ABSTRACT

Increased lipid mobilization in thyrotoxicosis is attributed to amplification of catecholamine action in fat cells by thyroid hormones. We investigated the adrenergic regulation of lipolysis in isolated sc abdominal fat cells obtained from 14 patients with thyrotoxicosis and 18 control subjects. Ten of the hyperthyroid subjects were also reinvestigated after antithyroid treatment. The thyrotoxic state was associated with a 3-fold increase in maximum norepinephrine-induced lipolysis (P < 0.005), unaltered sensitivity to dobutamine (selective \(\beta_1\)-adrenoceptor agonist) and clonidine (selective \(\alpha_2\)-adrenoceptor agonist), but 15 times enhanced sensitivity to terbutaline (selective \(\beta_2\)-adrenoceptor agonist; \(P < 0.01\)). Moreover, thyrotoxicosis was accompanied by a 3-fold increase in \(\beta_2\)-adrenoceptor number (\(P < 0.005\)), but unchanged \(\beta_1\)-adrenoceptor levels. Further, the lipolytic effects of dibutyryl cAMP (activating protein kinase A and thereby hormone-sensitive lipase) and forskolin (activating adenylate cyclase) were about 60% enhanced (\(P < 0.005\)). No change in the maximum activity of the hormone-sensitive lipase could be demonstrated in the hyperthyroid state compared to that in the euthyroid state. The observed abnormalities in lipolysis and \(\beta_2\)-adrenoceptor number were normalized after antithyroid treatment. It is concluded that in human hyperthyroidism, the interactions between thyroid hormone and catecholamines in adipocytes involve abnormalities at both receptor and postreceptor levels. The former mechanism seems to be a selective increase in the expression of the \(\beta_2\)-adrenoceptors. The latter mechanism involves increased ability of cAMP to activate hormone-sensitive lipase, but not a change in maximum enzyme capacity. (J Clin Endocrinol Metab 82: 159–166, 1997)

THYROID HORMONES amplify the actions of catecholamines in numerous target tissues (1). In thyrotoxicosis, several signs and symptoms suggest increased adrenergic activity, which can be efficiently inhibited by \(\beta\)-adrenoceptor blocking drugs. Moreover, in hypothyroidism, a large number of changes are opposite those in hyperthyroidism (1), mimicking a low adrenergic activity state. The mechanisms responsible for this potentiation of catecholamine activity by thyroid hormones have mainly been investigated in rodents. Little is known about the interactions of these hormones at the cellular level in humans with naturally occurring hyperthyreosis.

In hyperthyroidism, excess thyroid hormones have marked metabolic effects, such as enhancement of oxygen consumption and thermogenesis as well as increased lipid mobilization; all factors that promote weight loss. The enhanced lipolytic response to catecholamines found in fat cells of hyperthyroid subjects has been attributed to an increased total number of \(\beta\)-adrenergic (i.e. \(\beta_1\) plus \(\beta_2\)) receptors (2) as well as decreased phosphodiesterase activity (3). Both changes are accompanied by increased levels of cAMP after catecholamine stimulation. Three \(\beta\)-adrenoceptor subtypes are expressed in human fat cells: the \(\beta_1\) - and \(\beta_2\)-receptors, which are dominant in sc fat cells, and the \(\beta_3\)-receptor, mainly found (together with \(\beta_1\) and \(\beta_2\)-receptors) in visceral fat cells (4). In addition, catecholamine-induced lipolysis can be modified by antilipolytic \(\alpha_2\)-adrenoceptors (5).

Earlier detailed studies in laboratory animals have shown that treatment with thyroid hormones results in unaltered (6, 7) as well as increased (8) numbers of \(\beta\)-adrenoceptors. The \(\beta_1\)-adrenoceptor, but not the \(\beta_2\)-adrenoceptor, subtype is sensitive to up-regulation in adipose tissue (7, 9) as well as in ventricular myocytes (10). The expression of the \(\beta_2\)-receptor seems to be regulated in a tissue-specific manner, as lack of thyroid hormone resulted in up-regulation of this receptor in brown fat and down-regulation in white fat (11). On a postreceptor level, thyroid hormones regulate the signal transduction between the receptors and adenylate cyclase (7, 12). The demonstrated mechanism for this event is modulation of the steady state levels of specific G protein subunits, in particular the activity and abundance of the inhibitory G proteins, which couple antilipolytic receptors (6, 13). However, great care has to be taken when these findings in laboratory animals are extended to thyroid disorders in man. Species and tissue differences in adrenoceptor equipment as well as the use of clinically irrelevant study designs are some reasons why animal studies may differ from findings in man.

