Insulin secretion, adipocyte insulin binding and insulin sensitivity in thyrotoxicosis

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Abstract. The pattern of insulin secretion following an oral glucose load and the insulin receptor status and insulin sensitivity of adipocytes have been studied in patients with thyrotoxicosis and in matched controls. Thyrotoxic subjects showed normal basal and peak levels of serum immunoreactive insulin (peak, 69.0 ± 6.8 vs 54.3 ± 8.8 μU/l) and serum C-peptide (peak, 1.95 ± 0.13 vs 1.71 ± 0.12 nmol/l for thyrotoxic and control subjects, respectively). Peak serum proinsulin was higher in the thyrotoxic group (64.8 ± 7.3 vs 39.0 ± 3.7 pmol/l; \(P < 0.01\)). Maximum specific insulin binding to adipocytes was decreased in the thyrotoxic group (1.80 ± 0.18 vs 2.62 ± 0.27%; \(P < 0.025\)) and half-maximum displacement of tracer insulin was similar in the two groups, suggesting that reduced receptor number rather than reduced affinity accounted for the difference. However, adipocyte insulin sensitivity was normal as judged by half-maximal stimulation values of 13.9 ± 3.6 vs 11.4 ± 2.1 pmol/l, respectively for lipogenesis and 24.3 ± 2.2 vs 24.6 ± 3.6 pmol/l, respectively for glucose transport. Hence, thyroid hormone excess appears to affect adipocyte insulin receptor number directly, but change in receptor number is not associated with change in adipocyte insulin sensitivity in hyperthyroidism. The normal insulin secretion together with the failure to demonstrate abnormal insulin sensitivity of one of the major peripheral tissues suggests that disturbed hepatic rather than peripheral insulin responsiveness may be responsible for the glucose intolerance of hyperthyroidism.

Abnormalities of carbohydrate metabolism including fasting and post-prandial hyperglycaemia are well recognised in hyperthyroidism (Doar et al. 1969; McCulloch et al. 1982). The impairment of glucose tolerance in the face of normal serum insulin levels (Andreani et al. 1970; McCulloch et al. 1982) suggests resistance to the action of insulin. In vivo assessment of insulin resistance has given rise to conflicting results (Elrick et al. 1961; West et al. 1975).

Adipose tissue from hyperthyroid subjects has been reported to be resistant to the antilipolytic action of insulin (Wennlund et al. 1981). However, the adipocytes of rats rendered acutely hyperthyroid exhibited a large increase in insulin receptor number without change in insulin sensitivity (Heise et al. 1982). Thus tissue sensitivity to insulin and the state of tissue insulin receptors during chronic exposure to high serum thyroxine and triiodothyronine levels remain uncertain.

The present study was designed a) to elucidate the insulin sensitivity and insulin receptor status of adipocytes from patients with thyrotoxicosis, and b) to relate this to the pattern of insulin secretion in response to oral glucose in these subjects.

Patients and Methods

Thirteen patients referred to the clinic with a provisional diagnosis of hyperthyroidism were recruited and gave informed consent to the study which was approved by the Newcastle Area Health Authority ethical committee. The subjects were subsequently divided into thyrotoxic and control groups on the basis of total serum thyroxine and...
thyrotrophin-releasing hormone tests. The control subjects had no evidence of any organic disease which may have produced the presenting symptoms, and thus constituted a group of subjects who had similar symptoms of adrenergic overactivity but who did not have elevated serum thyroid hormone levels. The clinical and biochemical characteristics of the two groups are listed in Table 1.

**Chemicals**

Human serum albumin (> 99% electrophoretically pure) was obtained from Hoechst UK, Ltd. Collagenase from Clostridium histolyticum (Batch No. 504-22) was obtained from P-L Biochemicals Inc. Crystalline porcine insulin and mono-[²⁵¹I] (A₁₄ Tyr)-insulin were supplied by Novo. D-glucose (specific activity 270 mCi/mmol) was purchased from Amersham International PLC.

**Oral glucose tolerance test**

After an overnight fast 75 g glucose in 375 ml water was administered over a 5 min period. Blood was sampled via a plastic cannula inserted into a forearm vein under local anaesthetic. Patients remained seated throughout the test.

**Sampling and preparation of adipocytes**

Two—three g of subcutaneous adipose tissue was obtained from the lateral gluteal region by open biopsy after cutaneous infiltration with 1% lignocaine. All biopsies were taken between 8 and 9 a.m. after an overnight fast. The procedure was associated with minimal discomfort.

The adipocyte isolation procedure described by Pedersen et al. (1982) was used. Cell number and total cell surface area per incubation tube were derived knowing the mean of the individual cell volumes, the lipocrit of the final suspension and the mean of the individual cell surface areas. The coefficient of variation (CV) of the cell sizing procedure was 2.0% and that of the lipocrit determination was 2.5%.

