Insulin sensitivity and resistin levels in gestational diabetes mellitus and after parturition

Ana Megia, Joan Vendrell, Cristina Gutierrez, Modest Sabaté, Montse Broch, José-Manuel Fernández-Real and Inmaculada Simón
Endocrinology and Diabetes Research Department, University Hospital of Tarragona ‘Joan XXIII’, ‘Pere Virgili’ Institute, ‘Rovira i Virgili’ University, 43007, Tarragona, Spain; 1Laboratory Department, Hospital ‘St Pau i Sta. Tecla’, 43003, Tarragona, Spain and 2Endocrinology and Diabetes Unit, University Hospital ‘Josep Trueta’, 17007, Girona, Spain

(Correspondence should be addressed to A Megia who is now at Secció d’endocrinologia, Hospital Universitari ‘Joan XXIII’ de Tarragona, c/Mallafre´ Guasch, 4.43007 Tarragona, Spain; Email: amc@comt.es)

Abstract
Context: Resistin is expressed and secreted by the placenta during pregnancy. Increased serum resistin levels have been found in the second half of normal pregnancy, but its role in the pathogenesis of the insulin resistance of pregnancy is undetermined.
Objective: The objective of the study was to assess the relationship between circulating resistin levels and insulin sensitivity in gestational diabetes mellitus (GDM).
Design and setting: A case (n = 23)–control (n = 35) study was performed at the obstetrics and endocrinology clinic of a university hospital.
Patients: In total, 58 Caucasian women with a singleton pregnancy who had been referred for a 100 g oral glucose tolerance test were enrolled between the weeks 26 and 30, and 22 women with GDM were also evaluated after pregnancy.
Main outcome measures: Serum resistin and insulin sensitivity in GDM during and after pregnancy. The relationship of resistin to metabolic abnormalities was evaluated.
Results: Resistin levels were lower in GDM women than in pregnant women with normal glucose tolerance (NGT) (4.32 ± 1.56 vs 9.30 ± 1.32 ng/ml, P < 0.001), and experienced a further decrease after parturition (4.24 ± 1.56 vs 3.11 ± 1.63 ng/ml, P = 0.003). The association between low serum resistin levels and the diagnosis of GDM was independent of the degree of insulin sensitivity.
Conclusion: Lower resistin levels were observed in GDM than in NGT women and decreased after parturition, suggesting a role for resistin in the development of this disease. But we have failed to find an independent relationship between resistin levels and insulin sensitivity during pregnancy.

Introduction

Human pregnancy is characterised by a progressive decrease in insulin sensitivity, which parallels the growth of the foeto-placental unit and facilitates the diversion of glucose to the foetus. This situation is a physiological screening test for the insulin secretor capacity of β-cells and gestational diabetes mellitus (GDM) appears when pancreatic β-cells are unable to overcome this increase in insulin requirement. Debate exists over whether GDM represents a pregnancy-induced state of glucose intolerance or only the exacerbation of pre-existing insulin resistance state that will become evident in the future regardless of pregnancy. There are many similarities between GDM and the syndrome of insulin resistance in non-pregnant subjects (1), and GDM identifies a population of women at high risk of developing type 2 diabetes mellitus (T2 DM).

Therefore, understanding the factors involved in insulin resistance in pregnancy may open up more insights into insulin action and ultimately into understanding T2 DM (2).

Obesity is a risk factor for the development of T2 DM and GDM, and a relationship between mother obesity and systemic inflammatory response has been reported in GDM (3). Recently, interest has been focused on several adipose tissue-related mediators implicated in the pathogenesis of insulin resistance and inflammation, such as leptin, tumour necrosis factor-α (TNF-α) and adiponectin. Leptin and TNF-α have been related to insulin resistance during pregnancy (4), although no difference has been observed in TNF-α circulating among women with gestational diabetes and obese women with normal glucose tolerance (NGT) (5). Also, low first trimester and pre-pregnancy adiponectin levels have been reported in women with gestational diabetes (6), and adiponectin

European Journal of Endocrinology 158 173–178

© 2008 Society of the European Journal of Endocrinology

DOI: 10.1530/EJE-07-0671

Online version via www.eje-online.org

Downloaded from Bioscientifica.com at 08/10/2019 12:57:38AM via free access
levels during pregnancy have been negatively correlated with fasting insulin, glucose and pre-pregnancy body mass index (BMI) (2).

