

Retinol-binding Protein 4 in Rheumatoid Arthritis-related Insulin Resistance and β -cell Function

Iván Ferraz-Amaro, Miguel A. González-Gay, and Federico Diaz-González

ABSTRACT. Objective. Retinol-binding protein 4 (RBP4), an adipokine related to impaired glucose tolerance, has been associated with insulin resistance (IR) and β -cell function in subjects with obesity or diabetes. In our study we assessed RBP4 levels in patients with rheumatoid arthritis (RA). We also determined whether any correlation exists between RBP4 levels and the presence of IR in these patients.

Methods. Plasma RBP4, insulin, C-peptide concentrations, and homeostasis model assessment (HOMA)-IR were measured in 101 patients with RA and 115 sex-matched and age-matched controls. A multivariable analysis adjusted for IR classic cardiovascular risk factors and body mass index was performed to establish the correlation between RBP4 plasma concentrations and features of IR in RA. Data were adjusted for glucocorticoid intake in patients with RA.

Results. Patients had higher levels of insulin, C-peptide levels, HOMA-percentage of β -cell secretion (%B) index, and HOMA-IR index than controls. RBP4 levels were significantly lower in the whole group of patients than in controls [13.99 (9.78–19.88) vs 21.50 (10.28–32.59) $\mu\text{g/ml}$, $p < 0.01$]. However, only those who were glucocorticoid-naïve showed significant difference in RBP4 plasma concentration when compared to controls [11.88 (7.93–17.96) vs 21.50 (10.28–32.59) $\mu\text{g/ml}$, $p < 0.01$]. The HOMA-%B [log β coefficient 0.00 (0.00–0.01), $p < 0.01$] showed positive relationships regarding RBP4 in controls. That was not the case in patients with RA [log β coefficient 0.00 (–0.0–0.00), $p = 0.93$ for HOMA-%B].

Conclusion. RBP4 does not correlate with the presence of IR and β -cell function in patients with RA. The mechanisms leading to IR in RA may be different from those occurring in obesity or diabetes. (J Rheumatol First Release Feb 15 2014; doi:10.3899/jrheum.130834)

Key Indexing Terms:

RHEUMATOID ARTHRITIS

HUMAN RETINOL-BINDING PROTEIN 4

INSULIN-SECRETING CELLS

Rheumatoid arthritis (RA) is a chronic, systemic, inflammatory disorder of unknown etiology that can lead to the destruction and deformity of joints because of the erosion of cartilage and bone. Epidemiologic data suggest that patients with RA have increased prevalence of premature atherosclerosis even after adjusting for traditional cardiovascular (CV)

risk factors^{1,2,3}. This accelerated atherosclerosis and increased risk of CV disease in patients with RA may be influenced, at least in part, by the presence of insulin resistance (IR).

Inflammation and disease severity play important roles in the development of IR in RA. In this regard, association of markers of inflammation, such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and disease activity scores with IR has been observed in patients with RA⁴. Similarly, β -cell function has been found to inversely correlate with Disease Activity Score (DAS28) and with swollen and painful joints. Chung, *et al*⁵ studied IR in 104 patients with RA and 124 patients with systemic lupus erythematosus (SLE). Patients with RA had a higher IR index than did those with SLE. In RA, IR showed a positive correlation with the levels of proinflammatory cytokines, interleukin 6 (IL-6), and tumor necrosis factor (TNF)- α , CRP, ESR, and carotid atherosclerosis⁶. Other studies have also shown a high incidence of IR in RA. They emphasized the link between IR and inflammation in RA^{5,7,8,9}. However, other mechanisms may also account for the development of IR in RA¹⁰.

Retinol-binding protein 4 (RBP4) is a plasma protein

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released from adipocytes that has been found to correlate with the degree of IR in patients with obesity, impaired glucose tolerance, or type 2 diabetes, as well as in nonobese subjects with¹¹ or without¹² a strong family history of type 2 diabetes. Reduction of RBP4 levels has also been observed in individuals who experience improvement of IR with exercise. Studies in humans disclosed an inverse relationship between glucose transporter 4 (GLUT4) in adipocytes and serum RBP4¹¹. In addition, mice lacking adipocyte GLUT4 have increased RBP4 levels, leading to IR in muscle and liver¹³. Therefore, it is possible that increased serum RBP4 levels may contribute to impaired insulin-stimulated glucose uptake in the muscle and elevated hepatic glucose production, both of which are typical features of obesity and type 2 diabetes. However, the molecular mechanisms leading to IR mediated by RBP4 induction are unknown. Interestingly, RBP4 has been shown to induce the release of proinflammatory cytokines, including IL-6 by endothelial cells, in part through activation of NADPH oxidase and nuclear factor- κ B transcription factor¹⁴. Therefore, RBP4 may inhibit insulin signaling in adipocytes by inducing proinflammatory cytokines in macrophages¹⁵.

Because the presence of a chronic inflammatory state is of high importance in the process of accelerated atherogenesis observed in RA², it is plausible to think that inflammation may also play a key role in the mechanisms leading to IR in RA. This may be of singular importance to differentiate the development of IR in RA from that occurring in other conditions not associated with high chronic inflammation. In our study we aimed to assess whether serum levels of RBP4, a protein strongly associated with obesity and diabetes, correlate with the magnitude of IR observed in patients with RA.

