High Plasma Retinol-Binding Protein-4 Level is Associated with Impaired Glucose Metabolism in Obese Subjects

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ABSTRACT
Background: Retinol-binding protein 4 (RBP4) is a novel adipokine that induces insulin resistance in mice. Objective: Measurement of serum RBP4 concentration and relationship to insulin sensitivity and secretion in obese subjects with dysglycemia. Design and Participants: This case-control study consisted of 100 subjects, 20 of them were healthy lean subjects and 80 obese patients. They were divided into three groups: normal glucose tolerance (NGT; n = 20), impaired glucose tolerance (IGT; n = 20), and type 2 diabetes (n = 40). Laboratory and anthropometric measurements including, serum RBP4 levels, were assessed. Results: Plasma RBP4 levels were higher in obese IGT and T2DM subjects compared with obese NGT subjects (23.7±10, 26.3±10 vs. 16.6±8 μg/ml, P<0.001). In obese IGT and T2DM subjects, a positive correlations were observed between the plasma RBP4 and SBP and DBP (r = 0.358, P<0.001 and r = 0.332, P<0.001, respectively). A positive correlations were observed between the plasma RBP4, BMI (r = 0.618, P<0.001), 2-hour PG (r= 0.485, P<0.001) during OGTT, HbA1C (r = 0.387, P<0.001), fasting insulin (r = 0.417, P<0.001), HOMA-IR (r = 0.450, P<0.001), total cholesterol (r = 0.283, P<0.01), LDL-C (r = 0.232, P<0.05) and triglyceride (r = 0.437, P<0.001). A negative correlations were observed between plasma RBP4 and HOMA-B (r = -0.215, P<0.001) and HDL-C (r = -0.250, P<0.05). Conclusions: RBP4 concentrations were elevated in obese subjects with IGT or type 2 diabetes and was related to clinical parameters of insulin resistance.  

Keywords: Adipokines, RBP4, Insulin resistance, HOMA-IR, HOMA-B and Type 2 DM.

INTRODUCTION
Insulin resistance (IR) 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.(1)

In addition to its main metabolic role of storing energy in the form of fat, adipose tissue is now considered an active endocrine organ that secretes a variety of bioactive peptides. These peptides are known as "adipokines" and they coordinate biological processes such as energy metabolism, immune and neuroendocrine functions.(2)

Obesity is a condition that is associated with low-level chronic inflammation, IR, hyperlipidaemia and other metabolic disorders. There is now growing evidence that adipokines are important in the etiopathology of obesity-related disorders, either through a traditional (circulating) hormonal effect or by a local action in the adipose tissue.(3)

Since the circulating levels of a recently identified adipokine that acts as a carrier of retinol (vitamin A) in the blood, retinol binding protein-4 (RBP4), have been positively correlated with obesity and IR in an adipocyte-specific glucose transporter 4 knock-out mouse model,(4) it has been proposed that RBP4 may be behind the adipocyte-muscle connection that links obesity and IR. However, its role in human
obesity and IR physiopathology is still unclear.\(^1\)

Some \(^6\,7\), but not all \(^5\,8\) studies in humans have shown that plasma RBP4 levels are elevated in T2DM patients, and an inverse correlation has 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 predicts future diabetes\(^7\,9\).

However, these data have not been replicated in all populations. Recent studies failed to demonstrate any correlation between RBP4 levels and IR in T2DM patients \(^10\,11\). 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\(^12\). In obese women, neither plasma RBP4 levels nor RBP4 expression in adipose tissue was increased, and RBP4 levels did not correlate with insulin sensitivity\(^13\).

The main objective of this study was to examine whether plasma RBP4 levels was associated with IR in obese and/or glucose-intolerant nondiabetic and T2DM individuals.

SUBJECTS AND METHODS

Study population:
This case control study was conducted at diabetes and obesity outpatient clinic of Internal Medicine and Biochemistry Departments, Faculty of Medicine, Zagazig University Hospitals and Banha University Hospitals.

One hundred subjects were included in this study and received a 75-gm oral glucose tolerance test (OGTT). Subjects were divided into the following four groups: 20 lean subjects with normal glucose tolerance (NGT) (Lean NGT, BMI <25 kg./m\(^2\)) as control, 20 obese (BMI > 30 kg/m\(^2\)) IGT (IFG/IGT) subjects and 40 obese (BMI > 30 kg/m\(^2\)) subjects with T2DM according to American Diabetes Association Criteria\(^14\). 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 subjects was taken any medication known to affect glucose tolerance such as β-blockers, thiazide diuretics or statin therapy for at least last two weeks.

