Understanding the non-thyroidal illness syndrome from in vivo and in vitro studies

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Lacking TRβ gene does not influence alterations in peripheral thyroid hormone metabolism during acute illness

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Abstract

Downregulation of liver deiodinase type 1 (D1) is supposed to be one of the mechanisms behind the decrease in serum T3 observed during the nonthyroidal illness syndrome (NTIS). Liver D1 mRNA expression is positively regulated by T3, mainly via the thyroid hormone receptor (TR)-β1. One might thus expect that lacking the TRβ gene would result in diminished downregulation of liver D1 expression and a smaller decrease of serum T3 during illness. In this study, we used TRβ-/- mice to evaluate the role of TRβ in Lipopolysaccharide (LPS, bacterial endotoxin) induced changes in thyroid hormone metabolism. Our results show that the LPS-induced serum T3 and T4 and liver D1 decrease take place despite the absence of TRβ. Furthermore, we observed basal differences of liver D1 mRNA and activity between TRβ-/- and WT mice and TRβ-/- males and females which did not result in differences in serum T3. Serum T3 decreased rapidly after LPS, followed later on by decreased liver D1, indicating that the contribution of liver D1 during NTIS may be limited with respect to decreased serum T3 levels. Muscle deiodinase type 2 (D2) mRNA did not compensate for the low basal liver D1 observed in TRβ-/- mice and increased in response to LPS in TRβ-/- and WT mice. Other (TRβ-independent) mechanisms like decreased thyrooidal secretion and decreased binding to thyroid hormone binding proteins probably play a role in the early decrease of serum T3 observed in this study.
Introduction

Lipopolysaccharide (LPS, a bacterial endotoxin) administration is a well-established animal model for The NonThyroidal Illness Syndrome (NTIS) (Boelen et al. 1995) via induction of an acute phase inflammatory response (Palsson-McDermott & O’Neill 2004). This inflammatory response is accompanied by a decrease of liver type 1 Deiodinase (D1) mRNA and activity and a decrease of serum T3 and T4, all characteristic for NTIS (Boelen et al. 2004). Using this animal model, we have previously reported that liver TRβ1 mRNA decreased rapidly after LPS (Boelen et al. 2004), which was followed by a decrease of liver TRβ1 protein expression (Beigneux et al. 2003). In addition, in vitro studies in a hepatoma cell-line have shown that the proinflammatory cytokine IL-1β induces a decrease of TRβ1 mRNA, which is mediated via the Nuclear Factor (NF)κB inflammatory pathway. IL-1β also results in decreased D1 mRNA expression in vitro (Yu & Koenig 2000; Jakobs et al. 2002), which is mediated via the NFκB and Activator Protein (AP)-1 inflammatory pathways simultaneously, suggesting different regulatory mechanisms (Kwakkel et al. 2006). A possible mechanism thought to be responsible for the observed D1 mRNA decrease is competition for limiting amounts of Steroid Receptor Coactivator (SRC)-1 (Yu & Koenig 2000). The downregulation of liver D1 mRNA is supposed to be one of the mechanisms behind the LPS-induced decrease in serum T3 (Yu & Koenig 2006). D1 mRNA expression is positively regulated by T3 via the TRs which activates gene transcription by binding to two Thyroid Hormone Responsive Elements (TREs) in the promoter region of the human D1 gene (Jakobs et al. 1997; Toyoda et al. 1995). Although no TREs have been identified in the promoter region of the mouse D1 gene, liver D1 in mice is up regulated by T3 administration and down regulated in hypothyroidism (Amma et al. 2001). In liver, D1 is mainly regulated via the TRβ1. In the absence of TRβ, liver D1 mRNA expression is decreased, although TRα1 partly takes over the T3-mediated D1 mRNA expression (Macchia et al. 2001; Amma et al. 2001). It is however unknown whether lacking the TRβ gene results in diminished downregulation of liver D1 mRNA expression and activity induced by LPS. In this study, we used TRβ−/− mice to evaluate the role of TRβ in LPS-induced changes in thyroid hormone metabolism.

