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

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Polak J, Moro C, Klimeckova E, Kovacikova M, Bajzova M, Vitkova M, Kovacova Z, Sotornik R, Berlan M, Viguere 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 β3-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

PATEROLOGICAL 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-adrenoreceptors in vitro (10, 21, 30), but the influence of thyroid hormones on the density and function of antilipolytic α2-adrenoreceptors 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 hypothyroid state β-adrenoreceptor 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-adrenoreceptor, and G protein α2-subunit), whereas others are upregulated (β3-adrenoreceptors) 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).

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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-adrenergic 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 thryeostatic 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 achieved and maintained for 2 ± 2 mo [as defined by clinical findings and physiological levels of TSH, fT4, and free triiodothyronine (fT3)]. The time period between the first and second examination ranged from 4 to 6 mo.

**Experimental protocol.** The subjects entered the hospital at 8 AM after an overnight fast. After the subjects had voided their bladder, they underwent anthropometric and body composition assessments (Tanita TBF-410 GS Body Composition Analyzer; Tanita International, West Drayton, UK). Thereafter, they stayed in the semirecumbent position during the experimental period. After a 30-min rest, their resting metabolic rate was measured over 30 min using indirect calorimetry (Ymca; Sensor Medics, Yorba Linda, CA). Thereafter, an indwelling polyethylene catheter was inserted into the antecubital vein of one arm, and blood samples were drawn 30 min later. Following this, two microdialysis probes (Carnegie Medicine, Stockholm, Sweden) of 20 × 0.5 mm and a 20,000-MW cutoff were inserted percutaneously after epidural anesthesia (200 μl of 1% lidocaine; Roger-Bellon, Neuilly-sur-Seine, France) into the SCAAT at a distance of 10 cm to the right of the umbilicus. The probes were connected to a microperfusion pump (Harvard Apparatus, Les Ulis, France) and perfused with Ringer’s solution (139 meq/l sodium, 2.7 meq/l potassium, 0.45 meq/l calcium, 140.5 meq/l chloride, 2.4 meq/l bicarbonate, 101 mg/dl glucose) containing 1.7 g/l of ethanol to measure adipose tissue blood flow changes. The ethanol ratio was calculated as ethanol ratio (%) = (ethanol concentration in outgoing dialysate/ethanol concentration in ingoing perfusate) × 100. The variations of the ethanol ratio were taken as an index of variations in adipose tissue blood flow (6).

The perfusion rate was set at 2.5 μl/min for the experimental period. After a 30-min equilibration period, two 15-min fractions of the outgoing dialysate were collected at rest in each probe. Then, one probe was perfused with Ringer plus isoprenaline (0.002 mg/dl = 0.1 μmol/l, nonselective β-agonist) for 30 min and, subsequently, with Ringer plus isoprenaline (0.02 mg/dl = 1 μmol/l) for the next 30 min. The second probe was perfused with Ringer plus 3 mg/dl = 10 μmol/l human ANP for 60 min, as used previously (19, 24).

Six 10-min fractions of dialysate were collected during this 60-min period. The two probes were then perfused with Ringer solution for another 60 min, and four 15-min fractions were collected.

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 RNeasy kit and stored at −80°C until analysis. Total RNA concentrations were determined using a fluorimetric assay (Ribogreen; Fluoroscan 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′-ccagagtcgtagctaca-3′ and 5′-agccggctctagaagaa-3′; for PDE3B, 5′-gactgtattaaagacgaa-3′ and 5′-atggcagaaagattg-3′; for hormone-sensitive lipase, 5′-gtgaagagggacactca-3′ and 5′-gaagctggattgcttcc-3′; for uncoupling protein-2, 5′-ccagccatatattgagtttc-3′ and 5′-ccctggtagaactttg-3′; for NPR-A (ANP receptor), 5′-gagggactcaccctg-3′ and 5′-catatccggagagattg-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 an ultrasensitive radiometric technique (Biotrol kit; Merck-Cleveton, Nogent-s-Marne, France) [coefficient of variation (CV) 1.1–2.0%] and nonesterified fatty acids by an enzymatic procedure (Wako kit; Uniphar, Dardilly, France) (CV 2.7%). Plasma insulin concentrations were measured using RIA kits from Sanofi Diagnostics Pasteur (Marnes la Coquette, France) (CV 2.8–4%). 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 Mann-Whitney nonparametric test, whereas the impact of treatment was explored by the Wilcoxon Signed Rank Test.
using the same methods as described above. A level of $P < 0.05$ was considered statistically significant in all tests.

**RESULTS**

**Anthropometric, biochemical parameters, and energy expenditure.** Hyperthyroid patients had higher plasma levels of fT$_3$ and fT$_4$ 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 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 in both groups of subjects before as well as after the treatment. Higher EGC response to isoprenaline stimulation in EGC in both groups of subjects before as well as after the treatment (AUC: 211.9 vs. 88.7 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 patients compared with hypothyroid subjects (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 vs. 88.7 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$^{-1}$·60 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$^{-1}$·60 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$^{-1}$·60 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$^{-1}$·60 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 treatment (Table 3). The expression of other genes was not different between the groups before and after treatment.

In the hyperthyroid group gene expression of uncoupling protein-2 mRNA decreased by 37% ($P < 0.05$), in the hypothyroid 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 ($1.04 \pm 0.55$ vs. $0.6 \pm 0.46$ 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-

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**Fig. 1.** Response of extracellular glycerol concentration in subcutaneous abdominal adipose tissue to 60-min stimulation by isoprenaline [0.002 mg/dl (0.1 $\mu$mol/l) for 30 min and 0.02 mg/dl (1 $\mu$mol/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 $\mu$mol/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>
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<tr>
<td></td>
<td>Relative mRNA level</td>
<td>Relative mRNA level</td>
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<tr>
<td></td>
<td>(Hypothyroid)</td>
<td>(Hyperthyroid)</td>
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<tr>
<td></td>
<td>1.8 × 10^-5 ± 1.05 × 10^-5</td>
<td>1.88 × 10^-5 ± 1.6 × 10^-5</td>
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<tr>
<td>HSL</td>
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<td>1.53 × 10^-3 ± 1.83 × 10^-3</td>
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</tr>
<tr>
<td>UCP2</td>
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<td>1.22 × 10^-4 ± 1.7 × 10^-4</td>
<td>-1.59</td>
</tr>
<tr>
<td>PDE3B</td>
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<td>1.67 × 10^-5 ± 1.82 × 10^-4</td>
<td>1.88</td>
</tr>
<tr>
<td></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>β2-AR</td>
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<td>3.32 × 10^-4 ± 2.36 × 10^-4</td>
<td>-1.26</td>
</tr>
<tr>
<td>HSL</td>
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<tr>
<td>NPR-A</td>
<td>5.40 × 10^-5 ± 1.72 × 10^-5</td>
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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.
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.

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