Retinol-binding protein 4 is associated with impaired glucose tolerance but not with whole body or hepatic insulin resistance in Mexican Americans

Alberto O. Chavez,1 Dawn K. Coletta,1 Subhash Kamath,1 Douglas T. Cromack,1,2 Adriana Monroy,1 Franco Folli,1 Ralph A. DeFronzo,1,2 and Devjit Tripathy1,2

1Diabetes Division and 2South Texas Veterans Health Care System, University of Texas Health Science Center at San Antonio, San Antonio, Texas

Submitted 2 September 2008; accepted in final form 19 January 2009

Chavez AO, Coletta DK, Kamath S, Cromack DT, Monroy A, Folli F, DeFronzo RA, Tripathy D. Retinol-binding protein 4 is associated with impaired glucose tolerance but not with whole body or hepatic insulin resistance in Mexican Americans. Am J Physiol Endocrinol Metab 296: E758–E764, 2009. First published February 3, 2009; doi:10.1152/ajpendo.90737.2008.—Retinol-binding protein-4 (RBP4), a novel protein secreted mainly by adipose tissue, has been associated with insulin resistance in obese subjects and in individuals with type 2 diabetes mellitus (T2DM). We examined the relationship between plasma RBP4 levels, expression of RBP4 in skeletal muscle and adipose tissue, and insulin sensitivity in Mexican Americans with varying degrees of obesity and glucose tolerance. Seventy-two subjects [16 lean normal-glucose-tolerant (NGT), 17 obese NGT, and 39 subjects with impaired fasting glucose/impaired glucose tolerance/T2DM] received an oral glucose tolerance test (OGTT) and euglycemic-hyperinsulinemic clamp. Insulin secretion was measured as insulinogenic index during OGTT. In a subset of subjects, hepatic glucose production was measured by 3-[1-1H]glucose infusion, biopsies of the vastus lateralis muscle and subcutaneous adipose tissue were obtained under basal conditions, and quantitative RT-PCR was performed to measure the RBP4 mRNA gene expression. Plasma RBP4 was significantly elevated in impaired glucose tolerance/T2DM compared with NGT lean or obese subjects. Plasma RBP4 levels correlated with 2-h glucose, triglycerides, and hemoglobin A1c. There was no association between RBP4 levels and whole body insulin sensitivity measured with either the euglycemic insulin clamp or OGTT, basal hepatic glucose production rates, and the hepatic insulin resistance index. There was no correlation between plasma RBP4 levels and indexes of insulin secretion. RBP4 mRNA expression in skeletal muscle was similar in lean NGT subjects, obese NGT subjects, and T2DM subjects. There was no difference in RBP4 mRNA expression in adipose tissue between lean and obese NGT subjects or between NGT and T2DM individuals. Plasma RBP4 levels are elevated in T2DM and associated with impaired glucose tolerance, but not associated with obesity or insulin resistance or impaired insulin secretion in Mexican Americans.

insulin sensitivity; adipokines; pathogenesis; type 2 diabetes mellitus

INSULIN RESISTANCE IS A CHARACTERISTIC FEATURE of type 2 diabetes mellitus (T2DM), prediabetic (IGT) individuals, and normal glucose tolerant (NGT) subjects with a strong family history of diabetes (9, 12). Adipose tissue secretes several cytokines that affect, both positively and negatively, insulin sensitivity (7, 20, 32, 37). Retinol-binding protein-4 (RBP4), a fat-derived adipokine, has been shown to be associated with insulin resistance and decreased expression of GLUT4 (16, 18, 24, 38). In skeletal muscle, RBP4 causes insulin resistance by impairing insulin signaling, and in the liver RBP4 increases gluconeogenesis (38).

