Circulating Retinol-Binding Protein-4, Insulin Sensitivity, Insulin Secretion, and Insulin Disposition Index in Obese and Nonobese Subjects

MONTSERRAT BROCH, PHD[1,2]
JOAN VENDRELL, MD, PHD[1]
WIFREDO RICART, MD[3,4]
CRISTÓBAL RICHART, MD, PHD[1,2]
JOSÉ-MANUEL FERNÁNDEZ-REAL, MD, PHD[3,4]

OBJECTIVE — Recent investigations disclosed an upregulation of retinol-binding protein-4 (RBP4) in the adipose tissue of several insulin-resistant mouse models and increased serum RBP4 concentration in subjects with obesity and type 2 diabetes in association with insulin resistance. There is some experimental evidence that RBP4 also could been linked to insulin secretion.

RESEARCH DESIGN AND METHODS — We aimed to evaluate insulin secretion, insulin sensitivity, insulin disposition index (minimal model analysis), and circulating RBP4 (enzyme-linked immunosorbent assay) in nondiabetic men with a wide range of obesity (n = 107).

RESULTS — Serum RBP4 concentration was nonsignificantly different among lean, overweight, and obese subjects. Circulating RBP4 was not associated with age, BMI, waist-to-hip ratio, or metabolic parameters, including insulin sensitivity ($r = -0.03$, $P = 0.6$). On the contrary, circulating RBP4 was negatively associated with insulin secretion, especially in obese subjects ($r = -0.48$, $P = 0.007$), in whom RBP4 also was linked to insulin disposition index ($r = -0.44$, $P = 0.01$). On multiple regression analyses to predict insulin secretion (acute insulin response [AIR_g]), insulin sensitivity was the only factor that contributed to 17% of AIR_g variance in nonobese subjects. In obese subjects, however, RBP4 emerged as an independent factor that contributed independently to AIR_g variance (23%).

CONCLUSIONS — Our results suggest that oversecretion of RBP4 may negatively affect β-cell function directly or by preventing the binding of transthyretin to its receptor. These mechanisms could be behind the association between increased circulating RBP4 and type 2 diabetes. RBP4 could be one signal from insulin-resistant tissues that impacts on β-cell secretion.

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Serum retinol-binding protein-4 (RBP4) recently has been found to be increased in insulin-resistant subjects (1). Graham et al. (1) reported increased serum RBP4 concentration in subjects with obesity or type 2 diabetes compared with lean subjects. Insulin resistance was positively associated with serum RBP4 concentration and invoked to be causally related with type 2 diabetes. In fact, RBP4 is upregulated in the adipose tissue of several insulin-resistant mouse models (1,2). Transgenic expression or injections of RBP4 caused insulin resistance in mice, whereas experimentally decreased RBP4 levels ameliorated insulin resistance in diet-induced obesity. RBP4 augmented hepatic gluconeogenesis and attenuated insulin signaling in skeletal muscle (2). RBP4 was established as a rodent adipokine several years ago (3,4) and confirmed recently (5).

Recent findings in humans suggest that the increase in systemic RBP4 concentrations in insulin-resistant subjects or subjects with type 2 diabetes is not explained by increased RBP4 production in adipose tissue (5). In this study, the authors did not see a relationship between adipose tissue RBP4 expression and serum RBP4 levels in postmenopausal women. In fact, RBP4 mRNA was downregulated in subcutaneous abdominal adipose tissue, and circulating RBP4 concentrations were similar in the normal weight, overweight, and obese groups (5). Five percent weight loss improved the homeostasis model assessment index by 20%, whereas this change was associated with only a small decrease of adipose RBP4 expression and no significant change in RBP4 serum levels. In addition, a relationship between the homeostasis model assessment index and adipose RBP4 expression or with circulating RBP4 concentrations was not observed (5).

