The atrial natriuretic peptide- and catecholamine-induced lipolysis and expression of related genes in adipose tissue in hypothyroid and hyperthyroid patients

J. Polak, C. Moro, E. Klimcakova, M. Kovacikova, M. Bajzova, M. Vitkova, Z. Kovacova, R. Sotornik, M. Berlan, N. Viguerie, D. Langin, and V. Stich

1Franco-Czech Laboratory for Clinical Research on Obesity, Third Faculty of Medicine, Charles University, Prague, Czech Republic; 2Inserm, Unité de Recherches sur les Obésités; 3Université Paul Sabatier, Institut Louis Bugnard; 4Centre Hospitalier Universitaire de Toulouse, Toulouse, France; 5Second Internal Medicine Department of Kralovske Vinohrady Teaching Hospital, Prague, Czech Republic; and 6Center of Biomedical Sciences, Division of Cell and Molecular Biology, Third Faculty of Medicine, Charles University, Prague, Czech Republic

Submitted 16 December 2006; accepted in final form 26 March 2007

Polak J, Moro C, Klimcakova E, Kovacikova M, Bajzova M, Vitkova M, Kovacova Z, Sotornik R, Berlan M, Viguerie N, Langin D, Stich V. The atrial natriuretic peptide- and catecholamine-induced lipolysis and expression of related genes in adipose tissue in hypothyroid and hyperthyroid patients. Am J Physiol Endocrinol Metab 293: E246–E251, 2007. First published March 27, 2007; doi:10.1152/ajpendo.00688.2006.—Thyroid dysfunction is associated with several abnormalities in intermediary metabolism, including impairment of lipolytic response to catecholamines in subcutaneous abdominal adipose tissue (SCAAT). Atrial natriuretic peptide (ANP) is a powerful lipolytic peptide; however, the role of ANP-mediated lipolysis in thyroid disease has not been elucidated. The aim of this study was to investigate the role of thyroid hormones in the regulation of ANP-induced lipolysis as well as in the gene expression of hormone-sensitive lipase, phosphodiesterase 3B (PDE3B), uncoupling protein-2 (UCP2), natriuretic peptide receptor type A, and β2-adrenergic receptor in SCAAT of hyperthyroid and hypothyroid patients. Gene expression in SCAAT was studied in 13 hypothyroid and 11 hyperthyroid age-matched women before and 2–4 mo after the normalization of their thyroid status. A microdialysis study was performed on a subset of nine hyperthyroid and 10 hypothyroid subjects. ANP- and isoprenaline-induced lipolyses were higher in hyperthyroid subjects, with no differences between the groups following treatment. Hormone-sensitive lipase gene expression was higher in hyperthyroid compared with hypothyroid subjects before treatment, whereas no difference was observed following treatment. No differences in gene expression of other genes were observed between the two groups. Following treatment, the gene expression of UCP2 decreased in hyperthyroid, whereas the expression of PDE3B decreased in hypothyroid subjects. We conclude that thyroid hormones regulate ANP- and isoprenaline-mediated lipolysis in human SCAAT in vivo. Increased lipolytic subcutaneous adipose tissue response in hyperthyroid patients may involve postreceptor signaling mechanisms.

