The Effects of Thyroid Hormones on Gene Expression of Acyl-Coenzyme A Thioesterases in Adipose Tissue and Liver of Mice

Kerstin Krause¹ Juliane Weiner¹ Sebastian Hönes⁴ Nora Klöting³,⁴ Eddy Rijntjes⁶ John T. Heiker³ Claudia Gebhardt³ Josef Köhrle⁴ Dagmar Führer⁴ Karen Steinhoff⁵ Swen Hesse⁵,⁶ Lars C. Moeller⁴ Anke Tönjes¹

¹Division of Endocrinology and Nephrology, Department of Medicine, and ²Department of Nuclear Medicine, University of Leipzig, and ³IFB Adiposity Diseases, Leipzig University Medical Centre, Leipzig, ⁴Department of Endocrinology and Metabolism, University of Duisburg-Essen, Essen, and ⁵Institute for Experimental Endocrinology, Charité-Universitätsmedizin Berlin, Berlin, Germany

Key Words
Thyroid hormones · Acyl-CoA thioesterases · Fatty acids · Brown adipose tissue

Abstract
Background: Thyroid hormones (TH) exert pleiotropic effects on glucose and lipid homeostasis. However, it is as yet unclear how TH regulate lipid storage and utilization in order to adapt to metabolic needs. Acyl-CoA thioesterases (ACOTs) have been proposed to play a regulatory role in the metabolism of fatty acids. Objectives: We investigated the interaction between thyroid dysfunction and Acot expression in adipose tissues and livers of thyrotoxic and hypothyroid mice. Methods: Ten-week-old female C57BL/6NTac mice (n = 10/group) were made hyperthyroid by the application of L-thyroxine (2 μg/ml in drinking water) for 4 weeks. Hypothyroidism was induced in 10-week-old mice by feeding an iodine-free chow supplemented with 0.15% PTU for 4 weeks. We measured mRNA expression levels of Acot8, 11 and 13 in the liver and epididymal and inguinal white and brown adipose tissues (BAT). Furthermore, we investigated hepatic Acot gene expression in TRα- and TRβ-deficient mice. Results: We showed that the expression of Acot8, 11 and 13 is predominantly stimulated by a thyrotoxic state in the epididymal white adipose tissue. In contrast, hypothyroidism predominantly induces the expression of Acot8 in BAT in comparison with BAT of thyrotoxic and euthyroid mice (p < 0.01). However, no significant changes in Acot expression were observed in inguinal white adipose tissue. In liver, Acot gene expression is collectively elicited by a thyrotoxic state. Conclusions: These data suggest that ACOTs are targets of TH and are likely to influence 3,5,3′-triiodo-L-thyronine-or-chestrated mechanisms of lipid uptake, storage and utilization to adapt the regulation of metabolic demands.

Introduction
Thyroid hormones (TH) regulate a variety of physiological processes, including growth and development, and are potent regulators of metabolism throughout life [1, 2]. TH status correlates with body weight and energy

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expenditure [3–5]. Elevated circulating concentrations of
TH lead to a hypermetabolic state which is characterized
by increased resting energy expenditure, weight loss, ac-
celerated lipolysis and gluconeogenesis, and reduced se-
rum cholesterol concentrations. In contrast, the mani-
festation of a hypothyroid state, i.e. decreased serum TH
concentration, is associated with hypometabolism char-
acterized by cold intolerance, weight gain and increased
serum cholesterol concentrations [1].

It is well established that TH stimulate both a lipogen-
esis/lipolysis ‘futile cycle’ and that elevated TH concen-
trations lead to fat loss [6]. This is in particular regulated
by the liver-specific crosstalk of the active TH 3,5,3’-tri-
iodo-l-thyronine (T₃) by binding to its cognate receptors
TRα and TRβ and by TR crosstalk with other nuclear re-
ceptors, for instance PPARG, PGC1α and LXR [7]. Lipid
storage and synthesis are regulated by T₃ via an increased
expression of lipogenic genes such as fatty acid (FA) syn-
thase (Fas), Thsrp (Spot14) and acetyl-coA-carboxylase
(Acc1) [8]. Moreover, T₃ stimulates the shutting of free
FA (FFA) into mitochondria by increasing the expression
and activity of CPT1a, which is the rate-limiting step for
β-oxidation of FA in the mitochondria [9].