On the other hand, it is well known that retinol is pathophysiologically linked to β-cell function (6). Because abnormalities in insulin secretion contribute also to the development of the metabolic abnormalities observed in type 2 diabetes, we hypothesized that, besides insulin sensitivity, serum RBP4 levels also could be related to insulin secretion and the insulin disposition index.

RESEARCH DESIGN AND METHODS

— We studied 107 nondiabetic men who were consecutively enrolled in a prospective study of cardiovascular risk factors as described in Gubern et al. (7).

From the [1]Reseach Unit, Pere Virgili Institute for Biomedical Research, Tarragona, Spain; the [2]CIBER Fisiopatología Obesidad y Nutrición CB06/03/0011, Instituto de Salud Carlos III, Hospital Joan XXII, Tarrogona, Spain; the [3]Diabetes, Endocrinology and Nutrition Unit, Dr. Josep Trueta Hospital, Girona, Spain; and the [4]Girona Institute for Biomedical Research and CIBER Fisiopatología Obesidad y Nutrición CB06/03/0010, Instituto de Salud Carlos III, Girona, Spain.

Address correspondence and reprint requests to José Manuel Fernández-Real, MD, Unit of Diabetes, Endocrinology and Nutrition, Dr. Josep Trueta Hospital, Av. Francia s/n, 17007 Girona, Spain. E-mail: uden.jmfernandezreal@htrueta.scs.es.

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Abbreviations: AIR, acute insulin response; ELISA, enzyme-linked immunosorbent assay; RBP4, retinol-binding protein-4; TTR, transthyretin.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Clinical and laboratory variables in study subjects*

Characteristic	
n	107
Age (years)	50.4 ± 11.3
BMI (kg/m^2) (range)	27.8 ± 3.6 (19.4–39.8)
Waist-to-hip ratio	0.93 ± 0.06
Systolic blood pressure (mmHg)	126 ± 15
Diastolic blood pressure (mmHg)	80 ± 10
HDL cholesterol (mg/dl)	46 ± 13
Triglycerides (mg/dl)	102 (71.5–142.5)
Fasting glucose (mg/dl)	97 ± 10
Fasting insulin (mIU/l)	8.7 (6.1–11.9)
S_I (mIU · 1^{-1} · min^{-1} · 10^{-4})	2.1 (1.1–3.3)
S_G (min)	0.019 ± 0.006
AIR_g (mIU · 1^{-1} · min^{-1})	342 (176–536)
RBP4 (mg/dl)	3.8 ± 1.03

Data are means ± SD for Gaussian variables and median (interquartile range) for non-Gaussian variables. S_I, S_G, and AIR_g: from frequently sampled intravenous glucose tolerance tests.

Inclusion and exclusion criteria

All subjects reported that their body weight had been stable for at least 3 months before the study. A food frequency questionnaire was obtained from all subjects. None of the subjects was taking any medication or had any evidence of metabolic disease other than obesity. Inclusion criteria were *1*) BMI <40 kg/m^2, *2*) absence of any systemic disease, and *3*) absence of clinical symptoms and signs of infection in the previous month by structured questionnaire to the patient. Informed consent was obtained from all subjects. The local ethics committee approved the study.

Measurements

BMI was calculated as weight (in kilograms) divided by height (in meters) squared (kg/m^2). The subjects' waist was measured with a soft tape midway between the lowest rib and the iliac crest. The hip circumference was measured at the widest part of the gluteus region. The waist-to-hip ratio was then calculated. Blood pressure was measured in the supine position on the right arm after a 10-min rest. A standard sphygmomanometer of appropriate cuff size was used, and the first and fifth phases were recorded. Values used in the analysis are the average of three readings taken at 5-min intervals. Patients were requested to withhold alcohol and caffeine during at least 12 h before the different tests.