MATERIALS AND METHODS

Study participants. Two hundred sixteen subjects, 101 patients with RA, and 115 age-matched and sex-matched controls were recruited for a cross-sectional study. All patients with RA were 18 years old or older, and fulfilled the 2010 American College of Rheumatology/European League Against Rheumatism diagnostic criteria¹⁶. All of them were diagnosed by rheumatologists and were periodically followed at rheumatology outpatient clinics. For the purpose of inclusion in our study, RA disease duration was required to be ≥ 1 year. To minimize the potential effects of TNF- α blockers on IR¹⁷, patients with RA undergoing TNF- α antagonist therapy were not included in our study. However, because glucocorticoids are often used in the management of RA, patients taking prednisone or an equivalent dose (12.5 mg/day or less) were not excluded. Nevertheless, to determine the potential effect of glucocorticoid therapy, we established 2 groups within the cohort of patients with RA; those receiving prednisone therapy (including subjects taking prednisone therapy at the time of the assessment or who had been taking glucocorticoids within 3 months prior to onset of the study), and another group of glucocorticoid-naïve patients with RA. In patients included within the group of glucocorticoid-treated individuals, the glucocorticoid dose was measured as the equivalent prednisone dose administered during the previous 3 months (mg per day). Patients and controls with diabetes mellitus were not included in the study; therefore, none of the patients or controls were receiving glucose-lowering drugs or

insulin therapy. In addition, patients and controls were required to have a glycemia < 7 mmol/l. Patients and controls were excluded if they had a history of myocardial infarction, angina, stroke, a glomerular filtration rate < 60 ml/min/1.73 m², a history of cancer, any other chronic disease, or evidence of infection. None of the controls were receiving glucocorticoid treatment. Our study protocol was approved by the Institutional Review Committee at Hospital Universitario de Canarias (Spain), and all subjects provided written informed consent.

Data collection. Patient surveys for both the RA and control groups were identical, except for additional questions asked of patients with RA. Subjects completed a CV risk factor and medication use questionnaire and underwent physical examination to determine their anthropometrics and blood pressure. Medical records were reviewed to ascertain the specific diagnosis and medication. Waist circumference and hip circumference were measured and the waist-to-hip ratio was also estimated. In patients with RA, disease activity was measured using the DAS28¹⁸, whereas disease disability was determined by using the Health Assessment Questionnaire (HAQ)¹⁹. A subject's metabolic syndrome was defined based on the 2005 National Cholesterol Education Program (Adult Treatment Panel III) criteria²⁰.

Assessments. Serum RBP4 was measured using an ELISA (ALPCO Diagnostics). ELISA samples were run in duplicate; the coefficient of variation for interassay replicate samples was $< 7\%$. The homeostatic model assessment (HOMA) method was performed to determine IR; specifically, in this study, we used HOMA2: the updated-computer HOMA model²¹. In brief, this method consists of a structural computer model of the glucose-insulin feedback system in a homeostatic (overnight-fasted) state. It comprises a number of nonlinear empirical equations (and precludes an exact algebraic solution), which describes the functions of organs and tissues involved in glucose regulation. This model can be used to determine insulin sensitivity (%S) and percentage of β -cell secretion (%B) from paired fasting plasma glucose and specific insulin levels, or from C-peptide concentrations across a range of 1 to 2200 pmol/l for insulin and 1 to 25 mmol/l for glucose. In our study, we used C-peptide to calculate β -cell function, because the former is a marker of secretion. In addition, we used insulin data to calculate %S (since HOMA-%S is derived from glucose disposal as a function of insulin concentration). This computer model provides an insulin-sensitivity value expressed as HOMA2-%S (where 100% is normal). HOMA2-IR (IR index) is simply the reciprocal of %S. Insulin (Architect Abbott, 2000I) and C-peptide (Immulite 2000; Siemens), coefficient of variation $< 6\%$ in both, were determined using chemiluminescent immunometric assays. Standard techniques were used to measure plasma glucose, CRP, the Westergren ESR, and serum lipids.

Statistical analysis. The number of patients recruited for this research was based on the results of a preliminary study in which we assessed RBP4 in 15 patients with RA and 15 controls. RBP4 in the control group was 21.0 (18.0–24.3) μ g/ml, while in patients with RA it was 17.4 (14.3–23.5) μ g/ml. Based on this information, using a 1:1 relation, and using Student t test with an α level of 0.05 and a β level of 0.10, we estimated that we would have to enroll 170 subjects: 85 patients and 85 controls. A comparison of the demographic and clinical characteristics of patients with RA and controls, using a Pearson chi-square test for categorical variables or a Student t test for continuous variables (data described as mean \pm SD) is shown in Table 1. For noncontinuous variables, either a Mann-Whitney U test or a logarithmic transformation was carried out, and data were expressed as a median [interquartile range (IQR)]. Non-normally distributed variables were log-transformed when used in linear regression analysis. Multivariate analysis, adjusting for factors known to be associated with IR [sex, waist circumference, body mass index (BMI), and prednisone use], was performed to respectively assess the association of anthropometric measures with IR and IR with RBP4 in both controls and patients. All analyses used a 5% 2-sided significance level and were conducted using SPSS software, version 22 (IBM). A p value < 0.05 was considered statistically significant.

Table 1. Demographic data, metabolic risk factors, and disease-related characteristics of patients and controls.

