Abstract

A variety of illnesses that leads to profound changes in the hypothalamus–pituitary–thyroid (HPT) axis are collectively known as the nonthyroidal illness syndrome (NTIS). NTIS is characterized by decreased tri-iodothyronine (T₃) and thyroxine (T₄) and inappropriately low TSH serum concentrations, as well as altered hepatic thyroid hormone (TH) metabolism. Spontaneous caloric restriction often occurs during illness and may contribute to NTIS, but it is currently unknown to what extent. The role of diminished food intake is often studied using experimental fasting models, but partial food restriction might be a more physiologically relevant model. In this comparative study, we characterized hepatic TH metabolism in two models for caloric restriction: 36 h of complete fasting and 21 days of 50% food restriction. Both fasting and food restriction decreased serum T₄ concentration, while after 36-h fasting serum T₃ also decreased. Fasting decreased hepatic T₃ but not T₄ concentrations, while food restriction decreased both hepatic T₃ and T₄ concentrations. Fasting and food restriction both induced an upregulation of liver D3 expression and activity, D1 was not affected. A differential effect was seen in Mct10 mRNA expression, which was upregulated in the fasted rats but not in food-restricted rats. Other metabolic pathways of TH, such as sulfation and UDP-glucuronidation, were also differentially affected. The changes in hepatic TH concentrations were reflected by the expression of T₃-responsive genes Fas and Spot14 only in the 36-h fasted rats. In conclusion, limited food intake induced marked changes in hepatic TH metabolism, which are likely to contribute to the changes observed during NTIS.

Key Words

► liver
► nutrition
► rat
► thyroid hormone metabolism

Introduction

Profound changes occur in the hypothalamus–pituitary–thyroid (HPT) axis during illness and starvation. The non-thyroidal illness syndrome (NTIS) is characterized by decreased serum tri-iodothyronine (T₃) and thyroxine (T₄) concentrations, and inappropriately low thyroid-stimulating hormone (TSH) concentrations (Boelen et al. 2011). Cytokine release as part of the acute-phase response has been known to be important for the development of NTIS (Boelen et al. 1996). Spontaneous diminished food intake is known to be a part of a variety of illnesses,
but has received only little attention as a potential mediator of the illness-induced changes in thyroid hormone (TH) homeostasis, despite its sometimes profound impact. For example, in our earlier studies, we found that the low serum T₃ during chronic inflammation is completely explained by the diminished food intake, indicating that this is an important factor in the pathogenesis of NTIS, especially in prolonged illness (Boelen et al. 2006). Whether NTIS is an adaptive or maladaptive response is still a matter of debate.

Fasting leads to a central downregulation of the HPT axis, characterized by increased type 2 deiodinase expression in hypothalamic tanycytes which leads to local increased bioavailability of T₃ concentration that suppresses thyrotrophin-releasing hormone (TRH) expression in hypophysiotropic neurons of the paraventricular nucleus of the hypothalamus (Fekete et al. 2004, 2010). In addition, the peripheral handling of THs is also markedly altered during fasting (Vagenakis et al. 1977, Harris et al. 1978).

In the liver, an important site for TH metabolism, type 1 deiodinase (D1) and type 3 deiodinase (D3) are expressed. Type 1 deiodinase is able to deiodinate both the inner and outer ring of TH, and is regarded as a TH-activating enzyme, in addition it can also contribute to the clearance of TH by degrading sulfated T₃. D3 converts T₄ to rT₃ and T₃ to 3,3’diodothyronine (T₂) and it thus the main inactivating enzyme. Besides deiodination, sulfation by sulfotransferases and glucuronidation by UDP-glucuronosyltransferases enhance the metabolism of TH and its excretion via the bile and urine (Visser et al. 1990, 1998, Kester et al. 1999, 2003). Upstream of Sults and Ugts is the constitutive androstane receptor (CAR). CAR-target genes are the sulfotransferases Sult1b1 and Sult1c1 as well as the UDP-glucuronidase Ugt1a1, which are important for TH metabolism in the rat liver. Besides an enhanced metabolism of TH in peripheral tissues via altered deiodinases, sequestration of TH in tissues is also suggested to play a role in the decreased serum TH concentrations. In order to enter the hepatocyte, TH has to be transported over the cell membrane. This is mediated via two transporters: MCT8 and MCT10.