Insulin sensitivity and secretion

All subjects had fasting plasma glucose <7.0 mmol/l. Type 2 diabetes was ruled out by an oral glucose tolerance test according to criteria from the American Diabetes Association (8). Insulin sensitivity was measured using the frequently sampled intravenous glucose tolerance test. Insulin secretion was calculated as the insulin area during the first 10 min of the frequently sampled intravenous glucose tolerance test. This test also provides the insulin disposition index, a parameter emerging from the model that represents the ability of the pancreatic islets to compensate for insulin resistance. The disposition index is useful in the search of the causes of the failure of adequate β-cell compensation in type 2 diabetes and in the recognition of the nature of the signal(s) from insulin-resistant tissues that fail to elicit the appropriate β-cell increment in sensitivity to glucose and other stimuli (9).

In brief, the experimental protocol started between 8:00 and 8:30 A.M. after an overnight fast. A butterfly needle was inserted into an antecubital vein, and patency was maintained with a slow saline drip. Basal blood samples were drawn at −30, −10, and −5 min, after which glucose (300 mg/kg body wt) was injected over 1 min starting at time 0, and insulin (0.03 units/kg; Actrapid, Novo, Denmark) was administered at time 20. Additional samples were obtained from a contralateral antecubital vein up to 180 min, as previously described (7).

Analytical methods

Blood samples were drawn from each subject after an overnight fasting period. Serum was centrifuged at 4,000*g* for 10 min, immediately divided into aliquots, and frozen at −80°C until analysis. Serum glucose concentrations were measured in duplicate by the glucose oxidase method with the use of a Beckman Glucose Analyser II (Beckman Instruments, Brea, CA). The coefficient of variation was 1.9%. Serum insulin concentrations were measured in duplicate by a monoclonal immuno-radiometric assay (IRMA; Medgenix Diagnostics, Fleunes, Belgium). Intra-assay and interassay coefficients of variation were <7% (7,10). A1C was measured by high-performance liquid chromatography by means of a fully automated glycated hemoglobin analyser system (Hitachi L-9100).

Total serum cholesterol was measured through the reaction of cholesterol esterase/cholesterol oxidase/peroxidase. Total serum triglycerides were measured through the reaction of glycerol-phosphate-oxidase and peroxidase. Serum RBP4 concentrations were measured by nephelometry (Dade Behring, Marburg, Germany). Sensitivity of the method is 0.01 mg/ml. The intra-assay and interassay coefficients of variation were 3.1 and 2.2%, respectively.

Statistical methods

Descriptive results of continuous variables are expressed as means (±SD), if normally distributed, or as median and interquartile range. Before statistical analysis, normal distribution and homogeneity of the variances were evaluated using Kolmogorov–Smirnov' s test and Levene's test and then variables were given a log transformation if necessary. These parameters (insulin sensitivity, insulin secretion, insulin disposition index, and triglycerides) were analyzed on a log scale and tested for significance on that scale. The anti–log transformed values of the means are reported in the Tables. Differences between groups were tested by ANOVA's test for continuous variables. Relation between variables was tested using Pearson's test. Multivariate linear regression analysis was performed in a stepwise manner. A value of $P \leq 0.05$ was considered significant. Given this value of $P = 0.05$, the study had an 85% power to detect significant correlations (Pearson's coefficient of at least 0.3) between parameters in bilateral tests. The study also had a 67% power to detect significant differences of at least 1 SD in serum RBP4 concentration between obese and nonobese

subjects. Computations were carried out with SPSS version 11.0.

RESULTS — Main characteristics of study subjects are shown in Table 1. We studied 25 lean subjects (BMI ≤25 kg/m^2, mean 23.5 ± 1.04; mean age 46.2 ± 10.3 years), 52 subjects with overweight (BMI ≥25 and <30; mean BMI 27.2 ± 1.4 kg/m^2, mean age 51.3 ± 10.8 years), and 30 obese subjects (BMI ≥30 kg/m^2, mean 33.5 ± 2.4; mean age 52.3 ± 11.6 years). Age was not significantly different among these groups (ANOVA $P = 0.1$). In all subjects as a whole, circulating RBP4 was not found to be associated with age, BMI, waist-to-hip ratio, blood pressure, or circulating lipids. Serum RBP4 concentration was found to be similar among lean (3.6 ± 0.7 mg/dl), overweight (3.9 ± 0.9 mg/dl), and obese subjects (4.02 ± 1.1 mg/dl) ($P = 0.4$).