Characteristics	RA, n = 101	Glucocorticoid Naive, n = 46	Taking Glucocorticoids, n = 55	p [†]	Controls, n = 115	p
Demographic data						
Age, yrs	55 ± 10	53 ± 10	57 ± 9	0.05	56 ± 11	0.49
Female, n (%)	86 (85)	38 (83)	48 (87)	0.51	103 (90)	0.33
Metabolic risk factors						
Smokers, n (%)	19 (19)	6 (13)	13 (24)	0.19	11 (10)	0.05
Body mass index, kg/m ²	29.0 ± 5.8	28.0 ± 4.6	29.8 ± 6.6	0.12	30.1 ± 5.9	0.23
Waist circumference, cm	97 ± 16	94 ± 15	99 ± 16	0.11	93 ± 12	0.12
Male	106 ± 17	105 ± 12	107 ± 22	0.79	104 ± 12	0.77
Female	96 ± 15	92 ± 14	98 ± 15	0.08	91 ± 11	0.05
Hip circumference, cm	107 ± 16	106 ± 11	109 ± 20	0.37	102 ± 18	0.10
Male	109 ± 17	106 ± 9	112 ± 23	0.56	104 ± 5	0.50
Female	106 ± 17	106 ± 11	109 ± 19	0.46	102 ± 20	0.13
Waist to hip ratio	0.90 (0.85–0.94)	0.89 (0.83–0.94)	0.90 (0.86–0.94)	0.35	0.87 (0.83–0.92)	0.65
Male	0.97 (0.93–1.00)	0.99 (0.93–1.07)	0.97 (0.92–0.99)	0.38	1.01 (0.92–1.08)	0.71
Female	0.88 (0.84–0.93)	0.87 (0.82–0.93)	0.89 (0.85–0.93)	0.27	0.85 (0.81–0.91)	0.21
Hypertension, n (%)	36 (36)	14 (30)	22 (40)	0.51	44 (38)	0.96
Metabolic syndrome, n (%)	32 (32)	15 (33)	17 (31)	0.96	28 (24)	0.25
Total cholesterol, mg/dl	210 ± 40	204 ± 30	216 ± 46	0.08	207 ± 38	0.49
Triglycerides, mg/dl	118 (85–151)	122 (85–139)	135 (91–152)	0.28	104 (77–135)	0.10
HDL cholesterol, mg/dl	59 ± 15	57 ± 13	60 ± 16	0.62	55 ± 13	0.04*
LDL cholesterol, mg/dl	127 ± 33	128 ± 28	127 ± 37	0.66	129 ± 34	0.73
Cholesterol to HDL ratio	3.77 ± 1.14	3.74 ± 0.83	3.84 ± 1.34	0.53	3.88 ± 0.86	0.33
Non-HDL cholesterol, mg/dl	152 ± 42	148 ± 29	157 ± 50	0.21	152 ± 36	0.99
Systemic inflammation						
ESR, mm/1st h	25 (16–39)	25 (15–41)	26 (18–39)	0.78	19 (13–24)	0.00*
CRP, mg/l	3.8 (1.4–9.5)	4.5 (1.3–13.7)	3.2 (1.6–9.0)	0.60	1.9 (0.9–4.8)	0.00*
RA related data						
Disease duration, yrs	7 (2–11)	9 (5–12)	3 (1–10)	0.06		
DAS28-ESR	3.99 ± 1.41	4.11 ± 1.56	3.18 ± 1.27	0.44		
HAQ	0.625 (0.250–1.250)	0.690 (0.130–1.370)	0.600 (0.250–1.200)	0.93		
Positive rheumatoid factor, n (%)	64 (63)	21 (46)	43 (78)	0.01*		
Current nonbiologic DMARD, n (%)	76 (75)	41 (89)	47 (85)	0.22		
Current prednisone, n (%)	55 (54)					
Prednisone, mg/day/last 3 mos	6.5 ± 2.7					

*Denotes p value < 0.05 between controls and patients. †p refers to the difference between patients with and without glucocorticoids. Data are expressed as mean (± SD) or median (interquartile range). Dichotomous variables are expressed as n and percentage. ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; HAQ: Health Assessment Questionnaire; DAS28: 28-joint Disease Activity Score; DMARD: disease-modifying antirheumatic drug; HDL: high-density lipoprotein; LDL: low-density lipoprotein; RA: rheumatoid arthritis.

RESULTS

Clinical characteristics of patients and controls, and the relationship with IR and β-cell function. Table 1 summarizes the population data of our study. Age (55 ± 10 vs 56 ± 11 yrs, p = 0.49) and sex (85% and 90% of women, p = 0.33) in patients and controls, respectively, did not differ significantly. The frequency of hypertension, metabolic syndrome, waist and hip circumferences, waist-to-hip ratio split by sex, levels of total cholesterol, triglycerides, or low-density lipoprotein cholesterol did not differ significantly between patients and controls. Only high-density lipoprotein cholesterol levels showed significant differences (55 ± 13 mg/dl in controls vs 59 ± 15 mg/dl in patients, p = 0.04). As expected, ESR and CRP values showed statistically significant differences between patients and controls. Patients taking glucocorticoids did not differ from those not taking glucocorti-

coids in demographic data, BMI, waist and hip circumferences, and waist-to-hip ratio. That was also the case for waist and hip circumferences and waist-to-hip ratio differences when patients taking glucocorticoids or those not were stratified according to sex. Apart from a higher frequency of rheumatoid factor positivity in the subgroup of patients with RA taking glucocorticoids (78% vs 38%, p = 0.01), laboratory data (lipids and acute-phase reactants) did not differ between glucocorticoid-naive patients and patients taking glucocorticoids.

Table 2 summarizes the correlations between the demographic and laboratory data and between RA-related features and IR indexes in both controls and patients. In both controls and patients there were significant correlations between BMI, waist circumference, hypertension, and metabolic syndrome with HOMA-IR. Although waist

Table 2. Metabolic risk factors, systemic inflammation, and disease characteristics associated with insulin resistance (IR) indexes and retinol-binding protein 4.