Adrenergic lipolysis; thyroid disease; gene expression

Pathological thyroid hormone levels in hyperthyroid or hypothyroid patients have been associated with profound pathophysiological changes in resting energy expenditure (REE), lipid and glucose metabolism, and body composition. Dysregulations in basal and catecholamine-induced lipolysis in patients with thyroid dysfunction have been described in vitro and in vivo (10, 22, 29, 30). The hyperthyroid state has been documented to be accompanied by an increased lipolytic response to catecholamine stimulation connected with an increased number and density of lipolytic β2-adrenergoreceptors in vitro (10, 21, 30), but the influence of thyroid hormones on the density and function of antilipolytic α2-adrenergoreceptors in subcutaneous abdominal adipose tissue (SCAAT) is less evident (21). A decreased sensitivity to insulin antilipolytic effect was also documented in hyperthyroid state (7). No differences in β- or α2-receptor number were observed in hypothyroid subjects (15, 29). Major abnormalities in hyperthyroid subjects have also been detected in the responsiveness (maximal effect) of isolated adipocytes to adrenergic lipolysis (1, 29, 30). In the hyperthyroid state β-adrenergoreceptor responsiveness is decreased, and an impairment of phosphodiesterase 3B (PDE3B), adenylyl-cyclase, or protein kinase has been suggested to be involved in the dysregulation of adrenergic lipolysis in hypo- and hyperthyroidisms (10, 30). These results derive from in vitro experiments. The activity of the of PDE3B, a key cellular regulator of insulin’s antilipolytic action (3), has been found to be increased in hypothyroid patients and decreased in hyperthyroid subjects with a normalization of PDE3B activity after hyperthyroidism treatment (5). The downregulation of phosphodiesterase activity contributes to increased intracellular cyclic adenosine monophosphate levels and facilitates lipolysis (4). In animal experiments (rat), thyroid hormone exposure blunted antilipolytic effect of insulin in vitro (7). In human adipose tissue culture, triiodothyronine treatment led to a 1.6-fold downregulation of PDE3B mRNA (28).

A number of genes related to regulation of lipolysis and energy expenditure have been reported to be downregulated by thyroid hormones (including PDE3B, α2-adrenergic receptor, and G protein α2-subunit), whereas others are upregulated (β2-adrenergic receptors) in human subcutaneous adipose tissue (11, 27, 28). The dysregulation of REE is a common clinical finding in subjects with abnormal thyroid hormone levels (25). Among the possible mediators of REE impairment, uncoupling protein-2 and -3 attract interest, since their gene expression has been shown to be influenced by thyroid hormone levels (16).
Recently, a new lipolytic pathway involving atrial natriuretic peptide (ANP) has been identified specifically in humans. ANP is a powerful lipolytic agent on isolated adipocytes (24) as well as when administered intravenously (8) or in situ into SCAAT (19). The physiological effect of ANP is mediated by a membrane-bound natriuretic peptide receptor type A (NPR-A) with guanylyl cyclase activity (24) and is independent of adrenergic and insulin pathways. The potential role of thyroid hormones on ANP-mediated lipolysis has not yet been assessed in humans.

The purpose of this study was to investigate in situ modifications in SCAAT responsiveness to ANP or isoprenaline stimulation of lipolysis in hypothyroid and hyperthyroid patients before and after normalization of their thyroid status following treatment. Simultaneously, changes in the expression of genes involved in these lipolytic pathways were studied to provide some mechanistic explanations.

METHODS

Subjects. Thirteen hypothyroid subjects and 11 age-matched hyperthyroid women aged 52.3 ± 13.6 yr were recruited through referral from cooperating endocrinologists. A microdialysis study was performed on a subset of nine hyperthyroid and 10 hypothyroid subjects. Subjects were diagnosed as hyperthyroid or hypothyroid on the basis of clinical findings and plasma thyroid stimulating hormone (TSH) and free thyroxine (fT4) levels (normal values of TSH 0.25–5.0 mIU/l, fT4 0.77–2.0 ng/dl). All patients were newly diagnosed as having thyroid dysfunction. At the time of diagnosis they were not taking any medication that could interfere with the methodology of the study (e.g., β-adrenoceptor blocking agents). All patients gave their written informed consent before the study began. All aspects of the study were performed according to the Declaration of Helsinki and approved by the Ethics Committee of the Third Faculty of Medicine, Charles University (Prague, Czech Republic).

Design of the study. The subjects were examined prior to treatment (consisting of thyreostatic drugs or thyroid hormone replacement therapy, depending on the diagnosis), a few days after the diagnosis was carried out, and again once the euthyroid state was normalized with treatment (consisting of thyreostatic drugs or thyroid hormone replacement therapy, depending on the diagnosis). Two days after this investigation, at 9 AM, a needle biopsy (200–300 mg) of SCAAT was performed under local anaesthesia at a distance of 10–15 cm to the left of the umbilicus for mRNA evaluation (26).

