabetes Res Clin Pract* 2008; 79: 185–190
- 43 Seo JA, Kim NH, Park SY, Kim HY, Ryu OH, Lee KW, Lee J, Kim DL, Choi KM, Baik SH, Choi DS, Kim SG. Serum retinol-binding protein 4 levels are elevated in non-alcoholic fatty liver disease. *Clin Endocrinol* 2008; 68: 555–560
- 44 Romanowska A, Lebensztejn DM, Skiba E, Tarasów E, Kaczmarek M. Retinol binding protein-4 as a serum biomarker of intrahepatic lipid content in obese children – preliminary report. *Acta Biochim Pol* 2011; 58: 35–38
- 45 Perseghin G, Lattuada G, Cobelli FD, Esposito A, Belloni E, Canu T, Ragogna F, Scifo P, Maschio AD, Luzi L. Serum retinol binding protein 4, leptin and adiponectin concentrations are related to ectopic fat accumulation. *J Clin Endocrinol Metab* 2007; 92: 4883–4888
- 46 Shin MJ, Kang SM, Jang Y, Lee JH, Oh J, Chung JH, Chung N. Serum retinol binding protein 4 levels are associated with serum adiponectin levels in non-diabetic, non-obese subjects with hypercholesterolemia. *Clin Chim Acta* 2007; 378: 227–229
- 47 Choi SH, Lee YJ, Park YJ, Kim KW, Lee EJ, Lim S, Park do J, Kim SE, Park KS, Jang HC, Cho BY. Retinol binding protein-4 elevation is associated with serum thyroid-stimulating hormone level independently of obesity in elderly subjects with normal glucose tolerance. *J Clin Endocrinol Metab* 2008; 93: 2313–2318
- 48 Makino S, Fujiwara M, Suzukawa K, Handa H, Fujie T, Ohtaka Y, Komatsu Y, Aoki Y, Maruyama H, Terada Y, Hashimoto K, Sugimoto T. Visceral obesity is associated with the metabolic syndrome and elevated plasma retinol binding protein-4 level in obstructive sleep apnea syndrome. *Horm Metab Res* 2009; 41: 221–226
- 49 Lim S, Yoon JW, Choi SH, Park YJ, Lee JJ, Park JH, Lee SB, Kim KW, Lim JY, Kim YB, Park KS, Lee HK, Cho SI, Jang HC. Combined impact of adiponectin and retinol-binding protein 4 on Metabolic Syndrome in elderly people: The Korean Longitudinal Study on Health and Aging. *Obesity* 2010; 18: 826–832
- 50 Sasaki M, Otani T, Kawakami M, Ishikawa S. Elevation of plasma retinol-binding protein 4 and reduction of plasma adiponectin in subjects with cerebral infarction. *Metabolism* 2010; 59: 527–532
- 51 Rhie YJ, Choi BM, Eun SH, Son CS, Park SH, Lee KH. Association of serum retinol binding protein 4 with adiposity and pubertal development in Korean children and adolescents. *J Korean Med Sci* 2011; 26: 797–802
- 52 Usui S, Ichimura M, Ikeda S, Okamoto M. Association between serum retinol-binding protein 4 and small dense low-density lipoprotein cholesterol levels in young adult women. *Clin Chim Acta* 2009; 399: 45–48
- 53 Park SE, Kim DH, Lee JH, Park JS, Kang ES, Ahn CW, Lee HC, Cha BS. Retinol-binding protein-4 is associated with endothelial dysfunction in adults with newly diagnosed type 2 diabetes mellitus. *Atherosclerosis* 2009; 204: 23–25
- 54 Broch M, Auguet MT, Ramirez R, Olona M, Aguilar C, Megia A, Alcaide MJ, Pastor R, Martínez S, Caubet E, Garcia-España A, Richart C. Parallel downregulation of retinol-binding protein-4 and adiponectin expression in subcutaneous adipose tissue of non-morbidly obese subjects. *Eur J Endocrinol* 2009; 161: 87–94
- 55 Shim CY, Park S, Kim JS, Shin DJ, Ko YG, Kang SM, Choi D, Ha JW, Jang Y, Chung N. Association of plasma retinol-binding protein 4, adiponectin, and high molecular weight adiponectin with insulin resistance in non-diabetic hypertensive patients. *Yonsei Med J* 2010; 51: 375–384
- 56 Lim S, Choi SH, Jeong IK, Kim JH, Moon MK, Park KS, Lee HK, Kim YB, Jang HC. Insulin-sensitizing effects of exercise on adiponectin and retinol-binding protein-4 concentrations in young and middle-aged women. *J Clin Endocrinol Metab* 2008; 93: 2263–2268
- 57 Sell H, Eckel J. Regulation of retinol binding protein 4 production in primary human adipocytes by adiponectin, troglitazone and TNF-alpha. *Diabetologia* 2007; 50: 2221–2223