When previous studies of human thyrotoxicosis are summarized, it is still unknown whether this state in man is attributed to up-regulation of a specific \(\beta\)-adrenoceptor subtype. Further, it is uncertain whether distal postreceptor changes near the hormone-sensitive lipase, catalyzing the rate-limiting step of the lipolysis, occur. The aim of this study was, therefore, to investigate the influence of thyroid hormones on the adrenergic regulation of lipolysis in abdominal
isolated sc adipocytes of hyperthyroid patients before and after antithyroid treatment, with special focus on the roles of β₁- and β₂-adrenoceptor subtypes and distal postreceptor events. Data from the hyperthyroid state were also compared to those from a group of sex-, age-, and body mass index-matched euthyroid controls.

**Materials and Methods**

All patients with the diagnosis of hyperthyroidism based on clinical symptoms and laboratory findings but no ongoing medication, such as β-blockers, who were referred to the endocrine out-patient ward of the hospital (~20/yr) were asked to participate in the study, which included a fat biopsy on 2 different occasions. Initially, 14 patients (12 women and 2 men), with Grave’s disease but otherwise healthy were included in the study and investigated before treatment. All had undetectable serum TSH levels and highly elevated concentrations of T₃ and free T₄ and a duration of disease of at least 3–6 months at the time of the first investigation (based on the history and laboratory findings provided by the referring physicians). The patients received either radioiodine or antithyroid drug treatment. Then the nonselective β₂-adrenoceptor-selective agonist (before) and the selective β₂-adrenoceptor agonist (after) were compared to those from a control group consisting of 18 age-, gender-, and body mass index-matched volunteers (14 women and 4 men), all healthy and taking no medication. Clinical data from all participants are presented in Table 1.

Of the 14 hyperthyroid patients, only 10 subjects (9 women and 1 man) maintained their willingness to be restudied after treatment. At the time of reinvestigation they had been euthyroid for 4–10 months (mean, 7 ± 0.7 months) according to clinical symptoms and laboratory findings. Written consent was obtained from all subjects, and the study was approved by the ethics committee of the Karolinska Institute.

The subjects were investigated in the morning after an overnight fast. Height and body weight were measured. Venous blood samples were obtained for the determination of plasma catecholamine and thyroid hormone levels by the hospital’s routine chemistry laboratory. A sc fat biopsy (0.5–2 g) was obtained under local anesthesia from the abdominal region randomly to the right or the left of the umbilicus. The local anesthesia was given in a way that did not influence the function of the removed adipose tissue (14). It was not possible to remove larger pieces of adipose tissue because, first, all subjects were thin and, second, the tissue was hyperaemic in the hyperthyroid state.

**Isolation of fat cells and lipolysis experiments**

A piece of adipose tissue (~150 mg) was first removed and immediately frozen in liquid nitrogen for later analysis of hormone-sensitive lipase. Then, fat cells were isolated from the stroma by collagenase digestion according to the method of Rodbell (15). Fat cells were carefully washed three times in Krebs-Ringer phosphate buffer (pH 7.4) and filtered twice through a silk cloth to remove traces of stroma and broken fat cells. Fat cell diameter, weight, and volume as well as number of fat cells incubated were determined as previously described (16).

The lipolysis assay, performed in all subjects, has previously been described in detail (17). Briefly, a diluted suspension of fat cells (~5,000–10,000 cells/mL) was incubated for 2 h in duplicate samples with air as the gas phase at 37°C in Krebs-Ringer phosphate buffer (pH 7.4) supplemented with glucose (1 mg/mL), ascorbic acid (0.1 mg/mL), and BSA (20 mg/mL) in the absence (basal) or presence of increasing concentrations of different lipolysis agents. These were the natural catecholamine norepinephrine, which acts on all adrenoceptors present in human fat cells, i.e. β₂- and β₁- and β₂-adrenoceptors; the β₂-adrenoceptor-selective agonist terbutaline; the β₂-adrenoceptor-selective agonist, dobutamine; the adenylate cyclase-stimulating agent, forskolin; and the phosphodiesterase resistant CAMP analog, dibutyryl cAMP (dcAMP), stimulating the protein kinase A-/hormone-sensitive lipase complex and the selective β₂-adrenoceptor agonist clonidine.