	Log β Coefficient (95% CI), p			
	Controls, n = 115		RA, n = 101	
	HOMA-IR	HOMA-%B	HOMA-IR	HOMA-%B
Demographic data				
Age, yrs	0.01 (0.00–0.02), 0.02 [†]	0.00 (–0.01–0.01)	0.00 (–0.02–0.02), 0.83	0.01 (–0.03–0.00), 0.06
Male	0.16 (–0.21–0.54), 0.39	–0.09 (–0.41–0.24), 0.60	–0.24 (–0.76–0.27), 0.35	–0.52 (–0.92 to –0.12), 0.01 [†]
Metabolic risk factors				
Body mass index, kg/m ²	0.03 (0.01–0.06), 0.01 [†]	0.02 (–0.00–0.04), 0.07	0.06 (0.03–0.10), 0.00 [†]	0.02 (–0.01–0.05), 0.16
Waist circumference, cm*	0.03 (0.01–0.06), 0.02 [†]	0.02 (0.00–0.04), 0.02 [†]	0.02 (0.01–0.04), 0.00 [†]	0.00 (–0.01–0.02), 0.32
Hip circumference, cm*	0.01 (–0.01–0.02), 0.33	0.00 (–0.01–0.01), 0.89	0.03 (0.02–0.04), 0.00 [†]	0.01 (0.00–0.02), 0.02 [†]
Waist-to-hip ratio*	–0.03 (–0.23–0.16), 0.73	0.03 (–0.12–0.18), 0.65	–0.34 (–0.55 to –0.14), 0.00 [†]	–0.17 (–0.34 to –0.01), 0.04 [†]
Hypertension	0.39 (0.18–0.61), 0.00 [†]	0.18 (–0.01–0.38), 0.06	0.48 (0.11–0.86), 0.01 [†]	0.10 (–0.21–0.41), 0.53
Metabolic syndrome	0.56 (0.30–0.81), 0.00 [†]	0.15 (–0.08–0.39), 0.20	0.57 (0.17–0.97), 0.01 [†]	0.06 (–0.40–0.28), 0.73
Systemic inflammation				
ESR, mm/h	–0.01 (–0.02–0.01), 0.46	0.00 (–0.10–0.02), 0.65	–0.00 (–0.01–0.01), 0.66	0.00 (–0.01–0.01), 0.52
CRP, mg/l	0.03 (–0.00–0.07), 0.07	0.02 (–0.01–0.05), 0.21	–0.01 (–0.02–0.00), 0.05	0.01 (–0.02–0.01), 0.32
RA-related data				
Disease duration, yrs			–0.02 (–0.06–0.01), 0.19	0.02 (–0.01–0.04), 0.27
DAS28-ESR			–0.00 (–0.14–0.13), 0.96	–0.01 (–0.12–0.10), 0.81
HAQ			–0.01 (–0.30–0.29), 0.97	0.03 (–0.27–0.21), 0.83
Positive rheumatoid factor			0.00 (–0.39–0.39), 0.99	0.10 (–0.21–0.42), 0.52
Current nonbiologic DMARD			–0.28 (–1.35–0.79), 0.60	0.16 (–1.10–0.79), 0.74
Patients with RA taking glucocorticoids, n = 46				
Current prednisone			0.23 (–0.14–0.60), 0.22	0.01 (–0.28–0.31), 0.93
Prednisone, (mg/day/last 3 mos)			0.01 (–0.23–0.24), 0.96	0.02 (–0.20–0.24), 0.87

*Waist and hip circumference, and waist-to-hip ratio relation with HOMA-IR and HOMA-%B were adjusted for sex. β coefficients of HOMA-IR, HOMA-%B, and RB4 are expressed log-transformed. [†]Significant associations ($p < 0.05$). HOMA: homeostasis model assessment; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; DAS28: Disease Activity Score; DMARD: disease-modifying antirheumatic drug; HAQ: Health Assessment Questionnaire; RA: rheumatoid arthritis.

circumference was correlated with HOMA-%B in controls, this was not the case for patients. Additionally, BMI and waist circumference were not associated with HOMA-%B in patients, and waist-hip ratio was inversely related to the HOMA-IR and HOMA-%B only in patients with RA. Nevertheless, ESR and CRP in patients with RA and controls did not show any correlation with IR indexes. Similarly, DAS28 and HAQ scores, as well as positivity for rheumatoid factor or prednisone use, were not significantly associated with IR indexes in our series.

Differences between patients and controls regarding IR and β -cell function and RBP4 plasma concentration. Univariate analyses demonstrated significantly higher levels in RA patients than in controls of insulin [81.26 (47.23–111.12) vs 59.03 (40.28–78.48) pmol/l, $p < 0.01$], C-peptide [0.72 (0.63–0.89) vs 0.58 (0.54–0.68) nmol/l, $p < 0.01$], HOMA-%B [101% (67% to 133%) vs 81% (56% to 114%), $p < 0.01$], and HOMA-IR [1.66 (0.86–2.26) vs 1.09 (0.73–1.52), $p < 0.01$; Table 3]. When patients were split into those taking glucocorticoids and those not, the former maintained this trend (i.e., exhibited higher levels) compared to controls. However, in HOMA-IR between controls and glucocorticoid-naïve patients, no statistically significant difference was found [1.09 (0.73–1.52) in controls vs 1.30 (0.84–1.82) in patients with RA, $p = 0.21$].

RBP4 levels were significantly lower in the whole group of patients with RA group than in controls [13.99 (9.78–19.88) vs 21.50 (10.28–32.59) $\mu\text{g/ml}$, $p < 0.01$]. Nevertheless, when patients were stratified according to glucocorticoid intake, only those not taking these drugs (glucocorticoid-naïve patients) showed significant differences in RBP4 plasma concentration when compared with controls [11.88 (7.93–17.96) vs 21.50 (10.28–32.59) $\mu\text{g/ml}$, $p < 0.01$]. In the subgroup of patients with RA taking glucocorticoid therapy, RBP4 levels were also lower than in controls [17.34 (11.97–22.94) $\mu\text{g/ml}$ vs 21.50 (10.28–32.59)] but the difference was not statistically significant ($p = 0.35$; Figure 1).