Real-time RT-PCR. Following chloroform lipid extraction of adipose tissue biopsies, total RNA was extracted using the Qiagen RNaseasy kit and stored at −80°C until analysis. cDNA concentrations were determined using a fluorimetric assay (Ribogreen; Fluoroskan Ascent). Reverse transcription was performed using 1 μg of total RNA, Thermoscript reverse transcriptase (Invitrogen), and random hexamers as recommended by the manufacturer. Real-time quantitative RT-PCR was performed on GeneAmp 5700 Sequence Detection system using SYBR Green chemistry (Applied Biosystems, Courtaboeuf, France). A set of primers was designed for each gene using the software Primer Express 1.5 (Applied Biosystems). Ten nanograms of cDNA were used as template for real-time PCR in duplicate. A dissociation curve was generated at the end of the PCR cycles to verify that a single gene product was amplified. For each primer pair, a standard curve was obtained using serial dilutions of human adipose tissue cDNA. Messenger RNA levels were assessed using the following primers: for β2-AR, 5'-cgcgaagtcgctgaactg-3' and 5'-cagcgcgtgttgtagaatgaa-3'; for PDE3B, 5'-gacttgttgcatttgaaatggac-3' and 5'-atgacgcaagacagatgtgc-3'; for hormone-sensitive lipase, 5'-ttgctcaaacggaagggatca-3' and 5'-gaagctggctgtggtgcctct-3'; for uncoupling protein-2, 5'-cagcgaatctggtgtgct-3' and 5'-ccctgggtctgtagaatgtgca-3'; for NPR-A (ANP receptor), 5'-tcgacaaggcagtcactca-3' and 5'-gtcagctggctgtggtgcctct-3'; for adrenergic receptor, 5'-gacgtctcggagtttcccctcag-3' and 5'-ccatatcccagagggagaagtct-3'; for uncoupling protein-2, 5'-cagcgaatctggtgtgct-3' and 5'-ccctgggtctgtagaatgtgca-3'; for NPR-A (ANP receptor), 5'-tcgacaaggcagtcactca-3' and 5'-gtcagctggctgtggtgcctct-3'; for adrenergic receptor, 5'-gacgtctcggagtttcccctcag-3' and 5'-ccatatcccagagggagaagtct-3'; and for uncoupling protein-2, 5'-cagcgaatctggtgtgct-3' and 5'-ccctgggtctgtagaatgtgca-3'. All measurements were made using SYBR Green. We used 18S ribosomal RNA as control to normalize gene expression using the Ribosomal RNA Control TaqMan Assay kit (Applied Biosystems).

Drugs and analytical methods. Glycerol in dialysate (10 μl) and in plasma (20 μl) was analyzed with an ultrasensitive radiometric method (17). Plasma glucose was determined with a glucose-oxidase technique (Biotrol kit; Merck-Clevenot, Nogent-s-Marne, France) [coefficient of variation (CV) 1.1–2.0%] and nonesterified fatty acids by an enzymatic procedure (Wako kit; Unipath, Dardilly, France) (CV 1.1–2.0%). Plasma cortisol concentrations were determined using RIA kits from Sanofi Diagnostics Pasteur (Marnes la Coquette, France) (CV 2.7%). Plasma insulin concentrations were measured using RIA kits from Sanofi Diagnostics Pasteur (Marnes la Coquette, France) (CV 2.8–4.5%). Plasma epinephrine and norepinephrine were assayed in 1-ml aliquots of plasma by high-pressure liquid chromatography using electrochemical (amperometric) detection. The above-mentioned measurements were done in duplicate.