Subjects with T2DM were treated with diet alone (n=5), metformin (n=10), sulfonylurea (n=10), or a combination of both (n=15). No T2DM patients were on thiazolidinedione or insulin. Body weight was stable (±1.5 kg) of at least 3 months before study in all subjects. No subjects participated in any excessively heavy exercise program. The study protocol was approved by the local ethics committee and informed written consent was obtained from all study participants before entering the study.

Anthropometrical measurements and analytical methods

Body height and weight were measured with the patient standing in light clothes and without shoes. BMI was calculated as body weight divided by height squared (kg/m\(^2\)). Blood pressure was measured in the supine position on the right arm after 10 min of 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. The same physician performed all examination.

OGTT. A 75-gram glucose load was performed between 8.00 and 10.00 am after an 8-hour overnight fast and blood samples were collected for the measurement of fasting (FBG) and 2-hour post load plasma glucose (2-hour PBG) and fasting serum insulin levels.

Blood samples were drawn from each subjects after an overnight fasting
period. Serum was centrifugated at 4,000 g for 10 min, immediately divided into aliquots, and frozen at -80°C until analysis. The serum glucose and lipid profile parameters were determined using standard clinical biochemistry methods. The level of low-density lipoprotein (LDL)-cholesterol was estimated using the formula: total cholesterol-HDL-cholesterol-(Triglyceride÷5). Fasting serum insulin concentrations were measured in duplicate by a monoclonal immuno-radiometric assay (the bio source INS-EASIA). Intra-assay and inter assay coefficients of variation were <7%. HbA1C was measured by high-performance liquid chromatography by means of a fully automated glycated hemoglobin analyzer system (Hitachi L-9100). Fasting plasma RBP4 concentration was measured in duplicated by enzyme-linked immunoassay kit (R&D systems, Inc., Minneapolis, United State of America).

Analysis of insulin resistance parameters:

IR was estimated by homeostasis model assessment of IR (HOMA-IR). HOMA-IR was calculated using fasting glucose and insulin with the formula.

\[ \text{HOMA-IR} = \frac{\text{glucose} \times \text{insulin}}{405} \]

The cut-off value of 1.77 for HOMA-IR, as defined for subjects with metabolic syndrome (15), was also used in the present study. β-cell function was estimated by homeostasis model assessment of β-cell function (HOMA-B). HOMA%B was calculated using fasting glucose and insulin with the formula. (16):

\[ \text{HOMA%B} = \frac{20 \times \text{insulin} \times \text{glucose}}{0.055 \times 3.5} \]

Statistical analysis:

All collected data were first assessed before taken for analysis. The normality test of Kosmogorov-Smirnov was done to assess whether the data were normally distributed or not. Unless otherwise stated, data represent the means ±SD. Differences between parameters were tested using ANOVA or student's t-test, post hoc tests (least significant of difference) were performed for multiple comparisons between groups, while X² tests were used for categorical variable. Correlation between variables of interest was performed using Pearson's correlation. A multiple linear regression analysis were performed using statistical package of social sciences (SPSS) for windows version 17. A P-value ≤ 0.05 was considered statistically significant.

RESULTS

Patients characteristics

Lean/obese NGT, IGT and T2DM subjects were well matched for age and gender (Table 1). BMI was statistically higher in obese subjects with or without NGT as compared to lean subjects with NGT (F = 158.29, P-value<0.001). However, BMI was similar in obese NGT, IGT and T2DM subjects. Both systolic (SBP) and diastolic blood pressure (DBP) were statistically higher among obese subjects with or compared to lean NGT subjects (F = 5.50, P-value<0.001 and F = 4.27, P-value<0.001, respectively). However, no significant difference as regard to both SBP and DBP, is observed in obese groups. Subjects with IGT and T2DM had higher FBG and 2-hour PBG levels than lean NGT subjects (F = 136.2, P-value<0.001 and F = 93.6, P-value<0.001, respectively). In T2DM, both FBG and 2-hour PBG levels were significantly higher compared with IGT subjects (P-value<0.001).

