DECREASED INSULIN BINDING IN ASIAN INDIAN WOMEN WITH GESTATIONAL DIABETES MELLITUS

AMBADY RAMACHANDRAN, LAKSHMINARAYANAN SUSHEELA, VISWANATHAN MOHAN, DURGA A. S. KUZHALI, MOOPIL VISWANATHAN

Diabetes Research Centre and M.V. Hospital for Diabetes, Royapuram, Madras, India

Pregnancy is associated with increased resistance to insulin action and this has been implicated in the diabetogenic effect of the pregnant state. Decreased insulin sensitivity in pregnancy is suggested by the slow response of blood glucose to exogenously administered insulin, fasting hyperinsulinemia, and an exaggerated plasma insulin response to a glucose load seen in pregnancy. Increased levels of hormones such as human chorionic somatomammotropin, progesterone, estrogens and cortisol have been implicated in causing insulin resistance in pregnancy, although the exact mechanisms of these alterations in pregnancy are not yet clearly understood.

Several studies have documented decreased insulin binding to its receptors in insulin resistant states. All the previous studies in hyperglycemia in pregnancy were undertaken in pregnant subjects who were known diabetics under treatment. Hence, the present study was undertaken in order to assess insulin binding to erythrocyte insulin receptors in pregnant women in whom diabetes was first detected during pregnancy, and the results are compared with those of normal pregnant women.

MATERIALS AND METHODS

Subjects - The study groups comprised 10 normal pregnant women with no family history of diabetes and 10 pregnant women in whom diabetes was detected during pregnancy (gestational diabetes) and 10 age-matched healthy non-pregnant women, with no family history of diabetes, as controls. The hyperglycemia in these subjects was detected during the latter part of the second

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or in the third trimester of pregnancy. None of these subjects had received any treatment for diabetes before the study. The normal non-pregnant control subjects were studied during the luteal phase of the menstrual cycle in order to eliminate any possible variation in insulin receptor binding during the cycle. The mean (± SD) gestational age at the time of the study was 28 ± 3 weeks in non-diabetic pregnant women and 29 ± 3 weeks in gestational diabetic subjects. The body weight was evaluated by the body mass index (BMI) which was calculated using the formula: weight in kg/height² (m).

After thorough clinical examination, all subjects underwent an oral glucose tolerance test using a 100 g glucose load. The criteria used for diagnosis of diabetes were those of O'SULLIVAN and MAHAN. Fasting and post-glucose blood samples were collected for the estimation of glucose, plasma IRI and reticulocyte counts. The fasting samples were used for insulin binding studies.

The reticulocyte counts were determined in all the study subjects by taking fresh blood smears stained with brilliant cresyl blue. There were no significant differences in the reticulocyte counts in blood samples obtained from non-diabetic non-pregnant controls, non-diabetic pregnant women and subjects with gestational diabetes. The reticulocyte counts did not exceed 0.6% of the total erythrocyte counts.

Materials - Mono-l-Tyr-4-insulin (porcine) of specific activity 360 µCi/µg (Hoechst, Frankfurt, FRG) was used as tracer. Purified unlabelled porcine insulin was obtained from Boehringer, Mannheim, FRG. Ficoll-Hypaque was obtained from Pharmacia, Uppsala, Sweden.

Preparation of cells - Blood was collected in EDTA tubes and processed immediately. Whole blood was centrifuged for 10 min at 400 × g at 25°C. Plasma was separated and stored at -20°C for insulin assay. The resulting pellet was diluted with HEPES buffer (pH 8.0) and the erythrocytes were fractionated by passing twice through a Ficoll-Hypaque gradient. The final suspension of the cells in HEPES buffer consisted of 3.5 - 4.5 × 10⁸ cells/ml, and more than 98% of the cells were viable. The cell concentration was determined using the hematocytometer and by hematocrit.