**Adipocyte insulin binding**

Insulin binding to adipocytes was measured by incubating 300 µl aliquots of cell suspension with 100 µl A₁₄ labelled mono-[²⁵¹I]insulin (final concentration 4–16 pmol/l) and 100 µl buffer or unlabelled insulin (final concentration 120–21 000 pmol/l) in duplicate at 37°C for 60 min in a HEPES buffer according to Pedersen et al. (1982). The binding was stopped by adding 10 ml chilled 154 mmol/l NaCl. Silicone oil (density 0.97 g/ml), 1 ml, was layered on the surface and the tubes were spun at 1000 g for 3 min. The cell pellet was harvested using a pipette cleaner as described by Gliemann & Sonne (1978). Specific binding was calculated by subtracting the binding observed in the presence of 10⁻⁵ M insulin from the total binding for each insulin concentration. The non-specific binding averaged 1.5 ± 0.2% of cell bound insulin at a tracer concentration of 16 pmol/l. The intra-assay coefficient of variation of specific binding at a tracer concentration of 4 pm was 2.3%. Binding was expressed as per cent specific binding per 10 cm² adipocyte membrane (Pedersen & Hjollund 1982).

**Insulin stimulation of lipogenesis**

Stimulation of tracer glucose incorporation into total lipids was measured as described by Pedersen et al. (1982). Triplicate 200 µl aliquots of adipocytes in glucose-free HEPES buffer were pre-incubated at 37°C for 45 min with or without increasing amounts of insulin. 0.4 µCi U-[¹⁴C]β-glucose was added (final concentration 6 µmol/l) and the incubation was continued without shaking for 90 min. The reaction was stopped by adding 200 µl 1 M H₂SO₄ and the tubes were allowed to stand for 60 min before the total lipid fraction was extracted and separated. The amount of U-[¹⁴C]β-glucose incorporation into lipid for each triplicate set of tubes was calculated and expressed as pmol of glucose incorporated per 90 min per 10 cm² adipocyte membrane.

**Insulin stimulation of glucose transport**

Stimulation of tracer glucose incorporation into total lipids was measured as described by Pedersen et al. (1982). Initial rates of glucose transport were assessed using the method described by Whitesell & Gliemann (1979) and U-[¹⁴C]β-glucose as tracer (Zeuzem & Taylor 1983). Glucose transport was assessed in only three thyrotoxic and four control subjects.
Glucose, insulin, C-peptide and proinsulin assays

Blood glucose was measured using a Yellowsprings analyser. Serum was separated and stored at −20°C for assay of insulin and C-peptide by radioimmunoassay (Hales & Randle 1963; Heding 1975). Proinsulin was assayed by a modification of a previously described method (Rainbow et al. 1979) using flexible microtitre plates as solid phase and a 125I-labelled mouse monoclonal anti-rabbit immunoglobulin. Proinsulin extracted from human pancreas was extracted and calibrated for use as previously described (Rainbow et al. 1979). Insulin cross-reacted less than 10% in the proinsulin assay, but proinsulin displayed 50% cross-reactivity with the anti-insulin antibody used in the insulin assay. The specificity of the proinsulin assay was 100% for circulating proinsulin (65 A₁ and 32–33 split forms). The intra- and inter-assay variations for the proinsulin and insulin assays were 6% and 10%, and 4% and 7%, respectively.

Statistical analyses

Statistical analyses were carried out using Student's t-test and the Spearman rank correlation coefficient. Results are expressed as mean ± SEM.

Results

1. Oral glucose tolerance tests

Mean fasting blood glucose was 4.9 ± 0.3 mmol/l for the thyrotoxic groups compared with 4.4 ± 0.2
mmol/l for the control group \((P < 0.1 > 0.05)\). The rise in blood glucose was greater in the thyrotoxic group, and was significantly different at 10, 25, 30, 45 and 90 min \((P < 0.05)\) (Fig. 1 and Table 2).

Fasting serum insulin levels were similar in the two groups \((9.5 \pm 2.8 vs. 7.5 \pm 0.8 \text{ mU/l (60.8} \pm 17.9 \text{ vs} 48.0 \pm 5.1 \text{ pm})\) for thyrotoxic and control groups, respectively). Peak serum insulin was 69.0 \pm 6.8 \text{ mU/l (442} \pm 44 \text{ pm}) for the thyrotoxic group (at 45 min) and 54.3 \pm 8.8 \text{ mU/l (348} \pm 56 \text{ pm}) for the control group (at 60 min) \((P < 0.1 > 0.05)\). Fasting serum proinsulin was very similar in the two groups \((14.1 \pm 3.8 \text{ vs} 15.8 \pm 3.2 \text{ pm}, \text{ respectively})\) but rose more steeply to a higher peak in the thyrotoxic group \((64.8 \pm 7.3 \text{ vs} 39 \pm 3.7 \text{ pm}
\text{ at} 60 \text{ min}; \ P < 0.01)\) (Fig. 2). Serum C-peptide levels were similar both basally \((0.46 \pm 0.11 \text{ vs} 0.37 \pm 0.04 \text{ nmol/l, respectively})\) and throughout the oral glucose tolerance test (Fig. 1).