A prerequisite for nearly all FA metabolic pathways is
the activation of ligation of FA to acyl-CoA by acyl-coA
synthetases [10]. The high-energy acyl-CoA thioesters
have a broad CoA substrate specificity. Interestingly, it has
been demonstrated by Hunt et al. [16] that Acot8 activity
is inhibited by CoASH. Thus, it is anticipated that Acot8
plays a role in the regulation of intraperoxisomal CoA/
CoASH concentrations in order to balance the availabil-
ity of FA for β-oxidation [16].

In view of the unique role of ACOTs in directing lipid
storage and utilization we investigated the interaction be-
tween thyroid dysfunction and Acot gene expression in
adipose tissue and liver of mice. We show that gene ex-
pression of Acot8, 11 and 13 is stimulated by a thyrotoxic
state predominantly in epididymal white adipose tissue,
whereas a hypothyroid state enhances the expression of
Acot8, in particular in BAT. Likewise, hepatic Acot ex-
pression is elicited by thyrotoxicosis. These data suggest
that ACOTs are targets of TH and may provide a funda-
mental means by which T₃ integrates the mechanisms of
lipid uptake, storage and utilization to gauge the regula-
tion of metabolic demands.

Materials and Methods

Animal Care
Breeding and husbandry of all C57BL/6NTac mice (female,
n = 10 per group) was done in the Medical Experimental Center
of the University of Leipzig (Leipzig, Germany). All mice were
maintained in a room under pathogen-free conditions with a controlled
temperature (21 ± 1 °C) on a 12/12 h light/dark cycle. Water and
chow were provided ad libitum. The local ethics committee
(Regierungspräsidium Leipzig) of the state of Saxony (Germany)
approved the protocol of the animal experiments (approval No.
TVV04/12). TRα- and TRβ-deficient mice (male, 15 weeks old)
were obtained from the European Mutant Mouse Archive [17]
and the local ethics committee approved the study (Landesamt für
Umwelt, Natur und Verbraucherschutz Nordrhein-Westfalen Az.
84-02.04.2014.A092).

Animal Treatment
For the induction of thyrotoxicosis, mice received l-thyroxine
at a dose of 2 μg/ml diluted in drinking water for 4 weeks. Hy-
pothyroidism was induced in 10-week-old mice by feeding iodine-
free chow supplemented with 0.15% PTU (catalog TD 97061; Har-
lan Teklan, Madison, Wis., USA) for 4 weeks. Euthyroid mice fed
a normal chow diet (Altromin GmbH, Lage, Germany) served as
controls.

T₄ Serum Measurements
Total T₄ concentrations in serum were determined by radioim-
nunoassay using commercially available kits (RIA-4524; DRG In-
struments GmbH, Marburg, Germany). The samples and calibra-
tors were incubated in duplicate with radiolabeled tracer in anti-
body-coated tubes according to the manufacturer’s instructions.
After incubation the liquid was aspirated and the antibody-bound
radiolabeled tracer was counted in a gamma counter (1277 Gam-
mamaster; LKB Wallac, Turku, Finland). The limit of quantifica-
tion was 10 nM with an intra-assay coefficient of variation of 5.5%
at 36 nM, 5.9% at 81 nM and 2.2% at 169 nM.
RNA Extraction and RT-PCR

RNA was isolated from snap-frozen livers and adipose tissue using Trizol reagent (Invitrogen, Carlsbad, Calif., USA). In total, 1 μg of RNA per sample was reverse transcribed in a final mixture of 5× first-strand buffer (250 mM Tris-HCl, pH 8.3, 375 mM KCl, 15 mM MgCl₂), 0.5 mM of dNTPs, 5 mM of DTT (Promega, Madison, Wis., USA), 15 U of Prime RNase Inhibitor (ThermoFisher, Waltham, Mass., USA), 0.5 μg of random hexamer primers and 200 U of Moloney murine leukemia virus reverse transcriptase (Promega). Reverse transcription was performed at 37 °C for 60 min and 94 °C for 5 min.