Binding studies - Binding studies were performed as described by GAMBIH et al. with modifications. Erythrocytes (4 - 4.5 × 10⁹ cells/ml) were incubated at 15°C with (²⁰¹) insulin (20 µg in 50 µl) with or without varying amounts of unlabelled insulin (0 - 0.5 × 10⁹ ng) in a total volume of 0.5 ml. After 210 min of incubation, duplicate samples were placed in pre-chilled microfuge tubes along with the buffer and dibutyl phthalate. Cell bound and free insulin were separated by centrifugation at 7000 × g at 4°C for 10 min. The radioactivity in the cell pellet and supernatant was determined in a Gamma counter (FCIL, Hyderabad, India). Non-specific binding is defined as the amount of (²⁰¹) insulin that remains bound in the presence of 10⁹ ng/ml of unlabelled porcine insulin which amounted to less than 10% of the total binding. All binding data were corrected for this non-specific binding to represent the specific cell binding for purposes of comparison.

Plasma glucose was estimated by the glucose oxidase PAP method (Boehringer, Mannheim, FRG) and HbA, by a colorimetric method. Plasma IRI was assayed by the method of HERRETT et al. The sensitivity of the assay in
From specimens was 2 μU/ml. The intra- and inter-assay coefficients of variation were 5% and 7% respectively.

**Data analysis** - All values are expressed as mean ± SD. The insulin binding data were further analyzed by Scatchard analysis and by the method of average affinity profile. The x intercept in the Scatchard plots was obtained from the slope of the least square straight line drawn to fit the points at insulin concentrations between 20 to 1000 ng/ml. Individual data were analyzed by individual Scatchard plots and average affinity profiles. Statistical analysis was carried out by Wilcoxon’s two-tailed test for comparison of values between study groups. Spearman’s coefficient of rank was applied in correlation studies.

**RESULTS**

Figure 1 shows the plasma glucose values during the oral glucose tolerance test (panel A) and the concomitant insulin response (panel B) among the study groups. Fasting plasma glucose values were not significantly different in

<table>
<thead>
<tr>
<th></th>
<th>glucose</th>
<th>fasting insulin</th>
<th>post-glucose (90-120 min) glucose</th>
<th>fasting insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>non-pregnant controls vs non-diabetic pregnancy</td>
<td>n.s.</td>
<td>&lt;0.01</td>
<td>p&lt;0.01</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>non-diabetic pregnancy vs gestational diabetes</td>
<td>&lt;0.01</td>
<td>n.s.</td>
<td>p&lt;0.01</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

![Figure 1 - Plasma glucose (panel A) and insulin response (panel B) to a 100 g oral glucose load in 10 non-pregnant controls (○), 10 healthy non-diabetic pregnant women (★) studied at 28 ± 3 weeks of pregnancy and 10 gestational diabetic subjects (▲) studied at 29 ± 3 weeks of pregnancy. Value at each point represents mean ± SD.](image)
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<table>
<thead>
<tr>
<th></th>
<th>non-pregnant controls (n = 10)</th>
<th>normal pregnancy (n = 10)</th>
<th>gestational diabetes (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>age (years)</td>
<td>24 ± 1.7</td>
<td>26 ± 1.6</td>
<td>27 ± 2.7</td>
</tr>
<tr>
<td>gestational age (weeks)</td>
<td>-</td>
<td>28 ± 3</td>
<td>29 ± 3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25 ± 1.0</td>
<td>27.3 ± 2.0</td>
<td>28.2 ± 2.4</td>
</tr>
<tr>
<td>fasting plasma glucose (mmol/l)</td>
<td>4.6 ± 0.4</td>
<td>4.7 ± 0.7</td>
<td>6.7 ± 1.2*</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>n.s.</td>
<td>7.0 ± 0.6</td>
<td>8.7 ± 0.6**</td>
</tr>
<tr>
<td>fasting plasma IRI (µU/ml)</td>
<td>16 ± 2</td>
<td>22 ± 9</td>
<td>21 ± 6*</td>
</tr>
</tbody>
</table>

Tab. 1 - Clinical characteristics and biochemical parameters of the study groups. Values given are mean ± SD vs non-pregnant controls: *p<0.001; **p<0.01

The non-pregnant controls and non-diabetic pregnant women but elevated in subjects with gestational diabetes (p<0.01). Plasma glucose levels at 1h and 2h after glucose ingestion were higher in non-diabetic pregnant women as compared to non-pregnant controls (p<0.01) and increased to a greater extent in gestational diabetic subjects (vs non-diabetic pregnancy: p<0.001).