Obese IGT and T2DM subjects had lower fasting serum insulin levels compared
to lean/obese NGT subjects (F = 17.84, p-value<0.001). However, no significant difference was found between obese IGT and obese T2DM subjects (p-value>0.05). In T2DM, HbA1c was 7.4±0.5%, indicating to lesser extent good glycemic control.

Obese NGT, IGT and T2DM subjects had higher plasma triglyceride concentrations compared to lean NGT subjects (F = 8.8, p-value<0.001), however, no significant difference was found between obese NGT and IGT (p-value>0.05). Subjects with T2DM had higher plasma triglyceride and lower plasma high-density lipoprotein cholesterol concentrations compared with lean/obese NGT subjects (p-value<0.001) (Table 1).

**Insulin sensitivity.** As expected, obese NGT, IGT and T2DM subjects were more insulin resistant (measured by HOMA-IR) than lean NGT subjects (F = 46.3, p-value<0.001). Insulin sensitivity was decreased more in obese IGT and T2DM subjects compared with obese NGT subjects (p-value<0.001). Insulin secretion (measured by HOMA-B) was decreased in obese NGT, IGT and T2DM subjects compared with lean NGT subjects (F = 21.55, p-value<0.001). Obese T2DM subjects had lower insulin secretory function compared with obese NGT and IGT subjects (p-value<0.001) (Table 1).

**Circulating RBP4 concentrations in obese subjects with or without NGT**

Fasting plasma RBP4 levels were significantly higher in obese IGT and T2DM subjects compared with lean and obese NGT subjects (23.7±10, 26.3±10 vs. 6.1±1 and 16.6±8 μg/ml, F = 6.12, p-value<0.001). However, no significant difference was found between obese IGT and T2DM subjects (23.7±10 vs 26.3±10 μg/ml, p-value>0.05) (Table 2).

**Relationship between plasma RBP4, metabolic and anthropometric parameters**

Correlation between plasma RBP4 and various parameter in all three obese groups collectively, there was no correlation between plasma RBP4 and age (r = 0.143, p-value>0.05) or gender (r = 0.115, p-value>0.05). A highly significant positive correlation was observed between plasma RBP4 and BMI (r = 0.618, p-value<0.001) (Table 3).

In obese NGT and T2DM subjects, a highly significant positive correlations were observed between the plasma RBP4 concentration and systolic and diastolic blood pressure (r = 0.358, p-value<0.001 and r = 0.332, p-value<0.001, respectively) (Table 3).

Significant positive correlations were observed between the plasma RBP4 concentration and FBG (r = 0.506, p-value<0.001), 2-hour PBG (r = 0.485, p-value<0.001) during OGTT, HbA1C (r = 0.387, p-value<0.001), fasting serum insulin (r = 0.417, p-value<0.001), HOMA-IR (r = 0.450, p-value<0.001), serum total cholesterol (r = 0.283, p-value<0.01), serum LDL-C (r = 0.232, p-value<0.05) and serum triglyceride (r = 0.437, p-value<0.001). A negative correlations were observed between plasma RBP4 concentration and HOMA-B (r = -0.215, p-value<0.05) and HDL-C (r = -0.250, p-value<0.05) (Table 3 & Fig.1,2,3,4,5,6,7).