- 58 Guerre-Millo M. Adiponectin: an update. *Diabetes Metab* 2008; 34: 12–18
- 59 Volek JS, Phinney SD, Forsythe CE, Quann EE, Wood RJ, Puglisi MJ, Kraemer WJ, Bibus DM, Fernandez ML, Feinman RD. Carbohydrate restriction has a more favorable impact on the metabolic syndrome than a low fat diet. *Lipids* 2009; 44: 297–309
- 60 Zhang W, Della-Fera MA, Hartzell DL, Hausman D, Baile CA. Adipose tissue gene expression profiles in ob/ob mice treated with leptin. *Life Sci* 2008; 83: 35–42
- 61 Qi Q, Wang J, Li H, Yu Z, Ye X, Hu FB, Franco OH, Pan A, Liu Y, Lin X. Associations of resistin with inflammatory and fibrinolytic markers, insulin resistance, and metabolic syndrome in middle-aged and older Chinese. *Eur J Endocrinol* 2008; 159: 585–593
- 62 Yildiz BO, Bozdag G, Otegen U, Harmanci A, Boynukalin K, Vural Z, Kirazli S, Yarli H. Vsfatin and retinol-binding protein 4 concentrations in lean, glucose-tolerant women with PCOS. *Reprod Biomed Online* 2010; 20: 150–155
- 63 Goralski KB, Sinal CJ. Type 2 diabetes and cardiovascular disease: getting to the fat of the matter. *Can J Physiol Pharmacol* 2007; 85: 113–132
- 64 Christou GA, Tellis CC, Elisaf MS, Tselepis AD, Kiortsis DN. The Changes in Plasma Retinol-Binding Protein 4 Levels are Associated With Those of the Apolipoprotein B-Containing Lipoproteins During Dietary and Drug Treatment. *Angiology* 2011 May 20 Epub ahead of print
- 65 Chan DC, Watts GF, Ng TW, Yamashita S, Barrett PH. Effect of weight loss on markers of triglyceride-rich lipoprotein metabolism in the metabolic syndrome. *Eur J Clin Invest* 2008; 38: 743–751
- 66 Chan DC, Watts GF, Gan S, Wong AT, Ooi EM, Barrett PH. Nonalcoholic fatty liver disease as the transducer of hepatic oversecretion of very-low-density lipoprotein-apolipoprotein B-100 in obesity. *Arterioscler Thromb Vasc Biol* 2010; 30: 1043–1050
- 67 Ng TW, Watts GF, Barrett PH, Rye KA, Chan DC. Effect of weight loss on LDL and HDL kinetics in the Metabolic Syndrome: associations with changes in plasma retinol-binding protein-4 and adiponectin levels. *Diabetes Care* 2007; 30: 2945–2950
- 68 Reinehr T, Stoffel-Wagner B, Roth CL. Retinol-binding protein 4 and its relation to insulin resistance in obese children before and after weight loss. *J Clin Endocrinol Metab* 2008; 93: 2287–2293
- 69 Mitterberger MC, Mattesich M, Klaver E, Lechner S, Engelhardt T, Larcher L, Pierer G, Piza-Katzer H, Zwierschke W. Adipokine profile and insulin sensitivity in formerly obese women subjected to bariatric surgery or diet-induced long-term caloric restriction. *J Gerontol A Biol Sci Med Sci* 2010; 65: 915–923
- 70 Hermsdorff HH, Zulet MA, Abete I, Martínez JA. Discriminated benefits of a Mediterranean dietary pattern within a hypocaloric diet program on plasma RBP4 concentrations and other inflammatory markers in obese subjects. *Endocrine* 2009; 36: 445–451
- 71 Vitkova M, Klimcakova E, Kovacikova M, Valle C, Moro C, Polak J, Hanacek J, Capel F, Viguerie N, Richterova B, Bajzova M, Hejnova J, Stich V, Langin D. Plasma levels and adipose tissue messenger ribonucleic acid expression of retinol-binding protein 4 are reduced during calorie restriction in obese subjects but are not related to diet-induced changes in insulin sensitivity. *J Clin Endocrinol Metab* 2007; 92: 2330–2335
- 72 Kiortsis DN, Tzotzas T, Giral P, Bruckert E, Beucler I, Valsamides S, Turpin G. Changes in lipoprotein(a) levels and hormonal correlations during a weight reduction program. *Nutr Metab Cardiovasc Dis* 2001; 11: 153–157
- 73 Yu Z, Ye X, Wang J, Qi Q, Franco OH, Rennie KL, Pan A, Li H, Liu Y, Hu FB, Lin X. Associations of physical activity with inflammatory factors, adipocytokines, and metabolic syndrome in middle-aged and older Chinese people. *Circulation* 2009; 119: 2969–2977
