Increased serum retinol-binding protein-4 levels in pregnant women with and without gestational diabetes mellitus

Y.-X. Su\textsuperscript{a,1}, J. Hong\textsuperscript{a,1}, Q. Yan\textsuperscript{a}, C. Xu\textsuperscript{a}, W.-Q. Gu\textsuperscript{a}, Y.-F. Zhang\textsuperscript{a}, C.-F. Shen\textsuperscript{a}, Z.-N. Chi\textsuperscript{a}, M. Dai\textsuperscript{a}, M. Xu\textsuperscript{a}, Y.-W. Zhang\textsuperscript{a}, Q.-R. Liu\textsuperscript{a}, X.-Y. Li\textsuperscript{a,b}, G. Ning\textsuperscript{a,b}, W.-Q. Wang\textsuperscript{a,*}

\textsuperscript{a}State Key Laboratory of Medical Genomics, Shanghai Clinical Center for Endocrine and Metabolic Diseases, Shanghai Institute of Endocrine and Metabolic Diseases, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, 197 Ruijin Er Road, Shanghai 200025, PR China

\textsuperscript{b}Health Science Center, Shanghai Institute of Biological Sciences, Chinese Academy of Science and Shanghai Jiaotong University School of Medicine, Shanghai, PR China

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Abstract

Objective. – Retinol-binding protein 4 (RBP4) is thought to be associated with insulin resistance in humans, while pregnancy is normally characterized by progressive insulin resistance. Gestational diabetes (GDM) occurs when pancreatic beta-cell function is unable to compensate for insulin resistance. This study aimed to determine whether or not serum RBP4 levels are elevated in pregnancy, and to explore the relationship between RBP4 levels and insulin resistance during pregnancy.

Methods. – Serum RBP4 was measured at median gestational week 26 in 121 pregnant women, including 63 with GDM (GDM group) and 58 normal, glucose-tolerant pregnant women (P-NGT group), as well as 65 non-pregnant normal, glucose-tolerant women (NP-NGT group). Multiple stepwise regression analysis was used to explore the independent factors of RBP4.

Results. – Serum RBP4 levels in the P-NGT and GDM groups were significantly higher than in the NP-NGT group (34.50 ± 9.80 mg/L and 41.64 ± 12.21 mg/L vs 30.64 ± 9.46 mg/L, respectively; \(P < 0.05\)) after adjusting for age, body mass index (BMI) and blood pressure. Furthermore, RBP4 levels were much higher in the GDM vs P-NGT group. Spearman’s correlation analysis showed that serum RBP4 levels were positively correlated with triglycerides (TG), fasting plasma glucose, postprandial 2 h plasma glucose and HOMA-IR in pregnancy. Of these, TG and HOMA-IR (\(r^2 = 0.312\)) were independent factors of serum RBP4.

Conclusion. – Serum RBP4 levels are significantly increased in pregnancy, independent of age and BMI, and are also considerably higher in pregnant women with GDM than in those with normal glucose tolerance. In addition, serum RBP4 levels appear to be a valuable marker of insulin resistance and dysfunctional lipid metabolism in pregnancy.

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Keywords: Retinol-binding protein 4; Pregnancy; Gestational diabetes mellitus; Insulin resistance

Résumé

Élévation des concentrations sériques de retinol binding protein 4 chez des femmes enceintes avec et sans diabète gestationnel.

Objectif. – La retinol binding protein 4 (RBP4) est considérée comme associée à l’insulinorésistance chez l’homme. Une grossesse normale est caractérisée par une insulinorésistance progressive. Le diabète gestationnel (GDM) se développe lorsque les cellules β sont incapables de compenser cette insulinorésistance. Le but de cette étude était de chercher si les concentrations de RBP4 étaient élevées chez les femmes enceintes, et d’explorer les rapports entre concentrations de RBP4 et insulinorésistance pendant la grossesse.