Resistin is another adipose tissue-related protein that has been related with the obesity-induced insulin resistance (7). In humans, resistin levels have been found elevated in obesity (8–11) and diabetes (12–16), although not all reports have been consistent in this regard (17–20). In humans, resistin mRNA is detectable in adipose tissue but mononuclear cells seem to be the principal source. During human pregnancy, resistin is expressed and secreted by the placenta (20) and resistin levels increase by the third trimester (21). These changes parallel resistin gene expression found in placental tissues during pregnancy (22) and it has been postulated that it could be implicated in the pathogenesis of the insulin resistance state found in the second half of pregnancy and in the development of gestational diabetes. Recent reports have measured resistin levels during pregnancies complicated by gestational diabetes with inconsistent results (23–25), and a fall-off of resistin levels has been observed immediately after parturition (25).

To study the influence of resistin in the development of gestational diabetes, we evaluated resistin levels and insulin sensitivity in women with and without gestational diabetes in the second half of pregnancy. Resistin levels were also measured in the GDM group after parturition to test differences with the pregnancy state that could help explain the development of the insulin resistant state.

**Subjects and methods**

**Subjects**

Totally, 58 women with a singleton pregnancy were recruited for the study.

Study participants were Caucasian pregnant women attending outpatient obstetrics clinics, who had been referred for a 100 g oral glucose tolerance test (OGTT) between 26 and 30 weeks of gestation. Gestational age was calculated according to the date of the last menstrual period and was confirmed by ultrasound in early pregnancy. Women with pre-existing chronic medical conditions, including collagen vascular diseases, inflammatory bowel disease and chronic inflammatory, and on current use of corticosteroids were excluded. Participants were recruited prior to undergoing the 100 g OGTT. Women were diagnosed with GDM if two or more of the four OGTT glucose levels exceeded the American Diabetes Association criteria (26), and the women who did not fulfill these criteria were considered to have NGT. Twenty-three women were diagnosed with GDM and 35 were of NGT. Among the 23 women with GDM, 10 were treated with diet and insulin through pregnancy, while the remaining 13 were treated with diet alone. None of the studied women had hypertension defined by diastolic blood pressure > 90 mmHg. The study was done according to protocol approved by the Hospital Ethics Committee and informed consent was obtained from all participants.

**Study protocol**

**Baseline evaluation** On the day of the 100 g OGTT, demographic and historical information was collected. using a standard questionnaire that included patient demographic data, information regarding current pregnancy including illnesses, infections and medications, personal medical and obstetrical history and family history. Specific GDM risk factors were assessed including age, pre-pregnancy weight, personal history of GDM, previous delivery of a macrosomic infant or type 2 diabetes. Anthropometric measurements of height (measured nearest to 0.5 cm) and weight (measured nearest to 0.1 kg) were obtained using a medical scale. Glucose levels were assayed in baseline fasting plasma samples and in the three post-challenge plasma samples collected at 1-h intervals.

**Postpartum evaluation** Women diagnosed with GDM were re-evaluated after pregnancy. A 75 g OGTT was performed 6 months after parturition and at least 1 month after breast-feeding cessation. On the day of the OGTT, weight (measured nearest to 0.1 kg), waist circumference (measured nearest to 0.1 cm) and blood pressure were recorded.

The insulin sensitivity index from the OGTT was calculated according to the equation derived by Matsuda & De Fronzo (27) in which insulin sensitivity is estimated by dividing a constant (10 000) by the square root of the product of fasting glucose (FG) times, fasting insulin (FI) times, mean glucose (G) times and mean insulin (I). \( ISI_{OGTT} = \frac{10,000}{(FG \times FI) \times (G \times I)} \). Mean glucose and mean insulin were calculated using measurements at baseline, and after 60, 120 and 180 min during the 100 g OGTT and using measurements at baseline, and after 30, 60 and 120 min during the 75 g OGTT. The index has been validated in pregnant patients and shows a good correlation with insulin sensitivity derived using the euglycaemic clamp technique (28). The incremental area under the insulin curve (AUC\(_{\text{insulin}}\)) and the incremental area under the glucose curve (AUC\(_{\text{glucose}}\)) were calculated using the trapezoidal rule.