Quantitative Real-Time PCR

Exon/intron spanning primers were designed to quantitatively calculate the expression of the Acot8, 11 and 13 isoforms in mouse liver and adipose tissue. The primer sequences are presented in Table 1. Gene expression was analyzed in duplicate by reverse-transcriptase real-time PCR by using the commercial LightCycler-DNA Master SYBR Green I Kit (Roche, Mannheim, Germany) as previously described [18]. Gene expression was analyzed according to the ΔΔCt method normalized to housekeeping genes 36B4 (adipose tissue) or 18s (liver), and expression levels were calculated as 2 –ΔΔCt [19]. Data are expressed as the mean ± SEM. All statistical analyses and final presentations were performed using GraphPad Prism 6.0 software (GraphPad, San Diego, Calif., USA). One-way ANOVA followed by the Bonferroni’s post hoc test was used for multiple comparisons between groups. p values ≤ 0.05 were considered to be significant.

Histology and Oil Red O Staining

For oil red O staining, livers were shock frozen in liquid nitrogen, embedded in OCT cryostat sectioning medium (Fisher Scientific, Schwerte, Germany) and cut at 8 μm. Sections were subjected to oil red O staining as described and counterstained with hematoxylin [20].

Results

Characterization of Thyrotoxic and Hypothyroid Mice

Thyroid dysfunction was established as described in Materials and Methods. Hypothyroid female mice had significantly reduced T₄ concentrations (14.00 ± 1.2 nM) compared to thyrotoxic (41.33 ± 3.67 nM, p < 0.01) and euthyroid mice (37.5 ± 0.5 nM, p < 0.001; fig. 1a).

In liver, the expression of genes known to be TH responsive, Dio1 and Pdk4, were significantly downregulated in hypothyroid mice and upregulated in thyrotoxic mice compared to euthyroid controls (fig. 1b). Thyroxin-binding globulin, Tbg, was studied as an example of a hepatic gene that is negatively regulated by TH. There was a 12-fold increase in Tbg mRNA expression in hypothyroid mice (fig. 1b). Altogether, in addition to the T₄ serum concentration, the gene expression analysis of TH-responsive genes further confirms the thyrotoxic and hypothyroid phenotype of the T₄- and PTU-treated mouse cohorts.

Distinct Tissue-Specific Acot Gene Expression in Mice with Thyroid Dysfunction

To address the impact of thyroid dysfunction on Acot gene expression in major tissues relevant for lipid metabolism, we first investigated Acot gene expression in epididymal (eWAT) and inguinal (iWAT) white adipose tissue and interscapular BAT in thyrotoxic, hypo- and euthyroid mice. Interestingly, we found both (i) fat depot-specific differences in the expression of Acot and (ii) a switch of Acot expression associated with thyroid status (fig. 2). The Acot11 gene reached the significantly highest values in white fat depots, eWAT and iWAT of thyrotoxic mice (fig. 2a). There was a 6-fold increase in eWAT in these mice when compared to eWATs of hypothyroid and euthyroid mice (p < 0.001; fig. 2a). Similarly, Acot8 was overexpressed in the eWAT of thyrotoxic mice. However, in a hypothyroid state Acot8 expression was highest in the