Relationship of RBP4 with demographic data, RA features, and IR and β function. Table 4 depicts the relationship between RBP4 plasma concentration and IR in patients and controls. C-peptide levels, but not insulin concentrations, correlated with RBP4 plasma concentrations both in controls and patients with RA [log β coefficient 1.01 (0.10–1.91), $p = 0.03$ in controls and 0.88 (0.16–1.60), $p = 0.02$ in patients with RA, respectively]. ESR, CRP, and disease activity scores did not correlate with RBP4 levels. Only prednisone intake, when analyzed in a binary fashion, was associated with higher RBP4 levels [log β coefficient 0.39 (0.12–0.65), $p = 0.01$]. On the other hand, in controls,

Table 3. Insulin resistance (IR)-related measures in patients and controls (univariate model).

	Controls, n = 115	RA, n = 101	p	RA – GC, n = 55	p [#]	RA + GC, n = 46	p [†]
Glucose, mmol/l	5.39 (4.94–6.22)	5.28 (4.83–6.44)	0.60	5.28 (4.78–6.11)	0.31	5.33 (4.89–6.81)	0.86
Insulin, pmol/l	59.03 (40.28–78.48)	81.26 (47.23–111.12)	0.01*	66.67 (45.14–98.62)	0.22	99.66 (56.95–134.73)	0.00*
C-peptide, nmol/l	0.58 (0.54–0.68)	0.72 (0.63–0.89)	0.00*	0.67 (0.60–0.79)	0.00*	0.78 (0.65–0.97)	0.00*
HOMA-IR	1.09 (0.73–1.52)	1.66 (0.86–2.26)	0.00*	1.30 (0.84–1.82)	0.21	1.95 (1.08–2.51)	0.00*
HOMA-%B	81 (56–114)	101 (67–133)	0.02*	103 (70–118)	0.06	98 (64–157)	0.07
RBP4, g/ml	21.50 (10.28–32.59)	13.99 (9.78–19.88)	0.00	11.88 (7.93–17.96)	0.00	17.34 (11.97–22.94)	0.35

All data are expressed as median (interquartile range). p* differences between controls and total RA patients. †p differences between RA + GC and controls. #p differences between RA – GC and controls. RA: rheumatoid arthritis; RA + GC: rheumatoid arthritis patients taking glucocorticoids; RA – GC: naive glucocorticoids patients; RBP4: retinol-binding protein 4; HOMA: homeostasis model assessment; IR: insulin resistance.

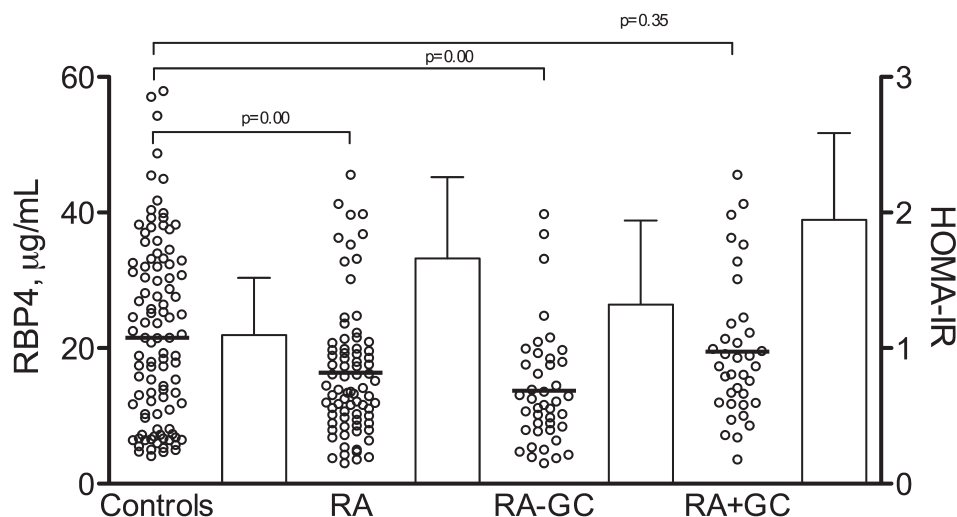


Figure 1. Retinol-binding protein 4 (RBP4; left axis) is expressed as dots (median) in controls, total number of patients with rheumatoid arthritis (RA), patients with RA not taking glucocorticoids (RA-GC), and patients with RA taking glucocorticoids (RA+GC). Homeostasis model assessment (HOMA)-insulin resistance (IR; right axis) is expressed as columns for the same groups (median \pm interquartile range). RBP4 levels were significantly lower in the whole group of patients with RA than in controls ($p < 0.01$). However, when patients were stratified according to glucocorticoid intake, only those who were not taking these drugs showed significant difference in RBP4 plasma concentration when compared with controls.

HOMA-%B [log β coefficient 0.00 (0.00–0.01), $p = 0.00$] showed a positive correlation with RBP4; this was not the case for patients with RA [log β coefficient 0.06 (–0.04–0.15), $p = 0.24$ for HOMA-IR and 0.00 (–0.0–0.00), $p = 0.93$ for HOMA-%B; Figure 2]. When this relationship was adjusted for sex, BMI, and waist circumference in controls; and then for sex, BMI, waist circumference, and prednisone intake in patients with RA, the relationship maintained the same trend. The interactions between prednisone intake and HOMA-IR and HOMA-%B, when a lineal regression was performed with RBP4, were all nonsignificant.

DISCUSSION

RBP4, an adipokine, was reportedly associated with obesity and its comorbidities, especially IR, type 2 diabetes, and certain components of the metabolic syndrome²². Circu-

lating RBP4 levels have also been positively correlated with intima media thickness, an established measure of atherosclerosis²³ that predicts the risk of CV events in patients with RA²⁴. To the best of our knowledge, RBP4 influence has not been explored in RA. Therefore our study constitutes the first attempt to determine the implication of this molecule in a disease where IR is closely linked to the presence of inflammation.