Materials & Methods

Animal experiments

Male and female TRβ−/− and wildtype (WT) (129Sv/Ev) mice were used at 6-12 weeks of age. TRβ−/− were generated as previously described (Gauthier et al. 1999). Homozygous TRβ−/− mice were derived from heterozygous mothers to prevent intra uterine effects of
the homozygous genotype. WT and TRβ⁻/⁻ mice were crossed and bred separately. The mice were kept in 12h light/dark cycles in a temperature controlled room. Acute illness was induced by an intraperitoneal (i.p.) injection of 200 μg LPS (Lipopolysaccharide, \textit{E.coli} O127:B8; Sigma, St. Louis, MO, USA) diluted in 0.5 ml saline. Control mice received 0.5 ml saline. Due to diurnal variations (Zandieh \textit{et al.} 2002) each time point had his own control and the experiment started at 9AM. At time points 0, 4, 8 and 24 hours after LPS or saline administration injection 2 female and 4 male mice per group (2 female and 2-4 male per group for t=24) were anaesthetized by i.p. injection of 100 mg/kg ketamine (Virbac) and 2 mg/kg xylazine (Bayer) and killed by cervical dislocation. Blood was taken by cardiac puncture and serum was stored at -20ºC until analyzed. Liver and hindlimb-muscle tissue were obtained and immediately stored in liquid nitrogen. The study was approved by the University Victor Segalen Animal Care and Use Committee.

**Thyroid hormone levels**

Serum T₃ and T₄ were measured with in-house RIAs (Wiersinga & Chopra 1982). To prevent inter-assay variation (T₃: 6.2% and T₄: 7.3%), all samples of one experiment were measured within the same assay (intra-assay variability T₃: 3.6% and T₄: 6.6%).

**RNA isolation and RT-PCR**

Liver and muscle mRNA was isolated on the Magna Pure (Roche Molecular Biochemicals, Mannheim, Germany) using the Magna Pure LC mRNA tissue kit and appr. 10 mg of tissue. The protocol and buffers supplied with the corresponding kit were followed. cDNAsynthesis was performed using the First Strand cDNA Synthesis Kit for RT-PCR with oligo d(T) primers (Roche Molecular Biochemicals, Mannheim, Germany). Real Time PCR was performed using the Lightcycler (Roche Molecular Biochemicals, Mannheim, Germany). For liver PCR’s Lightcycler DNA Master SYBR Green I kit (Roche Molecular Biochemicals, Mannheim, Germany) was used, adding 3mM MgCl₂ and 50ng primers (Biolegio, Nijmegen, The Netherlands) each. For muscle PCR’s Lightcycler FastStart DNA Master\textsuperscript{Plus} SYBR Green I kit (Roche Molecular Biochemicals, Mannheim, Germany) was used, adding 50ng primers each (Biolegio, Nijmegen, The Netherlands). Primer pairs for hypoxanthine phosphoribosyl transferase (HPRT), TRα1, D1 and IL-1β were previously described (Sweet \textit{et al.} 2001) (Bakker 2001)(Boelen \textit{et al.} 2004)(Bouaboula \textit{et al.} 1992). We designed primer pairs for D2 (D2-forward: 5’- GCT TCC TCC TAG ATG CCT ACA A - 3’, D2-Reverse: 5’- CCG AGG CAT AAT TGT TAC CTG - 3’). Primers were intron-spanning or genomic DNA contamination was tested using a cDNA synthesis reaction without the addition of Reverse Transcriptase. PCR programs were as follows: denaturation 30 sec 95°C, 40-45 cycles of 0-10 sec 95°C, 10 sec annealing temperature, 15-20 sec 72°C. For PCRs using FastStart, denaturation time was extended to 10 min. Annealing
temperatures were: 54°C for HPRT, 64°C for TRα1, 52°C for D1, 55°C for D2 and 60°C for IL-1β. For quantification a standard curve was generated of a sequence-specific PCR-product ranging from 0.01 fg/μl until 100 fg/μl (measurements taken during the exponential phase of the amplification). Samples were corrected for their mRNA content using HPRT as a housekeeping gene. Samples were individually checked for their PCR-efficiency (Ramakers et al. 2003). The median of the efficiency was calculated for each assay, samples that differed more than 0.05 of the efficiency median value were not taken into account (0% for liver TRα1, 2.5% for liver IL-1β and muscle D2 and 4% for liver D1). Aberrant PCR-efficiencies occurred randomly and therefore did not bias the results.