- 74 Ku YH, Han KA, Ahn H, Kwon H, Koo BK, Kim HC, Min KW. Resistance exercise did not alter intramuscular adipose tissue but reduced retinol-binding protein-4 concentration in individuals with type 2 diabetes mellitus. *J Int Med Res* 2010; 38: 782–791

- 75 Choi KM, Kim TN, Yoo HJ, Lee KW, Cho GJ, Hwang TG, Baik SH, Choi DS, Kim SM. Effect of exercise training on A-FABP, lipocalin-2 and RBP4 levels in obese women. *Clin Endocrinol (Oxf)* 2009; 70: 569–574
- 76 Bradley RL, Jeon JY, Liu FF, Maratos-Flier E. Voluntary exercise improves insulin sensitivity and adipose tissue inflammation in diet-induced obese mice. *Am J Physiol Endocrinol Metab* 2008; 295: E586–E594
- 77 Ryan AS. Insulin resistance with aging: effects of diet and exercise. *Sports Med* 2000; 30: 327–346
- 78 Derosa G, Maffioli P, Ferrari I, D'Angelo A, Fogari E, Palumbo I, Randazzo S, Cicero AF. Orlistat and L-carnitine compared to orlistat alone on insulin resistance in obese diabetic patients. *Endocr J* 2010; 57: 777–786
- 79 Derosa G, Maffioli P, Salvadeo SA, Ferrari I, Gravina A, Mereu R, D'Angelo A, Palumbo I, Randazzo S, Cicero AF. Sibutramine and L-carnitine compared to sibutramine alone on insulin resistance in diabetic patients. *Intern Med* 2010; 49: 1717–1725
- 80 Mohapatra J, Sharma M, Singh S, Pandya G, Chatterjee A, Balaraman R, Patel PR, Jain MR. Involvement of adipokines in rimonabant-mediated insulin sensitivity in ob/ob mice. *J Pharm Pharmacol* 2009; 61: 1493–1498
- 81 Swarbrick MM, Stanhope KL, Austrheim-Smith IT, Van Loan MD, Ali MR, Wolfe BM, Havel PJ. Longitudinal changes in pancreatic and adipocyte hormones following Roux-en-Y gastric bypass surgery. *Diabetologia* 2008; 51: 1901–1911
- 82 Broch M, Gómez JM, Auguet MT, Villarsa N, Pastor R, Elio I, Olona M, García-España A, Richart C. Association of retinol-binding protein-4 (RBP4) with lipid parameters in obese women. *Obes Surg* 2010; 20: 1258–1264
- 83 Gómez-Ambrosi J, Rodríguez A, Catalán V, Ramírez B, Silva C, Rotellar F, Gil MJ, Salvador J, Frühbeck G. Serum retinol-binding protein 4 is not increased in obesity or obesity-associated type 2 diabetes mellitus, but is reduced after relevant reductions in body fat following gastric bypass. *Clin Endocrinol (Oxf)* 2008; 69: 208–215
- 84 Tschoner A, Sturm W, Engl J, Kaser S, Laimer M, Laimer E, Weiss H, Patsch JR, Ebenbichler CF. Retinol-binding protein 4, visceral fat, and the metabolic syndrome: effects of weight loss. *Obesity* 2008; 16: 2439–2444
- 85 Lin CC, Lai MM, Li TC, Li CI, Liu CS, Chen CC, Wu MT. Relationship between serum retinol-binding protein 4 and visfatin and the metabolic syndrome. *Diabetes Res Clin Pract* 2009; 85: 24–29
- 86 Yoshida A, Matsutani Y, Fukuchi Y, Saito K, Naito M. Analysis of the factors contributing to serum retinol binding protein and transthyretin levels in Japanese adults. *J Atheroscler Thromb* 2006; 13: 209–215
- 87 Mallat Z, Simon T, Benessiano J, Clement K, Taleb S, Wareham NJ, Luben R, Khaw KT, Tedgui A, Boekholdt SM. Retinol-binding protein 4 and prediction of incident coronary events in healthy men and women. *J Clin Endocrinol Metab* 2009; 94: 255–260
- 88 Von Eyatten M, Lepper PM, Liu D, Lang K, Baumann M, Nawroth PP, Bierhaus A, Dugi KA, Heemann U, Alolio B, Humpert PM. Retinol-binding protein 4 is associated with components of the metabolic syndrome, but not with insulin resistance, in men with type 2 diabetes or coronary artery disease. *Diabetologia* 2007; 50: 1930–1937
- 89 Schaefer EJ, Lamon-Fava S, Ordovas JM, Cohn SD, Schaefer MM, Castelli WP, Wilson PW. Factors associated with low and elevated plasma high density lipoprotein cholesterol and apolipoprotein A-I levels in the Framingham Offspring Study. *J Lipid Res* 1994; 35: 871–882
