CHARACTERISATION OF MECHANISMS RESPONSIBLE FOR URAEMIC INSULIN RESISTANCE: IN VITRO EXPERIMENTS

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Summary

In an attempt to define the cellular basis of the uraemic insulin resistance we studied insulin action in adipocytes from eight patients with undialysed chronic uraemia and from eight matched healthy controls. (\textsuperscript{125}I)-insulin binding to fat cells from uraemic patients was normal. In contrast (\textsuperscript{14}C)-D-glucose transport exhibited decreased sensitivity to insulin. The concentrations of insulin that elicited a half-maximal response were 422±95pmol/L in uraemic patients and 179±38pmol/L in normals (p<0.01). The non-insulin and the maximally insulin stimulated glucose transport of adipocytes from uraemic patients was normal. The lipogenesis of fat cells from uraemic patients had depressed sensitivity to insulin (half-maximal stimulation at 38±8pmol/L in uraemic patients and at 11±3pmol/L in normals, p<0.01) with unchanged non-insulin and maximally insulin stimulated lipogenesis. Taken together these results suggest that the insulin resistance of adipocytes from patients with chronic uraemia may be primarily accounted for by post-binding defects localised to glucose transport and metabolism.

Introduction

Numerous experiments have demonstrated that the glucose intolerance of uraemic patients is primarily due to insulin resistance of peripheral tissues [1,2]. Moreover, it has been shown that the liver of uraemic man retains normal sensitivity to insulin at least with respect to the inhibitory effect on hepatic glucose release [1,2]. To examine the cellular mechanisms behind the insulin resistance of peripheral tissue from uraemic man we have measured (\textsuperscript{125}I)-insulin binding and non-insulin and insulin stimulated (\textsuperscript{14}C)-D-glucose transport and metabolism in isolated human adipocytes from healthy controls and from uraemic patients not yet on chronic dialysis treatment.
TABLE 1. Clinical and laboratory data of patients

<table>
<thead>
<tr>
<th>Patient No</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Obesity index</th>
<th>Renal disease</th>
<th>Plasma</th>
<th>Serum</th>
<th>Total CO₂</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Insulin μU/ml</td>
<td>Glucose mmol/L</td>
<td>Creatinine μmol/L</td>
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<td>1</td>
<td>M</td>
<td>52</td>
<td>1.04</td>
<td>Chronic glomerulonephritis</td>
<td>19</td>
<td>6.5</td>
<td>884</td>
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<tr>
<td>2</td>
<td>M</td>
<td>53</td>
<td>0.94</td>
<td>Polycystic kidney disease</td>
<td>8</td>
<td>4.2</td>
<td>887</td>
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<tr>
<td>3</td>
<td>F</td>
<td>55</td>
<td>1.00</td>
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<td>5.1</td>
<td>766</td>
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<tr>
<td>4</td>
<td>M</td>
<td>58</td>
<td>0.97</td>
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<td>929</td>
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<tr>
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<td>F</td>
<td>55</td>
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<td>678</td>
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<tr>
<td>6</td>
<td>F</td>
<td>31</td>
<td>0.94</td>
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<td>M</td>
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<td>5.3</td>
<td>559</td>
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<td>Mean</td>
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<td>1.04</td>
<td></td>
<td>14</td>
<td>5.0</td>
<td>793</td>
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<tr>
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<td></td>
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<td>0.2</td>
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</table>
Materials and methods

Subjects

Eight adult, ambulatory non-diabetic uraemic patients were studied and compared with sex, age and weight-matched healthy volunteers. Pertinent clinical data for the patients appear in Table I. All the patients had an oral glucose tolerance test (1g of glucose/kg body weight). Patient No. 2 had a two-hour value plasma glucose of 11.6mmol/L and patient No. 7 had a two-hour value of 9.8mmol/L, whereas the rest of the group all had two-hour values below 7.5mmol/L.

Four healthy males and four healthy females served as control subjects. Their mean age was 44 years (range 58–26 years) while their mean obesity index was 1.05 (range 0.85–1.15).

Insulin receptor binding

Adipose tissue (about 10g) was obtained by open biopsy from the upper one-fourth of the right gluteal region after a square field had been anaesthetised with an epidermal injection of 1% lidocaine without epinephrine. Details about fat cell isolation as well as determination of fat cell size and number have been published previously [3]. Insulin binding to fat cells (about 10^5 cells per ml of cell suspension) was measured in a Hepes buffer at 37°C, after incubation for 60 minutes with tyrosine-A_{14}-labelled (125I)-insulin with or without increasing concentrations of unlabelled insulin [3]. Specific insulin binding to adipocytes was expressed per 30cm^2 of surface area per ml.