Deiodinase activity

D1: Deiodinase type 1 activity was analysed as previously described (Peeters et al. 2003). Samples were homogenized on ice in 10 volumes of PED10 buffer (0.1M sodium phosphate, 2 mM EDTA and 10 mM DTT pH 7.2) using a Polytron (Kinematica, Luzern, Switzerland). Homogenates were snap frozen and stored at -80°C until use. Protein concentration was measured with the Bio-Rad protein assay using bovine serum albumin (BSA) as the standard following the manufacturer’s instructions (Bio-Rad Laboratories, Veenendaal, The Netherlands). Liver D1 activity was measured in duplicate, using 50 μl homogenate incubated in a final volume of 0.1ml with 0.1 μM rT3 with the addition of approximately 1*10⁵cpm [3’5’-125I]rT3 in PED10. Reactions were stopped by adding 0.1ml of 5% BSA on ice. The protein-bound iodothyronines were precipitated by the addition of 10% (w/v) trichloroacetic acid. After centrifugation, 125I was separated from the supernatant by chromatography on Sephadex LH-20 columns with a bed volume of 0.25ml, equilibrated and eluted with 0.1M HCl. Released 125I was counted using the Packard Cobra Auto-Gamma Counting System (Canberra Packard, Zürich, Switzerland) in the eluate. D1 activity was expressed as 125I pmol released per minute per mg liver protein.

D2: Muscle Deiodinase type 2 activity was measured as previously described (Mebis et al. 2007). Samples were homogenized on ice in 10 volumes of PED50 buffer (0.1M sodium phosphate, 2 mM EDTA and 25 mM DTT pH 7.2) using a Polytron (Kinematica, Luzern, Switzerland). Homogenates were used immediately. Protein concentration was measured with the Bio-Rad protein assay using bovine serum albumin (BSA) as the standard following the manufacturer’s instructions (Bio-Rad Laboratories, Veenendaal, The Netherlands). D2 activity was measured in duplicate, using 50 μl (≈100μg protein) homogenate incubated 4 hours at 37°C in a final volume of 0.1ml with 1 nM T4 with the addition of approximately 1*10⁵cpm [3’5’-125I]T4 in PED25. Reactions were stopped by adding 0.1 ml ice-cold methanol. After centrifugation, 0.1 ml of the supernatant was added
to 0.1 ml 0.02 M ammonium acetate (pH 4), and 0.1 ml of the mixture was applied to 4.6 x 250 mm Symmetry C18 column connected to an Alliance HPLC system (Waters, Etten-Leur, The Netherlands). The column was eluted with a linear gradient of acetonitrile (28-42\% in 15 min) in 0.02 M ammonium acetate (pH 4.0) at a flow of 1.2 ml/min. The activity of T_4 and T_3 in the eluate was measured on-line using a Radiomatic Z-500 flow scintillation detector (Packard, Meriden, CT, USA). D2 activity was expressed as fmol generated T_3 per minute per gram muscle tissue.

Statistics

Normal distribution of the data was tested using the Shapiro-Wilk test. Statistical significance between treatments and genotypes were evaluated using two-way ANOVA with two grouping factors (time and treatment) (time and genotype) (SPSS, Chicago, IL, USA). P-values in the figures represent the significant effect of treatment or genotype. To test pair-wise comparisons ANOVA was followed by students t-test (Excel Microsoft, Redmond, WA, USA) when data was normally distributed or Mann-Whitney U tests (SPSS, Chicago, IL, USA) when not normally distributed. Symbols in the figures represent the pair-wise P-values. P-values < 0.05 were considered statistically significant.

Results

Basal levels of thyroid hormones, deiodinases and TRα mRNA (Table 1)

For the analysis of the basal values t=0h and t=24h saline groups were pooled. Basal serum T_3 and T_4 levels were significantly higher in TRβ^{-/-} mice compared to WT. Sex-

<table>
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<th>wildtype</th>
<th>TRβ^{-/-}</th>
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<td></td>
<td>♀ (♀)</td>
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<tr>
<td>Serum T_3 (nmol/L)</td>
<td>1.55 ± 0.34</td>
<td>2.53 ± 0.24</td>
</tr>
<tr>
<td>Serum T_4 (nmol/L)</td>
<td>67 ± 6</td>
<td>133 ± 14</td>
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<tr>
<td>Liver D1 mRNA (a.u.)</td>
<td>2.48 ± 0.17</td>
<td>0.98 ± 0.08</td>
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<tr>
<td>Liver D1 activity (pmol/min/mg protein)</td>
<td>5.34 ± 0.5</td>
<td>2.5 ± 0.3</td>
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<tr>
<td>Muscle D2 mRNA (a.u.)</td>
<td>0.091 ± 0.028</td>
<td>0.216 ± 0.064</td>
</tr>
<tr>
<td>Liver TRα1 mRNA (a.u.)</td>
<td>0.75 ± 0.06</td>
<td>0.52 ± 0.00</td>
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Mean values ± SEM are given. Significances were evaluated by Students-t-test or Mann Whitney U test when appropriate. ^{a} p≤ 0.05 and ^{b} p≤ 0.01 TRβ^{-/-} compared to wildtype, ^{c} p≤ 0.01 females compared to males.
Lacking TRβ gene does not influence alterations