In the multivariate analysis, in a model including BMI and, as confounding factors, age, gender, FBG, fasting serum insulin, and HOMA-1R, we found that BMI (B = 0.868, p-value<0.001) and FBG level (B = 0.185, p-value<0.01) were independently associated with the RBP4 systemic levels (Table 4).
Table (1): Demographic data and clinical parameters of studies groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Lean NGT Group 1 (n=20)</th>
<th>Obese NGT Group 2 (n=20)</th>
<th>Obese IGT Group 3 (n=20)</th>
<th>Obese T2DM Group 4 (n=40)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>10/10</td>
<td>5/15</td>
<td>7/13</td>
<td>4/26</td>
<td>2.43</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Age (years)</td>
<td>39.3±9.5</td>
<td>43.1±8.8</td>
<td>46.4±8.39</td>
<td>45.9±11.1</td>
<td>158.29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.05±1</td>
<td>35.3±2.7^a</td>
<td>43.5±1.9^b</td>
<td>34.8±2.4^c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Systolic</td>
<td>116.5±339</td>
<td>129.4±13^a</td>
<td>134±13.5^b</td>
<td>133.6±13^c</td>
<td>5.50</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>- Diastolic</td>
<td>74.25±0.37</td>
<td>81±6.198^a</td>
<td>86.9±8.9^b</td>
<td>86.7±9.5^c</td>
<td>4.27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glycemic parameters:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- FBG (mg/dl)</td>
<td>87.9±3.5</td>
<td>92.6±4.5</td>
<td>107±11^bd</td>
<td>176.9±2939^ee</td>
<td>136.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>- PBG (mg/dl)</td>
<td>117.9±7.6</td>
<td>119±8.6</td>
<td>145±17^bd</td>
<td>211.5±35.6^ce</td>
<td>93.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>- HbA1C (%)</td>
<td>6.3±0.3</td>
<td>6.6±4^a</td>
<td>6.9±0.5^b</td>
<td>7.4±0.5^ce</td>
<td>25.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>- Fasting insulin (u/U/ml)</td>
<td>17.10±6.2</td>
<td>14.52±4.6</td>
<td>11.09±3.5^bd</td>
<td>8.15±1.6^c</td>
<td>17.84</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting lipid profiles:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Total Cholesterol (mg/dl)</td>
<td>196.8±22.7</td>
<td>207.1±34</td>
<td>220.9±25</td>
<td>208.6±38.7</td>
<td>1.81</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>- HDL-cholesterol (mg/dl)</td>
<td>41.95±6.3</td>
<td>42.5±6.5</td>
<td>41.15±6.75</td>
<td>34.78±5.08^ce</td>
<td>11.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>- LDL-cholesterol (mg/dl)</td>
<td>119.25±4.7</td>
<td>122.7±34.6</td>
<td>135.90±5.4</td>
<td>128.3±38</td>
<td>0.992</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>- Triglycerides (mg/dl)</td>
<td>178±23.9</td>
<td>209.1±40.4^d</td>
<td>219±29.9^b</td>
<td>229±42.7^ce</td>
<td>8.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.7±0.3</td>
<td>2.5±0.7^a</td>
<td>3.8±1.3^bd</td>
<td>7.4±3.0^ce</td>
<td>46.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-B</td>
<td>119±27.8</td>
<td>54.6±12^a</td>
<td>48.8±10.9^b</td>
<td>38.2±6^ce</td>
<td>21.55</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD
a = P-value<0.001 in comparison with group1 and 2
b = P-value<0.05 in comparison between group 1 and 3.
c = P-value>0.05 in comparison between group 1 and 4.
d = P-value>0.05 in comparison between group 2 and 3
e = P-value>0.05 in comparison between group 3 and 4

Table (2): Study of plasma RBP-4 (ug/ml) among the studied groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1 (n=20)</th>
<th>Group 2 (n=20)</th>
<th>Group 3 (n=20)</th>
<th>Group 4 (n=40)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBP-4 (ug/ml)</td>
<td>6.1±1</td>
<td>16.64±8^a</td>
<td>23.7±10^bd</td>
<td>26.3±10^c</td>
<td>6.12</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD
a = P-value<0.001 in comparison with group1 and 2
b = P-value<0.05 in comparison between group 1 and 3.
c = P-value>0.05 in comparison between group 1 and 4.
d = P-value>0.05 in comparison between group 2 and 3
e = P-value>0.05 in comparison between group 3 and 4
Table (3): Correlation of plasma RBP4 level with various metabolic variables in obese subjects with or without impaired glucose metabolism (n = 80).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Plasma RBP4 level</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.143</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>0.115</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.618</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Blood pressure (mmHg):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Systolic</td>
<td>0.358</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>- Diastolic</td>
<td>0.332</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Glycemic parameters:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- FBH (mg/dl)</td>
<td>0.506</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>- PBG (mg/dl)</td>
<td>0.485</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>- HbA1C (%)</td>
<td>0.387</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>- Fasting insulin (u/U/ml)</td>
<td>0.417</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.450</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>HOMA-B</td>
<td>-0.215</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Fasting lipid profile</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Total cholesterol (mg/dl)</td>
<td>0.283</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>- LDL-cholesterol (mg/dl)</td>
<td>0.232</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>- HDL-cholesterol (mg/dl)</td>
<td>-0.250</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>- Triglycerides (mg/dl)</td>
<td>0.437</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