Some (6, 33, 38), but not all (23, 35, 39), studies in humans have shown that plasma RBP4 levels are elevated in T2DM patients, and an inverse correlation has been observed between the plasma RBP4 concentration and insulin sensitivity. Insulin resistant subjects with T2DM, hypertension, and polycystic ovarian syndrome have increased serum RBP4 levels and elevated RBP4 levels predict future diabetes (18, 33, 36). Interventions, such as weight loss and gastric banding, that lead to improved insulin sensitivity have been shown to decrease RBP4 levels (2, 18, 19, 22). However, these data have not been replicated in all populations. Recent studies failed to demonstrate any correlation between RBP4 levels and insulin resistance in T2DM patients (22, 29, 31, 39). In lean NGT subjects, no difference in RBP4 levels was seen between insulin-resistant and insulin-sensitive subjects with and without a family history of T2DM (28). In obese women, neither plasma RBP4 levels nor RBP4 expression in adipose tissue was increased, and RBP4 levels did not correlate with insulin sensitivity (22).

Mexican Americans have one of the highest prevalence rates of T2DM (14, 26, 27). The main objective of this study was to examine whether plasma RBP4 levels and skeletal muscle and adipose tissue RBP4 expression was associated with insulin resistance in obese and/or glucose-intolerant nondiabetic and T2DM individuals of Mexican American decent. We report that, in this ethnic population, plasma RBP4 concentrations are elevated in T2DM and do not correlate with measures of insulin resistance or insulin secretion.

EXPERIMENTAL DESIGN

Subjects. Seventy-two Mexican American subjects received a euglycemic-hyperinsulinemic clamp and a 75-g oral glucose tolerance test (OGTT). Subjects were divided into the following three groups: 16 lean subjects with NGT [lean NGT, body mass index (BMI) <25 kg/m²], 17 obese (BMI >30 kg/m²) NGT subjects, and 39 subjects with IGT/T2DM (IGT/IGT, n = 15 and T2DM, n = 24) according to American Diabetes Association criteria (25). Nine of the lean NGT subjects had a strong family history of T2DM. All subjects had normal liver, cardiopulmonary, and kidney function as determined by medical history, physical examination, screening blood tests, electrocardiogram, and urinalysis. No NGT or IGT subject was taking any medication known to affect glucose tolerance.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Subjects with T2DM were treated with diet alone (n = 5), sulfonylurea (n = 4), metformin (n = 10), or a combination of both (n = 5). No T2DM patients were on thiazolidinedione or insulin. Body weight was stable (±1.5 kg) for at least 3 mo before study in all subjects. No subject participated in any excessively heavy exercise program. The study protocol was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio, and informed written consent was obtained from all subjects before their participation. All studies were performed at the General Clinical Research Center of the University of Texas Health Science Center at 0800 following a 10- to 12-h overnight fast.

OGTT. Before the 75-gram OGTT, a small polyethylene catheter was placed in an antecubital vein, and blood samples were collected at −30, −15, 0, 15, 30, 45, 60, 75, 90, 105, and 120 min for the measurement of plasma glucose and insulin concentrations.

Euglycemic insulin clamp. Before the start of the insulin clamp, a catheter was placed in an antecubital vein for the infusion of all test substances. A second catheter was inserted retrogradely in a vein on the dorsum of the hand for blood withdrawal, and the hand was placed in a thermoregulated box heated to 70°C. At 0800, all subjects received a primed-continuous infusion of insulin at 480 pmol (80 mU)·min⁻¹·m⁻² for 240 min. During the 30 min before the start of insulin, plasma samples were taken at 5- to 10-min intervals for the determination of plasma glucose and insulin concentrations. During the insulin infusion, plasma glucose concentration was measured every 5 min, and a variable infusion of 20% glucose was adjusted, based on the negative feedback principle, to maintain the plasma glucose concentration at each subject’s fasting plasma glucose (FPG) level with a coefficient of variation <5% (11). In diabetic subjects, the plasma glucose concentration was allowed to decline to 100 mg/dl, at which level it was maintained for the duration of the study. A subset (n = 35) received a primed (25 μCi)-continuous (0.25 μCi/min) infusion of 3-[³H]glucose (DuPont NEN Life Science Products, Boston, MA), which was continued for 2 h. After the basal tracer equilibration period, subjects received a primed-continuous insulin infusion. During the last 30 min of the basal equilibration period, plasma samples were taken at 5- to 10-min intervals for the determination of plasma glucose and insulin concentrations and tritiated glucose radioactivity. Vastus lateralis muscle biopsies were taken at the start of the insulin clamp in the lean NGT (n = 9), obese NGT (n = 6), and T2DM individuals (n = 13). Subcutaneous adipose tissue biopsies also were performed on a separate day under fasting conditions in lean NGT (n = 7), obese NGT (n = 6), and T2DM individuals (n = 16).