![Graph showing competition curves of I-insulin binding to erythrocytes from 10 normal non-pregnant women. The experimental points represent specific insulin binding normalised to a cell concentration of 4.5 x 10^7 cells/ml. Maximal binding at tracer concentration (not shown in the figure): 150 ± 14%. Inset in the figure shows Scatchard analysis of the binding data. *us gestational diabetes (shown in fig. 3) p<0.01.](image)

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Fig. 3 - Competition curves of specific insulin binding (mean ± SD) to erythrocytes from 10 normal pregnant women (○) and 10 pregnant women with gestational diabetes (●). Maximal specific binding at tracer concentration: normal pregnancy: 12.0 ± 2.2 vs gestational diabetes: 9.0 ± 1.1; p<0.01. Inset in the figure shows Scatchard analysis of the binding data for normal pregnancy (○) and gestational diabetes (●). * vs gestational diabetes: p<0.001. * vs non-diabetic non-pregnant controls shown in fig. 2: not significant.

Pregnant women had significantly higher mean fasting and post-glucose plasma IRI at all time intervals (30 to 120 min) in comparison to non-pregnant controls (p<0.001). There was no significant difference in fasting and post-glucose plasma IRI levels between normal pregnant women and gestational diabetic subjects, although the plasma glucose was elevated in the latter. The

<table>
<thead>
<tr>
<th>Binding parameters</th>
<th>Non-pregnant controls (n = 10)</th>
<th>Normal pregnancy (n = 10)</th>
<th>Gestational diabetes (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal specific binding of tracer (%)</td>
<td>13.0 ± 1.4</td>
<td>12.0 ± 2.2</td>
<td>9.0 ± 1.1*</td>
</tr>
<tr>
<td>Insulin for 50% inhibition - ED₅₀ (ng/ml)</td>
<td>5.0 ± 0.8</td>
<td>6.0 ± 1.3</td>
<td>8.7 ± 0.9*</td>
</tr>
<tr>
<td>Average affinity constant Kᵣ (empty sites) × 10⁶ × M⁻¹</td>
<td>2.03 ± 0.2</td>
<td>2.32 ± 0.6</td>
<td>1.44 ± 0.5*</td>
</tr>
</tbody>
</table>

Tab. 2 - Summary of the binding data of the study groups. Values expressed as mean ± SD. * vs non-pregnant controls: *p<0.001.
clinal characteristics and the biochemical parameters of the groups studied are summarised in tab. 1.

**Insulin binding studies** - Competition curves of insulin binding to erythrocyte insulin receptors in non-pregnant controls are shown in fig. 2 and those of pregnant women are shown in fig. 3. At tracer insulin concentration, maximal specific binding was lower in women with gestational diabetes in comparison to both normal pregnant women $(p<0.01)$ and normal non-pregnant controls $(p<0.001)$. Scatchard analysis of the binding data (shown as inset in figs 2 and 3) suggested that the decreased insulin binding observed in women with gestational diabetes was significant only at lower insulin concentrations $(0.2-2$ ng/ml) as compared to normal pregnant women $(p<0.01)$. The curves converged at higher insulin concentrations suggesting decreased receptor affinity with no change in the number of receptors. This was further substantiated by an increased concentration of native insulin required for 50% inhibition of maximal tracer binding $(ED_{50})$ in gestational diabetes.

Analysis of the binding data by DeMeys' average affinity profiles also indicated a significant decrease in receptor affinity in gestational diabetes. The average affinity constant $K$, for empty sites was $2.63 \times 10^6$ in non-pregnant controls, $2.32 \times 10^6$ in normal pregnancy and $1.44 \times 10^6$ in gestational diabetes. The salient features of the binding data are summarised in tab. 2.

**DISCUSSION**

The aim of the present study was to evaluate insulin binding to its receptors in women in whom hyperglycemia was first detected during gestation and to compare it with that of normal pregnant women and non-pregnant controls. A number of reports have appeared on insulin binding to receptors in the pregnant state. **Beck-Nielsen et al.** reported decreased insulin binding to monocytes in normal women during late pregnancy as compared to non-pregnant controls, which was due to a decrease in the receptor number per cell with no change in receptor affinity. On the contrary, **Paavilainen et al.** found no change in insulin binding to monocytes in normal pregnant women and concluded that gestational insulin resistance was not correlated with impaired binding of insulin to receptors on circulating monocytes.