Statistical analysis. Data are presented as means ± SD. All statistical analyses were performed using SPSS 12.0 for Windows (SPSS, Chicago, IL). Differences in the mean plasma values and gene expression between the two groups (hyperthyroid and hypothyroid) were explored using the Mann-Whitney nonparametric test, whereas the impact of treatment was explored by the Wilcoxon Signed Rank Test. Lipolytic response to pharmacological stimulation was assessed by evaluating the incremental area under curve (AUC) of the concentration of glycerol in dialysate over a 60-min stimulation period (calculated as the area under the curve above the basal values using the linear trapezoidal rule). Differences in the lipolytic responses between the two groups and the impact of treatment were studied using the linear trapezoidal rule). Differences in the lipolytic responses between the two groups and the impact of treatment were studied.
RESULTS

Anthropometric, biochemical parameters, and energy expenditure. Hyperthyroid patients had higher plasma levels of fT₃ and fT₄ and lower plasma thyroid-stimulating hormone compared with hypothyroid patients. Thyroid hormone and thyroid-stimulating hormone levels were normalized after treatment (Table 1). Compared with hypothyroid patients, hyperthyroid patients presented with higher resting energy expenditure and lower body weight and adiposity. No difference in fasting plasma glucose or insulin was detected between the two groups. Plasma norepinephrine concentrations were lower in the hyperthyroid group, whereas plasma epinephrine concentrations showed no difference between the two groups at the beginning of the study. Treatment of thyroid dysfunction had no effect on body weight or adiposity; however, REE increased in the hyperthyroid group and decreased in the hypothyroid group following treatment (Table 2).

Lipolytic response to catecholamines and ANP. The hyperthyroid group showed higher basal plasma glycerol (Table 2) and SCAAT extracellular glycerol concentration (EGC) compared with the hypothyroid group (3.54 ± 1.02 vs. 2.18 ± 1.05 mg/dl, P < 0.05). Isoprenaline perfusion induced an increase in EGC in both groups of subjects before as well as after the treatment. Higher EGC response to isoprenaline stimulation was observed in hyperthyroid subjects compared with hypothyroid patients (Fig. 1), whereas no difference between the two groups was found after the respective treatments. In hyperthyroid subjects the lipolytic response to isoprenaline decreased (AUC: 211.9 µg/mg/dl, P < 0.05). Isoprenaline-induced lipolysis in SCAAT were higher in hyperthyroid group compared with the hypothyroid group before (194.5 vs. 88.7 µg/mg/dl, P < 0.05) after treatment. In the hypothyroid group, the change of the lipolytic response was not significant (AUC: 121.1 ± 194.5 vs. 88.7 ± 44.3 mg/dl·min⁻¹, P = 0.63).

The effects of 3 mg/dl ANP on EGC in SCAAT are depicted in Fig. 2. ANP perfusion induced a significant increase in EGC in both groups of subjects before as well as after the treatment. ANP-stimulated EGC AUC was higher in the hyperthyroid compared with hypothyroid group before the treatment (AUC: 230.4 ± 132.9 vs. 102.4 ± 80 mg/dl·min⁻¹, P < 0.05) and dropped to levels not different from the hypothyroid group following treatment (AUC: 114.1 ± 39.8 vs. 112.2 ± 196.2 mg/dl·min⁻¹, P = 0.88). The induction of lipolysis by ANP perfusion in the hyperthyroid group was not modified by treatment (AUC: 102.4 ± 80 vs. 112.2 ± 196.2 mg/dl·min⁻¹, P > 0.05).

Adipose tissue blood flow. Regional adipose tissue blood flow, estimated using the ethanol clearance ratio, increased (ethanol ratio decreased) during isoprenaline and ANP perfusions in subjects of both groups before as well as after the treatment (data not shown).

Gene expression studies. Gene expression of hormone-sensitive lipase was higher in the hyperthyroid group compared with the hypothyroid group before and after the treatment (Table 3). The expression of other genes was not different between the groups both before and after treatment.

In the hyperthyroid group gene expression of uncoupling protein-2 mRNA decreased by 37% (P < 0.05), in the hyperthyroid group expression of uncoupling protein-2 increased by 88% (P = 0.07), and expression of PDE3B decreased by 20% (P < 0.05) after thyroid hormone substitution therapy. No treatment-induced changes of hormone-sensitive lipase, β₂-adrenergic receptor, or NPR-A occurred in either group.

DISCUSSION

In this study, we have demonstrated that ANP and isoprenaline-induced lipolysis in SCAAT were higher in hyperthyroid compared with hypothyroid subjects. Differences in lipolytic
responses between groups were not detectable following treatment.