Analytical procedures. Plasma glucose concentration was determined by the glucose oxidase reaction (Glucose Oxidase Analyzer; Beckman, Fullerton, CA), and plasma insulin concentration was measured by radioimmunoassay (Coat A Coat; Diagnostic Products, Los Angeles, CA). The intra-assay and interassay coefficients of variation were 4.5 and 5.2%, respectively. Plasma samples were frozen immediately and stored at −80°C until analyzed. Fasting plasma RBP4 concentration was measured by radioimmunoassay (Coat A Coat; Beckman, Fullerton, CA). Muscle expression of RBP4 was determined using a one-step QRT-PCR from total RNA (Applied Biosystems 7900HT). The amount of RBP4 mRNA in each sample was normalized to the amount of 18S ribosomal RNA using the comparative (2⁻ΔΔCT) method.

Calculations. Endogenous glucose production (EGP) was calculated as the tritiated glucose infusion rate (dpm/min) divided by the plasma tritiated glucose specific activity (dpm/mg). During the insulin clamp, non-steady-state conditions for tritiated glucose specific activity prevail, and the rate of glucose appearance (Ra) was calculated with Steele’s equation (30). The rate of residual EGP during the insulin clamp was calculated by subtracting the rate of exogenous glucose infusion rate from the tracer-derived Ra. Insulin sensitivity was calculated as the mean glucose infusion rate (Ra) (mg·kg⁻¹·min⁻¹) necessary to maintain euglycemia during the last 40 min of the euglycemic-hyperinsulinemic clamp. Insulin sensitivity index (ISI) from the OGTT was estimated using the Matsuda index (25):

\[
\text{ISI} = 10,000 / \left( \text{Ins}_{\text{mean}} \times \text{Gluc}_{\text{mean}} \right) \times \left( \text{Ins}_0 \times \text{Gluc}_0 \right)
\]

where \( \text{Ins}_{\text{mean}} \) and \( \text{Gluc}_{\text{mean}} \) are the mean insulin and glucose concentrations, respectively, and \( \text{Ins}_0 \) and \( \text{Gluc}_0 \) are the respective basal insulin and glucose concentrations.

Insulin secretion indexes were calculated as the ratio of the incremental insulin-to-glucose response during the first 30 min (ΔI₀₋₃₀/ΔG₀₋₃₀) and during the 0–120 min (ΔI₀₋₁₂₀/ΔG₀₋₁₂₀) of the OGTT (13). The insulin secretion/insulin resistance (disposition) index from the OGTT was calculated as the ΔI₀₋₁₂₀/ΔG₀₋₁₂₀ × 1/Ra.

Statistical analysis. Unless otherwise stated, data represent the means ± SE. Differences between parameters were tested using ANOVA or Student’s t-test (for comparison between subjects with and without a family history of diabetes), and correlation between variables of interest was performed using either Pearson’s or Spearman’s correlation where appropriate. Plasma RBP levels were log transformed for normality. A P value <0.05 was considered statistically significant. The statistical software package SPSS (SPSS, Chicago, IL) was used.

RESULTS

Lean/obese NGT and T2DM subjects were well matched for age and gender (Table 1). BMI was similar in obese NGT and T2DM subjects. Subjects with T2DM had higher plasma triglyceride and lower plasma high-density lipoprotein cholesterol concentrations compared with NGT subjects. In T2DM, hemoglobin A₁c (HbA₁c) was 6.5 ± 0.2%, indicating reasonably good glycemic control.