There are a few reports on insulin binding in pregnancy with diabetes. **Pagano et al.** studied insulin binding and insulin action on adipocytes isolated during Caesarian section from obese pregnant women with NIDDM and found decreased receptor number and affinity suggesting that the higher insulin levels during pregnancy caused down-regulation of insulin receptors leading to peripheral insulin resistance. **Pedersen et al.** studied insulin binding to monocytes and erythrocytes in pregnant women with insulin-dependent diabetes mellitus and found no significant change when compared with normal non-pregnant women. Insulin receptor binding remained unchanged during the third trimester even in the face of significantly increased insulin requirement and concomitant hyperinsulinemia. Their findings suggested that changes in insulin receptors were not primarily involved in the alterations of diabetic control during pregnancy.

There are a number of problems regarding the studies described above which were carried out in pregnant subjects that were already known diabetics
and undergoing treatment with insulin. It is well known that the administration of exogenous insulin can influence peripheral insulin binding. Moreover, the hyperglycemic state itself (especially NIDDM) is associated with changes in peripheral insulin binding. Differences in food intake between diabetic and non-diabetic subjects may also contribute to differences in insulin binding. The present study was carried out in women in whom hyperglycemia was detected for the first time during pregnancy and in whom no treatment was started prior to the study. Thus, the results obtained were not influenced by the factors mentioned above and represent true changes in insulin binding occurring during pregnancy.

In this study, we found no significant difference in insulin binding in normal pregnant women compared to non-pregnant controls studied in the luteal phase of their menstrual cycle. Thus, our results in normal pregnant women are in agreement with the findings of Tsirbas et al. and Poulakis et al. Nevertheless, the most interesting finding is that specific insulin binding was significantly decreased only in women with gestational diabetes. Thus, it is possible that the impairment of insulin binding may be one of the factors responsible for causing insulin resistance and hyperglycemia in gestational diabetes.

Our finding in this study that pregnancy with normal glucose tolerance did not manifest changes in insulin binding to its receptor as against decreased insulin binding and affinity in gestational diabetes suggested a pathogenetic role for impaired insulin binding. The mechanism responsible for the genesis of hyperglycemia in gestational diabetes may be an increase in peripheral insulin resistance caused by modulations at the receptor binding level.

One of the limitations of this study is that erythrocytes have been used instead of a target tissue for insulin action. There are a number of recent studies to show that there are structural similarities in insulin receptors from erythrocytes and other target tissues. Further studies with target tissues for insulin action in gestational diabetics are needed to throw light on our findings in this study.

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SUMMARY

Insulin binding to erythrocyte insulin receptors was studied in 10 women with gestational diabetes and compared with 10 matched, normal, pregnant women and 10 normal, non-pregnant controls, with no family history of diabetes. Pregnant women had higher mean fasting and post-glucose plasma immunoreactive insulin (IRI) compared to non-pregnant controls (p<0.001). Women with gestational diabetes had higher mean fasting and post-glucose plasma glucose levels and a lower mean specific binding of insulin when compared with the other two groups (p<0.001). The decreased insulin binding was significant only at lower insulin concentrations (0.2-2.0 ng/ml) when compared with those of normal pregnant women (p<0.01), suggesting decreased receptor affinity with no change in receptor number. In addition, an increased mean ED₅₀ value for 50% inhibition of maximal binding and a lower mean average affinity constant Kₐ (empty site) obtained in gestational diabetes in comparison to the other two groups also suggested decreased affinity of the receptor. The finding that pregnancy with normal glucose tolerance
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was not accompanied by changes in insulin binding as against decreased insulin binding and affinity observed in gestational diabetes suggested a pathogenetic role for impaired insulin binding as one of the factors responsible for insulin resistance and hyperglycemia in gestational diabetes.

REFERENCES


A. RAMACHANDRAN et al.


Requests for reprints should be addressed to:

Ambady Ramachandran
Diabetes Research Centre
5, Main Road, Royapuram
Madras - 600 013 - India