During fasting, the activity of hepatic type 3 deiodinase (D3) is increased (Boelen et al. 2012) while the activity of type 1 deiodinase (D1) is decreased (Omara et al. 1993, Araujo et al. 2008, 2009). Theoretically, these changes would result in an enhanced inactivation of TH and less conversion of T₄ to T₃. However, recent studies has indicated that the fasting-induced changes in TH serum concentrations persist in D1-knockout (KO) mice, and are only mildly affected in D3-KO mice (Galton et al. 2014). The precise role of sulfotransferases and UDP-glucurononidases during fasting and illness is unclear at present and seems to be species dependent, because increased hepatic expression of Sults and Ugts (Maglich et al. 2004, Vella et al. 2011) in mice, and normal or decreased expression in rats (de Jong et al. 1992, Kester et al. 2003) were reported.

A recent study in a rabbit model for prolonged critical illness has revealed that the hepatic increase in D3 and decrease in D1 could be reverted by parenteral feeding (Mebis et al. 2012). By contrast, the central changes in Trh mRNA expression in the PVN were not affected by parenteral feeding, which is in line with earlier findings during chronic inflammation in mice, showing that, hypothalamic expression of Dio3 and Trh in the hypothalamus is not primarily modulated by nutrient restriction (Boelen et al. 2006).

Effects of caloric restriction on TH metabolism are often studied in relative short-term fasting models such as an overnight fast or a 24–48 h fasting period. However, prolonged caloric deprivation might be a physiologically more relevant model, especially when prolonged critical illness is studied. To gain more insights into the effects of caloric restriction on TH homeostasis in the liver we compared two models. Hepatic TH metabolism was assessed in 36-h fasted rats, and in rats that received only 50% of their baseline caloric intake during 3 weeks. In both experiments, food-restricted rats were compared with rats fed ad libitum.

Materials and methods

Animal experiments

Male Wistar rats weighing 250–350 g and at the age of 8–12 weeks (Charles River breeding Laboratories, Sulzfeld, Germany) were housed individually in a 12 h light:12 h darkness cycle, lights were on at 0700 h. Standard laboratory chow (CRM (E) chow from Special Diet Services, Essex, UK) and water were provided ad libitum unless stated otherwise. All procedures were approved by the Animal Welfare Committee of the Academic Medical Center (AMC) of the University of Amsterdam.

For the short-term fasting, experiment food was removed in the evening. After 36 h (two nights, 1 day) of food deprivation, rats (n = 6 per group) were killed at ±0900 h with an overdose of pentobarbital (120 mg/kg body weight). Trunk blood samples was collected, spun down, and serum was stored at −20 °C until analysis. The right liver lobe was dissected, snap frozen in liquid
nitrogen, and stored in −80 °C until further use. For the food restriction experiment, 24-h food intake was monitored for 4 days (baseline value). Subsequently, rats daily received 50% of their individual baseline 24-h intake for 21 days at ±1700 h. After 21 days, rats (n=6 per group) were killed as described above at ±0900 h. Both experiments had their own ad libitum fed control group.

Deiodinase measurements

For measurements of deiodinase activity, samples were homogenized on ice in ten volumes of PE buffer (0.1 M sodium phosphate, 2 mM EDTA pH 7.2) using a polytron (Kinematica, Luzern, Switzerland). 10 mM dithiothreitol (DTT) was added to the PE buffer (PED10) for measurement of D1 activity and 50 mM for D3 (PED50). Homogenates were snap frozen in aliquots and stored in −80 °C until further use. Protein concentration was measured with the Bio-Rad protein assay using BSA as the standard following the manufacturer’s instructions (Bio-Rad Laboratories).