In the experiments with clonidine, the incubation buffer was supplemented with adenosine deaminase (1 U/mL) to eliminate all traces of adenosine, as this substance may interfere with the antilipolytic effects that are mediated through β₂-adrenoergic receptors. In a diluted incubation system, there is only minimal leakage of adenosine, which does not influence the action of the presently used lipolysis-stimulating drugs (17).

After incubation, an aliquot of the medium was removed, and glycerol, which was used as a measurement of the lipolysis rate, was analyzed using a bioluminescence method (18). The concentration-response curves showing glycerol release were analyzed for all lipolysis agents. A plateau was reached with each agent at the highest drug concentrations in all experiments. The maximum rate of glycerol release was calculated at the maximum effective concentration of the different agents (i.e. responsiveness). These values (with the basal lipolysis subtracted) for norepinephrine, dcAMP, and forskolin represent the maximum activation of lipolysis at the levels of all β-receptors, adenylate cyclase, and the protein kinase A-/hormone-sensitive lipase complex, respectively, as discussed in detail previously (16, 19). The responsiveness was also calculated for the selective adrenergic agonists dobutamine, terbutaline, and clonidine. However, for these agents, the main focus was made to evaluate adrenergic subtype sensitivity, as expressed by half-maximum effective concentration (EC₅₀). The EC₅₀ was determined by log-log transformation and linear regression analysis of the dose-response curves, as previously described (20). At least six different concentrations of the selective adrenergic agonists were used, which covered the full concentration-response relationship in each experiment. The lipolysis rates were related to the number of incubated cells and expressed per cell number. Glycerol release is linear with time for at least 3 h in the absence or presence of lipolytic agents when abdominal sc fat cells are incubated under the present conditions (21).

**Radioligand binding**

The receptor binding studies were performed on isolated fat cells as described in detail previously (16, 20). This assay was performed in all 18 controls, because of lack of adipose tissue, it could only be performed in 10 of the entire group of 14 hyperthyroid subjects and in 8 of the subset of 10 hyperthyroid patients who were reinvestigated after antithyroid drug treatment. The nonselective β₁- and β₂-adrenergic antagonist [¹²⁵I]cyanopindolol was used in saturation and displacement experiments. Fat cells were incubated for 60 min in a concentration of

**TABLE 1.** Clinical characteristics and laboratory findings in controls and hyperthyroid patients before and after antithyroid treatment

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Control (n = 18)</th>
<th>All (n = 14)</th>
<th>Hyperthyroid patients</th>
<th>Before treatment (n = 10)</th>
<th>After treatment (n = 10)</th>
<th>P (before vs. after)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>22.5 ± 0.6</td>
<td>22.9 ± 1.6</td>
<td>NS</td>
<td>22.1 ± 1.9</td>
<td>24.0 ± 1.9</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Fat cell vol (pl)</td>
<td>524 ± 41</td>
<td>536 ± 39</td>
<td>NS</td>
<td>570 ± 49</td>
<td>654 ± 41</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>S-TSH (0.4–4.0 mU/L)</td>
<td>1.3 ± 0.3</td>
<td>1.0 ± 0.1</td>
<td>&lt;0.001</td>
<td>6.7 ± 0.8</td>
<td>1.7 ± 0.3</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>S-T₃ (15–65 pmol/L)</td>
<td>55.4 ± 6.7</td>
<td>61.8 ± 1.5</td>
<td>&lt;0.01</td>
<td>1.6 ± 0.2</td>
<td>1.2 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>P-Norepinephrine (0.7–2.3 nmol/L)</td>
<td>0.24 ± 0.03</td>
<td>0.24 ± 0.05</td>
<td>NS</td>
<td>0.22 ± 0.06</td>
<td>0.17 ± 0.03</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are the mean ± SE. Data were compared using Student’s unpaired t test for controls vs. all hyperthyroid patients and Student’s paired t test for hyperthyroid patients before vs. after antithyroid treatment.
about 20,000 cells/mL at 37°C in a buffer composed as described above, except that the albumin content was 5 g/L. In the saturation experiments the fat cells were incubated with 10, 50, 100, 250, 500, and 750 pmol/mL [125I]yanopindolol. Nonspecific binding (30–40%) was determined by the addition of propranolol (0.1 μmol/L) in parallel incubations. The cell-bound and free radioactivities were separated by the addition of ice-cold saline and vacuum-filtering through Whatman GF/C filters (Whatman, Clifton, NJ) with a pore size of 1 μm, which retains intact fat cells but not small cell fragments on the filter. The total maximum binding capacity and ligand affinity were determined by linear regression analysis of Scatchard plots (22). In the displacement experiments, [125I]yanopindolol binding (100 pmol/mL) competed with the highly selective β2-adrenoceptor antagonist ICI 118,551 in 12 increasing concentrations (10−11-10−4 mol/L). Nonspecific binding (30–30%) was defined as the binding not displaced by 10−4 mol/L ICI 118,551 and was not different from nonspecific binding determined using 0.1 μmol/L propranolol.

