A reduced number of insulin receptors in patients with Prader-Willi syndrome

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Abstract. The Prader-Willi syndrome is among other features characterized by obesity and a high prevalence of glucose intolerance. The fasting plasma insulin concentration and the insulin response to glucose are often increased, indicating some insulin resistance in this disease. To investigate whether this could be due to an insulin receptor defect 7 patients with Prader-Willi syndrome, 10 normal weight subjects and 8 obese subjects were tested for the binding of [¹²⁵I]insulin to monocytes. Monocytes from patients with Prader-Willi syndrome bound significantly less insulin than cells from normal subjects (P < 0.01). However, no difference was found between Prader-Willi patients and the obese controls (P > 0.1). It is concluded that the insulin resistance found in Prader-Willi patients, similar to that found in obese subjects, in part, may be explained by an insulin receptor defect on target cells for insulin action.

Prader-Willi syndrome (PW) was first described in 1956 (Prader et al. 1956) and is thought to be caused by a hypothalamic abnormality. The cardinal features are neonatal hypotonia, hypogonadism, mental retardation and obesity. Untreated this obesity causes most of the complaints and also the mortality seen in PW syndrome (Laurance et al. 1981). Carbohydrate intolerance in PW patients has been reported in several studies (Laurance et al. 1981; Hall & Smith 1972; Jeffcoate et al. 1980). Diabetes mellitus was earlier thought to be a part of the syndrome, but the prevalence seems now to be lower than earlier described (Tze et al. 1981).

Glucose intolerance and diabetes mellitus is a common feature in obese subjects. The changes of glucose homeostasis in PW patients may thus be secondary to their obese state (Tze et al. 1981). Compatible with this, the fasting plasma insulin concentration and the insulin response to glucose are often increased in PW patients as compared to normals (Tze et al. 1981; Parra et al. 1973), which indicate that PW patients are insulin resistant.

The first step in the cellular insulin action is the binding to a specific receptor on the cell membrane (Beck-Nielsen 1980). It is well known that the insulin resistance in obese subjects mainly is due to an insulin receptor defect (Beck-Nielsen 1980).

The purpose of the present study was to investigate whether the insulin resistance in patients with PW syndrome is associated with a reduced cellular number of insulin receptors like that in obese state. As the monocyte insulin receptors mirror the receptors on target cells in obese subjects (Beck-Nielsen et al. 1977), we have studied the [¹²⁵I]insulin binding to monocytes isolated from patients with PW syndrome and as controls we have studied obese and normals.

Materials and Methods

Subjects
The study included 7 patients with the clinical features of PW syndrome. They were otherwise healthy and none...
had frank diabetes mellitus. Ten healthy normal-weight subjects and 8 obese, otherwise healthy subjects comprised the control groups. None were receiving any drug during the study period known to affect glucose or insulin metabolism. Tables 1 and 2 shows the clinical data of the study groups. Informed consent of the parents were obtained.

**Analysis of chemical quantities in plasma and serum**

Glucose in plasma was analysed with a glucose dehydrogenase method (Merck enzymatic kit). Insulin in serum was measured with radioimmunoassay (Heding 1972). Free fatty acids (FFA's) were measured according to the method of Duncombe (1964).

**Glucose tolerance test**

Glucose tolerance test was performed orally in 4 patients and iv in 2 patients. 1.0 g glucose per kg body weight was given. Blood samples for estimation of plasma glucose were obtained at 0, 30, 60 and 120 min.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Sex</th>
<th>Age years</th>
<th>Bodyweight % of ideal</th>
<th>Glucose mmol/l</th>
<th>Insulin µU/ml</th>
<th>Specific monocyte bound fraction × 10⁻²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>5</td>
<td>182</td>
<td>4.9</td>
<td>16</td>
<td>2.32</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>14</td>
<td>102</td>
<td>4.9</td>
<td>16</td>
<td>2.95</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>4</td>
<td>158</td>
<td>4.8</td>
<td>47</td>
<td>3.16</td>
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<tr>
<td>4</td>
<td>M</td>
<td>8</td>
<td>142</td>
<td>16</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>15</td>
<td>192</td>
<td>4.8</td>
<td>38</td>
<td>2.97</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>13</td>
<td>147</td>
<td>5.0</td>
<td>3</td>
<td>3.92</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>29</td>
<td>153</td>
<td>—*</td>
<td>—*</td>
<td>3.96</td>
</tr>
</tbody>
</table>

* Plasma samples were destroyed.

**Table 1.**

The clinical data, and fasting plasma glucose, fasting plasma insulin and specific monocytes bound fractions in patients with Prader-Willi syndrome.