Glucose transport

All studies were carried out at 37°C. Forty microlitres of adipocyte suspension with a volume fraction of 0.4 (about 6 x 10^5 cells/ml) were placed in polypropylene tubes and preincubated with or without insulin for 45 minutes. Twelve microlitres (0.24 μCi) of tracer D-U-(14C)-glucose (final glucose concentration 20μmol/L) was added at time zero and uptake was determined after 10 seconds by adding 3ml of phloretin (0.3mmol/L). Silicone oil (0.8ml of 0.99g/ml) was layered on the top, and the tubes were spun within two minutes at 2500G. The cells were collected from the top of the oil and placed in scintillation vials with 5ml of scintillation fluid [4].

Lipogenesis

Lipogenesis was measured as the conversion of D-U-(14C)-glucose to (14C)-total lipids [5]. Isolated adipocytes (about 7 x 10^4 cells/ml) were prepared in a 10mmol/L Hepes buffer containing 0.5mmol/L glucose. The cells were preincubated for 45 minutes at 37°C with or without insulin in increasing concentrations. Then 0.4μCi D-U-(14C)-glucose was added to each tube (final glucose concentration 0.5mmol/L) and the incubation was continued for 90 minutes.
H₂SO₄ was added and a Dole extraction was performed. A sample for liquid scintillation counting was taken from the upper phase.

**Analytical methods**

Plasma glucose was analysed with a glucose dehydrogenase method (Merck enzymatic kit) and plasma insulin was measured with a RIA technique [6].

**Statistical methods**

In text, tables and figures data are given as the mean ± 1 SEM. Significant differences between groups were assessed by Mann Whitney's test. In correlation studies Spearman's test was employed.

![Graphs](image)

**Figure 1.** Glucose transport of fat cells. Upper panel: adipocytes from eight uraemic patients (○) and eight normal subjects (○) were preincubated with or without insulin in the for 45 minutes at 37°C. Initial transport rate was then measured during the first 10 seconds after (¹⁴C)-D-glucose had been added to reach a final concentration of 20μmol/L (mean ± 1 SEM).

Lower panel: glucose transport data are expressed as percentage of the response to insulin in maximally effective concentrations.
Results

The uraemic condition did not influence the binding of $\left(^{125}\text{I}\right)$-insulin or the ability of unlabelled insulin to complete for binding over a concentration range of 0.55–245 nmol/L. When $\left(^{14}\text{C}\right)$-D-glucose transport was measured in the absence or the presence of maximally effective insulin concentrations adipocytes from uraemic patients responded similarly to adipocytes from healthy volunteers (Figure 1). However, the insulin-dose response relationship of glucose transport was altered by uraemia. In patients with renal failure the dose-response curve for insulin stimulated glucose uptake was markedly shifted to the right (Figure 1) suggesting an impaired sensitivity to insulin. For example, the concentrations of insulin that elicited a half-maximal effect was 422±95 pmol/L in uraemic patients and 179±38 pmol/L in healthy subjects (p<0.01).

![Graph showing glucose transport and insulin sensitivity](image-url)

Figure 2. Upper panel: adipocytes from eight uraemic patients (●) and eight normal subjects (○) were preincubated with or without insulin in the indicated concentrations for 45 minutes at 37°C. Then $\left(^{14}\text{C}\right)$-D-glucose was added (total glucose concentration in the final preparation was 0.5 mmol/L) and the incubation was terminated after 90 minutes (mean ± 1 SEM).

Lower panel: lipogenesis data are expressed as percentage of the response to insulin in maximally effective concentrations.
The effect of uraemia on glucose metabolism by human adipocytes was studied by measuring \(^{14}\text{C})\text{-D-glucose conversion to total lipids (Figure 2). Both non-insulin and maximally insulin stimulated lipogenesis were normal in uraemic cells. However, consistent with our observations in glucose transport studies the insulin concentrations giving half-maximal lipogenesis were significantly higher in adipocytes from uraemic patients (38±8 pmol/L in uraemic patients versus 11±3 pmol/L in normals, \(p<0.01\)).

Discussion

Our results indicate that long-term uraemia has no significant impact on adipocyte insulin receptor binding. Previous investigations of blood cell insulin receptors in human uraemia have shown normal monocyte binding and decreased or normal erythrocyte binding [2,7–10]. Uraemic defects in adipocyte insulin action were found in the insulin-dose response studies of glucose transport as well as glucose metabolism. Both cellular functions were in uraemic cells characterised by impaired sensitivity to submaximal insulin stimulation but unaltered basal and maximal responses.

The in vivo studies of the insulin resistance to human uraemia using the euglycaemic clamp technique with more steady state plasma insulin values have demonstrated both a rightward shift of insulin dose response curves and a decreased maximal insulin responsiveness of the glucose disposal to peripheral tissues [1,2]. Under the insulin clamp study conditions the major site of glucose uptake is muscle. Provided our finding of normal adipocyte insulin receptor binding can be extrapolated to muscles the clamp results are most compatible with post-binding defects in insulin-mediated in vivo glucose utilisation. Finally, since we could find no depression of maximal adipocyte glucose metabolism in vitro the in vivo finding of impaired maximal responsiveness of the glucose disposal [1,2] may reflect additional defective post-binding steps of insulin action in skeletal muscles.

Acknowledgment

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