Méthodes. – Les concentrations sériques de RBP4 ont été mesurées chez 121 femmes enceintes à la 26\textsuperscript{e} semaine de la grossesse, 63 atteintes de diabète gestationnel (groupe GDM) et 58 femmes enceintes dont la tolérance au glucose était normale (groupe P-NGT), ainsi que chez 65 femmes non-enceintes à tolérance au glucose normale (groupe NP-NGT). Les facteurs indépendants déterminants les concentrations de RBP4 ont été étudiés par analyse de régression multivariée.

* Corresponding author. Tel.: +86 21 64370045 ext. 665345; fax: +86 21 64373514.
E-mail address: wqingw@hotmail.com (W.-Q. Wang).
\textsuperscript{1} These authors contributed equally to this work.

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1. Introduction

Pregnancy is characterized by a progressive increase in nutrient-stimulated insulin responses, despite only minor deterioration of glucose tolerance, which is consistent with progressive insulin resistance. Also, hyperinsulinaemic–euglycaemic glucose clamp and intravenous glucose tolerance tests have indicated that insulin action in late normal pregnancy is 50–70% lower than in normal, non-pregnant women [1–3]. To maintain euglycaemia in a normal pregnancy, insulin secretion increases by 200–250% [3]. Metabolic adaptations, however, do not fully compensate in some pregnancies, and glucose intolerance ensues. Women with gestational diabetes mellitus (GDM) present with various features of the metabolic syndrome [4], such as obesity, low-grade inflammation [5] and dyslipidaemia [6,7], and have an increased risk of developing type 2 diabetes mellitus (T2DM) [8]. Although various placent al hormones have been suggested to reprogramme maternal physiology to meet fetal needs, the mechanism behind the change in insulin resistance during gestation remains obscure [9].

The expression of glucose transporter 4 (GLUT4), an insulin-regulated glucose transporter, is greatly reduced in the adipocytes of both rodents and humans who are obese and have insulin resistance [10,11]. In the adipocytes of women with GDM, GLUT4 levels may be reduced or the insulin-stimulated recruitment of GLUT4 to the plasma membrane may be impaired [11]. As shown by Yang et al. [12], the decrease in GLUT4 expression that occurs in the fatty tissue of obese animals is accompanied by an increased expression and secretion of the fat-derived factor retinol-binding protein 4 (RBP4). Experimentally raised levels of RBP4 have been demonstrated to impair insulin signaling and to induce gluconeogenic enzymes in the liver [12]. Recently, studies have indicated that patients with obesity and T2DM have elevated serum RBP4 concentrations [13,14], but contrary results have also been found by others [15,16]. Thus, the correlation between serum RBP4 and insulin resistance has been inconsistent. In addition, so far, the data for serum RBP4 levels in pregnant women are limited. For this reason, the present study aimed to measure serum RBP4 levels in pregnant women with and without GDM, and in non-pregnant healthy women. Our goal was to clarify the role of serum RBP4 in pregnant women, and to investigate whether or not there is a relationship between insulin resistance and serum RBP4 levels during pregnancy.

2. Subjects and methods

2.1. Subjects

All of the study participants (aged 24–34 years) were unrelated Han Chinese women living in Shanghai, including 121 pregnant women and 65 non-pregnant normal, glucose-tolerant women who were matched for age and body mass index (BMI). The study protocol was approved by the Research Ethics Board of Ruijin Hospital, and all participants gave their written informed consent. Pregnant women were recruited from July 2005 to July 2007 at the Department of Gynecology and Obstetrics of Ruijin Hospital when they visited for a routine prenatal examination, and were referred to undergo a 100 g oral glucose tolerance test (OGTT) following an abnormal result (plasma glucose > 7.8 mmol/L at 1 h postchallenge) on a screening 50 g glucose challenge test (GCT) at gestational week 24–28. Of these women, 63 were prescribed with GDM and 58 were not, according to the criteria of the American Diabetes Association (ADA); all were also matched for age and prepregnancy BMI [17]. Exclusion criteria included women with multiple pregnancies, fetal anomalies, preexisting hypertension, or DM or other chronic diseases, or who were currently using corticosteroids.