Successful treatment of thyroid dysfunction was demonstrated by the normalization of plasma levels of thyroid-stimulating hormone and free thyroid hormone (T3 and T4) levels in both groups. Together with the normalization of the thyroid status, REE decreased in hyperthyroid and increased in hypothyroid patients so that there was no difference in REE between the two groups after the treatment period.

An original finding of our study is that ANP-stimulated lipolysis in SCAAT is dependent on the thyroid status; it is increased with hyperthyroidism and reduced upon the normalization of plasma thyroid hormone levels. ANP-stimulated lipolysis was related to plasma levels of thyroxine \( P < 0.06 \), suggesting a novel regulatory pathway for ANP-induced lipolysis by thyroid hormones. Plasma glycerol concentration decreased after treatment in hyperthyroid subjects (1.04 ± 0.55 vs. 0.6 ± 0.46 mg/dl). Identical changes were observed in adipose tissue, where a fall in basal, ANP-, and catecholamine-stimulated EGC (index of lipolysis) after the antithyroid treatment was demonstrated. No significant changes in basal, ANP-, or catecholamine-stimulated lipolysis were observed during treatment in hypothyroid subjects.

In our study, we demonstrated, for the first time, the decrease of catecholamine-induced lipolysis after the thyroid dysfunction treatment in hyperthyroid subjects. No treatment-induced changes in catecholamine-stimulated lipolysis were observed in hypothyroid patients. The gene expression of the \( \beta_2 \)-adrenoreceptor was unchanged at the end of the treatment protocol, although changes in \( \beta_2 \)-adrenoreceptor number and binding capacity in thyroid dysfunction have been described by others (10, 21, 29). This might be explained by the sequential response over time from gene expression to protein translation. It cannot be excluded that induction of \( \beta_2 \)-adrenoreceptor gene expression might have occurred early during the treatment period and normalized at the end.

The expression of selected genes was measured in SCAAT in both groups before and after the normalization of thyroid dysfunction. Gene selection was based on previous data, suggesting their role in SCAAT lipolysis as well as their potential modulation by thyroid hormones from in vitro experiments (28). Gene expression of hormone-sensitive lipase in SCAAT was higher in the hyperthyroid group compared with the hypothyroid group in our study. Hormone-sensitive lipase is a major intracellular lipolytic enzyme; however, published data on the role of thyroid hormones on hormone-sensitive lipase gene expression in adipose tissue are scarce. In vitro studies describe no thyroid hormone-induced change of hormone-sensitive lipase gene expression in rat (13) or human adipocytes (28). To the best of our knowledge, no in vivo longitu-

![Fig. 1. Response of extracellular glycerol concentration in subcutaneous abdominal adipose tissue to 60-min stimulation by isoprenaline [0.002 mg/dl (0.1 μmol/l) for 30 min and 0.02 mg/dl (1 μmol/l) for another 30 min] in hyperthyroid and hypothyroid subjects before and after thyroid dysfunction treatment. \( P < 0.05 \) before vs. after treatment. Data are presented as means ± SD; hyperthyroid group \( n = 9 \), hypothyroid group \( n = 10 \). To convert mg/dl to mmol/l, multiply glycerol concentration by 108.59.](#)

![Fig. 2. Response of extracellular glycerol concentration in subcutaneous abdominal adipose tissue to 60-min stimulation by 3 mg/dl (10 μmol/l) atrial natriuretic peptide (ANP) in hyperthyroid and hypothyroid subjects before and after thyroid dysfunction treatment. \( P < 0.05 \) before vs. after treatment, ANP. Data are presented as means ± SD; hyperthyroid group \( n = 9 \), hypothyroid group \( n = 10 \). To convert mg/dl to mmol/l, multiply glycerol concentration by 108.59.](#)
Table 3. Relative mRNA levels in hyperthyroid and hypothyroid group before and after normalization of their thyroid status following treatment