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**Table 1. Primer sequences used for qPCR**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer (5’→ 3’)</th>
<th>Reverse primer (3’→ 5’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acot8</td>
<td>GGTCTGGGAGATGCTCATGG</td>
<td>CCCAGTAATGCTTCTCTCTGT</td>
</tr>
<tr>
<td>Acot11</td>
<td>CAGAATGTGGGGACCCACTTG</td>
<td>GCATGCTCTCCGGCTGT</td>
</tr>
<tr>
<td>Acot13</td>
<td>GAGTTTTGGAAAAAGGTGACGT</td>
<td>TGGAGGTTGCCAGTATT</td>
</tr>
<tr>
<td>Acox1</td>
<td>TCGCAGACCCTGAAGAAATC</td>
<td>CCGGATTCAAGAGAGAAGCC</td>
</tr>
<tr>
<td>Acc1</td>
<td>TACAGATGTTTTGGGCTITT</td>
<td>CAAATCTGCGAGGACAAAGCC</td>
</tr>
<tr>
<td>Cpt1a</td>
<td>TGGATGGCTATGTTCAAAGGT</td>
<td>TCTCCCTCTCTTCATAGTGG</td>
</tr>
<tr>
<td>Cpt2</td>
<td>TGGCTGAGTGCTCCAAATACC</td>
<td>GCCGATACGGTAGAGCGCAAACA</td>
</tr>
<tr>
<td>Scd1</td>
<td>GAGGCCTGTACGGGGATCATA</td>
<td>CAGCCGAGCCTTGTAGTT</td>
</tr>
<tr>
<td>Fasn</td>
<td>AGATCCTGGAACGAGACGAT</td>
<td>GAGACGTTGACTCCTGGACATTG</td>
</tr>
</tbody>
</table>

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**Fig. 1.** Characterization of thyrotoxic and hypothyroid mice. Serum T4 concentrations in thyrotoxic, hypothyroid and euthyroid female mice (a) and mRNA expression of TH responsive genes in liver (b) confirm the thyrotoxic and hypothyroid phenotype of the T4- and PTU-treated mouse cohorts. Statistical analysis was performed by one-way ANOVA followed by Bonferroni’s post hoc test. Data are presented as means ± SEM. * p < 0.05, ** p < 0.01, *** p < 0.001.

**Fig. 2.** Effect of altered TH status on Acot gene expression in adipose tissue. The mRNA expression of Acot11 (a), Acot13 (b) and Acot8 (c) genes was determined in WAT (eWAT, iWAT) and BAT of thyrotoxic, hypothyroid and euthyroid female mice. Statistical analysis was performed by one-way ANOVA followed by Bonferroni’s post hoc test. Data are presented as means ± SEM. * p < 0.05, ** p < 0.01, *** p < 0.001.
Regulation of ACOT Expression by TH

BAT, which was 3-fold higher compared to Acot8 expression in the BAT of thyrotoxic and euthyroid mice (p < 0.01; fig. 2c). Collectively, the data suggest that gene expression of Acot8, 11 and 13 are stimulated by a thyrotoxic state predominantly in eWAT, whereas low TH concentrations elevate expression of the Acot8 isoform in BAT.

Increased Hepatic Acot Gene Expression in Thyrotoxic Mice

In order to elucidate a potential role of TH-induced Acot expression on hepatic lipid metabolism, we first examined the mRNA expression of genes associated with lipid metabolism in livers of thyrotoxic or hypo- and euthyroid female mice (fig. 3). In the livers of hypothyroid mice, expression of genes involved in FA oxidation as well as lipogenic genes is decreased (p < 0.001 for Cpt1α and p < 0.05 for Scd1; fig. 3a). Although these genes encode for proteins which promote or reduce hepatic triglyceride content, no triglyceride accumulation in the liver was observed in the thyrotoxic, hypo- or euthyroid mice (fig. 3b). Similar to the observed increase in transcript concentrations of Acot in eWAT in a thyrotoxic state, in livers of thyrotoxic mice a trend towards collective upregulation of all analyzed Acot transcripts was evident when compared to the livers of hypothyroid and euthyroid mice (fig. 3c). Collectively, these data suggest the induction of hepatic Acot gene expression by TH.