In addition, 65 non-pregnant normal glucose-tolerant women (NP-NGT) were recruited as the control group. The control subjects were all healthy volunteers who had neither previous GDM nor a family history of T2DM. Furthermore, all of the controls had undergone a 75 g OGTT to exclude impaired glucose tolerance.

2.2. Baseline evaluation

On the day of the OGTT, demographic and clinical-history information was collected by an interviewer-administered questionnaire. The data collected included: (1) patient demographics; (2) information regarding the current pregnancy, including illnesses, infections and medications; (3) personal medical, obstetric and smoking history; and (4) family history. Specific GDM risk factors were assessed, such as age, prepregnancy weight and weight-gain during pregnancy, personal history of GDM and family history of GDM or T2DM. Height (to the nearest 0.5 cm), weight (to the nearest 0.1 kg) and blood pressure (to nearest 1 mmHg) were also measured by the same interviewer.
2.3. Laboratory measurements

Plasma glucose was determined by a glucose-oxidase electrode method (Beckman Coulter Inc, Fullerton, CA, USA); serum insulin was measured by radioimmunoassay (DSL Inc, Webster, TX, USA); serum total cholesterol (TC) and triglycerides (TG) were measured by the enzymatic method, and high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol were measured, using a specific precipitation method (Beckman Coulter LX20, Brea, CA, USA).

For enzyme-linked immunosorbent assay (ELISA) of RBP4, serum RBP4 levels were measured in duplicate by a sandwich ELISA protocol developed in house, using affinity-chromatography-purified polyclonal and monoclonal antibodies generated against recombinant human RBP4 protein [16]. The assay system was subsequently cross-validated by Western blotting [18]. The intra-assay coefficient of variation (CV) was 1.8–7.6% and the interassay CV was 3.7–8.8%.

2.4. Oral glucose tolerance test and homoeostasis model assessment

Subjects were fasted overnight for at least 10 h prior to the OGTT test. After a blood sample was taken for fasting plasma glucose measurement, the OGTT was performed with the standard glucose load for pregnant women (100 g) and the standard glucose load for the non-pregnant controls (75 g). Insulin resistance was determined by homoeostasis model assessment of insulin resistance (HOMA-IR), which was calculated based on fasting insulin and glucose according to the following equation: HOMA-IR = fasting serum insulin (\(\mu\)IU/mL) \times fasting plasma glucose (mmol/L)/22.5. In addition, insulin secretion was determined by HOMA-\(\beta\) index, which was calculated using the following formula: [20 \times fasting serum insulin (\(\mu\)IU/mL)]/[fasting plasma glucose (mmol/L) – 3.5].