differences were not observed between male and female mice. Liver D1 mRNA and activity was significantly lower in TRβ⁻/⁻ mice compared to WT. The lower expression of D1 mRNA was more pronounced in male TRβ⁻/⁻ mice than in female TRβ⁻/⁻ mice, while no sex-difference was observed in WT mice. In WT mice liver TRα1 mRNA expression was higher in females than in males, whereas this difference was absent in the TRβ⁻/⁻, resulting in a significant difference between female TRβ⁻/⁻ compared to WT mice. Basal muscle D2 mRNA levels were not significantly different between TRβ⁻/⁻ and WT mice, furthermore, no sex-difference was observed. Muscle D2 activity was not detectable.

Effects of LPS administration on serum thyroid hormone levels

LPS treated WT mice were compared to saline treated WT mice. As expected, serum T₃ and T₄ significantly decreased after LPS administration (T₃; P<0.05 and T₄; P<0.01). LPS administration in TRβ⁻/⁻ mice also resulted in decreased serum T₃ and T₄ compared to saline treated TRβ⁻/⁻ mice (T₃; P<0.01 and T₄; P<0.05). Serum T₃

Figure 1. Relative values of serum T₃ and T₄ after 0, 4, 8 and 24 hours in A) saline (■) and LPS (●) treated WT mice, B) saline (□) and LPS (○) treated TRβ⁻/⁻ mice and C) LPS treated WT (●) and TRβ⁻/⁻ (○) mice. Mean values ± SEM (n=4/6) are shown. P values indicate differences between groups by ANOVA. Significances of separate time-points were evaluated by students t-tests, * P≤0.05, ** P≤0.01.
Chapter 4

significantly decreased (only TRβ−/−) 8 and 24h after LPS, whereas T4 decreased significantly after 8h. No significant difference was observed in the relative serum T3 and T4 decrease after LPS between TRβ−/− and WT mice. Results are shown in figure 1.

Effects of LPS administration on liver D1, TRα1 and IL-1β expression

No significant difference was observed in liver IL-1β mRNA expression after LPS between the WT and TRβ−/− mice, indicating a similar inflammatory response after LPS administration (data not shown). Liver D1 mRNA expression decreased in WT

Figure 2. Relative values of liver D1 mRNA, liver D1 activity and liver TRα1 mRNA expression after 0, 4, 8 and 24 hours in A) saline (■) and LPS (●) treated WT mice, B) saline (□) and LPS (○) treated TRβ−/− mice and C) LPS treated WT (●) and TRβ−/− (○) mice. Mean values ± SEM (n=4/6) are shown. P values indicate differences between groups by ANOVA. Significances of separate time-points were evaluated by students t-tests for TRα1 and Mann-Whitney U tests for D1, * P≤0.05, ** P≤0.01.
and TRβ−/− mice 8 and 24 hours after LPS administration (P < 0.01). Liver D1 activity decreased significantly 24h after LPS administration in WT mice compared to saline treated controls. Liver D1 activity in TRβ−/− mice decreased 8h but not 24h after LPS which is not in agreement with the observed decrease in mRNA levels. The discrepancy between WT and TRβ−/− mice at 24h is caused by the large variation in D1 activity levels in TRβ−/− mice which results in an abnormal distribution. Median values were not different (t=24; TRβ−/− mice: 77% of basal value and WT mice: 67% of basal value). The overall decrease in liver D1 mRNA expression and activity was not significantly different between TRβ−/− and WT mice.

Liver TRα1 mRNA was significantly decreased in the LPS treated WT and TRβ−/− mice after 4, 8 and 24h (P < 0.01). Relative liver TRα1 mRNA returned to normal levels after 8 and 24h of LPS treatment in TRβ−/− mice while WT mice TRα1 mRNA levels were still decreased (P < 0.01). Results are shown in figure 2.

