TITLE: Insulin sensitivity and Resistin levels in Gestational Diabetes Mellitus and after parturition.

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ABSTRACT

Context: Resistin is expressed and secreted by the placenta during pregnancy. Increased serum Resistin levels have been found in the second half of 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: Fifty-eight caucasian women with a singleton pregnancy who had been referred for a 100 g OGTT 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 decreased 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: No relationship was observed between resistin and insulin sensitivity in GDM. Resistin was lower in GDM than in NGT women and decreased after parturition in the GDM group.

Human pregnancy is characterised by a progressive decrease in insulin sensitivity, which parallels the growth of the feto-placental unit and facilitates the diversion of glucose to the foetus. This situation is a physiological screening test for the insulin secretor capacity of ß-cells and, gestational diabetes mellitus (GDM) appears when pancreatic ß-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 type 2 diabetes mellitus and Gestational diabetes mellitus, 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, 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 gestational diabetes women and obese women with normal glucose tolerance (5). Also low first trimester and pre-pregnancy adiponectin levels have been reported in women with gestational diabetes (6),

and adiponectin levels during pregnancy have been negatively correlated with fasting insulin, glucose and pre-pregnancy 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 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 to explain the development of the insulin resistant state.

Subjects and Methods

Subjects

Fifty-eight 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 OGTT between 26 and 30 weeks' 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 (ADA) criteria (26), and the women who not fulfilled these criteria were considered to have normal glucose tolerance (NGT). Twenty-three women were diagnosed of GDM and 35 were 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 mm Hg. 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 one 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 and 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}= 10.000/_ (FPG * FPI)* (G * I). Mean glucose and mean insulin were calculated using measurements at baseline, and after 60, 120 and 180 minutes during the 100 g OGTT and using measurements at baseline, and after 30, 60 and 120 minutes during the 75 g OGTT. The index has been validated in pregnant patients and shows a good correlation with insulin sensitivity derived using the euglycemic clamp technique (28). The incremental area under the insulin curve ($AUC_{insulin}$) and the incremental area under the glucose curve ($AUC_{glucose}$) 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 immunoradiometric assay (IRMA, Medgenix Diagnostics, Fleunes, Belgium) in which proinsulin did not cross-reactivity. 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 enzyme-linked immunosorbent assay (BioVendor Laboratory Medicine, Inc Palackeho, Check Republic). The sensitivity was 0.2 ng/mL. The intra and inter-assay coefficients of variation were 5,8% and 14,7% respectively.

Statistical Analysis

SPSS 11.5 (SPSS Inc., Chicago, IL) was used in all analysis. $P<0,05$ was considered statistically significant. Data are reported as mean $\pm$ SD for continuous variables and number of cases (percentage) for categorical variables. The distribution of Insulin, ISI_{OGTT}, HOMA-R and resistin were skewed, thus data were log-transformed to normalize their distribution for t-test and multivariate analysis, with back-transformed results expressed in the text and corresponding tables. GDM and NGT groups were compared by using independent samples *t* test and X_2 analysis with Yates' correction or Fisher exact test when appropriate and the independence of variables was explored using binary logistical regression. GDM groups during and after pregnancy were compared by using paired samples *t* test. Univariate associations were

examined using Sperman's correlation analysis. Multiple linear regression analysis of dependent variable logarithmically transformed ISI_{OGTT} was used to determine factors that were independently associated with insulin sensitivity. In a prior power 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 of GDM and 35 women were 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 (18,89 to 38,37 kg/m^2 vs 17,27 to 32,27 kg/m^2 and 21,94 to 40,47 kg/m^2 vs 23,75 to 34,78 kg/m^2) 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 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 ISI_{OGTT} was lower in the GDM women (2,55±1,43 vs. 7,68±1,05, p<0,001). Furthermore, in the GDM group, the $AUC_{glucose}$ and the $AUC_{insulin}$ were also higher (1574,57±149,98 vs. 1207,43±89,64 mmol ·L^{-1}·h^{-1}, p<0,001 and 107937,45±11,81 vs. 43508,34±10,97 pmol ·L^{-1}·h^{-1}, p<0,001). Because 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 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, twenty-two of the twenty-three women with GDM were also evaluated 6 months after parturition, and at least one 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±1,45 vs. 3,54±1,46, p=0,001). Resistin levels decreased significantly after pregnancy (4,24±1,56 vs. 3,11±1,63 ng/mL, p=0,003). Fasting glucose levels increased significantly in the postpartum evaluation (4,84±0,62 vs. 5,44±0,53 mmol/L, p<0,001, p<0,001) while no difference was found in fasting insulin levels (78,81±9,24 vs. 72,44±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.