2.5. Statistical analysis

Data were evaluated using SPSS software for Windows® (version 11.0; SPSS Inc, Chicago, IL, USA). Normally distributed data were expressed as means ± SD. Skewed variables (RBP4, insulin, HOMA-IR, TG and HbA1c) were log-transformed for all statistical analyses and reported as medians with interquartile ranges. Characteristics of subjects between groups were compared by one-way analysis of variance (ANOVA) and covariance (ANCOVA). Spearman’s correlation and multiple-regression analyses were carried out to determine the relationship between the variables. All reported \(P\) values were two-tailed, and \(P\) values < 0.05 were considered statistically significant.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>NP-NGT</th>
<th>P-NGT</th>
<th>GDM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28.1 ± 3.4</td>
<td>28.4 ± 2.4</td>
<td>28.8 ± 1.8</td>
</tr>
<tr>
<td>BMI-1 (kg/m²)</td>
<td>20.6 ± 1.7</td>
<td>20.7 ± 1.9</td>
<td>21.1 ± 1.9</td>
</tr>
<tr>
<td>BMI-2 (kg/m²)</td>
<td>–</td>
<td>24.9 ± 2.1</td>
<td>25.5 ± 2.6</td>
</tr>
<tr>
<td>Gestational age at GCT (week)</td>
<td>–</td>
<td>26.4 ± 2.1</td>
<td>26.9 ± 1.9</td>
</tr>
<tr>
<td>SBP-1 (mmHg)</td>
<td>107.1 ± 12.1</td>
<td>108.5 ± 7.8</td>
<td>107.9 ± 9.7</td>
</tr>
<tr>
<td>DBP-1 (mmHg)</td>
<td>69.1 ± 7.5</td>
<td>70.6 ± 5.0</td>
<td>70.7 ± 7.8</td>
</tr>
<tr>
<td>SBP-2 (mmHg)</td>
<td>–</td>
<td>114.2 ± 8.5</td>
<td>115.6 ± 12.1</td>
</tr>
<tr>
<td>DBP-2 (mmHg)</td>
<td>–</td>
<td>73.5 ± 7.6</td>
<td>76.1 ± 8.8</td>
</tr>
<tr>
<td>1 h GCT glucose (mmol/L)</td>
<td>–</td>
<td>8.1 ± 1.6</td>
<td>10.1 ± 1.6 ²</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.75 (0.64–1.0)</td>
<td>2.6 (2.1–3.4) ²</td>
<td>2.9 (2.3–3.6) ²</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.3 ± 0.8</td>
<td>6.1 ± 1.1 ²</td>
<td>6.0 ± 1.2 ²</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.5 ± 0.3</td>
<td>1.9 ± 0.3 ²</td>
<td>1.9 ± 0.4 ²</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>2.5 ± 0.7</td>
<td>3.1 ± 0.9 ²</td>
<td>3.0 ± 0.9 ²</td>
</tr>
<tr>
<td>Fasting PG (mmol/L)</td>
<td>4.8 ± 0.5</td>
<td>4.4 ± 0.6 ²</td>
<td>5.2 ± 1.3 ²</td>
</tr>
<tr>
<td>2 h OGTT PG (mmol/L)</td>
<td>–</td>
<td>7.4 ± 0.8 ²</td>
<td>10. 5 ± 1.7 ²</td>
</tr>
<tr>
<td>Fasting insulin(\mu)IU/L)</td>
<td>6.7 (4.5–9.6)</td>
<td>9.6 (5.8–12.8)</td>
<td>9.4 (7.17–13.4)</td>
</tr>
<tr>
<td>2 h OGTT insulin ((\mu)IU/L)</td>
<td>–</td>
<td>64.80 (41.5–100.0)</td>
<td>88.1 (49.8–125.0) ²</td>
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<td>HbA1c (%)</td>
<td>5.17</td>
<td>5.47 ²</td>
<td>5.47 ²</td>
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<tr>
<td>HOMA-IR(\beta)</td>
<td>1.5 (1.2–1.7)</td>
<td>1.9 (1.7–2.2) ²</td>
<td>2.6 (2.2–3.0) ²</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>116.4 (69.7–156.3)</td>
<td>172.9 (111.9–342.3) ²</td>
<td>146.6 (86.0–209.6) ²</td>
</tr>
<tr>
<td>RBP4 (mg/L)</td>
<td>30.6 ± 9.5</td>
<td>34.5 ± 9.8 ²</td>
<td>41.6 ± 12.2 ²</td>
</tr>
</tbody>
</table>

\(^*P<0.05\) vs NP-NGT group; \(^\circ P<0.05\) vs P-NGT group.

BMI-1: prepregnancy body mass index (BMI) in pregnant women and BMI in NP-NGT women at the time of recruitment; BMI-2: BMI in pregnant women at the time of glucose challenge test (GCT); SBP-1/DBP-1: prepregnancy systolic/diastolic blood pressure in pregnant women and in NP-NGT women at the time of recruitment; SBP-2/DBP-2: SBP/DBP in pregnant women at the time of GCT; 2 h OGTT PG: postprandial 2 h plasma glucose at the time of oral glucose tolerance test (OGTT); 2 h OGTT insulin: postprandial 2 h serum insulin at the time of OGTT; HDL: high-density lipoprotein; LDL: low-density lipoprotein; HOMA-IR: homoeostasis model assessment of insulin resistance; HOMA-\(\beta\): homoeostasis model assessment of insulin sensitivity.