As the displacing ligand ICI 118,551 binds to β2-receptors with high affinity and to β1-receptors with low affinity, the displacement experiments gave shallow biphasic curves. A nonlinear least squares regression analysis of the displacement curves was performed (23). From the best-fitted two binding sites, it is possible to determine the binding affinity for the displacing ligand, respectively, as well as the binding affinities of ICI 118,551 to these two sites. The results of the saturation and displacement experiments taken together provide an estimate of the total binding capacity of β2- and β1-receptor binding sites, respectively, for each subject, which represents the β-receptor subtype number. As discussed in detail previously (16, 19), β1- and β2-adrenergic receptor binding cannot be detected under the present experimental conditions.

Assay of hormone-sensitive lipase activity

This assay was performed in 10 control subjects, 11 thyrotoxic patients, and 10 subjects reinvestigated after antithyroid therapy. It was conducted exactly as described previously (21). In brief, adipose tissue that had been stored in liquid nitrogen was homogenized at 4°C in 0.25 mol/L sucrose, 1 mmol/L ethylenediamine tetraacetate, 1 mmol/L di-thiothreitol, and 20 mmol/L each of the protease inhibitors antipain and leupeptin. The homogenate was centrifuged at 100,000 × g, and the fat-free infranatant was recovered for analysis of maximum hormone-sensitive lipase activity using the diacylglycerol analog 1(3)Holeoyl-2-0-oleylglycerol as substrate (24). The extract was obtained from the same source of production (Department of Medical and Physiological Chemistry, Lund University, Lund, Sweden) as in the original methodological studies (24, 25). The substrate for hormone-sensitive lipase has only one hydrolyzable ester bond at the I(3) position. Therefore, the substrate and its hydrolysis products were not hydrolyzed by monoacylglycerol lipase, which is abundant in adipose tissue. Furthermore, under the present incubation conditions (pH 7.0 and no apolipoprotein CII present), lipoprotein lipase activity was negligible (24). As the phosphorylated and dephosphorylated forms of the enzyme have the same activity toward the substrate, only the total amount of activatable enzyme in the sample was measured (25). Hormone-sensitive lipase hydrolyzes tri- and diacylglycerols at a relative ratio of 1:10 (24). Therefore, the sensitivity of the assay was enhanced by the use of a diacylglycerol analog as substrate. All samples were incubated at 37°C for 30 min on one occasion. One unit of enzyme activity equals 1 nmol fatty acid produced/min at 37°C. Enzyme activity was related to the total protein concentration of the sample, which was measured using a commercial protein assay kit (BCA, Pierce Chemical Co., Rockford, IL). The enzyme activity and total protein concentration of the fat free infranatant were proportional to the wet weight of the tissue specimen when multiple samples from one subject were analyzed (r = 0.95; P < 0.005). The within-run coefficient of variation was 7%. The intrasubject coefficient of variation, examined by multiple samples of adipose tissue from one subject, was 11%.