**Insulin binding to monocytes in mononuclear leukocyte suspensions**

Insulin binding to monocytes was performed as described previously (Beck-Nielsen et al. 1977) with minor modifications. For the analysis 50 ml of blood was used. Mononuclear cells were incubated for 100 min at 5°C in a Hepes buffer (100 mmol/l, pH 7.8 at 15°C) with [¹²⁵I]insulin (Α₁₄-labelled, specific activity about 200 µCi/µg) at a final concentration of 51 pmol/l. Monocytes were identified by morphological and cytochemical criteria (Beck-Nielsen et al. 1977) using a blind counting technique, i.e. the observer had no knowledge of the identified smears. There were no statistically significant difference between the monocyte concentrations in the mononuclear leucocyte suspension isolated from the three groups. All binding values were corrected for insulin binding to lymphocytes (Beck-Nielsen et al. 1977) and for non-specific binding which averaged 20 ± 9% of total monocyte binding. There were no statistically significant difference of the non-specific binding between the groups.

**Table 2.**

The clinical data and fasting plasma glucose, fasting plasma FFA and fasting plasma insulin in the normal-weight group, the obese group and the Prader-Willi group (mean ± SD).

<table>
<thead>
<tr>
<th></th>
<th>Age years</th>
<th>Sex</th>
<th>Bodyweight % of ideal</th>
<th>Glucose mmol/l</th>
<th>FFA µmol/l</th>
<th>Insulin µU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normals</td>
<td>14.9 ± 7.2</td>
<td>6M / 4F</td>
<td>102 ± 6</td>
<td>5.0 ± 0.4</td>
<td>0.26 ± 0.10</td>
<td>10 ± 6</td>
</tr>
<tr>
<td>Obese</td>
<td>11.8 ± 4.5</td>
<td>3M / 5F</td>
<td>157 ± 21</td>
<td>4.9 ± 0.5</td>
<td>0.25 ± 0.10</td>
<td>18 ± 11</td>
</tr>
<tr>
<td>Prader-Willi</td>
<td>12.6 ± 8.4</td>
<td>3M / 4F</td>
<td>154 ± 29</td>
<td>4.9 ± 0.1</td>
<td>0.45 ± 0.24</td>
<td>23 ± 14</td>
</tr>
</tbody>
</table>
Specific monocyte bound fractions were adjusted to 10⁷ monocytes/ml, as previously described by us (Beck-Nielsen et al. 1977). In 2 of the patients with PW syndrome the insulin binding was estimated also after 24 h of fasting.

**Procedures for studying serum effect**

Mononuclear leucocytes from a normal subject were incubated with serum from the PW patients or with control serum at dilutions 1:2 for 15 min at 37°C and then washed three times in Hepes buffer. The concentrated cells were resuspended in 8 ml of buffer and incubated at 37°C at a pH of 7.4 for 1 h so that the dissociation of serum factors not tightly bound to cells could occur. At the end of the dissociation period the cells had a final wash before they were used in the assay for insulin binding.

**Statistical methods**

Wilcoxon's test for unpaired data and Spearman's rho test were used.

**Results**

Only 1 patient with PW syndrome (No. 5) had impaired glucose tolerance curve with an elevated 2-h value at 7.9 mmol/l (Table 3). The other patients had normal glucose tolerance. The fasting plasma glucose concentration was normal in all patients. The fasting plasma insulin level was significantly higher in PW patients than in normals ($P < 0.01$), but not significantly different from the obese controls ($P < 0.1$, Table 2).

In Fig. 1 [¹²⁵I]insulin binding to mononuclear leukocytes from the three groups studied and the inhibitory effect of native insulin (mean ± SEM).

### Table 3

Plasma glucose values in patients with Prader-Willi syndrome after intake of 1 g glucose per kg body weight. In 2 of the patients* the glucose was given iv.

<table>
<thead>
<tr>
<th>Patient</th>
<th>mmol/l</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
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<tr>
<td>1*</td>
<td>3.4</td>
<td>12.8</td>
<td>6.3</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>4.3</td>
<td>7.5</td>
<td>8.6</td>
<td>7.1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5.0</td>
<td>8.8</td>
<td>10.8</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>4*</td>
<td>4.7</td>
<td>15.1</td>
<td>7.3</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5.0</td>
<td>6.6</td>
<td>8.7</td>
<td>7.9</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>4.1</td>
<td>6.1</td>
<td>7.0</td>
<td>6.0</td>
<td></td>
</tr>
</tbody>
</table>

*Patients who received intravenous glucose.
shown. At each insulin concentration monocytes from PW patients bound significantly less insulin than those from normals \((P < 0.01)\). However, no difference was found between PW patients and obese \((P < 0.1)\). Scatchard analysis of the data from Fig. 1 indicates that the reduced binding ability of monocytes from patients with PW syndrome and from obese controls was due to a reduced receptor number per cell, as the plots are fairly parallel (Fig. 2).