A total of 5 lean, 9 overweight, and 12 obese subjects showed glucose intolerance (2-h oral glucose tolerance test serum glucose between 7.8 and 11.1 mmol/l). After excluding these subjects from the analyses, the results remained essentially the same. Serum RBP4 concentration was not significantly different between subjects with normal or impaired glucose tolerance, both in nonobese subjects (3.8 ± 0.8 vs. 3.8 ± 1.08 mg/dl, $P = 0.6$, in normal and glucose-intolerant subjects, respectively) and obese subjects (3.9 ± 1 vs. 4.06 ± 1.2 mg/dl, $P = 0.3$, in normal and glucose-intolerant subjects, respectively).

Circulating RBP4 tended to be positively linked to glycated hemoglobin ($r = 0.16$, $P = 0.08$) and did not show association with insulin sensitivity ($r = -0.03$, $P = 0.6$). On the contrary, circulating RBP4 was negatively associated with insulin secretion ($r = -0.27$, $P = 0.006$) and tended to be associated with insulin disposition index ($r = -0.18$, $P = 0.06$). When we evaluated these associations separately in obese and nonobese subjects, the relationships of RBP4 with insulin secretion and insulin disposition index were especially significant among obese subjects (Fig. 1). In these subjects, RBP4 also was negatively associated with insulin at 30 min after oral glucose load, a surrogate of insulin secretion ($r = -0.39$, $P = 0.03$).

Circulating RBP4 concentrations were not associated with S_G (glucose effectiveness) in all subjects as a whole or in obese and nonobese subjects separately (r coefficients from 0.0005 to 0.02, P = NS). On multiple regression analyses to predict insulin secretion (acute insulin response [AIR_g]), insulin sensitivity was the only factor that contributed to 17% of AIR_g variance in nonobese subjects (Table 2). In obese subjects, however, RBP4