Type 1 deiodinase Liver D1 activity was measured by duplicate incubations of 75 μl of 300 times diluted homogenate (approximately between 5 and 15 μg of total protein) for 30 min at 37 °C in a final volume of 0.15 ml with 0.1 μM rT3 with the addition of ~1×10^5 c.p.m. [3^125I]rT3 in PE buffer. One sample of each group was incubated in the presence of 500 nM PTU in order to inhibit D1 activity representing a tissue blank. Reactions were stopped by the addition of 0.15 ml ice-cold ethanol. After centrifugation, 0.125 ml of the supernatant was added to 0.125 ml 0.02 M ammonium acetate (pH 4.0), and 0.1 ml of the mixture was applied to 4.6×250 mm Symmetry C18 column connected to a waters HPLC system (model 600E pump, model 717 WISP autosampler, Waters, Etten-Leur, The Netherlands). The column was eluted with a linear gradient of acetonitrile (28–42%) for 15 min in 0.02 M ammonium acetate (pH 4.0) at a flow of 1.2 ml/min. The activity of rT3 and T2 in the eluate was measured online using a Radiomatic 150 TR flow scintillation analyzer (Perkin Elmer, Waltham, MA, USA). D1 activity can be calculated by subtracting the activity measured in the tissue blank from the activity measured without PTU. D1 activity was expressed as femtomole 3,3′,5′-triiodothyronine (T3) per minute per milligram protein, with intra-assay variation being 5.5%.

Type 3 deiodinase D3 activity was measured with the same method as D1 with the following modifications. Duplicate incubations of 75 μl of homogenate (approximately between 0.5 and 1.5 mg of total protein) were incubated for 120 min at 37 °C with the addition of 1 nM unlabeled T3 and 2×10^5 c.p.m. [3^125I]rT3 in a final volume of 0.15 ml PE buffer. One sample of each group was incubated in the presence of 500 nM unlabeled T3 to inhibit D3 activity representing a tissue blank. The activity measured with 1 nM T3 minus the incubation with 500 nM T3 represent true D3 activity. D3 activity was expressed as femtomole 3,3′,5′-triiodothyronine (T3) per minute per milligram protein, with intra-assay variation being 5.5%.

Thyroid hormones

Serum T3 and T4 were measured by in-house RIAs (Wiersinga & Chopra 1982). All samples of one experiment were measured within the same assay (intra-assay variation T3: 3.6% and T4: 6.6%).

THs in tissue

Liver concentrations of T4 and T3 were measured by an LC–MS method as described previously (De Escobar et al. 1985, Ackermans et al. 2012), with some modifications. Briefly, for the determinations of T4 and T3 in tissues, 50 mg frozen tissue were added to a plastic 2 ml polypropylene (PP) tube containing 150 mg zirconia beads, 400 μl methanol, and 40 μl 13C6-labeled internal standards (Ackermans et al. 2012). The samples were homogenized using a Magna Lyser (Roche Molecular Biochemicals) and transferred to 5 ml glass tubes. The plastic tubes were rinsed with 500 μl methanol and this was added to the homogenate. Chloroform (1.8 ml) was then added in a volume double that of the amount of methanol contained in the tissue homogenate. The samples were mixed on a Vortex for 15 s. The extraction was carried out in two steps, interspaced with centrifugations for 10 min at 1841 g. In the seconds step, a mixture of 0.9 ml chloroform-methanol (2:1) was added to the pellet. The final volume of extract was about 40 times the weight of the tissue plus the volume of the internal standard, as described previously (Reyns et al. 2002). The chloroform–methanol extracts were transferred to glass tubes for back-extraction of the iodothyronines into an aqueous phase with 0.05% CaCl₂, using the following calculation: 0.05% CaCl₂ (ml)=final volume extracts (ml)×1/4−(tissue weight (g)×0.8)−volume internal standard (ml). The value of 0.8 represents the estimated amount of water per gram tissue in milliliter per gram.

DOI: 10.1530/JOE-14-0533
Followed by a second extraction with pure upper phase (chloroform:methanol:0.05% CaCl₂ 3:49:48).

The iodothyronines in the pooled aqueous phases were concentrated and further purified using a small Bio-Rad AG 1X2 resin column (bed volume 0.5 ml) in a Pasteur pipette, as described previously (Mallol et al. 1982) and eluted in a volume of 2 ml 70% acetic acid. The eluates were then evaporated to dryness and taken up in 200 μl 0.1% NH₄OH. The iodothyronines were measured on an Acquity UPLC – Xevo TQ-S tandem mass spectrometer system equipped with a Z-Spray ion source operated in positive electrospray ionization mode (Waters, Milford, MA, USA). All aspects of system operation and data acquisition were controlled using MassLynx version 4.1 software with automated data processing using the QuanLynx Application Manager (Waters).