Hepatic Acot Expression in TRα and TRβ Knockout Mice

In liver, T3 exerts its effects on lipid and carbohydrate metabolism by binding to its cognate nuclear receptors...
TRα and TRβ. Prolonged treatment with T₃ promotes FA catabolism [6]. Having shown that expression of ACOTs is coordinately upregulated by TH in livers in vivo, we next aimed to assess whether this effect is mediated by TR. We used male TRα and TRβ knockout (KO) mice and compared the hepatic Acot gene expression to that in wild-type mice. As shown in figure 4, deficiency of TRα had no effect on hepatic Acot expression. However, in livers of TRβ-deficient mice there was a significant upregulation of Acot8 compared to the wild-type mice (1.5-fold, p < 0.05). This effect was even stronger for Acot11 with a 5-fold increase in TRβ-deficient mice versus wild-type controls (p < 0.05; fig. 4). The highly elevated serum and liver TH concentrations in TRβ-deficient mice [8, 21] possibly increase the hepatic expression of Acot8 and 11 via compensation by TRα. It therefore appears as if both TRs can mediate Acot expression in liver, at least for Acot8 and 11.

Discussion

TH influence a wide variety of physiological processes, including growth and differentiation as well as thermogenesis. To date little is known about the molecular mechanisms by which TH orchestrates energy storage and expenditure within the cell. Lipogenesis as well as lipolysis are regulated by T₃, and it is well known that T₃ stimulates the shuttling of FFA into mitochondria [22]. In recent years, a number of ACOTs have been identified with distinct roles in lipid metabolism. It has been demonstrated that Acot11 is upregulated in BAT when mice are exposed to cold and it has been proposed that Acot11 supports the transition of this tissue towards increased metabolic activity, most likely through alteration of intracellular fatty acyl-CoA concentration [23]. In our present work we found a significant upregulation of Acot11 in eWAT from thyrotoxic mice but not in BAT. Depot-specific differences in adipose tissue lipolysis are well known, for instance in the context of FFA release from the adipocyte. As an example, visceral (omentum) cultured differentiated preadipocytes display a greater FFA flux than subcutaneous adipocytes, suggesting the contribution of a partially cell-autonomous mechanism [24]. Whether or not visceral Acot11 expression in thyrotoxic mice represents such a regulatory point in visceral FA metabolism by balancing lipid storage and FA oxidation needs to be addressed in further functional studies.

A second finding of the present work was that the lack of TH triggers Acot8 expression in BAT. Acot8 is a peroxisomal ACOT whose activity is strongly inhibited by CoASH, suggesting a role of Acot8 in the regulation of peroxisomal CoA/CoASH concentrations. Peroxisomes optimize the flux of FA through β-oxidation and also participate in the synthesis of bile acids [14, 25]. It is worth noting that bile acids stimulate deiodinase 2 activity in BAT, thereby promoting energy expenditure and preventing resistance to insulin and obesity [26]. However, the contribution of Acot8 in the circuit of bile acids/cAMP/deiodinase 2 in BAT is thus far elusive. Additionally, it has been demonstrated that in BAT Dio2 may regulate the local availability of T₃. This results not only in an increase of the intracellular but also the extracellular T₃ pool [27–30]. In fact, 30–40% of T₃ is produced by PTU-insensitive pathways in adult athyroid T₄-supplemented [29], cold-exposed [28], preweaning [31] or hypothyroid rats [29, 30]. Since Dio2 is insensitive to PTU [29], it is likely that the compensatory local BAT T₃ concentration contributes to enhanced Acot8 gene expression in hypothyroid BAT.

TH regulation of lipid metabolism in liver is primarily dependent on direct actions of T₃ on TR as well as indirect crosstalks with nutrient-activated nuclear receptors, such as PPARα and LXR. LXR and TRβ control the expression of key enzymes involved in FA oxidation as well as lipogenesis, such as Cpt1α, Fasn and Acc1 [1]. In the livers of our hypothyroid mice these enzymes were
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In summary, our study provides the first evidence that ACOTs are targets of TH and that a disturbed TH status is associated with alterations in Acot gene expression in the liver and both white and brown fat depots in mice. In the liver the changes in Acot expression are not primarily dependent on the direct actions of TH on TR. However, our observations may add to the knowledge of TH-regulated hepatic lipid metabolism. Finally, the finding of increased expression of Acot in the BAT of hypothyroid mice might provide further insight into the oxidative metabolism in BAT under TH-deprived conditions.

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Disclosure Statement

The authors have nothing to disclose.

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Regulation of ACOT Expression by TH