Insulin sensitivity. As expected, obese NGT and T2DM subjects were more insulin resistant (measured both with the euglycemic clamp and ISI from OGTT) than lean NGT subjects. Insulin sensitivity was decreased more in T2DM subjects compared with obese NGT subjects. The insulin secretion/
Table 1. Clinical characteristics

<table>
<thead>
<tr>
<th></th>
<th>Lean NGT</th>
<th>Obese NGT</th>
<th>DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>16</td>
<td>17</td>
<td>39</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>8/8</td>
<td>12/5</td>
<td>17/12</td>
</tr>
<tr>
<td>Age, yr</td>
<td>38±2</td>
<td>43±2</td>
<td>48±2</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.8±0.5</td>
<td>32.4±1.1</td>
<td>34.2±0.4</td>
</tr>
<tr>
<td>FPG, mg/dl</td>
<td>91±1</td>
<td>89±2</td>
<td>131±6</td>
</tr>
<tr>
<td>2-h PG, mg/dl</td>
<td>97±4</td>
<td>110±4</td>
<td>197±10</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.2±0.1</td>
<td>5.3±0.1</td>
<td>6.5±0.2</td>
</tr>
<tr>
<td>Fasting PI, mU/l</td>
<td>2.77±0.5</td>
<td>5.2±1.2</td>
<td>12.5±1.9</td>
</tr>
<tr>
<td>2-h Insulin, mU/l</td>
<td>19.4±4.1</td>
<td>40±8*</td>
<td>61.3±8*</td>
</tr>
<tr>
<td>Rd, g·kg⁻¹·min⁻¹</td>
<td>10.05±0.5</td>
<td>8.6±0.4</td>
<td>5.8±0.4</td>
</tr>
<tr>
<td>EGP, mg·kg⁻¹·min⁻¹</td>
<td>2.1±0.4</td>
<td>1.9±0.1</td>
<td>2.3±0.1</td>
</tr>
<tr>
<td>Hepatic insulin resistance index</td>
<td>4.5±1.4</td>
<td>8.9±4.4</td>
<td>23.7±3.3*</td>
</tr>
<tr>
<td>ISI⁰⁻¹⁰⁻¹⁰⁻¹²⁰</td>
<td>14.9±1.9</td>
<td>8.5±1.9*</td>
<td>4.1±0.6*</td>
</tr>
<tr>
<td>ΔL₀⁻₃₀/ΔG₀⁻₃₀ Rd⁻¹</td>
<td>14.8±3.8</td>
<td>6.5±3.0</td>
<td>3.6±1.2</td>
</tr>
<tr>
<td>ΔL₁₀⁻₁₂⁰/ΔG₀⁻₁₂⁰ Rd⁻¹</td>
<td>15.8±3.5</td>
<td>12.1±2.9</td>
<td>4.2±1.5</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>183±7</td>
<td>179±7</td>
<td>180±6</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dl</td>
<td>115±6</td>
<td>116±7</td>
<td>111±5.2</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>46±2</td>
<td>46±3</td>
<td>38±2*</td>
</tr>
</tbody>
</table>
| (so called disposition) index declined progressively from lean NGT to obese NGT to obese T2DM. Plasma RBP4 levels (Fig. 1) were significantly higher in T2DM subjects compared with lean and obese NGT subjects (21.9 ± 2.7 vs. 26.6 ± 1.1 μg/ml, P = 0.02).

Correlation between insulin sensitivity and plasma RBP4. In all three groups collectively, there was no correlation between plasma RBP4 concentration and insulin sensitivity measured with the euglycemic insulin clamp (r = −0.061, P = 0.646; Fig. 2) or Matsuda insulin sensitivity index from OGTT (r = −0.042, P = 0.845). No correlation was observed between BMI and plasma RBP4 concentration (r = 0.044, P = not significant (NS)). Significant correlations were observed between the plasma RBP4 concentration and 2-h glucose during OGTT (r = 0.237, P = 0.05), HbA1c (r = 0.266, P = 0.02), and plasma triglyceride (r = 0.314, P = 0.007) concentration (Fig. 3, A-D and Table 2).