- 90 Friedewald WT, Levy RI, Frederickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; 18: 499–502
- 91 National Cholesterol Education Program. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 2002; 106: 3143–3421
- 92 Berneis KK, Krauss RM. Metabolic origins and clinical significance of LDL heterogeneity. *J Lipid Res* 2002; 43: 1363–1379
- 93 Muggeo M, Zenti MG, Travia D, Sartori A, Trimloni S, Grigolini L, Graziani MS, Cigolini M. Serum retinol levels throughout 2 years of cholesterol-lowering therapy. *Metabolism* 1995; 44: 398–403
- 94 Szendroedi J, Anderwald C, Krssak M, Bayerle-Eder M, Esterbauer H, Pfeiler G, Brehm A, Nowotny P, Hofer A, Waldhausl W, Roden M. Effects of high-dose simvastatin therapy on glucose metabolism and ectopic lipid deposition in nonobese type 2 diabetic patients. *Diabetes Care* 2009; 32: 209–214
- 95 Takebayashi K, Suetsugu M, Matsumoto S, Aso Y, Inukai T. Effects of rosuvastatin and colestimide on metabolic parameters and urinary monocyte chemoattractant protein-1 in type 2 diabetic patients with hyperlipidemia. *South Med J* 2009; 102: 361–368
- 96 Wu H, Wei L, Bao Y, Lu J, Huang P, Liu Y, Jia W, Xiang K. Fenofibrate reduces serum retinol-binding protein-4 by suppressing its expression in adipose tissue. *Am J Physiol Endocrinol Metab* 2009; 296: 628–634
- 97 Piscator M. Early detection of tubular dysfunction. *Kidney Int Suppl* 1991; 34: S15–S17
- 98 Chan DC, Watts GF, Gan SK, Ooi EM, Barrett PH. Effect of ezetimibe on hepatic fat, inflammatory markers, and apolipoprotein B-100 kinetics in insulin-resistant obese subjects on a weight loss diet. *Diabetes Care* 2010; 33: 1134–1139
- 99 Ingelsson E, Lind L. Circulating retinol binding protein 4 and subclinical cardiovascular disease in the elderly. *Diabetes Care* 2009; 32: 733–735
- 100 Solini A, Santini E, Madec S, Rossi C, Muscelli E. Retinol-binding protein-4 in women with untreated essential hypertension. *Am J Hypertens* 2009; 22: 1001–1006
- 101 Bobbert T, Raila J, Schwarz F, Mai K, Henze A, Pfeiffer AF, Schweigert FJ, Spranger J. Relation between retinol, retinol-binding protein 4, transthyretin and carotid intima media thickness. *Atherosclerosis* 2010; 213: 549–551
- 102 Christensen EI, Moskaug JO, Vorum H, Jacobsen C, Gundersen TE, Nykjaer A, Blomhoff R, Willnow TE, Moestrup SK. Evidence for an essential role of megalin in transepithelial transport of retinol. *J Am Soc Nephrol* 1999; 10: 685–695
- 103 Raila J, Willnow TE, Schweigert FJ. Megalin-mediated reuptake of retinol in the kidneys of mice is essential for vitamin A homeostasis. *J Nutr* 2005; 135: 2512–2516
- 104 Ziegelmeier M, Bachmann A, Seeger J, Lossner U, Kratzsch J, Blüher M, Stumvoll M, Fasshauer M. Serum levels of adipokine retinol-binding protein-4 in relation to renal function. *Diabetes Care* 2007; 30: 2588–2592
- 105 Chang YH, Lin KD, Wang CL, Hsieh MC, Hsiao PJ, Shin SJ. Elevated serum retinol-binding protein 4 concentrations are associated with renal dysfunction and uric acid in type 2 diabetic patients. *Diabetes Metab Res Rev* 2008; 24: 629–634
- 106 Graham TE, Wason CJ, Blüher M, Kahn BB. Shortcomings in methodology complicate measurements of serum retinol binding protein (RBP4) in insulin-resistant human subjects. *Diabetologia* 2007; 50: 814–823
- 107 Promintzer M, Krebs M, Todoric J, Luger A, Bischof MG, Nowotny P, Wagner O, Esterbauer H, Anderwald C. Insulin resistance is unrelated to circulating retinol binding protein and protein C inhibitor. *J Clin Endocrinol Metab* 2007; 92: 4306–4312
- 108 Gavi S, Qurashi S, Stuart LM, Lau R, Melendez MM, Mynarcik DC, McNurlan MA, Gelato MC. Influence of age on the association of retinol-binding protein 4 with metabolic syndrome. *Obesity (Silver Spring)* 2008; 16: 893–895