In our study we found that patients with RA, in particular those who are glucocorticoid-naive, have lower RBP4 levels than controls. Also, HOMA indexes correlate with RBP4 in controls but not in patients with RA, and RBP4 levels do not correlate with disease activity in patients with RA. Based on these findings, we hypothesize that IR, when present in patients with RA, is RBP4-independent and that low levels of RBP4 in these patients may be the result of a negative influence mediated by another factor associated with the

Table 4. Metabolic risk factors, systemic inflammation, and disease characteristics association with RBP4.

	RBP4, Log β Coefficient (95% CI), p			
	Controls, n = 115	Adjusted model*		RA, n = 101
		Adjusted model*		Adjusted model#
IR and β-cell function				
HOMA-IR	0.10 (-0.04-0.24), 0.15	0.08 (-0.04-0.20), 0.17	0.06 (-0.04-0.15), 0.24	-0.00 (-0.10-0.10), 0.99
HOMA-%B	0.00 (0.00-0.01), 0.00 [†]	0.00 (0.00-0.01), 0.01 [†]	0.00 (-0.00-0.00), 0.93	0.00 (-0.00-0.00), 0.76
Demographic data				
Age, yrs	0.00 (-0.01-0.02), 0.91		0.01 (-0.00-0.03), 0.06	
Male	-0.39 (-0.86-0.08), 0.10		0.22 (-0.16-0.61), 0.24	
Metabolic risk factors				
BMI, kg/m ²	-0.02 (-0.05-0.01), 0.20		0.03 (0.00-0.05), 0.02 [†]	
Waist circumference, cm	-0.00 (-0.02-0.01), 0.67		0.01 (0.00-0.02), 0.02 [†]	
Hip circumference, cm	-0.01 (-0.03-0.01), 0.16		0.00 (-0.01-0.01), 0.83	
Waist to hip ratio	0.48 (-1.39-2.36), 0.60		0.09 (-0.07-0.24), 0.27	
Hypertension	0.19 (-0.09-0.48), 0.18		0.18 (-0.12-0.47), 0.23	
Metabolic syndrome	0.02 (-0.33-0.37), 0.91		0.12 (-0.19-0.42), 0.45	
Glucose, mmol/L	-0.14 (-0.25 to -0.02), 0.02 [†]		0.06 (-0.01-0.13), 0.09	
Insulin, pmol/L	0.00 (0.00-0.00), 0.12		0.00 (-0.00-0.00), 0.30	
C-peptide	1.01 (0.10-1.91), 0.03 [†]		0.88 (0.16-1.60), 0.02 [†]	
Systemic inflammation				
ESR, mm/h	-0.00 (-0.02-0.02), 0.78		-0.00 (-0.01-0.01), 0.52	
CRP, mg/l	-0.02 (-0.07-0.03), 0.52		0.00 (-0.01-0.01), 0.51	
RA-related data				
Disease duration, yrs			-0.02 (-0.05-0.02), 0.32	
DAS28-ESR			0.07 (-0.03-0.18), 0.18	
HAQ			-0.08 (-0.30-0.13), 0.44	
Positive rheumatoid factor			0.28 (-0.01-0.57), 0.06	
Current nonbiologic DMARD			0.21 (-0.43-0.84), 0.51	
Patients with RA taking glucocorticoids, n = 46				
Current prednisone			0.39 (0.12-0.65), 0.01 [†]	
Current prednisone \times HOMA-IR interaction			-0.05 (-0.24-0.15), 0.63	
Current prednisone \times HOMA-%B interaction			-0.00 (-0.01-0.00), 0.66	
Prednisone, (mg/day/last 3 mos)			0.06 (-0.05-0.17), 0.24	

*Adjusted model for sex, BMI, and waist circumference. #Adjusted model for sex, BMI, waist circumference, and current prednisone. [†]Significant associations ($p < 0.05$). β coefficients of HOMA-%IR, HOMA-%B, and RBP4 are expressed log-transformed. RBP4: retinol binding protein 4; HOMA: homeostasis model assessment; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; HAQ: Health Assessment Questionnaire; DAS28: 28-joint Disease Activity Score; DMARD: disease-modifying antirheumatic drug; IR: insulin resistance; RA: rheumatoid arthritis.

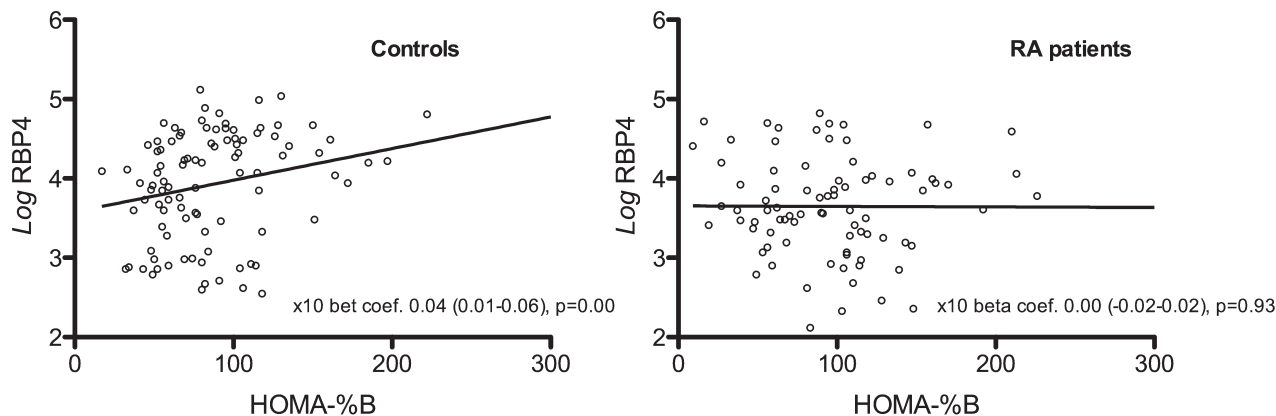


Figure 2. Retinol-binding protein 4 (RBP4) and homeostasis model assessment (HOMA)-%B univariate analysis for controls and patients with RA. Results were expressed as $\times 10 \beta$ coefficient (95% CI). RA: rheumatoid arthritis.

disease (namely, the presence of a chronic inflammatory state).