<table>
<thead>
<tr>
<th>Gene</th>
<th>Before</th>
<th>After</th>
<th>Fold Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSL</td>
<td>1.8 × 10⁻²⁺ ± 1.05 × 10⁻⁵</td>
<td>1.88 × 10⁻²⁺ ± 1.6 × 10⁻⁵</td>
<td>1.03</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>1.05 × 10⁻²⁺ ± 1.1 × 10⁻³⁺</td>
<td>1.53 × 10⁻²⁺ ± 1.83 × 10⁻⁵</td>
<td>1.46</td>
</tr>
<tr>
<td>UCP2</td>
<td>1.93 × 10⁻⁴⁺ ± 2.6 × 10⁻⁴</td>
<td>1.22 × 10⁻⁴⁺ ± 1.7 × 10⁻⁴⁺</td>
<td>-1.59</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>8.87 × 10⁻⁵⁺ ± 9.4 × 10⁻⁵</td>
<td>1.67 × 10⁻⁵⁺ ± 1.82 × 10⁻⁴⁺</td>
<td>1.88</td>
</tr>
<tr>
<td>PDE3B</td>
<td>3.82 × 10⁻⁴⁺ ± 2.86 × 10⁻⁴</td>
<td>2.98 × 10⁻⁴⁺ ± 1.26 × 10⁻⁴⁺</td>
<td>-1.29</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>4.17 × 10⁻⁴⁺ ± 2.58 × 10⁻⁴</td>
<td>3.32 × 10⁻⁴⁺ ± 2.36 × 10⁻⁴⁺</td>
<td>-1.26</td>
</tr>
<tr>
<td>β₂-AR</td>
<td>7.77 × 10⁻⁵⁺ ± 6.24 × 10⁻⁵</td>
<td>8.58 × 10⁻⁵⁺ ± 5.06 × 10⁻⁵</td>
<td>1.1</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>5.40 × 10⁻⁵⁺ ± 1.72 × 10⁻⁵</td>
<td>5.77 × 10⁻⁵⁺ ± 3.16 × 10⁻⁵</td>
<td>1.07</td>
</tr>
<tr>
<td>NPR-A</td>
<td>1.77 × 10⁻⁴⁺ ± 1.47 × 10⁻³</td>
<td>1.93 × 10⁻⁴⁺ ± 1.29 × 10⁻⁴</td>
<td>1.09</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>2.42 × 10⁻¹⁺ ± 1.56 × 10⁻¹</td>
<td>2.44 × 10⁻¹⁺ ± 2.41 × 10⁻¹</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD; hyperthyroid group n = 11, hypothyroid group n = 13. HSL, hormone-sensitive lipase; UCP2, uncoupling protein-2; PDE3B, phosphodiesterase 3B; β₂-AR, β₂-adrenergic receptor; NPR-A, natriuretic peptide receptor type A. *P < 0.05 hyperthyroid vs. hypothyroid group; †P < 0.05 before vs. after treatment. Data are expressed as cycle threshold values obtained after normalization by the 18S ribosomal RNA subunit. Fold change in the gene expression analysis was calculated by dividing the gene expression values after treatment by values before intervention for the increases and by dividing the gene expression values before treatment by values after intervention for reductions.

Dinal study on the role of thyroid hormones in the regulation of hormone-sensitive lipase gene expression in humans has been published so far. Gene expression of hormone-sensitive lipase remained statistically unchanged in both groups following the treatment in our study; however, a 46% upregulation in hypothyroid subjects was observed after normalization of the thyroid status (P = 0.72). Our findings of increased hormone-sensitive lipase gene expression in SCAAT of hyperthyroid subjects fits well with the physiological findings, i.e., the presence of increased basal (as well as catecholamine- and ANP-stimulated) lipolysis in hyperthyroid subjects and plasma levels of glycerol, both being elevated in hyperthyroid patients with no difference between groups following treatment. Enhanced basal and stimulated lipolysis in hyperthyroid subjects might be partly explained by a parallel increase in expression of hormone-sensitive lipase and decrease in expression of the lipolysis-inhibiting enzyme PDE3B. In our study, we demonstrated a decrease of PDE3B gene expression after thyroid hormone substitution, which supports previous findings and extends prior in vitro findings (28) to in vivo clinical settings. SCAAT gene expression of NPR-A, mediating ANP actions at the cellular membrane, has not been studied in hyperthyroid/hypothyroid subjects so far; however, a role of thyroid hormones could be hypothesized (9). In this study we show that the gene expression of NPR-A was not influenced by initial thyroid hormone status; furthermore, no treatment-induced changes of its gene expression were detectable. These findings suggest that the regulation of ANP-induced lipolysis by thyroid hormones could be mediated by postreceptor signaling mechanisms independently of change in NPR-A gene expression.