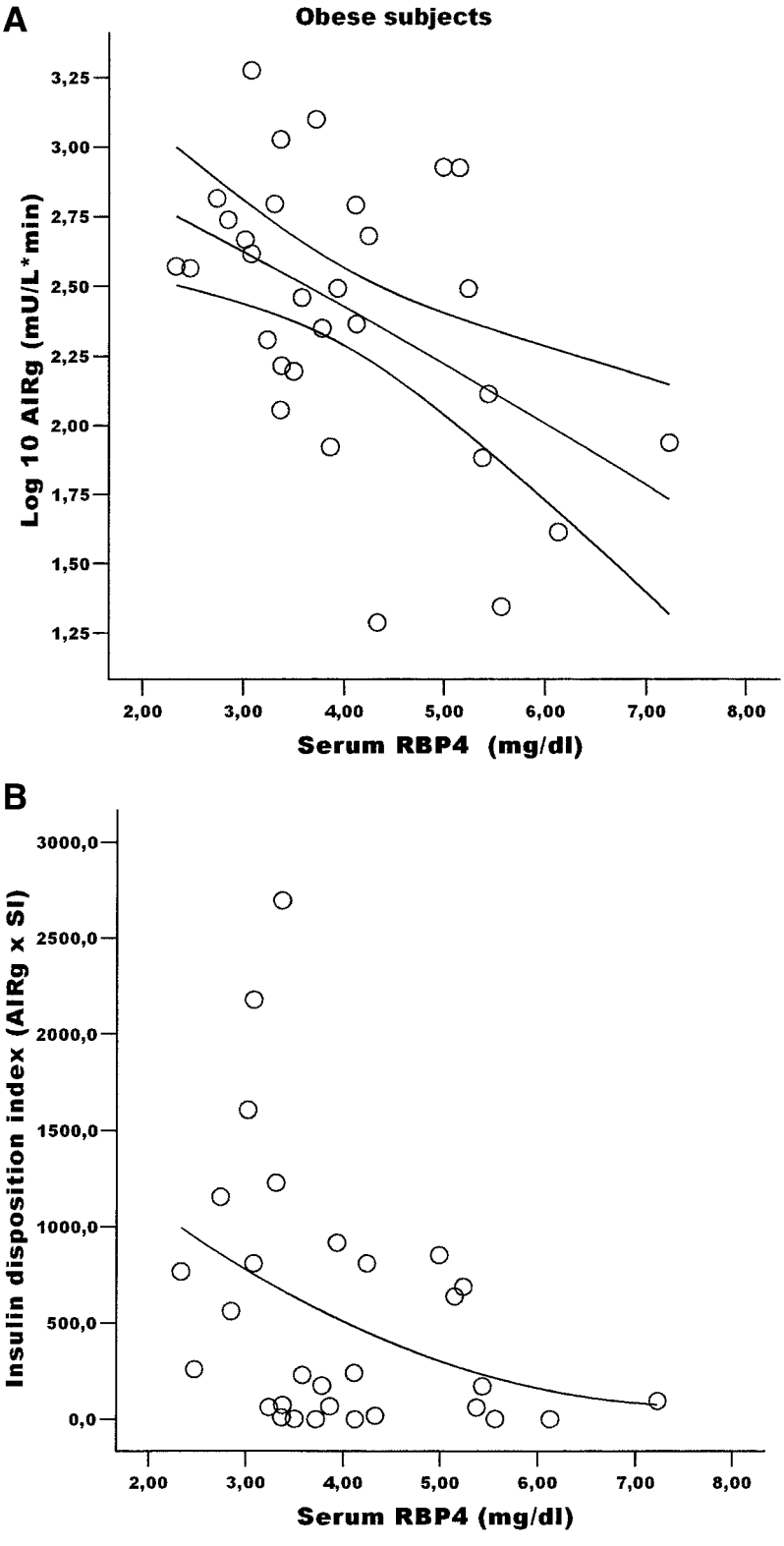

Figure 1—*Correlation graph of serum RBP4 and log_{10}-transformed AIR_g (from frequently sampled intravenous glucose tolerance test studies)* (A) *and insulin disposition index* (B) *in obese men.* r *and* P *values are shown from Pearson's analysis (nonadjusted data).* A: r = *−0.48;* P = *0.007.* B: r = *−0.44;* P = *0.01.*

Table 2—*Linear multivariate regression analysis with AIR_g as a dependent variable*

	Coefficients				
	Unstandardized coefficients		Standardized coefficients		
	β	SE	β	*t*	*P*
Nonobese subjects					
Independent variables (constant)	2.297	0.653		3.519	0.001
Insulin sensitivity	−0.522	0.231	−0.311	−2.257	0.028
BMI	0.023	0.020	0.152	1.103	0.275
Age	0.000	0.004	−0.002	−0.019	0.985
RBP4	−0.023	0.038	−0.074	−0.614	0.542

	Coefficients				
	Unstandardized coefficients		Standardized coefficients		
	β	SE	β	*t*	*P*
Obese subjects					
Independent variables (constant)	2.644	1.262		2.095	0.048
Insulin sensitivity	0.539	0.424	0.208	1.273	0.216
BMI	0.026	0.033	0.131	0.789	0.439
Age	−0.011	0.007	−0.248	−1.442	0.163
RBP4	−0.175	0.073	−0.413	−2.408	0.025

emerged as an independent factor that contributed independently to AIR_g variance (23%) after controlling for BMI, age, and insulin sensitivity (Table 2, *lower panel*).