**Effects of LPS administration on muscle D2 expression**

LPS administration in WT and TRβ−/− mice resulted in increased muscle D2 mRNA expression compared to saline treated WT and TRβ−/− mice (P < 0.01). In WT mice D2 mRNA significantly increased after 4, 8 and 24 hours after LPS. In TRβ−/− mice

![Figure 3](image)

**Figure 3.** Relative values of muscle D2 mRNA expression after 0, 4, 8 and 24 hours of LPS treatment in A) saline (■) and LPS (●) treated WT mice, B) saline (□) and LPS (○) treated TRβ−/− mice and C) LPS treated WT (●) and TRβ−/− (○) mice. Mean values ± SEM (n=4/6) are shown. P values indicate differences between groups by ANOVA. Significances of separate time-points were evaluated by Mann-Whitney U tests, * P ≤ 0.05, ** P ≤ 0.01.

D2 mRNA significantly increased 24h after LPS. The relative increase of muscle D2 mRNA was more pronounced in WT mice compared to TRβ−/− mice (P < 0.01). Results are shown in figure 3. Muscle D2 activity was not detectable.
Discussion

In this study we aimed to investigate whether the TRβ gene is involved in altered thyroid hormone metabolism during illness. To this end we studied TRβ−/− and WT mice in an established animal model of NTIS. TRβ−/− mice have a disturbed pituitary and hypothalamic feedback mechanism, which results in high serum T3 and T4 levels, without decreased Thyroid Stimulating Hormone (TSH) and Thyrotropin Releasing Hormone (TRH) levels (Forrest et al. 1996; Gauthier et al. 1999; Nikrodhanond et al. 2006). Furthermore, it has been shown that these mice have low basal liver D1 mRNA expression and D1 activity (Macchia et al. 2001; Amma et al. 2001). The elevated basal serum T3 and T4 levels and low liver D1 mRNA expression and activity in TRβ−/− mice observed in our experiments are in line with these findings.

Despite differences in basal levels, LPS administration resulted in similar relative decreases in serum T3 and T4 and liver D1 decrease in TRβ−/− and WT mice. WT and TRβ−/− mice had similar liver IL-1β mRNA levels, indicating that the inflammatory response to LPS might be the same in TRβ−/− and WT mice. Part of the illness-induced alterations might be due to diminished food-intake, as it is known that prolonged fasting influences both peripheral and central thyroid hormone metabolism (Boelen et al. 2006). However we study a model of acute illness which takes place within 24 hours. LPS-experiments using pair-fed control mice have shown that although T3 and T4 decrease after reduced food intake, T3 and T4 serum levels were significantly lower after LPS. Furthermore it was shown that liver D1 mRNA was not or only marginally affected after 24 hours of starvation (Boelen et al. 1996). Therefore we conclude that the acute alterations shown in this study can not be attributed to decreased food-intake due to LPS administration.

No difference in time-course of the relative serum T3 and T4 decrease after LPS could be observed between TRβ−/− and WT mice. We concluded that the absence of TRβ had no effect on the illness-induced serum T3 and T4 decrease. Because TRβ1 regulates D1 gene expression (Amma et al. 2001; Macchia et al. 2001), which is thought to be responsible for the decrease of serum T3 during illness (Yu & Koenig 2006), liver D1 mRNA expression and activity were evaluated. LPS administration resulted in a similar overall decrease of relative liver D1 mRNA expression and activity in both genotypes. However, LPS administration resulted in decreased D1 activity levels after 24h in WT while we did not observed this in TRβ−/− mice. This is caused by biological variation in D1 levels in TRβ−/− mice. In addition, LPS also induced a more pronounced decrease in D1 activity levels in TRβ−/− mice compared to saline treated controls at 8h. Absolute D1 activity levels were low in TRβ−/− mice and despite the fact that the relative decrease was not different we can not exclude that the effect of LPS on D1 activity might be also related to the absolute amount of D1 present.

From these observations we concluded that the decrease of liver D1 mRNA and activity observed during illness is not mediated via the TRβ1. Although we cannot
Lacking TRβ gene does not influence alterations

Chapter 4

exclude the possibility that the TRβ-mediated declines in the WT are mimicked by compensatory mechanisms in the TRβ knock-out mice.

Additionally, liver TRα1 mRNA expression was evaluated because TRα1 might compensate for the lack of TRβ1 in D1 regulation (Macchia et al. 2001; Amma et al. 2001). However, in TRβ−/− mice TRα1 seemed to return to normal levels more quickly after LPS, while D1 mRNA remained low although we cannot exclude that TRα1 protein levels were still low 24h after LPS. Recently it has been shown that D1 is not essential as a determinant of serum T₃ during euthyroidism in mice (Schneider et al. 2006). We observed a significant difference in liver D1 between TRβ−/− and WT mice and between TRβ−/− males and females, which was not reflected in serum T₃ levels, supporting the observation made by Schneider et al.