**Laboratory measurements** The 100 g OGTT and the 75 g OGTT were performed in the morning after an overnight fast. During pregnancy, venous blood samples were drawn at baseline, 60, 120, and 180 min after ingestion of a standard 100 g glucose load and, in the postpartum evaluation, at baseline, 30, 60 and 120 min after ingestion of a standard 75 g glucose load. Serum glucose was measured with a glucose oxidase method.
using a Hitachi auto analyser. Specific insulin was measured at each of the four time points of the OGTT using monoclonal IRMA (Medgenix Diagnostics, Leuven, Belgium) in which proinsulin did not cross-react. The intra- and interassay coefficients of variation were 6 and 7% respectively. Sensibility was 4.1 μU/ml.

**Plasma resistin concentrations**

Resistin levels were measured by sandwich ELISA (BioVendor Laboratory Medicine Inc., Palackého, Czech Republic). The sensitivity was 0.2 ng/ml. The intra- and interassay coefficients of variation were 5.8 and 14.7% respectively.

**Statistical analysis**

SPSS 11.5 (SPSS Inc., Chicago, IL, USA) was used in all analysis. *P*<0.05 was considered statistically significant. Data are reported as mean±s.d. for continuous variables and number of cases (percentage) for categorical variables. The distribution of insulin, ISIOGTT, HOMA-R and resistin were skewed, thus data were log-transformed to normalise their distribution for calculation analysis we established, the population included in the analysis provided a power of 90% to detect a 20% difference in serum resistin.

**Results**

**Demographic and metabolic characteristics of study subjects**

Clinical and laboratory characteristics of subjects in the NGT and GDM groups are outlined in Table 1. Twenty-three women were diagnosed with GDM and 35 women were of NGT. Both groups were well matched with respect to age, parity and gestational week at testing. Despite a wider range of pre-pregnancy and current BMI in the GDM group (18.89–38.37 kg/m² vs 17.27–32.27 kg/m² and 21.94–40.47 kg/m² vs 23.75–34.78 kg/m²), no differences were observed when we compared both groups. Women in the GDM group reported more frequently first-degree family history of diabetes (2 vs 16%, *P*<0.001) and gestational diabetes in previous pregnancy (1 vs 4%, *P*<0.001).

Resistin levels were higher in the NGT women than in GDM group (9.30 ± 1.32 vs 4.32 ± 1.56 ng/ml, *P*<0.001). In the subset of women with GDM, no significant differences were observed between the group treated with nutrition therapy and those with additional insulin therapy (4.59 ± 1.47 vs 4.00 ± 1.70 ng/ml). As expected, basal glucose and insulin levels were significantly higher in the GDM than those in the NGT group (4.86 ± 0.62 vs 4.51 ± 0.32 mmol/l, *P*<0.001 and 77.09 ± 9.03 vs 30.91 ± 12.92 pmol/l, *P*<0.01 respectively). Insulin sensitivity determined by the ISIOGTT was lower in the GDM women (2.55 ± 1.43 vs 7.68 ± 1.05, *P*<0.001). Furthermore, in the GDM group, the AUCglucose and the AUCinsulin were also higher (1574.57 ± 149.98 vs 1207.43 ± 89.64 mmol/l per h, *P*<0.001 and 107 937.45 ± 11.81 vs 43 508.34 ± 10.97 pmol/l per h, *P*<0.001). Because