Intra-assay was variation <9%, total-assay variation was <12% for all metabolites. LOD T₃ 0.20; T₄ 0.39 pg on column. LOQ T₃ 1.43; T₄ 1.32 pg on column. Recovery T₃ 83% and T₄ 77%.

RNA isolation and qPCR

RNA was isolated using the Magna Pure apparatus (Roche Molecular Biochemicals) and the Magna pure tissue III total RNA kit (Roche Molecular Biochemicals). RNA yield was determined using the Nano drop (Nanodrop, Wilmington, DW, USA) and cDNA was synthesized with equal RNA input with the First-strand cDNA synthesis kit for qPCR with oligo d(T) primers (Roche Molecular Biochemicals). As a control for genomic DNA contamination, we included a cDNA synthesis reaction without reverse transcriptase. Quantitative PCR was carried out using the Lightcycler 480 and Lightcycler480SybrGreen I Master mix (Roche Molecular Biochemicals). Quantification was carried out using the LinReg software. PCR efficiency of each sample was calculated and samples that had a deviation of

### Table 1

<table>
<thead>
<tr>
<th>Primer sequences used for qPCR</th>
<th>Forward</th>
<th>Reverse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophilin</td>
<td>5′-GAGACTTCACCAGGGG-3′</td>
<td>3′-CTACCTCTTGCCGCAATCTCC-3′</td>
</tr>
<tr>
<td>Tbp</td>
<td>5′-TGCACACCATTTCCAGAACA-3′</td>
<td>3′-TGCCCTTCCCTTCTGCCACAC-3′</td>
</tr>
<tr>
<td>Dio1</td>
<td>5′-GAAGTGAAGTCTGCCGAAATGTG-3′</td>
<td>3′-AGGAGGAGCCGAGAAGTGTCTGGG-3′</td>
</tr>
<tr>
<td>Dio3</td>
<td>5′-AGCCCGAGAAAGTACTCCGACT-3′</td>
<td>3′-GCCGCTCTCATTGGCCAGGACC-3′</td>
</tr>
<tr>
<td>Car</td>
<td>5′-TCCTCCTTCCCTTCTGCCACAC-3′</td>
<td>3′-GCCGCTCTCATTGGCCAGGACC-3′</td>
</tr>
<tr>
<td>Sult1b1</td>
<td>5′-ACGGGCGAGTTCGTGAGTGT-3′</td>
<td>3′-AGGAGGAGCCGAGAAGTGTCTGGG-3′</td>
</tr>
<tr>
<td>Sult1c1</td>
<td>5′-CTCTGTTCGGAGACCTGCCATCTG-3′</td>
<td>3′-AGGAGGAGCCGAGAAGTGTCTGGG-3′</td>
</tr>
<tr>
<td>Ugt1a1</td>
<td>5′-AGCCCGCTTTCATCCACCTG-3′</td>
<td>3′-AGGAGGAGCCGAGAAGTGTCTGGG-3′</td>
</tr>
<tr>
<td>Spot14</td>
<td>5′-CTGAGGCTTTAGTCTGATCTGC-3′</td>
<td>3′-AGGAGGAGCCGAGAAGTGTCTGGG-3′</td>
</tr>
<tr>
<td>T Ra1</td>
<td>5′-CTCTGTTCGGAGACCTGCCATCTG-3′</td>
<td>3′-AGGAGGAGCCGAGAAGTGTCTGGG-3′</td>
</tr>
<tr>
<td>T Ra2</td>
<td>5′-CTCTGTTCGGAGACCTGCCATCTG-3′</td>
<td>3′-AGGAGGAGCCGAGAAGTGTCTGGG-3′</td>
</tr>
<tr>
<td>T Rb1</td>
<td>5′-CTCGGcíaAGTGCTGAGTCTGGG-3′</td>
<td>3′-AGGAGGAGCCGAGAAGTGTCTGGG-3′</td>
</tr>
<tr>
<td>Fas</td>
<td>5′-AGCCGCTTTCATCCACCTG-3′</td>
<td>3′-AGGAGGAGCCGAGAAGTGTCTGGG-3′</td>
</tr>
<tr>
<td>Pepck</td>
<td>5′-AGCCGCTTTCATCCACCTG-3′</td>
<td>3′-AGGAGGAGCCGAGAAGTGTCTGGG-3′</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Body weight (g)</th>
<th>Pre</th>
<th>Post</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad libitum</td>
<td>250.3 ± 2.2</td>
<td>262.7 ± 2.7</td>
<td>+4.9 ± 0.3</td>
</tr>
<tr>
<td>Fasting 36 h</td>
<td>250.7 ± 2.7</td>
<td>225.3 ± 2.2</td>
<td>−10.1 ± 0.3</td>
</tr>
<tr>
<td>Ad libitum</td>
<td>358.3 ± 2.6</td>
<td>406.2 ± 3.7</td>
<td>+13.4 ± 1.0</td>
</tr>
<tr>
<td>Food restriction</td>
<td>360.2 ± 1.4</td>
<td>298.5 ± 2.1</td>
<td>−17.1 ± 0.3</td>
</tr>
</tbody>
</table>
more than 5% of the mean efficiency value of the assay were excluded. We tested de 
T3-responsive metabolic genes fatty acid synthase (Fas), phosphoenolpyruvate 
carboxykinase (Pepek (Pck1)), and Spot14 as a readout of 
metabolic status in the liver. Calculated values were 
normalized by the expression of housekeeping genes 
(Hprt, Cyclophilin, and Tbp), which were selected to be 
stable among different groups. Published primer 
sequences were used for Hprt (Boelen et al. 2006); Mct8, 
Mct10 (van Beeren et al. 2012); other primer sequences 
are displayed in Table 1.