A positive association between uncoupling protein-2 gene expression and thyroid hormone levels has been documented in human adipose tissue in vitro and in vivo (2, 11). In agreement with these studies, the gene expression of uncoupling protein-2 in hyperthyroid subjects was 2.4 times higher compared with hypothyroid subjects in our study; however, this difference did not reach statistical significance (P = 0.2). We also found a regulatory effect of thyroid status on uncoupling protein-2 gene expression, as evidenced by an 88% upregulation and a 37% downregulation of uncoupling protein-2 gene expression following treatment of hypothyroid and hyperthyroid patients, respectively. However, these findings should be interpreted with caution, as SCAAT protein level of uncoupling protein-2 was not measured in our study, and some authors (14) were unable to show changes in uncoupling protein-2 protein despite changes in uncoupling protein-2 mRNA. It is noteworthy, however, that the expression of the uncoupling protein-2 gene is very sensitive to thyroid status both in vitro and in vivo. Body weight and fat mass remained unchanged after the treatment of thyroid disease in both groups, allowing us to interpret the changes in SCAAT gene expression as the effect of changes in thyroid status, rather than changes in the adiposity of subjects.

Changes in the adipose tissue blood flow might influence EGC in SCAAT. Thus, the ethanol ratio method was used to determine changes in local blood flow. Although this method (being semiquantitative) does not allow comparison of adipose tissue blood flow response between groups, it may assess relative changes in local or regional blood flow during infusion of pharmacological compounds. The ethanol ratio decreased during ANP and isoprenaline perfusion, suggesting an increase in local blood flow consecutive to vasodilatation. These data are congruent with findings of other studies documenting direct vasodilating effect of ANP (12, 23). In addition, it cannot be excluded that, in addition to the direct ANP effect, the higher lipolytic response in hyperthyroid subjects, releasing more metabolic products such as adenosine, may induce the higher vasodilatation.

Plasma insulin levels were in the range of normal values in both groups before as well as after the treatment. A tendency toward higher insulin level in hyperthyroid subjects did not appear significant due to interindividual variability in both groups. No effect of treatment on plasma insulin levels was observed in either group. It is to be noted here that insulin is a potent antilipolytic agent interfering with catecholamine-mediated induction of lipolysis (17). We have observed higher...
lipolytic response in hyperthyroid compared with hypothyroid subjects despite the possible antilipolytic contribution of insulin. ANP-mediated lipolytic response has been shown to be independent of insulin action (20).

In conclusion, the present study demonstrated that ANP-simulated lipolysis (together with catecholamine-stimulated lipolysis) is enhanced in hyperthyroid patients and decreases during antithyroid treatment. These effects might take place at a postreceptor level since gene expression of NPR-A and β2-adrenergic receptors were unchanged after treatment. Enhanced hormonal stimulation of lipolysis was associated with increased gene expression of hormone-sensitive lipase in hyperthyroid patients. Increased ANP-mediated lipolysis in hyperthyroid patients provides a new mechanism whereby hyperthyroidism contributes to fat and weight loss in humans.

ACKNOWLEDGMENTS

We greatly thank Zuzana Parizkova for generous and efficient technical assistance.

GRANTS

This study was supported by the Grant Agency of the Ministry of Health of the Czech Republic (NB6832-2/2001 and NR8066-3/2004) and by the Research Project of the Ministry of Education of the Czech Republic (MSM 0021620814). This work is part of the project “Hepatic and Adipose Tissue and Functions in the Metabolic Syndrome” (HEPADIP; see http://www.hepadip.org), which is supported by the European Commission as an Integrated Project under the 6th Framework Programme (Contract LSHM-CT-2005-018734).

REFERENCES