Our data are in keeping with other reports that disclosed the presence of IR in patients with RA^{7,9,10,25,26}. However, in contrast to other studies^{4,27}, we did not find that the levels of ESR, CRP, or the disease activity scores assessed at the time of the study had a correlation with the presence of IR in our series. It is possible that the presence of lower levels of inflammation in our study, when compared with other studies, may explain these results. Therefore, low levels of inflammation, as shown in our study, may minimize the well-established effects that a proinflammatory state has on the development of IR in RA. In the study performed by Dessein, *et al*⁴, the median CRP level was 19.2 mg/l in a series of 81 patients with RA in whom high-grade inflammation showed a correlation with IR, while in our cohort of 101 patients the median CRP at the time of the study was only 3.8 mg/l. Therefore, as pointed out by the same authors, apart from inflammation, other mechanisms may be implicated in the increased risk of IR observed in patients with RA²⁸.

As mentioned, serum RBP4 levels not only exist at higher levels in subjects with impaired glucose tolerance or type 2 diabetes, but also inversely correlate with insulin sensitivity in nondiabetic subjects^{11,12,29}. In this regard, our finding that shows a correlation of RBP4 with IR indexes in controls is in agreement with these studies. Although we did not find a correlation between RBP4 and insulin levels, we were able to find a relationship with C-peptide levels in both patients and controls, effectively showing that this molecule is related to IR indexes. Interestingly, although C-peptide also correlated with RBP4 in patients with RA, HOMA indexes did not correlate, suggesting that HOMA indexes in RA may not be dependent on RBP4. In the same way, serum RBP4 levels have been found to be elevated and correlated positively with BMI in obese subjects with and without diabetes^{11,13}. Consistent with this finding, we found a correlation, albeit only in patients with RA, between RBP4 and BMI and abdominal obesity. When we analyzed the relationship of RBP4 with disease features, we found no association with disease duration or activity scores. Further, we did not observe any correlation between CRP or ESR with RBP4 levels, although in another study, higher RBP4 levels were associated with markers of systemic inflammation³⁰.

To our knowledge, the influence of glucocorticoids on RBP4 levels has not been studied in RA. In our study, patients with RA not exposed to glucocorticoids were the subgroup of patients with lower levels of RBP4. Patients with RA taking glucocorticoids and controls did not show statistically significant differences in RBP4 levels. The reason that glucocorticoids induce IR is not completely understood, but it seems to occur as a result of peripheral metabolic effects on skeletal muscle, liver, and adipose

tissue, thus leading to hepatic gluconeogenesis, inhibition of glucose uptake in adipose tissue, and alterations in receptor and postreceptor functionality³¹. As RBP4 levels have been found to decrease with weight loss and exercise, it is reasonable to think that patients not exposed to glucocorticoids may have lower levels of RBP4 than those who were following such a regimen. Interestingly, in our series, patients who had not used glucocorticoids also exhibited lower levels of RBP4 than did controls. This means that these patients, in contrast to healthy controls, may have an inhibitory mechanism against this molecule stemming from the IR related to the disease itself. Our findings that showed that glucocorticoids induce higher levels of RBP4 could link them with a specific mechanism that is related to an alteration of the glucose metabolism.

To better understand the relationship between IR and RBP4 in patients with RA taking glucocorticoids, we constructed a multivariate analysis of the relationship of IR indexes, with RBP4 adjusted for glucocorticoid intake. We expected to find a significant relationship after the adjustment. However, this was not the case, because the relationship of IR indexes with RBP4, after adjusting for adiposity and glucocorticoids, was not significant. Therefore, an effect of interaction between glucocorticoids and IR indexes associated with RBP4 was not confirmed in our study. A plausible explanation for that may be related to the presence of a chronic inflammatory burden in our series of patients. In this regard, because inflammation plays a pivotal role in the mechanisms associated with IR and accelerated atherogenesis in RA, it is possible that our results indicate that the effect mediated by chronic inflammation prevails over those of glucocorticoids, adiposity, or RBP4 levels in the development of IR in RA.

We found that IR in patients with RA was not associated with RBP4, a molecule that has been found to correlate with IR in other situations, such as obesity or diabetes. We believe that chronic inflammation or other unknown mechanisms implicated in the pathogenesis of RA may account for the development of IR in patients with RA. Although our study involved a relatively large number of subjects, the actual effect of RBP4 in the IR of patients with chronic inflammatory diseases warrants further investigation.

REFERENCES

1. Chung CP, Oeser A, Raggi P, Gebretsadik T, Shintani AK, Sokka T, et al. Increased coronary-artery atherosclerosis in rheumatoid arthritis: relationship to disease duration and cardiovascular risk factors. *Arthritis Rheum* 2005;52:3045-53.
2. Gonzalez-Gay MA, Szekanecz Z, Popa CD, Dessein P. Atherosclerosis in rheumatoid arthritis. *Mediators Inflamm* 2012;2012:489608.
3. Gonzalez-Gay MA, Gonzalez-Juanatey C, Martin J. Rheumatoid arthritis: a disease associated with accelerated atherogenesis. *Semin Arthritis Rheum* 2005;35:8-17.
4. Dessein PH, Joffe BI. Insulin resistance and impaired beta cell function in rheumatoid arthritis. *Arthritis Rheum* 2006;54:2765-75.