Statistical analysis

Data are presented as the mean ± se. The data were compared using Student’s paired and unpaired t tests and linear regression analysis.

Results

The clinical characteristics of all participants are presented in Table 1. All thyrotoxic patients reported weight reduction before treatment; the mean decrease in body weight was about 5 ± 2 kg, as judged by the patient’s own history. After treatment, there was a significant rise in mean body weight by about 6 kg (P < 0.005, by paired t test); in other words, the patients had then approximately returned to their usual weight. After treatment, there was also a significant increase in fat cell volume, of about 13% (P < 0.05). Thyroid status did not influence the levels of circulating catecholamines, which is in accordance with the results of earlier studies (1).

Marked differences in lipolysis were found between hyper-thyroid and euthyroid subjects. When lipolysis was stimulated by the naturally occurring catecholamine norepinephrine as well as by the postreceptor-acting drugs forskolin and dcAMP, there was a pronounced increase in the rate of lipolysis in the thyrotoxic patients compared to that in the healthy controls (Fig. 1). These rates were normalized and almost identical to those in the control group after antithyroid therapy (Fig. 2).

The maximum response for these agents, i.e., maximum glycerol value with the basal rate subtracted, was 2- to 3-fold higher in the hyperthyroid compared to the control group, whereas no significant difference in the basal lipolysis rate, i.e., without lipolytic agents present in the incubation medium, was recorded between the groups (Table 2). Antithyroid therapy almost normalized responsiveness. As shown in Table 2, lipolysis induced by dcAMP and forskolin was slightly higher in hyperthyroid subjects after treatment compared to that in controls. However, when values from the latter groups were compared using unpaired t test, no significant difference was found.

Adrenoceptor subtype function was also evaluated. The maximal lipolytic response (micromoles of glycerol per 107 cells/2 h) at the maximum effective concentration in the hyperthyroid state (n = 14) was 30.3 ± 3.1 for terbutaline and 29.0 ± 2.7 for dobutamine. The corresponding values for controls were 16.0 ± 1.7 for terbutaline and 16.6 ± 1.9 for dobutamine. These values in the controls differed significantly from those in the hyperthyroid subjects (P < 0.0005).

Antithyroid therapy reduced responsiveness for terbutaline and dobutamine to values similar to those in the control group (data not shown). The maximum inhibiting effect of clonidine in the hyperthyroid state was 1.4 ± 0.2; it was 1.8 ± 0.3 for controls and 1.7 ± 0.3 for antithyroid drug-treated patients. These values did not differ in a significant way. However, the principal aim of the use of selective agonists was to investigate adrenoceptor subtype sensitivity. Human fat cells contain sparse β1- and δ2-adrenoceptors. Therefore, an increase in receptor number is accompanied only by an increase in hormone sensitivity, whereas an increased maximum lipolytic response is mainly due to alterations at the postreceptor level. The evaluation of these concentration-response curves was, therefore, expressed as a percentage of the maximum lipolysis to enlighten right- or leftward shifts in the curve, which reflects receptor sensitivity, as discussed in detail previously (27). The concentration-response curves for dobutamine and clonidine were almost superimposed when control and hyperthyroid subjects were compared (Fig. 3), and they were not influenced by antithyroid...
FIG. 1. Mean concentration-response curves for norepinephrine (upper graph) and the postreceptor acting drugs, forskolin (middle graph) and dcAMP (lower graph). The lipolytic effect was determined in fat cells obtained from 14 thyrotoxic patients (filled symbols) and 18 healthy controls (open symbols) and expressed as glycerol release per cell number. The different drug concentrations used in the experiment are depicted in the graph. Values are the mean ± SE.

FIG. 2. Lipolysis induced by norepinephrine (upper graph), forskolin (middle graph), and dcAMP (lower graph) in 10 hyperthyroid patients before (filled symbols) and after (open symbols) antithyroid treatment. See Fig. 1 for additional details.
The individual EC$_{50}$ values were calculated for each concentration-response curve with selective agonists. The mean EC$_{50}$ values for dobutamine and clonidine did not differ between hyperthyroid and control subjects and were not influenced by antithyroid therapy (Table 3). As also shown in Table 3, the EC$_{50}$ for terbutaline was 1.2 log unit lower in hyperthyroid than control subjects, which represents a more than 15-fold difference in sensitivity. The EC$_{50}$ for terbutaline was normalized after antithyroid therapy, whereas the effect of clonidine was not affected by thyroid status, with no observed changes in EC$_{50}$ values (Table 3) and an inhibition of basal lipolysis of about 70% (Figs. 3 and 4).