Statistical analyses

Differences between groups were evaluated using 
Student’s t-tests. To correct for multiple testing, we 
employed the Holm’s sequential Bonferonni method. All 
tests were performed using GraphPad prism 5 (GraphPad 
Software, Inc., La Jolla, CA, USA).

Results

Effects of fasting and caloric restriction on body weight

In the fasting experiment, 36 h of fasting led to a 10.1% 
body weight loss compared with the pre-fasting body 
weight. Ad libitum fed control animals gained 4.9% 
body weight during the same period. Three weeks of 
a 50% caloric restriction led to a 17.1% body weight 
loss, compared with a 13.4% body weight gain in the 
ad libitum fed control rats (Table 2).

TH concentrations in serum and liver

Serum T3 and T4 levels both decreased after 36 h of 
fasting compared with control rats (T3: −36 %, T4: −26 %), 
while only serum T4 level was decreased (−19%) after food 
restriction for 3 weeks (Fig. 1). Serum rT3 concentrations 
were not affected by fasting or food restriction (data 
not shown). Fasting resulted in decreased hepatic T3 
(−24%) but not T4 concentrations, while both hepatic 
T4 (−24%) and T3 concentrations (−24%) were decreased 
in the food-restricted rats compared with controls (Fig. 1). 
Hepatic rT3 concentrations were below the detection 
limit of our assay.

Hepatic deiodinase activity and expression

D3 activity and mRNA expression were significantly 
increased in both 36-h fasted and food-restricted rats 
compared with ad libitum fed controls. While Dio1 mRNA 
expression decreased after 36 h fasting, no significant 
decrease was observed in D1 activity. D1 activity and 
expression were not affected by 3 weeks of food restriction 
(Fig. 2).

Figure 1

Serum T4 and T3 concentrations (upper panel) and hepatic thyroid 
hormone concentrations (lower panel) in the fasting (circles) and 
restriction (squares) experiment. Open circles and squares represent 
ad libitum fed control rats, closed circles the 36-h fasted rats, and closed 
squares the rats that received 50% of their baseline food intake for 21 days. 
Mean values ± S.E.M. are shown. Symbols indicate differences between 
fed vs fasted/restricted groups (*P ≤ 0.05, **P ≤ 0.01) as evaluated by 
Student’s t-tests.
Expression of transporters and TRs

We observed an upregulation of hepatic Mct10 mRNA expression after 36 h fasting compared with ad libitum fed controls, but no changes after food restriction. Mct8 mRNA expression was not affected by fasting or food restriction. Likewise, no effects of fasting or food restriction were observed on the expression of the TH receptors TRα1, TRα2, and TRβ1 (Fig. 3).