Muscle D2 has been proposed to play a significant role in regulating serum T₃ (Maia et al. 2005). Therefore, we evaluated whether muscle D2 might be responsible for the serum T₃ decrease during NTIS as was previously suggested (Peeters et al. 2005). However, LPS administration did not result in a D2 mRNA decrease, on the contrary, D2 mRNA increased in response to LPS in WT and TRβ−/− mice. D2 activity could not be detected. Our results were confirmed in a recent study by Mebis et al., who describe an increase in muscle D2 mRNA and activity in muscle tissue of ICU patients (Mebis et al. 2007). The muscle D2 mRNA increase was more pronounced in WT mice, indicating that the increase in response to LPS might be partly mediated by TRβ.

The question remains whether a decrease of liver D1 is responsible for the observed decrease of serum T₃. The rapid decrease in serum T₃, followed later on by decreased liver D1, suggests that other mechanisms play a role. It is known that 50% of the serum T₃ in rodents is derived from the thyroid and 50% from peripheral conversion (Chanoine et al. 1993). We previously observed early effects on thyroidal gene expression in LPS treated mice (Boelen et al. 2004) but this could only partly be responsible for the rapid serum T₃ decrease observed in this study because the half-life of serum T₃ is 10 hours. Effects of LPS on thyroid hormone binding proteins might also influence serum T₃ levels shortly after LPS. It is known in humans that during the acute phase response serum levels of thyroid hormone binding proteins quickly decrease; thereby also decreasing serum total thyroid hormone levels (Wiersinga 2005; Afandi et al. 2000). However, it is known that 24h after LPS fT₃ decreases, so the thyroid hormone binding protein effect can only account for the early T₃ decrease (Boelen et al. 1995).

Interestingly, we observed that the low basal liver D1 mRNA expression and enzyme activity was more pronounced in male TRβ−/− mice than in female TRβ−/− mice, while no sex-difference was observed in WT mice. Although sex differences in liver D1 mRNA and activity have been described in rats (Harris et al. 1979; Miyashita et al. 1995) and mice (Riese et al. 2006), we only observed a sex-difference in TRβ−/− mice and not in WT mice. Furthermore, no discrepancy was observed between D1 mRNA expression and D1 enzyme activity as previously described by Riese et al (Riese et al. 2006), which could be due to the different genetic background of the mice. The sex
difference observed in the TRβ/-/- mice might be the result of other transcription factors involved in the regulation of the D1 gene via the TRE when the TRβ is absent. The estrogen receptor (ER) seems a logical candidate for mediating sex-differences. The ER and TR share a common half site in the consensus DNA binding sequence. Furthermore, it has been reported for several genes that the ER and TR influence each other’s transcriptional activity, both inhibitory and stimulatory (Vasudevan et al. 2002). This regulatory mechanism might be impaired when the TR is absent, which is clearly evident from the disturbed lordosis behaviour of female TRβ/-/- mice (Dellovade et al. 2000). In addition, it has been shown for the human growth-hormone promoter, that when the TR is absent, the ER can activate gene-transcription by binding to the TRE (Graupner et al. 1991). It might be possible that when the TRβ is absent, the ER binds the TRE and regulates D1 mRNA expression in female TRβ/-/- mice.

From this study we can conclude that the illness-induced serum T₃ and T₄ and liver D1 decrease take place despite the absence of TRβ. Furthermore, the observed basal differences of liver D1 mRNA and activity did not result in differences in serum T₃. Serum T₃ decreased rapidly after LPS, followed later on by decreased liver D1, indicating that the contribution of liver D1 during NTIS may be limited with respect to decreased serum T₃ levels. It could be that the decrease of liver D1 might only be involved in the serum T₃ decrease 24h after LPS. Muscle D2 mRNA did not compensate for the low basal liver D1 observed in TRβ/-/- and increased in response to LPS. Therefore muscle D2 cannot be responsible for the observed serum T₃ decrease during NTIS. In this mouse model it is most likely that decreased thyroidal secretion and the decreased binding of thyroid hormone to thyroid hormone binding proteins are involved in the early decrease of T₃ and T₄ after LPS, whereas the liver D1 decrease and downregulation of the HPT-axis might be involved later in time.

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