To further characterize the mechanisms underlying the differences in agonist sensitivity, radioligand competition-inhibition experiments were performed. As shown in Table 3, there was a significant up-regulation of the b$_2$-receptors in thyrotoxicosis. The maximum radioligand binding capacity for this receptor in the thyrotoxic state was more than 300% higher before compared to after treatment and compared to healthy controls (P < 0.005 or less by paired and unpaired t tests). A 30–40% increase in the number of b$_2$-adrenoceptors occurred during the disease, but this difference was not statistically different. b$_1$- and b$_2$-adrenoceptor subtype affinities were not altered by hyperthyroidism (data not shown). All Scatchard curves obtained before and after treatment were linear, and there was no difference in [125I]cyanopindolol binding affinity between the investigations (data not shown).

To further characterize postreceptor mechanisms, measurements of hormone-sensitive lipase activity were performed. The enzyme activity, expressed as milliunits per mg protein, was 1.76 ± 0.25 in the entire group of thyrotoxic patients before treatment and 1.47 ± 0.22 in the control group (P = 0.4, by unpaired t test). In the group of thyrotoxic subjects who were investigated twice, the values were 1.84 ± 0.27 before and 1.46 ± 0.29 after (P = 0.11, by paired t test) treatment, respectively.

The possible influence of changes in body weight and fat cell size on the changes in lipolysis were investigated. The net differences in lipolytic responsiveness, b$_2$-adrenoceptor binding and terbutaline EC$_{50}$ dependent variables, and fat cell size as well as body weight (independent variables) were calculated. Independent and dependent variables were compared by linear regression analysis. No significant relationship between these variables was obtained (r = −0.2 to 0.2).

Discussion

The findings in this study demonstrate that the adrenergic regulation of lipolysis in human thyrotoxicosis occurs at both receptor and postreceptor levels. The data also suggest for the first time that in the hyperthyroid state in man, the b$_2$-adrenoceptor number is increased by a selective up-regulation of the b$_2$-receptor, and that postreceptor changes occur that are located distal in the lipolytic chain. These alterations, which were marked, appear not to be secondary to the small changes in body weight and fat cell size.

The present results are different from what has been previously observed in studies of experimental hyperthyroidism in laboratory animals. When all animal experiments are considered together, it appears that the interactions between thyroid hormones and catecholamine-induced lipolysis are located at the b$_1$- and b$_2$-adrenoceptors, adenylate cyclase, and G proteins (6–13, 28).

Because of the severity of the symptoms associated with naturally occurring human thyrotoxicosis, detailed studies on lipolysis in this condition are often not feasible. Little is known, therefore, about the adrenergic regulation of lipolysis in human hyperthyroidism. However, an increase in the total number of b$_2$-adrenoceptors (b$_2$ plus b$_3$) (2, 29), unaltered function of the a$_2$-receptor (29), and enhancement of the lipolytic response to catecholamines (30) have been demonstrated in gluteal fat. Human hyperthyroidism has also been associated with increased activity of the phosphodiesterase enzyme (3), which may elevate cAMP and thereby activate lipolysis at the postreceptor level.

The present findings of the increased ability of norepinephrine to stimulate lipolysis in fat cells in untreated hyperthyroidism are in accordance with earlier observations. However, divergent from previous studies in thyrotoxic animals (7, 9, 10), we here demonstrate a selective enhancement of the b$_2$-adrenoceptor subtype function. This was indicated by a more than 15-fold increase in sensitivity for the b$_2$-receptor agonist terbutaline and was further confirmed by radioligand binding, which revealed a 3-fold increase in b$_2$-adrenoceptor number in the hyperthyroid compared to the euthyroid state. The b$_1$-adrenoceptor, whether measured with radioligand binding or functionally with EC$_{50}$ for dobutamine, was not significantly altered by the hyperthyroid state. The present data clearly show that the increased lipolytic responsiveness induced by norepinephrine as well as the enhanced b$_2$-adrenoceptor subtype function observed in the hyperthyroid state decrease after treatment to levels al-
To illustrate sensitivity to agonist action, data are expressed as a percentage of glycerol release at the maximum effective agonist concentration with basal lipolysis subtracted. The EC\textsubscript{50} concentration is indicated with a line. The antilipolytic effect of clonidine is shown as the percent inhibition of basal lipolysis.
LIPOLYSIS IN HYPERTHYROIDISM