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Clinical and metabolic characteristics of the women studied during pregnancy.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGT group</td>
<td>GDM group</td>
</tr>
<tr>
<td>Age (years)</td>
<td>31.48±4.47</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>27.34±5.49</td>
</tr>
<tr>
<td>Pre-pregnancy BMI (kg/m²)</td>
<td>24.02±3.72</td>
</tr>
<tr>
<td>Current BMI (kg/m²)</td>
<td>27.49±3.24</td>
</tr>
<tr>
<td>Weight gain in pregnancy (kg)</td>
<td>7.16±3.74</td>
</tr>
<tr>
<td>Previous GDM (N %)</td>
<td>1 (2.9)</td>
</tr>
<tr>
<td>Fam Hx of GDM (N %)</td>
<td>2 (5.7)</td>
</tr>
<tr>
<td>Parity</td>
<td>1.86±1.31</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/l)</td>
<td>4.51±0.32</td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)</td>
<td>30.91±12.92</td>
</tr>
<tr>
<td>ISIOGTT</td>
<td>7.68±1.05</td>
</tr>
<tr>
<td>Resistin (ng/ml)</td>
<td>9.30±1.32</td>
</tr>
<tr>
<td>AUCinsulin (pmol/l per h)</td>
<td>43 508.34±10.97</td>
</tr>
<tr>
<td>AUCglucose (mmol/l per h)</td>
<td>1207.43±89.64</td>
</tr>
</tbody>
</table>

Fam Hx of GDM, familiar history of gestational diabetes mellitus; ISIOGTT, insulin sensitivity index; weight gain in pregnancy, amount of weight gain from the beginning of pregnancy until the day of evaluation; AUCglucose, area under the glucose curve; AUCinsulin, area under the insulin curve.

www.eje-online.org
insulin sensitivity may influence resistin levels, binary logistic regression was used to evaluate whether resistin levels and insulin sensitivity were independent in relation to the diagnosis of GDM. Before \( (B: -0.813, P<0.001) \) and after controlling for insulin sensitivity \( (B: -0.634, P<0.05 \) for resistin and \( B: -0.866, P=0.01 \) for ISI_OGTT), the highest concentrations of resistin were associated with women in the NGT group.

We observed a correlation between insulin sensitivity and resistin during pregnancy \( (r: 0.558, P<0.001) \). To study the compound effect of other variables known to affect insulin sensitivity, we performed a multiple linear regression analysis with insulin sensitivity as the dependent variable and resistin, pre-pregnancy BMI, age and the dichotomy variable GDM/no GDM as independent variables. BMI, age and the diagnosis of GDM were related independently to insulin sensitivity, but resistin was not (Table 2).

**Postpartum period**

To test differences between pregnancy and non-pregnant state, 22 of the 23 women with GDM were also evaluated 6 months after parturition, and at least 1 month after breast-feeding cessation. One of the women was lost during follow-up and was excluded from the statistical study. Insulin sensitivity improved significantly after pregnancy \( (ISI_{OGTT} 2.59 \pm 1.45 \) vs \( 3.54 \pm 1.46, P=0.001) \). Resistin levels decreased significantly after pregnancy \( (4.24 \pm 1.56 \) vs \( 3.11 \pm 1.63 \) ng/ml, \( P=0.003) \). Fasting glucose levels increased significantly in the postpartum evaluation \( (4.84 \pm 0.62 \) vs \( 5.44 \pm 0.53 \) mmol/l, \( P<0.001, P<0.001) \), while no difference was found in fasting insulin levels \( (78.81 \pm 9.24 \) vs \( 72.44 \pm 9.93 \) pmol/l).

A correlation was observed between postpartum ISI_OGTT and resistin levels in the postpartum period \( (r: 0.520, P<0.05) \), but when we adjusted for possible confounding variables (postpartum BMI and age), this association lost its significance.

**Discussion**

In this study, we report lower resistin levels in GDM women compared with NGT women during pregnancy and we confirm a decrease in resistin levels in the postpartum period in the GDM group. Nevertheless, we have failed to find an independent relationship between insulin sensitivity and serum resistin concentrations during pregnancy in our population. The relationship found between insulin sensitivity and resistin when we studied the whole group of pregnant women was lost when we considered the diagnosis of GDM as co-variable, supporting differences in the regulation of resistin levels in pregnancies complicated by diabetes.