\(^a\) Log-transformed values used for analyses; data are expressed as means ± SD or medians (interquartile range).
3. Results

3.1. Baseline characteristics

Based on the pregnancy and OGTT results, study participants were stratified into the following three groups: non-pregnant and normal glucose tolerance (NP-NGT) \((n=65)\); normal, glucose-tolerant pregnant women (P-NGT) \((n=58)\); and pregnant women with GDM \((n=63)\). As shown in Table 1, there were no significant differences among the three groups in age, BMI-1 or systolic/diastolic blood pressure (SBP/DBP; prepregnancy SBP/DBP in pregnant women, and SBP/DBP at the time of recruitment in NP-NGT group). Also, no significant differences were found for gestational week, BMI at the time of the glucose challenge test (GCT; GCT-BMI), weight gain during pregnancy, SBP at GCT, DBP at GCT, and parity between the P-NGT and GDM groups. However, a family history of T2DM was significantly more prevalent in the GDM vs P-NGT group \((46.4\% \text{ vs } 23.3\%, \text{respectively}; P=0.008)\).

Pregnancy with or without GDM had higher levels of fasting insulin, HOMA-IR and HOMA-\(\beta\) than those in the NP-NGT group (Table 1). Moreover, the GDM group had significantly higher 1 h glucose after 50 g glucose loading, fasting glucose, and 2 h postload glucose and 2 h postload insulin levels, as well as higher HOMA-IR, but a lower HOMA-\(\beta\), than the P-NGT group (Table 1). TG and TC increased significantly in pregnant women with or without GDM than in the NP-NGT group, although there was no difference between the P-NGT and GDM groups in terms of lipid profiles.

Serum RBP4 levels were significantly higher in the P-NGT and GDM groups vs the NP-NGT group \((34.50 \pm 9.80 \text{ mg/L and } 41.64 \pm 12.21 \text{ mg/L vs } 30.64 \pm 9.46 \text{ mg/L, respectively}; \text{ all } P<0.05)\), even after adjusting for age, BMI and blood pressure. Furthermore, it was significantly higher in the GDM group than in P-NGT group \((P<0.001); \text{ after adjusting for age, BMI before and during pregnancy, blood pressure and gestational week } (P=0.011; \text{Table 1})\).

3.2. Associations of RBP4 and markers of insulin sensitivity and metabolic parameters in pregnancy

Spearman’s correlation analysis showed that serum RBP4 was positively correlated with fasting plasma glucose \((r=0.259, P=0.005)\), 1 h GCT glucose \((r=0.182, P=0.049)\), postprandial 2 h plasma glucose \((r=0.206, P=0.041)\), fasting insulin \((r=0.259, P=0.005)\), HbA1c \((r=0.293, P=0.005)\), HOMA-IR \((r=0.345, P<0.001)\), and TG \((r=0.341, P<0.001)\) in pregnant women (Table 2). No correlation was found between serum RBP4 and BMI (prepregnancy BMI in pregnant women). Moreover, stepwise linear-regression analyses revealed that TG and HOMA-IR \((r^2=0.312)\) were independent predictors of serum RBP4 concentrations in pregnant women (Table 3).