Hepatic insulin resistance and RBP4. The basal EGP tended to be higher in subjects with T2DM compared with NGT subjects. Because the FPG concentration primarily is determined by the rate of hepatic glucose production (8, 10), the product of the EGP × fasting plasma insulin provides a surrogate measure of hepatic insulin resistance (25). The hepatic insulin resistance index was significantly higher in T2DM subjects compared with NGT subjects (P < 0.05). No association was observed between plasma RBP4 levels and EGP (r = −0.040, P = NS) and hepatic insulin resistance index (r = 0.067, P = NS; Fig. 2B).

Insulin secretion and RBP4. A recent report indicated that increased plasma RBP4 concentrations were related to impaired insulin secretion rather than insulin sensitivity (5). Insulin secretion estimated as ΔL₀⁻₃₀/ΔG₀⁻₃₀ and ΔL₁₀⁻₁₂⁰ [area under the curve (AUC)/ΔG₀⁻₁₂⁰ (AUC)] did not correlate with the plasma RBP4 concentration (r = −0.013, P = NS). The insulin secretion/insulin resistance (so called disposition) index (Δ/ΔG ÷ Rd) also did not correlate with the plasma RBP4 concentration (r = −0.032, P = NS).
RBP4 mRNA expression in skeletal muscle and adipose tissue. Skeletal muscle RBP4 mRNA expression was not significantly different between lean and obese NGT subjects (1.02 ± 0.2 vs. 0.87 ± 0.3 relative units; \( P = \text{NS} \); Fig. 4A) and between NGT and T2DM (1.02 ± 0.2 vs. 1.71 ± 0.5 relative units; \( P = \text{NS} \)). Adipose tissue mRNA expression was not different between lean and obese NGT subjects (1.53 ± 0.7 vs. 0.48 ± 0.2 relative units; \( P = \text{NS} \)) or between NGT and T2DM subjects (1.53 ± 0.7 vs. 1.13 ± 0.2 relative units; \( P = \text{NS} \)).

**DISCUSSION**

Our results demonstrate that in Mexican Americans plasma RBP4 levels are elevated in T2DM subjects and correlate with measures of glycemia, but do not correlate with insulin sensitivity measured with two different techniques, euglycemic insulin clamp and Matsuda index from the OGTT.

The present findings are contrary to those of earlier publications that reported association of plasma RBP4 levels and insulin resistance in obese subjects (18, 38). Compared with these previous studies, the current study has significant differences and several strengths: 1) the population, Mexican Americans, represents a homogeneous group; 2) we measured plasma RBP4 levels and examined their relationship to whole body and hepatic insulin sensitivity over a wide spectrum of glucose tolerance (lean and obese NGT, and T2DM); and 3) RBP4 mRNA expression was measured in two insulin target tissues (skeletal muscle and adipose tissue). It is possible that different ethnic backgrounds (Mexican Americans in the current study compared with Caucasians in earlier reports) could explain the observed differences in plasma RBP4 levels. Al-

![Fig. 3. Correlation between plasma RBP4 concentration and fasting plasma glucose (A), 2-h glucose (B), hemoglobin A1c (HbA1c; C), and triglycerides (D).](image)
though plasma RBP4 levels were increased in T2DM, no association was observed between RBP4 levels and hepatic or whole body insulin sensitivity. Consistent with the latter observation, previous studies in different populations also failed to observe any association between circulating RBP4 levels and insulin sensitivity (29, 35, 39). A recent study in postmenopausal women also failed to show any difference in plasma RBP4 levels or mRNA expression in adipose tissue between obese and lean NGT subjects (22). Moreover, RBP4 mRNA expression has been shown to be reduced in the adipose tissue of obese women (22). We also examined the expression of RBP4 in the adipose tissue in NGT and T2DM subjects. We did not find any difference between lean and obese NGT subjects and subjects with T2DM. It is possible that adipose tissue in humans is not the major source of RBP4 and, as in rodents, liver could be the major source of circulating RBP4 (34). However, lack of association of RBP4 with hepatic glucose production and hepatic insulin resistance suggests against a role of RBP4 in either skeletal muscle or hepatic insulin resistance in Mexican Americans.