2. Adipocyte insulin binding

The binding displacement curve is shown in Fig. 3. Maximum specific insulin binding was 1.80 \pm 0.18\% for the thyrotoxic group and 2.62 \pm 0.27\% for the control group \((P < 0.025)\). The difference in specific insulin binding was significant at all points up to an insulin concentration of \(2.2 \times 10^{-9} \text{ M insulin}\). Half-maximum displacement of tracer insulin was observed at similar insulin concentrations \((145 \pm 16 \text{ and} 120 \pm 8 \text{ pm, respectively})\), suggesting that the difference in binding was secondary to reduced receptor number rather than alteration of affinity.

Within the thyrotoxic group there was a negative correlation between maximum specific binding and total serum thyroxine \((R_S = 0.71, P < 0.05)\) (Fig. 4) but not between maximum specific binding and total serum triiodothyronine.

3. Adipocyte insulin sensitivity

Basal rates of adipocyte lipogenesis were 52.0 \pm
16.0 \text{ and} 87.0 \pm 14.4 \text{ pmoles/10 cm}^2/90 \text{ min for the thyrotoxic and control groups (P} > 0.1)\). Maximum rates of lipogenesis were 121.5 \pm 28.0 \text{ and 175.8} \pm 25.4 \text{ pmoles/10 cm}^2/90 \text{ min, respectively (P} > 0.1)\). Insulin stimulation achieved similar percentage increases above basal levels in each group and the insulin concentrations required to achieve half-maximal stimulation were similar \((13.9 \pm 3.6 \text{ vs} 11.4 \pm 2.1 \text{ pmoles/10 cm}^2/90 \text{ min, respectively})\) (Fig. 5).

Basal rates of glucose transport were 0.75 \pm 0.22 \text{ pmoles/10 cm}^2/20 \text{ sec for the thyrotoxic subjects studied (n = 3) and 1.00} \pm 0.22 \text{ pmoles/10 cm}^2/20 \text{ sec for the control subjects studied (n = 4) (P} > 0.2)\). Maximum rates of glucose transport were 1.77 \pm 0.76 \text{ and} 2.31 \pm 0.42 \text{ pmoles/10 cm}^2/20 \text{ sec (P} > 0.3)\). The insulin concentrations required to achieve half-maximal stimulation were almost identical \((24.3 \pm 2.2 \text{ vs} 24.6 \pm 3.6 \text{ pmoles/10 cm}^2/20 \text{ sec, respectively})\) (Fig. 6).

![Fig. 3. Specific insulin binding to adipocytes from thyrotoxic (●—●) and control (○—○) subjects at increasing total insulin concentrations.](Bioscientifica.com)
Relationship between specific insulin binding to adipocytes and total serum thyroxine in the thyrotoxic group (●).

\[ R_s = 0.71; \ P < 0.05. \]
Control data is shown as mean ± SEM (○).

Discussion
In previous studies of hyperthyroid patients we have found increased fasting blood glucose concentrations (McCulloch et al. 1982) and glucose production rates (McCulloch et al. 1983b). Hyperglycaemia following oral glucose administration was observed in this as in many previous studies. However, serum immunoreactive insulin levels were not significantly different in the two groups. Previous workers have suggested that much of the insulin measured by standard radioimmunoassay...
in hyperthyroidism may in fact be proinsulin (Sestoft & Heding 1981). We confirm hyperproinsulinaemia in thyrotoxicosis which may be secondary to either hypersecretion or impaired hepatic extraction. In our insulin assay, proinsulin exhibits 50% cross-reactivity with insulin (unpublished data). It is apparent that the observed peak immunoreactive insulin levels would be affected by the proinsulin present, and that the true insulin levels are closer in the two groups. The finding of normal C-peptide responses to oral glucose in the present study confirms that the insulin secretion itself was normal, insulin and C-peptide being secreted in equimolar amounts. Hence, the thyrotoxic subjects displayed resistance to insulin action either in promoting hepatic glucose uptake, in inhibiting hepatic gluconeogenesis or in promoting glucose uptake into the peripheral tissues, muscle and fat.