Table (4): Multivariate analysis for the relationship between metabolic parameters and plasma RBP4 (ug/ml).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unstandardized coefficients</th>
<th>Standardized coefficients</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std. error</td>
<td>B</td>
</tr>
<tr>
<td>Constant</td>
<td>-25.008</td>
<td>10.117</td>
<td>-2.472</td>
</tr>
<tr>
<td>Age</td>
<td>-8.053E-02</td>
<td>0.088</td>
<td>-0.075</td>
</tr>
<tr>
<td>Gender</td>
<td>-4.385</td>
<td>3.171</td>
<td>-0.114</td>
</tr>
<tr>
<td>FBG</td>
<td>0.185</td>
<td>0.060</td>
<td>0.721</td>
</tr>
<tr>
<td>Fasting insulin</td>
<td>1.101</td>
<td>0.562</td>
<td>0.560</td>
</tr>
<tr>
<td>BMI</td>
<td>0.868</td>
<td>0.204</td>
<td>0.392</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>-2.948</td>
<td>1.498</td>
<td>-0.802</td>
</tr>
</tbody>
</table>
Fig (1): Plasma RBP4 concentrations (μg/ml) were highly correlated with BMI (kg/m²).

\[ r = 0.618 \]

Fig (2): Plasma RBP4 concentrations (μg/ml) were highly correlated with fasting blood glucose (mg/dl).

\[ r = 0.506 \]
Fig (3): Plasma RBP4 concentrations (μg/ml) were highly correlated with two hours post load blood glucose (mg/dl).

\[ r = 0.485 \]

Fig (4): Plasma RBP4 concentrations (μg/ml) were highly correlated with fasting serum Insulin (µU/ml).

\[ r = 0.417 \]
Fig (5): Plasma RBP4 concentrations (μg/ml) were highly correlated with HOMA .IR.

\[ r = 0.45 \]

Fig (6): plasma RBP4 concentrations (μg/ml) were highly correlated with HbA1c.

\[ r = 0.387 \]
Fig (7): Plasma RBP4 concentrations (μg/ml) were highly correlated with serum triglycerides (mg/dl) and serum cholesterol (mg/dl), while plasma RBP4 concentrations were modestly correlated with serum LDL.C (mg/dl) and serum HDL-C (mg/dl).

DISCUSSION

In the present study, we demonstrated that serum RBP4 levels were elevated in obese IGT and T2DM subjects compared to lean and obese NGT subjects and correlate with both measures of glycemia and insulin sensitivity.

Our results agreed with different clinical studies (4,17) that demonstrated elevated serum RBP4 levels in subjects with impaired glucose tolerance, T2DM and correlate inversely with insulin sensitivity in non-diabetic subjects with a family history of T2DM. Circulating RBP4 levels correlate with the degree of insulin resistance in these subjects and the relationship is independent of obesity. (18)

Our analysis between the circulating RBP4 protein levels and parameters of obesity revealed an increase in serum RBP4 levels in obese subjects with impaired glucose tolerance and T2DM. This positive relationship between serum RBP4 and obesity has been reported by other studies. (4,18).

In addition to increased BMI and whole-body fat content, increased circulating RBP4 levels are linked to increased visceral adipose tissue content (19). In a recent study, higher waist circumference and waist-to-hip ratio were associated with higher RBP4 levels and markers of systemic inflammation. (20).

Significant decrease in weight, achieved by diet, exercise, or bariatric surgery, leads to a decrease in circulating and/or adipose tissue RBP4 levels. (21) A decrease in serum RBP4 levels predicts the improvement in insulin sensitivity with greater specificity than leptin, adiponectin, interleukin-6 or C-reactive protein. (18).

Obesity is strongly associated with insulin resistance and impaired glucose-mediated insulin secretion. Adipose tissue is an endocrine organ secreting biologically active factors called adipokines. RBP4 is a recently identified adipokine suggested to
link obesity with its comorbidities, especially insulin resistance and T2DM.\(^{(22)}\)

Several mechanisms link RBP4 to IR and T2DM. Increasing serum RBP4 induces hepatic expression of the glyconeogenic enzyme phosphoenolpyruvate carboxykinase and impairs insulin signaling in skeletal muscle.\(^{(4)}\) Insulin signaling in primary human adipocytes was affected by RBP4 through blocking the insulin-stimulated phosphorylation of insulin receptor-1 at serine in position 307.\(^{(23)}\) In addition to decreased insulin sensitivity, a negative effect of RBP4 on the secreting function of the beta cell in suggested. It is well known that retinol, the ligand for RBP4, in pathophysiologically linked to B-cell function. 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 B-cell stimulus-secretion coupling. TTR inhibits the binding of RBP to the receptor. It is thus possible that increased serum RBP4 prevents transthyretin from exerting its B-cell stimulus-secretion effects.\(^{(24)}\)