Different assays have been used to measure RBP4 levels, and this could account for the varied results reported by different laboratories (17). However, the plasma RBP4 levels estimated by the current assay are well within the range of values reported in the literature (22). A recent study reported a strong correlation between RBP4 measured by Western blot and by ELISA, but neither method was able to detect a difference in plasma RBP4 concentrations between insulin-sensitive and insulin-resistant individuals (35). In the present study, we did not employ Western blotting to measure plasma RBP4 concentrations. However, based on the results of recent studies (23, 35), one would not expect to observe a difference between insulin-sensitive and insulin-resistant groups even if the Western blot method was employed.

Our results differ from two recent studies that demonstrated elevated plasma RBP4 levels in IGT and type 2 diabetic subjects (6, 33). In addition to the difference in ethnic background (Japanese and Chinese), another important difference between these studies and the present study is the severity of obesity. Thus the diabetic subjects in these two previous studies (6, 33) were very lean (BMI \( \sim 24 \text{ kg/m}^2 \)), which is not the typical phenotype in Caucasian, African American, or Mexican American IGT/diabetic subjects in the United States.

Therapeutic interventions, like weight loss, exercise, and gastric bypass surgery, have been shown to reduce RBP4 levels in subjects with morbid obesity and T2DM (2, 18, 19). However, data concerning the effects of oral antidiabetic agents on plasma RBP4 levels are less clear. Treatment of IGT subjects with pioglitazone improved whole body insulin sensitivity, yet increased RBP4 gene expression in adipose tissue (39). Similarly, treatment with metformin, while leading to improvement in insulin sensitivity, was not associated with a significant decrease in plasma RBP4 in patients with polycystic ovarian syndrome (21).

We observed a positive correlation between the plasma RBP4 concentration and measures of glycemia (2-h plasma glucose, and HbA1c). A strong inverse association was observed between measures of hyperglycemia and indexes of insulin secretion (and insulin sensitivity). However, no significant relationship was found between plasma RBP4 levels and impaired insulin secretion. It is possible that the correlation between plasma RBP4 and glucose concentrations represents a secondary, rather than a primary, phenomenon. One way to distinguish between acquired, i.e., hyperglycemia, vs. genetic etiologies is to study subjects at high risk of developing diabetes but who have NGT. Thus Graham et al. (18) reported that NGT subjects with a family history of T2DM had elevated plasma RBP4 levels.

RBP4 is secreted by liver as well as by adipose tissue (4). In mice treated with recombinant RBP4, increased expression of the key gluconeogenic enzyme, phosphoenolpyruvate carboxykinase, has been reported (38). Thus it is possible that plasma RBP4 could be related to hepatic insulin resistance. To our knowledge, this is the first study in which the relationship between RBP4 and hepatic insulin resistance in humans has been assessed. We failed to find any relationship between the index of hepatic insulin resistance and plasma RBP4 levels \((r = 0.067, P = NS)\). Again, there could be a species-specific effect of RBP4 in mice.

One limitation of our study is that we have not measured plasma retinol concentrations, since RBP4 is the transport protein for retinol. However, studies in patients with T2DM have shown similar plasma retinol concentrations compared with NGT subjects (1, 3). Therefore, even though retinol concentrations are measured in the current study, it is unlikely to affect the results.

In summary, our results indicate that, in Mexican American individuals with IGT and T2DM, plasma RBP4 levels are elevated but are not associated with insulin resistance or diabetic dyslipidemia.
impaired insulin secretion. However, a correlation between the plasma RBP4 concentration and hyperglycemia was observed. To our knowledge, no previous report has examined the association between plasma RBP4 levels, insulin sensitivity, and insulin secretion in Mexican Americans. The failure to observe any correlations among these variables stands in contrast to some previous publications (15, 16, 18, 31) and suggests the presence of significant ethnic differences in the regulation of plasma RBP4 levels. We conclude that, in Mexican Americans, elevated plasma RBP4 may not play a role in the development of insulin resistance and type 2 diabetes mellitus.

REFERENCES