In this study, we report lower resistin levels in GDM women compared to 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 related to sampling time during pregnancy. Concerning clinical variables, our study cohort was quite homogeneous and despite gestational diabetes mellitus is more frequent among obese women both groups were comparable according to BMI. This is an important aspect, thus an excess of adipose tissue could be responsible for an increased production of resistin as has been postulated in morbid obese patients (29). Also, changes in placental resistin expression and serum concentrations are know to occur during pregnancy. Sagawa and co-workers (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 (22,21). Therefore results obtained in studies performed at different time during pregnancy could yield different results. In addition, differences in molecular forms of resistin that could justify the discrepancy observed can not be rule out.

The cause of low resisitin 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 yield 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 and co-workers (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 down regulation of resistin expression in a high insulin medium. We postulate that this biphasic action of insulin could be responsible of the low resistin levels observed in the GDM group that had a richer insulin environment shown by the significantly higher AUCinsulin.

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 weeks after delivery in obese women with normal glucose tolerance (35). We studied GDM women up to 6 months after delivery and at least one 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. This data confirmed that despite the low third trimester resistin levels observed in GDM women, these levels were still high compare to the non-pregnant state in this subgroup of women. This difference could be due to an increase secretion by adipose tissue and/or to placental production. However, as Chen et al has shown a rapid fall-off in

resistin levels after parturition, 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 can not confirm a fall off in the NGT group and we have not been able to test differences between both groups in the postpartum period.

In summary, GDM is associated with lower resistin levels than that 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, although further research is needed to elucidate the exact role of resistin in human pregnancy.

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Table1. Clinical and metabolic characteristics of the women studied during pregnancy.

	NGT group N=35	GDM group N=23	P
Age (y)	31,48±4,47	31,22±4,63	ns
Gestational Age (wk)	27,34±5,49	27,30±4,25	ns
Prepregnancy BMI (kg/m^2)	24,02±3,72	24,84±4,78	ns
Current BMI (kg/m^2)	27,49±3,24	27,99±4,72	ns
Weight gain in pregnancy (kg)	7,16±3,74	6,15±2,77	ns
Previous GDM (N %)	1 (2,9)	4 (17,4)	<0,001
Fam Hx of GDM (N %)	2 (5,7)	16 (69,6)	<0,001
Parity	1,86±1,31	1,86±0,82	ns
Fasting blood glucose (mmol/L)	4,51±0,32	4,86±0,62	<0,01
Fasting insulin (pmol/L)	30,91±12,92	77,09±9,03	<0,01
ISI_{OGTT}	7,68±1,05	2,55±1,43	<0,001
Resistin (ng/mL)	9,30±1,32	4,32±1,56	<0,001
$AUC_{insulin}$ (pmol $\cdot L^{-1} \cdot h^{-1}$)	43508,34±10,97	107937,45±11,81	<0.001
$AUC_{glucosa}$ (mmol $\cdot L^{-1} \cdot h^{-1}$)	1207,43±89,64	1574,57±149,98	<0.001

Fam Hx of GDM: familiar history of gestational diabetes. ISI_{OGTT}:insulin sensitivity index. Weight gain in pregnancy: amount of weight gain from the beginning of pregnancy until the day of evaluation. $AUC_{glucose}$: Area under the glucose curve. $AUC_{insulin}$: Area under the insulin curve.

Table 2.-Multiple linear regression model with log ISI_{OGTT} as dependent variable.

	Beta	t	Sig
Log resistin	0.66	0.589	0.562
Age	0.173	2.264	<0.05
Prepregnancy BMI	-0.188	-2.244	<0.05
GDM/ no GDM	-0.782	-6.940	<0.001

R: 0.897 R^2: 0.805