TABLE 3. β-Adrenoceptor status in controls and hyperthyroid patients before and after antithyroid treatment

<table>
<thead>
<tr>
<th></th>
<th>ED50 (log mol/L)</th>
<th>Radioligand binding capacity (pmol/10^7 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dobutamine</td>
<td>Terbutaline</td>
</tr>
<tr>
<td>All hyperthyroid subjects (n = 14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (n = 18)</td>
<td>-7.78 ± 0.14</td>
<td>-8.61 ± 0.34</td>
</tr>
<tr>
<td>P (all vs. controls)</td>
<td>NS</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Before treatment (n = 10)</td>
<td>-7.66 ± 0.18</td>
<td>-8.25 ± 0.37</td>
</tr>
<tr>
<td>After treatment (n = 10)</td>
<td>-7.29 ± 0.12</td>
<td>-7.45 ± 0.21</td>
</tr>
<tr>
<td>P (before vs. after)</td>
<td>NS</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are the mean ± SE. They were compared using Student’s unpaired t test for controls vs. all hyperthyroid patients and Student’s paired t test for hyperthyroid patients before vs. after antithyroid treatment.

most identical to those observed in the control group. Lipolysis induced by postreceptor-acting drugs was somewhat greater in the hyperthyroid patients after treatment compared to controls; however, the values did not significantly differ. One explanation for these slightly higher values in hyperthyroid subjects an average of 7 months after treatment might be that postreceptor pathways are not yet normalized, but they might also be explained by the small number of subjects compared in this group. In healthy individuals, lipolytic parameters are stable in longitudinal experiments, as discussed in detail previously (17, 31). Taken together, these findings strongly indicate that the observed abnormalities in adrenergic regulation in hyperthyreosis are due to the disease itself and do not reflect spontaneous changes.

The enhanced β3-adrenoceptor expression could only in part explain the increased norepinephrine function. The augmented maximal lipolytic response to norepinephrine mirrors changes beyond as well as at or near the adrenoceptor level. A postreceptor alteration could be located at any step in the lipolytic cascade beyond receptor binding. In experiments with drugs acting at selective postreceptor levels, forskolin as well as cAMP significantly increased the maximal glycerol response in the hyperthyroid state. The latter drugs enhanced lipolysis to the same extent, and as cAMP is resistant to the phosphodiesterase enzyme, the data indirectly indicate that the most important changes in the post-receptor events probably not are located at the level of the phosphodiesterase enzyme as indicated by previous studies (3), but presumably reside in the lipolytic chain near the level of the hormone-sensitive lipase. They do not, however, seem to be due to variations in the maximum enzyme capacity of the hormone-sensitive lipase, as no differences could be demonstrated between the hyperthyroid and the euthyroid state in this respect. The regulation of hormone-sensitive lipase activity is not known in detail. The present assay used only measures the total amount of the enzyme and cannot separate the phosphorylated, active form from the dephosphorylated, inactive form (25). The discrepancies between lipolysis and enzyme data in the thyrotoxic state could be due to increased phosphorylation of the enzyme, i.e., changes could be located at the level of the cAMP-dependent protein kinase A. They could also be explained by other enzymes involved in the phosphorylation-dephosphorylation reactions of the hormone-sensitive lipase, which were not able to be measured here.

The existence of postreceptor as well as receptor alterations in thyrotoxicosis is further strengthened by comparing maximum lipolytic responses in Table 2. For norepinephrine, acting at the initial step in the lipolytic cascade (i.e., receptors), there was a 300% increase in the effect in the hyperthyroid state. For forskolin and cAMP (acting below receptors), the corresponding augmentation was only about 30–60%. In an earlier study in gluteal fat in humans, cAMP exhibited similar effects in hyperthyroid and euthyroid subjects (2). One explanation for the differences in results might be that gluteal fat is far less lipolytic active than abdominal fat, which was used in the present study, as previously discussed (17).