The adipocyte insulin binding studies demonstrated that insulin receptor number was decreased in thyrotoxicosis corroborating recently reported observations (Arner et al. 1984). This finding is in sharp contrast to the demonstration that the adipocytes of rats rendered acutely thyrotoxic almost doubled their insulin receptor number (Heise et al. 1982). The possibility of a species difference cannot be dismissed, but studies after more prolonged periods of thyroxine administration to rats would be of interest. The time course of the cellular insulin receptor response to continued stimulation by thyroid hormones requires further study in view of the possibility of initial increase in synthesis rate

### Table 2.

Incremental areas under the curves for blood glucose, serum immunoreactive insulin, proinsulin, insulin corrected for proinsulin cross-reactivity, and C-peptide after a 75 g oral glucose load.

<table>
<thead>
<tr>
<th></th>
<th>Thyrotoxic</th>
<th>Control</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Glucose (mM min)</td>
<td>373 ± 27</td>
<td>218 ± 51</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td>Immunoreactive insulin (mU/l min)</td>
<td>5606 ± 313</td>
<td>4714 ± 622</td>
<td>&gt; 0.2</td>
</tr>
<tr>
<td>Proinsulin (pM min)</td>
<td>4748 ± 665</td>
<td>2616 ± 197</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td>Corrected insulin (mU/l min)</td>
<td>5092 ± 448</td>
<td>3819 ± 798</td>
<td>&gt; 0.2</td>
</tr>
<tr>
<td>C-peptide (nM min)</td>
<td>246 ± 60</td>
<td>178 ± 9</td>
<td>&gt; 0.2</td>
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or recruitment from a latent pool of insulin receptors followed by compensatory downregulation. To date, few factors have been shown to be involved in the physiological control of insulin receptor number, insulin itself being far the most powerful influence (Marshall & Olefsky 1980). The results of the present study suggest that thyroid hormones may be involved in the control of insulin receptor number. The inverse correlation between total serum thyroxine and adipocyte insulin binding, which was also observed by Arner et al. 1984, adds weight to this hypothesis.

Adipocyte insulin resistance is usually present in those clinical states associated with reduced adipocyte insulin receptor number, such as obesity, diabetes and cirrhosis (Pedersen et al. 1981; Pedersen & Hjollund 1982; Taylor et al. 1985). However, the insulin concentrations required to achieve half-maximal stimulation of both glucose transport and lipogenesis were similar for the adipocytes of the thyrotoxic and control groups. The adipocytes from the thyrotoxic subjects thus exhibited not only receptor downregulation but also compensation for the receptor alteration at the post-receptor level. A dissociation of biological effects from change in adipocyte insulin receptor number was also observed after acute thyroxine administration to rats (Heise et al. 1982), although in this case insulin receptor number increased without change in adipocyte insulin sensitivity. The explanation for this phenomenon is obscure but fuels the concept that the linkage between receptor binding and insulin action is not simple. The observation that adipose tissue from thyrotoxic subjects did not exhibit normal sensitivity to insulin inhibition of lipolysis and stimulation of total glucose oxidation (Wennlund et al. 1981) suggests that the regulation of these post-receptor pathways in hyperthyroidism may differ from that of the glucose transport and lipogenesis pathways.

The apparently normal sensitivity of adipocytes from thyrotoxic subjects to insulin stimulation of glucose transport and lipogenesis accords with the recent demonstration of normal peripheral tissue sensitivity to exogenously administered insulin in man as assessed by the euglycaemic clamp technique (McCulloch et al. 1983a). The elimination of peripheral tissue resistance to glucose disposal as a possible cause of post-prandial hyperglycaemia makes defects of hepatic insulin sensitivity in hyperthyroidism more likely. Indirect support for this hypothesis is provided by the observation that hepatic glycogen stores are markedly reduced in hyperthyroidism (Maracek & Feldman 1973) and do not increase after oral glucose (Mirsyky & Broh-Kahn 1936). The only study of insulin binding to liver membranes from thyrotoxic rats showed a 22% reduction in maximum binding, although small numbers prevented statistical significance from being achieved (DeRuyter et al. 1982).

In conclusion, normal insulin secretory responses but relative hyperglycaemia following oral glucose have been observed in hyperthyroidism. No abnormality of adipocyte insulin sensitivity could be detected in vitro but adipocyte insulin receptor number was reduced. These results suggest that thyroid hormones could play a role in the control of insulin receptor number, this being modulated in such a manner that cellular insulin sensitivity remains unchanged. The normal insulin sensitivity of adipocytes, together with the recent in vivo observation of normal peripheral tissue insulin sensitivity indicative that disturbed hepatic insulin responsiveness may play a major role in the glucose intolerance of hyperthyroidism.

**Acknowledgments**

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**References**


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