The correlation between the fasting insulin and the specific monocyte bound fraction of all three groups.
A significantly negative correlation was found between fasting plasma insulin and insulin binding, when all three groups were taken together ($r = -0.45, P < 0.05$, Fig. 3).

In 2 of our patients (Nos. 1 and 3) the insulin binding study was repeated after 24 h of fasting. The bound fraction increased 18 and 30%, respectively.

The preincubation of normal monocytes with serum from the PW patients did not change the insulin binding fraction (Fig. 4) indicating the absence of antireceptor antibodies in serum from the PW patients.

**Discussion**

**Glucose tolerance**

Impaired glucose tolerance has been found in several earlier studies. Hall and coworkers found that 5 out of 14 patients with PW syndrome had a diabetic glucose tolerance curve, and one had frank diabetes (Hall & Smith 1972). In the material of Laurance and coworkers 3 out of 23 patients developed diabetes mellitus and 4 had a diabetic glucose tolerance curve (Laurance et al. 1981). All our patients had normal fasting plasma glucose and only 1 (No. 5) was glucose intolerant. The differences between the materials presented might be due to different age and body weight of the patients. Most of our patients were children.

The glucose tolerance in obesity and in the PW syndrome seems to be identical. In both conditions the prevalence of diabetes mellitus is higher than in normal subjects (Hall & Smith 1972; Beck-Nielsen 1980).

**Plasma insulin concentration**

In earlier studies fasting plasma insulin concentrations had been found to be normal, or elevated to levels similar to that found in obese subjects (Tze et al. 1981). In our study we have found that the patients as a group were hyperinsulinaemic. However, the group was heterogenic with a large variation in insulin concentrations (Table 1). The cause of the hyperinsulinism seems most likely to be the increase in food intake, but of course hypothalamic alterations cannot be excluded, since the PW syndrome is characterized by a defect in the hypothalamic hormonal regulation. In fact 1 patient (No. 2) was hyperinsulinaemic despite his normal body weight.

The insulin response to oral glucose has been found to be higher in PW patients compared to normals (Parra et al. 1973). Thus, the glucose intolerance and the diabetic state which develops in PW syndrome cannot primarily be due to hypoinsulinism.
Insulin resistance and insulin binding

As indicated by the high plasma insulin concentrations the PW patients seem to be insulin resistant. The finding of high FFA concentrations (Table 2) and a high lipoprotein lipase activity (Schwartz et al. 1979) shows that the insulin resistance also affects the fat metabolism. In accordance to this we found a reduced number of insulin receptors on monocytes, which seem to mirror the major target cells for insulin action in hyperinsulinaemic insulin resistant states (Beck-Nielsen 1980). We therefore consider that the receptor defect is general.

Cellular insulin resistance may be due to a receptor defect or post-receptor defect. The results of the present study indicate that a reduced receptor number may be of importance for the insulin resistant state in PW syndrome.

In 2 of our patients the insulin binding was increased after 24 h of fasting. This suggests that the receptor defect is not due to a genetic failure. No evidence for receptor antibodies was found. The receptor defect seems to be secondary to hyperinsulinism, such as previously demonstrated in obese with and without diabetes mellitus (Beck-Nielsen 1978). This conclusion fits with the negative correlation found between insulin binding and fasting plasma insulin concentration.

Insulin receptor defects have previously been demonstrated in other clinical syndromes such as generalized lipodystrophy (Oseid et al. 1977), ataxia telangiectasia (Bar et al. 1978), periodic hypokalaemic paralysis (Johnson & Beck-Nielsen 1979), Klinefelter's syndrome (Breyer et al. 1981) and acanthosis nigricans (Bar et al. 1980). The finding in this paper add PW syndrome to the groups of syndromes characterized by insulin resistance and an insulin receptor defect.

Conclusion

Patients with PW syndrome are often glucose intolerant, and frank diabetes develops more often in these patients than in normals. The metabolic findings seem to be secondary to their obesity, since identical changes in plasma insulin, insulin secretion and in monocyte insulin binding have been demonstrated in PW patients and their obese controls.

The glucose intolerance seems to be caused by insulin resistance, which at least in part, is explained by the finding of a reduced insulin receptor number on target cells. No data are available concerning post-receptor defects in these patients.

Acknowledgments

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References


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