CONCLUSIONS — In this article, we describe that circulating RBP4 was not significantly associated with insulin sensitivity or obesity status in middle-aged men. These findings appear to contradict a previous publication (1). This could be related to differences concerning the relatively small population studied. Graham et al. (1) evaluated seven obese men and nine obese men with type 2 diabetes compared with five lean subjects in study 1. The differences also could be due to the RBP4 assay. Western blotting and an enzyme-linked immunosorbent assay (ELISA) sandwich assay produced RBP4 measurements that distinguished normal individuals from insulin-resistant individuals and correlated with magnitude of insulin resistance (1). However, that ELISA is no longer available (11). These authors compared different methods measuring plasma RBP4 in a very recent study (11). One of the conclusions was that competitive ELISAs may selectively underestimate serum RBP4 levels in the setting of insulin resistance due to assay saturation. In addition, commercially available sandwich ELISA reports RBP4 concentrations that inversely correlate with insulin resistance, but values in normal subjects are higher than expected (11). The authors also stated that other assay methods, especially nephelometry, deserve testing (11). This was precisely the method we used.

In contrast to this recent observation (1), Janke et al. (5) also found that RBP4 was not associated with insulin resistance evaluated using homeostasis model assessment. The authors argued that "detection of RBP4-mediated changes in insulin sensitivity may require more accurate measurements of insulin sensitivity and patients with a wider range of insulin sensitivities than used in our study." We have used a relatively strong measure of insulin sensitivity in subjects with a wide range of adiposity, and we also did not observe associations between RBP4 and insulin sensitivity. In agreement with the findings by Janke et al. (5) in postmenopausal women, we did not detect differences in serum RBP4 concentration according to obese status in middle-aged men.

In contrast, we observed a significant association between circulating RBP4 and insulin secretion in all subjects as a whole. This association was especially remarkable in obese subjects in whom RBP4 also was negatively linked to insulin disposition index. The insulin disposition index represents the ability of the pancreatic islets to compensate for insulin resistance. The disposition index is useful in the search for the causes of the failure of adequate β-cell compensation in type 2 diabetes and in the recognition of the nature of the signal(s) from insulin-resistant tissues that fail to elicit the appropriate β-cell increment in sensitivity to glucose and other stimuli. Importantly, circulating RBP4 was independently associated with insulin secretion even after accounting for insulin sensitivity, BMI, and age among obese subjects. Our data suggest that obese subjects with increased circulating RBP4 show incapacity to adapt to low insulin sensitivity by enhancing insulin secretion (Fig. 1), and, in keeping with this, they also show a reduced insulin disposition index. We should point out that the negative relationship between RBP4 and insulin secretion was observed after an oral glucose bolus and also after an intravenous load, indicating that the associations detected do not depend on the route of glucose administration.

It is well known that retinol is pathophysiologically linked to β-cell function (6). On the other hand, retinol-binding protein circulates in serum, forming a complex with transthyretin (TTR), a transport protein for thyroxine. A recent investigation disclosed that TTR constitutes a functional component in pancreatic β-cell stimulus-secretion coupling (12). TTR inhibits the binding of RBP to the receptor (13). It thus is possible that increased serum RBP4 prevents TTR from exerting its β-cell stimulus-secretion effects.

In fact, circulating RBP4 is highly bound to TTR in a one-for-one stoichiometric ratio, and there is little or no "free RBP4" in circulation (14). In a very recent study (11) using gel filtration chromatography to analyze the RBP4-TTR complex, the authors found that increased serum RBP4 remains bound to TTR in insulin-resistant states. Because the affinity of RBP4-TTR binding is very strong (14), the relative stoichiometry and affinity of the two proteins in serum could conceivably influence kinetics of RBP4-antibody binding.

In summary, our results suggest that oversecretion of RBP4 may negatively affect β-cell function directly or by preventing the binding of TTR to its receptor. These mechanisms could be behind the association between increased circulating RBP4 and type 2 diabetes (1). To our

knowledge, this is the first report of an association between serum RBP4, insulin secretion, and insulin disposition index.

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