Insulin receptor tyrosine-kinase activity is altered in both muscle and adipose tissue from non-obese normoglycaemic insulin-resistant subjects

G. Grasso¹, L. Frittitta¹, M. Anello¹, P. Russo¹, G. Sesti², V. Trischitta¹

¹ Cattedra di Endocrinologia e Patologia Costituzionale, Università di Catania, Catania, Italy
² Dipartimento di Medicina Interna, Università di Roma “Tor Vergata”, Rome, Italy

Summary We performed i.v. insulin tolerance test in 30 non-obese (BMI < 30 male and < 28 female) non-diabetic (by oral glucose tolerance test) subjects and subdivided them into three groups of 10 subjects each, according to their insulin sensitivity (Kim values). Then we compared the tyrosine-kinase activity of immunopurified insulin receptors (using ³²P-ATP and poly-glu-tyr (4:1) from both muscle and adipose tissue in 7 of the most insulin-sensitive and 7 of the most insulin-resistant subjects. No difference was observed between the two groups in the basal (no insulin) receptor tyrosine-kinase activity from both tissues. In contrast, tyrosine-kinase activity response to insulin was significantly higher (p < 0.05 by 2-way ANOVA test) in receptors from both tissues of insulin-sensitive subjects. In addition, a decreased tyrosine-kinase sensitivity to insulin was observed in muscle, but not adipose, tissue of insulin-resistant subjects (insulin ED₅₀, being 0.87 ± 0.05 nmol/l vs 2.03 ± 0.07, p < 0.05 in insulin-sensitive and -resistant subjects). Insulin ED₅₀ of muscle receptor tyrosine-kinase significantly (p = 0.001) correlated to both Kim values (r = –0.79) and plasma insulin values at 120 min during OGTT (r = +0.80). Insulin receptor content, as assessed by radioimmunoassay, was similar in both muscle (7.9 ± 1.3 and 9.2 ± 1.9 ng/mg protein) and adipose tissue (8.2 ± 1.3 and 7.5 ± 1.4) of insulin-sensitive and -resistant subjects. Exon 11+ isoform of insulin receptor was similarly represented in muscle specimens from six insulin-sensitive (80 ± 8% of total receptor content) and six resistant (78 ± 6%) subjects. In conclusion, a defective insulin stimulation of receptor tyrosine-kinase activity is present in both muscle and adipose tissue of euglycaemic non-obese insulin-resistant subjects. This defect is, therefore, an early event in the development of insulin resistance. [Diabetologia (1995) 38: 55–61]

Key words Insulin receptor, tyrosine-kinase, insulin resistance, muscle tissue, adipose tissue.

The molecular mechanisms and the primary defects responsible for insulin resistance, a well-known characteristic of non-insulin-dependent diabetes mellitus NIDDM [1–2], are not completely understood. One possible molecular site of the defective insulin action is the insulin receptor and, in fact, several studies indicate that insulin receptor tyrosine-kinase activity is altered in obese and in NIDDM insulin-resistant patients [3–7]. However, whether this defect is either primary or secondary to obesity or NIDDM remains to be elucidated.

Insulin resistance is also present in approximately 25% of non-obese normoglycaemic subjects [1] who represent, therefore, a natural model to verify whether or not the defective insulin receptor tyrosine-kinase precedes the appearance of, and is independent from, the metabolic alterations associated with obesity and NIDDM.

In order to clarify this issue, we first quantified insulin sensitivity in 30 non-obese, normoglycaemic volunteers, then subdivided these subjects into three groups according to their in vivo sensitivity to insu-
Table 1. Clinical features of the studied subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Sex</th>
<th>Age (years)</th>
<th>BMI (kg/m²)</th>
<th>FPG (mmol/l)</th>
<th>IRI (µU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin-sensitive</td>
<td>5/2</td>
<td>31 ± 11</td>
<td>23 ± 1</td>
<td>4.9 ± 0.3</td>
<td>12 ± 3</td>
</tr>
<tr>
<td>Insulin-resistant</td>
<td>7/0</td>
<td>44 ± 13</td>
<td>25 ± 3</td>
<td>5.2 ± 0.7</td>
<td>11 ± 2</td>
</tr>
</tbody>
</table>

BMI, body mass index; FPG, fasting plasma glucose; IRI, fasting plasma immunoreactive insulin. Data indicate mean ± SEM.

Finally, we compared the content and the tyrosine kinase activity of insulin receptor in muscle and adipose tissues from seven of the most insulin-sensitive subjects and seven of the most insulin-resistant.

Subjects, materials and methods

Crystalline porcine insulin, Triton X-100, Bacitracin, p-methyl sulphonyl-fluoride (PMSF) and poly Glu-Tyr (4 : 1) were purchased from Sigma Chemical Co. (London, UK). 125I-A14-insulin (340-360 µCi/µg) was kindly provided by Dr. L. Benzi (Pisa, Italy). [Gamma 35P] ATP was obtained from Amersham Ltd (London, UK).

Subjects. Thirty healthy non-obese subjects, undergoing elective abdominal surgery, were studied, in accordance with the declaration of Helsinki, after giving informed consent. According to the World Health Organization criteria none of them was diabetic as judged by an oral glucose tolerance test (OGTT). All subjects were fed a weight-maintaining diet (50% carbohydrate, 30% lipid, and 20% protein) for 8 days preceding our study.