5. Chung CP, Oeser A, Solus JF, Gebretsadik T, Shintani A, Avalos I, et al. Inflammation-associated insulin resistance: differential effects in rheumatoid arthritis and systemic lupus erythematosus define potential mechanisms. *Arthritis Rheum* 2008;58:2105-12.
6. Dessein PH, Tobias M, Veller MG. Metabolic syndrome and subclinical atherosclerosis in rheumatoid arthritis. *J Rheumatol* 2006;33:2425-32.
7. Ferraz-Amaro I, Gonzalez-Juanatey C, Lopez-Mejias R, Riancho-Zarrabeitia L, Gonzalez-Gay MA. Metabolic syndrome in rheumatoid arthritis. *Mediators Inflamm* 2013;2013:710928.
8. La Montagna G, Cacciapuoti F, Buono R, Manzella D, Mennillo GA, Arciello A, et al. Insulin resistance is an independent risk factor for atherosclerosis in rheumatoid arthritis. *Diab Vasc Dis Res* 2007;4:130-5.
9. Ferraz-Amaro I, Garcia-Dopico JA, Medina-Vega L, Gonzalez-Gay MA, Diaz-Gonzalez F. Impaired beta cell function is present in non-diabetic rheumatoid arthritis patients. *Arthritis Res Ther* 2013;15:R17.
10. Ferraz Amaro I, Diaz Gonzalez F, Gonzalez Juanatey C, Gonzalez Gay MA. Resistencia a la insulina y la artritis reumatoide (Spanish). Insulin resistance and rheumatoid arthritis. *Reumatol Clin* 2011;7:124-9.
11. Graham TE, Yang Q, Blucher M, Hammarstedt A, Ciaraldi TP, Henry RR, et al. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. *N Engl J Med* 2006;354:2552-63.
12. Gavi S, Stuart LM, Kelly P, Melendez MM, Mynarcik DC, Gelato MC, et al. 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;92:1886-90.
13. Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature* 2005;436:356-62.
14. Farjo KM, Farjo RA, Halsey S, Moiseyev G, Ma JX. Retinol-binding protein 4 induces inflammation in human endothelial cells by an NADPH oxidase- and nuclear factor kappa B-dependent and retinol-independent mechanism. *Mol Cell Biol* 2012;32:5103-15.
15. Norseen J, Hosooka T, Hammarstedt A, Yore MM, Kant S, Aryal P, et al. Retinol-binding protein 4 inhibits insulin signaling in adipocytes by inducing proinflammatory cytokines in macrophages through a c-Jun N-terminal kinase- and toll-like receptor 4-dependent and retinol-independent mechanism. *Mol Cell Biol* 2012;32:2010-9.
16. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO 3rd, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum* 2010;62:2569-81.
17. Gonzalez-Gay MA, Gonzalez-Juanatey C, Vazquez-Rodriguez TR, Miranda-Filloo JA, Llorca J. Insulin resistance in rheumatoid arthritis: the impact of the anti-TNF-alpha therapy. *Ann N Y Acad Sci* 2010;1193:153-9.
18. Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum* 1995;38:44-8.
19. Pincus T, Swearingen C, Wolfe F. Toward a multidimensional Health Assessment Questionnaire (MDHAQ): assessment of advanced activities of daily living and psychological status in the patient-friendly health assessment questionnaire format. *Arthritis Rheum* 1999;42:2220-30.
20. Executive summary of the 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). *JAMA* 2001;285:2486-97.
21. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. *Diabetes Care* 2004;27:1487-95.
22. Kotnik P, Fischer-Posovszky P, Wabitsch M. RBP4: a controversial adipokine. *Eur J Endocrinol* 2011;165:703-11.
23. Bobbert T, Raila J, Schwarz F, Mai K, Henze A, Pfeiffer AF, et al. Relation between retinol, retinol-binding protein 4, transthyretin and carotid intima media thickness. *Atherosclerosis* 2010; 213:549-51.
24. Gonzalez-Juanatey C, Llorca J, Martin J, Gonzalez-Gay MA. Carotid intima-media thickness predicts the development of cardiovascular events in patients with rheumatoid arthritis. *Sem Arthritis Rheum* 2009;38:366-71.
25. Wasko MC, Kay J, Hsia EC, Rahman MU. Diabetes mellitus and insulin resistance in patients with rheumatoid arthritis: risk reduction in a chronic inflammatory disease. *Arthritis Care Res* 2011;63:512-21.
26. Dessein PH, Stanwix AE, Joffe BI. Cardiovascular risk in rheumatoid arthritis versus osteoarthritis: acute phase response related decreased insulin sensitivity and high-density lipoprotein cholesterol as well as clustering of metabolic syndrome features in rheumatoid arthritis. *Arthritis Res* 2002;4:R5.
27. Dessein PH, Joffe BI, Stanwix A, Botha AS, Moomal Z. The acute phase response does not fully predict the presence of insulin resistance and dyslipidemia in inflammatory arthritis. *J Rheumatol* 2002;29:462-6.
28. Dessein PH, Joffe BI, Stanwix AE. Inflammation, insulin resistance, and aberrant lipid metabolism as cardiovascular risk factors in rheumatoid arthritis. *J Rheumatol* 2003;30:1403-5.
29. Cho YM, Youn BS, Lee H, Lee N, Min SS, Kwak SH, et al. Plasma retinol-binding protein-4 concentrations are elevated in human subjects with impaired glucose tolerance and type 2 diabetes. *Diabetes Care* 2006;29:2457-61.
30. Hermsdorff HH, Zulet MA, Puchau B, Martinez JA. Central adiposity rather than total adiposity measurements are specifically involved in the inflammatory status from healthy young adults. *Inflammation* 2011;34:161-70.
31. Schacke H, Docke WD, Asadullah K. Mechanisms involved in the side effects of glucocorticoids. *Pharmacol Ther* 2002;96:23-43.