4. Discussion

Glucose metabolism disorder is a common medical complication of pregnancy, and its pathogenesis is associated with insulin resistance and deficiency of insulin secretion \([19,20]\); this situation is even worse in women with GDM. It has long been presumed that hormones secreted by the placenta might enhance insulin resistance. However, recent studies have demonstrated that increased circulating leptin \([21]\), reduced adiponectin \([22]\) and mild inflammation \([23,24]\) could lead to exaggerated insulin resistance in GDM \([25,26]\). RBP4, secreted by adipocytes and the liver, is a newly identified adipokine that has recently been shown to contribute to insulin resistance in several mouse models. Subsequent studies in humans have shown that serum RBP4 is elevated in obesity and in T2DM patients, and is a contributor to insulin resistance in such conditions.
cases. However, so far, there are limited data on RBP4 levels in pregnancy, and the relationship between serum RBP4 and insulin resistance has been inconsistent.

In the present study, serum RBP4 was measured in pregnancy with and without GDM as well as in non-pregnant controls. Our results demonstrate that serum RBP4 levels increased in pregnancy compared with non-pregnant women, which suggests that pregnancy itself may be correlated with RBP4 secretion. This result is partly in accordance with a study by Ueland et al. [27], which found that serum RBP4 levels increased with advancing gestation. On the other hand, our results also showed that serum RBP4 was significantly raised in women with GDM compared with healthy pregnant women. This result is similar to the previously published data from Lewandowski et al. [28]. Furthermore, a significant negative association between serum RBP4 and insulin sensitivity was confirmed by our present study, and this association persisted even after adjusting for age and BMI. This result is consistent with previous observations in non-pregnant individuals [12–14,29,30]. RBP4 in pregnancy was more strongly correlated with insulin resistance than with measures of adiposity. Our present findings suggest that higher RBP4 levels in GDM may be an early marker in the natural history of T2DM, with potential implications for the screening and prevention of the disease.

However, Krzyzanowska et al. [31] found that pregnant women with GDM had lower RBP4 levels than did healthy pregnant controls. They also discovered that women with GDM had higher RBP4-to-retinol molar ratios than did their pregnant controls. The reason for this was thought to be pregnancy-associated alterations in blood volume that induced lower retinol levels. RBP4 has to be bound to retinol to be secreted from hepatocytes, so that lower retinol levels could lead to lower serum RBP4 levels. In addition, gestational week and higher prepregnancy BMI are factors that can influence blood volume during pregnancy, but these two factors were not similar in the Krzyzanowska et al. and the present studies.

Dysfunction of lipid metabolism is a risk factor for cardiovascular disease (CVD) [32,33]. Recently, studies have demonstrated a possible role of RBP4 in lipid metabolism and the metabolic syndrome. von Eynatten et al. [15] found significant positive correlations between RBP4 and LDL cholesterol, very low-density lipoprotein (VLDL) cholesterol, TG and hepatic lipase activity in patients with T2DM and CVD. In the present study, TG levels were significantly higher in pregnant compared with non-pregnant women, but there was no difference between pregnant women with or without GDM. Our findings indicate that TG is an independent predictor of serum RBP4 concentration in pregnancy.

As far as we could ascertain, this was the first study to demonstrate and compare RBP4 concentrations between pregnant and non-pregnant women. Our results showed that serum RBP4 was significantly increased in pregnant women with or without GDM, and that serum RBP4 was correlated with HOMA-IR and TG. However, the present study has some limitations. Serum RBP4 was measured by ELISA and not by Western blot tests, the gold standard for RBP4 determinations, although some studies have determined that the correlation between the two RBP4 quantification techniques is highly significant ($r = 0.75; P < 0.001$) [15]. Also, the present study did not measure retinol and transthyretin, both of which are bound to RBP4 in plasma, nor did it measure the effect of RBP4 concentration on sex hormones or whether serum RBP4 changed acutely under particular conditions. Furthermore, the cross-sectional design of the study limited the ability to ascertain a causal relationship between increased serum RBP4 concentrations and pregnancy. Further studies are needed, especially those specifically designed to investigate the relationship between RBP4 and sex hormones in pregnant women. Also, studies investigating the association between placental RBP4 expression and insulin resistance may be informative, and might help to clarify the role of RBP4 in pregnancy.

Disclosure information

All authors have nothing to declare.

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