Increased serum RBP4 levels have been reported in subjects with obesity, IR, and T2DM\(^{(18)}\) and in other insulin-resistant states, such as non alcoholic fatty liver disease and the metabolic syndrome.\(^{(25)}\)

In our study, there is a correlation between serum RBP4 and different parameters of metabolic syndrome such as BMI, blood pressure and fasting serum lipids in agreement with other studies.\(^{(29)}\)

In contrary other studies showed no association between circulating RBP4 concentration and parameters of IR.\(^{(10,24)}\) 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.\(^{(30)}\)

It is possible that the correlation between RBP4, glucose concentrations and HOMA-B could suggest that over-secretion of RBP4 may negatively affect B-cell function in agreement to Broch et al.\(^{(24)}\) who concluded that RBP4 could be one signal from insulin-resistant tissues that impacts on \(\beta\)-cell secretion and this mechanism could be behind the association between increased circulating RBP4 and type 2 diabetes.

However, Cho et al.\(^{(6)}\) reported no association between circulating RBP4, glucose concentrations and B-cell function.

Several explanations could be postulated to explain the controversy of different studies: different ethnic populations in the studies, RBP4 genetic variation, different methods of measurement of RBP4, and sex-specific dimorphism of RBP4.

The present findings are contrary to those of different ethnic backgrounds (Caucasian in the current study compared with Chinese and Japanese in earlier reports), could explain the observed differences in serum RBP4 levels. T2DM individuals of Mexican American decent reported that, in this ethnic population, serum RBP4 concentrations are elevated and do not correlate with measures of insulin resistance or insulin secretion.\(^{(30)}\)

Several RBP4 genetic variants are associated with insulin resistance and type 2 DM, possibly through an effect on RBP4 expression. The RBP4 haplotypes were
related to an increased risk of type 2DM and are associated with a higher visceral and subcutaneous RBP4 mRNA expression. However, the association among RBP4 levels, RBP4 gene expression, and measures of obesity or insulin resistance have not been universally described.\(^{(31)}\)

Different assays have been used to measure RBP4 levels, and this could account for the varied results reported by different laboratories. 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.\(^{(10)}\) In the present study, we did not employ western blotting to measure serum RBP4 concentrations. However, based on the results of other studies\(^{(32)}\) one would not expect to observe a difference between insulin-sensitive and insulin-resistant groups even if the western blot method was employed. The serum RBP4 levels estimated by the current assay are well within the range of values reported in the literature.\(^{(30)}\)

It is known that certain adipokines are expressed differently depending on the gender. Higher circulating levels of leptin and adiponectin are found in women.\(^{(33)}\) On the contrary, higher levels of circulating RBP4 are described in men.\(^{(34)}\) This dimorphism was, however, not shown in all studies.\(^{(10,35)}\)

It is suggested that sex-specific dimorphisms in circulating levels of adipokines can be related to direct effects of sex hormones on adipocyte expression and secretion. In case of RBP4, this possible mechanism is not supported by the fact that serum RBP4 levels are similar in pubertal and prepubertal subjects that significantly differ in levels of circulating sex hormones.\(^{(36)}\) A possible direct role of gonadotropins on the expression of RBP4 is also proposed, as circulating RBP4 levels are higher in post menopausal compared with premenopausal women and in women older than 50 years when compared with those younger than 50 years.\(^{(6)}\)

One limitation of our study is that we have not measured serum retinol concentration, since RBP4 is the transport protein for retinol. However, studies in patients with T2DM have shown similar serum retinol concentrations compared with NGT subjects.\(^{(37)}\) Therefore, even though retinol concentrations are not measured in the current study, it is unlikely to affect the results.

In summary, our results indicate that serum RBP4 levels are elevated in obese IGT and T2DM subjects, compared with lean subject and are associated with metabolic parameters of IR. Therefore, the elevated serum RBP4 is related to decreased insulin sensitivity. Our data indicate that over-secretion of RBP4 may negatively affect B-cell function and this mechanism could be behind the association between increased circulating RBP4 and type 2 diabetes.

We conclude that elevated serum RBP4 may play a role in the development of IR and B-cell dysfunction in obese subjects with abnormal glucose metabolism.

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