The antilipolytic effect of clonidine was similar before as well as after antithyroid treatment. This normal function of the inhibitory α2-receptor pathway in the hyperthyroid state indicates an absence of changes at the level of the inhibitory component of the G protein (i.e., Gi). This indirect finding is in contrast to the results of earlier studies in rodents (13), but supports previous investigations in humans (29, 32).

One question is whether these in vitro data also have impact in vivo. Ligget et al. (33) found unchanged β-adrenoceptor-mediated responsiveness to epinephrine in vitro in experimental thyrotoxicosis induced in healthy volunteers. However, they also observed no alterations in isoprenaline-stimulated cAMP production in vitro in gluteal adipocytes despite a 60% increase in the total number of β-adrenoceptors. The discrepancy between this earlier study and the present one might be due to different study designs. In the study by Ligget et al., gluteal fat was investigated, and normal humans were studied before and during short term experimental thyrotoxicosis, inducing elevated T3 levels but decreased serum T4 levels. Our patients had a duration of thyrotoxic disease of 3–6 months, with markedly elevated serum levels of both T3 and T4. Furthermore, subjects with natural hyperthyroidism may differ in other important ways from those with experimental thyrotoxicosis. The clinical importance of our in vitro findings can, thus, only be determined by in vitro lipolysis studies in patients with established thyrotoxicosis. For ethical reasons, infusing catecholamines in naturally occurring thyrotoxicosis to study in vivo sensitivity was not possible. We initially tried to evaluate in vivo lipolysis during physical exercise on a bicycle. However, these studies were not continued because of difficulties in achieving a sufficient work load in most of the patients. Alternatively, it might be possible to study lipolysis by microdialysis; unfortunately, this method is not yet quantitative.

In this study, it was only possible to study adipose tissue from the sc abdominal region. One question is whether the present results also apply to other regional fat depots. As discussed above, gluteal fat seems to be metabolically less active in the hyperthyroid state. We cannot determine from
the present results whether the well known differences in lipolytic response to catecholamines between sc and visceral fat are even more augmented in thyrotoxicosis. Unfortunately, visceral fat is not available for the present type of experiments for ethical reasons. With regard to lipolytic sensitivity, an earlier study demonstrated a strong relationship between β2-adrenergocceptor sensitivities in sc and omental fat (17). Taken together, regional variation in lipolysis regulation during hyperthyroidism cannot be excluded; however, concerning the overall fuel homeostasis, sc fat is of major interest, as it constitutes about 80% of the total fat depot in man. Another important question is the possible occurrence of changes in the expression of the β3-adrenergocceptor in human thyrotoxicosis. Little is known about the regulation of this receptor during thyroid hormone excess. Unfortunately, when the present study was initiated about 5 yr ago, no appropriate β3-adrenergocceptor drugs were available.

It is of interest to compare the present findings regarding the β3-adrenergocceptor with those of other studies in our laboratory using identical methods. Lipolytic catecholamine resistance, with corresponding variations in β2-adrenergocceptor expression and function, but no change in the β1-adrenergocceptor, has been demonstrated in abdominal sc adipocytes of apparently normal subjects, in obese women, and in men with the so-called insulin resistance syndrome (17, 16, 19). When considered together, the present and earlier data strongly indicate that catecholamine action at the receptor level in human sc abdominal fat cells is mainly regulated by the β3-adrenergocceptor subtype.

Thyrotoxicosis is a catabolic state, like, for example, starvation. The latter condition is also accompanied by increased catecholamine-induced lipolysis. However, the most prominent finding with adipocytes obtained during long term fasting is an increase in basal lipolysis (20), which, however, was not influenced by thyrotoxicosis.

In summary, we demonstrated that adrenergic regulation of lipolysis in hyperthyroidism in man is associated with alterations at both receptor and distal postreceptor levels. The former is indicated by a selective up-regulation of the β3-adrenergocceptor, and the latter mechanism involves an increased ability of cAMP to activate hormone-sensitive lipase, but not a change in the maximum enzyme capacity.

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References