As we expected, our pregnant women with GDM were more insulin resistant than the NGT group; but contrary to previous reports (23-25) that have observed similar or higher resistin levels in GDM women, we found lower resistin levels in this group. The discrepancy of these findings is unclear, but may be related to differences in the population studied or to sampling time during pregnancy. Concerning clinical variables, our study cohort was quite homogeneous and despite GDM being more frequent among obese women, both groups were comparable according to BMI. This is an important aspect and thus an excess of adipose tissue could be responsible for an increased production of resistin, as has been postulated in morbidly obese patients (29). Also, changes in placental resistin expression and serum concentrations are known to occur during pregnancy. Sagawa et al. (20) reported greater resistin gene expression in term placenta than in chorionic villi of early pregnancy and resistin levels have been found to be higher at term pregnancy than in non-pregnant women (21, 22). Therefore, results obtained in studies performed at different times during pregnancy could yield different results. In addition, differences in molecular forms of resistin that could justify the discrepancy observed cannot be ruled out.

The cause of the low resistin levels observed is not directly accessible from this study. Although, as suggested by the name, resistin impairs glucose tolerance and insulin action, a number of human studies have yielded conflicting results (8-20). Several reports have shown an association between insulin sensitivity and low resistin levels in obesity (30, 32), and the role of resistin regulating insulin sensitivity is still unclear. Insulin is a key regulator of resistin gene expression and both inhibitory and stimulatory effects have been reported (30, 31). In human placentas, in vitro studies performed by Lappas et al. (33) have showed a biphasic effect of insulin in the release of resistin. At low concentrations, insulin significantly increases the release of resistin, whereas it returns to basal levels when placenta is exposed to higher insulin concentrations suggesting a downregulation of resistin expression in a high insulin medium. We postulate that this biphasic action of insulin could be responsible for the low resistin levels observed in the GDM group that had a richer insulin environment shown by the significantly higher AUC_insulin.

Postpartum period is accompanied by a restoration of insulin sensitivity. Shortly after delivery, insulin sensitivity begins to increase (34) and it is normal at 15–16

<table>
<thead>
<tr>
<th>Table 2 Multiple linear regression model with log insulin sensitivity index (ISI_OGTT) as dependent variable.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>β</strong></td>
</tr>
<tr>
<td>Log resistin</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Pre-pregnancy BMI</td>
</tr>
<tr>
<td>GDM/no GDM</td>
</tr>
</tbody>
</table>

\( R: 0.897, R^2: 0.805. \)
weeks after delivery in obese women with NGT (35). We studied GDM women up to 6 months after delivery and at least 1 month after lactation cessation to avoid any situation that could modify insulin sensitivity. Consistent with previous reports, a decrease in resistin levels was observed, whereas the insulin sensitivity improved. These data confirmed that despite the low third trimester resistin levels observed in GDM women, these levels were still high when compared with the non-pregnant state in this subgroup of women. This difference could be due to an increase in secretion by adipose tissue and/or to placental production. However, as Chen et al. have shown a rapid fall-off in resistin levels after parturition, the placenta seems to be the most reliable source (25).

The lack of a control group in the postpartum evaluation limits the conclusions that we could have obtained about the role of resistin in pregnancy. We have not been able to test differences in resistin levels between both groups in the postpartum period and we cannot confirm a fall-off in the NGT group, although resistin levels during pregnancy in this group were clearly higher than the levels that we have observed in our healthy women in a previous report (36). Therefore, we can hypothesise that the difference observed between both groups during pregnancy could be due to an inability to increase resistin levels in the GDM group.

In summary, GDM is associated with lower resistin levels than those found in healthy women during pregnancy. We confirm a decrease in resistin levels after pregnancy in GDM, suggesting the contribution of the placental tissue to resistin levels observed in pregnancy. And, although we have failed to find an independent relationship between resistin levels and insulin sensitivity, further research is needed to elucidate the exact role of resistin in human pregnancy and its possible contribution to the development of gestational diabetes.

Acknowledgements
This work was supported by ‘REDMET’ 06/0015/011 and grants 05/1377 and PI03/1322 from the Instituto de Salud Carlos III.

References
27 Matsuda M & DeFronzo R. Insulin sensitivity indices obtained from oral glucose tolerance testing. Diabetes Care 1999 22 1462–1470.
32 Nagaev I & Smith U. Insulin resistance and type 2 diabetes are not related to resistin expression in human fat cells or skeletal muscle. Biochemical and Biophysical Research Communications 2001 285 561–564.

Received 28 September 2007
Accepted 12 November 2007