After an overnight fast, insulin sensitivity was assessed by i.v. insulin tolerance test (ITT), performed by injecting as a single bolus 0.1 IU per kg of body weight of regular insulin [8]. Blood samples were collected 15 and 5 min before and 3, 6, 9, 12, and 15 min after insulin injection. Glucose was injected at 15 min to stop the glycaemic fall. The constant rate for plasma glucose disappearance (Km) was calculated according to the formula 0.693/t1/2. The plasma glucose t1/2 was calculated from the slope of least square analysis of the plasma concentrations from 3-15 min after i.v. insulin when the glucose concentration declined linearly. In order to verify the reproducibility of Km values, a second ITT was carried out in four subjects within 1-3 weeks from the first test. In all four cases the Km value obtained with the second test was within 8% of the value obtained at the first one (data not shown).

By this method we confirmed previous observations, obtained by glucose clamp studies, indicating that insulin sensitivity may vary in a wide range in non-obese normoglycaemic subjects [1]. In details, Km values ranged between 2.1 and 8.3 with a median of 6.0 and a mean ±SEM of 5.69 ± 0.24. According to Km values, subjects were subdivided in three groups with group 1 and 3 including the 10 most insulin-sensitive and the 10 most insulin-resistant subjects, respectively. Seven out of ten subjects from both groups 1 and 3 volunteered to allow muscle and adipose tissue biopsy during abdominal surgery in order to perform in vitro studies on insulin-receptor content and function. No significant differences were observed in age, body mass index, and fasting plasma glucose and insulin levels between the two groups, although insulin-resistant subjects were somewhat older and heavier (Table 1). A significant (p = 0.01) negative correlation was observed between plasma insulin values at 120 min during the OGTT and Km values when either all 30 subjects (r = -0.57) or only the 14 subjects who entered the study (r = -0.69) were considered. Plasma insulin values at 120 min during OGTT were significantly higher (p < 0.05) in insulin-resistant subjects (85 ± 20 µU/ml) as compared to insulin-sensitive subjects (34 ± 8). No significant difference in total cholesterol (177 ± 15 vs 219 ± 19 mg/dl) HDL cholesterol (46 ± 4 vs 42 ± 5 mg/dl) and triglyceride (116 ± 21 vs 111 ± 19) was observed in insulin-sensitive and resistant subjects, respectively. One insulin-sensitive and one insulin-resistant subject were first degree relatives of NIDDM patients.

Tissue specimens. Specimens from the external oblique muscle and abdominal subcutaneous adipose tissue were obtained at elective abdominal surgery (cholecystectomy) 3-5 days after ITT and OGTT, and immediately frozen in liquid nitrogen. Tissues were first powdered by mortar and pestle in the presence of liquid nitrogen and then homogenized (at 70 mg/ml for muscle and 200 mg/ml for adipose tissue) at 4°C by Polytron homogenizer (20 s at medium speed) in 50 mmol/l Hepes buffer pH 7.6, containing 150 mmol/l NaCl, 5 mmol/l EDTA, 5 mmol/l EGTA, 20 mmol/l sodium pyrophosphate, 1 mmol/l phenylmethylsulphonylfluoride, 1 mmol/l sodium-vanadate, 20 mmol/l sodium fluoride, 1 mg/ml bacitracin. When using adipose tissue the homogenized material was centrifuged at 200 x g for 2 min in order to remove the fat cake. Tissues were then solubilized by adding 1% Triton X 100 (final concentration) and continuous shaking for 1 h at 4°C. Solubilized material was then centrifuged at 10,000 x g and the supernatant was used for the measurement of insulin receptor content and its tyrosine kinase activity.

Tyrosine kinase activity. This activity was assessed on immunopurified insulin receptors by a modification [9] of the previously described procedure [10]. Briefly, 96-well microtitre polystyrene plates (Falcon) were coated with 50 µl of anti-mouse IgG (10 µg/ml final concentration, in 20 mmol/l NaHCO3, pH 9.6) and incubated overnight at 4°C. The wells were then washed three times with 50 mmol/l Hepes buffer, pH 7.6, containing 0.15 mol/l NaCl, 0.1% Triton X-100, 0.1% Tween-20, and 0.1% bovine serum albumin (BSA), coated with 50 µl of anti-human insulin receptor mouse monoclonal antibody 29 B4, 10 µg/ml final concentration, incubated overnight at 4°C and finally washed again three times with the 50 mmol/l Hepes buffer previously described. The solubilized tissue (40 µl, containing 1-1.5 ng insulin receptor) was then added in the wells and incubated overnight at 4°C. Wells were then washed three times with 50 mmol/l Hepes buffer and immunopurified insulin receptors preincubated for 30 min at 22°C in the absence or the presence of increasing insulin concentrations in 50 mmol/l Hepes buffer, pH 7.4, containing 0.1% Triton X-100, 2 mmol/l MnCl2 and 10 mmol/l MgCl2. Next 10 µmol/l ATP; gamma-32P ATP (2 BxCi/well) and the synthetic substrate poly-Glu: Tyr 4 : 1 (1 mg/ml final concentration) were added, the incubation continued for a further 60 min at 22°C and the radioactivity incorporated into the exogenous substrate measured. Non-specific incorporation was determined in parallel wells not coated with the anti-insulin receptor monoclonal antibody. Results were normalized for insulin binding values measured in parallel wells where immunosorbed insulin receptors from the same specimens were incubated at 22°C with 1 mmol/l 125I-A14 Insulin (in the absence or presence of 10 µmol/l native insulin) in 50 mmol/l Hepes binding buffer (pH 7.8, 1% BSA). After 2 h wells were washed five times with chilled 50 mmol/l Hepes binding buffer and then cut and bound radioactivity counted. No difference was