Sult and Ugt mRNA expression

A striking difference between the two feeding models was observed in Car mRNA expression. While hepatic Car mRNA expression increased in the 36-h fasted rats, it did not change after food restriction. This was also reflected in the upregulation of the Car downstream target gene Ugt1a1, which was significantly higher in the 36-h fasted rats, but not in the food restricted rats. Sulfotransferases Sult1b1 and Sult1c1 were differentially regulated. Sult1b1 decreased after 36 h fasting but not after food restriction, while the opposite was observed for Sult1c1 (Fig. 4).

Metabolic status in liver

Clear differences were observed in hepatic expression of the key T3-regulated genes and metabolic enzymes Pepck and Fas. Pepck was increased after 36 h fasting but not after food restriction. Fas was decreased after 36 h fasting but not after food restriction. The TH-responsive gene Spot14 (Thrsp) was downregulated after 36 h fasting, but was unaffected by food restriction (Fig. 5).

Discussion

In this study, we compared the changes in hepatic TH metabolism in two caloric restriction models, i.e., 36-h fasting or 50% caloric restriction for 3 weeks. As caloric restriction is a major part of illness, insights into the differences between these models are helpful in understanding the contribution of caloric restriction to the pathogenesis of NTIS.

Fasting for 36 h led to a significant decrease in both serum T3 and T4 concentrations (36 and 26% respectively), while food restriction only decreased serum T4 concentration by 19%. This may indicate that a rigorous 36-h starvation period requires strict adaptations, including a limitation of energy expenditure, mediated by the low serum T3 concentrations, while food restriction for a longer period may enable the organism to adapt to the new situation. Interestingly, the group of Carvalho did find significant effects on serum T3 (−53%) and T4 (−38%) in 25-day 40% food-restricted male rats (Araujo et al. 2008, 2009). As feeding schedules from their study...
and the this study are more or less similar (21 days 50% restriction vs 25 days 40% restriction), these differences are difficult to explain. The timing of feeding and timing of killing the animal might be relevant. We gave access to the food at the beginning of the dark period and killed the animals at the beginning of the light period, but it is not known when this was done in the studies from the Carvalho group (Araujo et al. 2008, 2009). In addition to serum TH concentrations, we measured hepatic TH concentrations after 36-h fasting and 50% food restriction. Hepatic $T_3$, but not $T_4$, concentration, decreased after 36 h fasting while both hepatic $T_3$ and $T_4$ decreased after 3-week food restriction. This suggests a hypothyroid state of the liver after longtime diminished food intake.

In order to explain the observed changes in liver TH concentrations, we evaluated hepatic deiodinase, transporter, and TR expression in both models. As observed before, $D3$ mRNA expression and activity were markedly increased after 36 h fasting. A similar pattern was present after food restriction, although during food restriction the increase in mRNA expression and activity was lower compared with the fasting condition. We have observed a fasting-induced increase in liver $D3$ activity before in mice after 24 and 48 h of fasting, and this was shown to be dependent on the drop in serum leptin (Boelen et al. 2012). As the decreased body weight in both our models is likely to represent a decrease in adiposity, and thus in leptin secretion, this may explain the increased $D3$ activity in both models.

$Dio1$ mRNA expression was only decreased after 36 h of fasting, but neither fasting nor food restriction decreased $D1$ activity. The 36-h fasting period is likely to be too short to observe decreases in $D1$ activity. Araujo et al. (2008, 2009) observed a decrease in $D1$ activity after 25 days of 40% food restriction, but this might be due to the decrease in $T_3$ serum concentration, which was absent in our study. Although $D1$ was thought to be important for the fasting-induced decrease in TH concentrations (Harris et al. 1978), it is more likely that $D1$ regulation is secondary to the serum TH concentrations, because changes in $D1$ occur later than the changes in serum TH concentrations (Omara et al. 1993), and $D1$-KO mice show no altered response to fasting (Galton et al. 2014).

We observed a striking increase in $Mct10$ expression after 36-h fasting, but not after food restriction. MCT10 has been shown to be an effective bidirectional aromatic amino acid transporter, which has an affinity for $T_4$ that is even higher than MCT8 (Ramadan et al. 2006). Transfection of hMCT10 in cells enhances the intracellular degradation of $T_3$ by $D3$ (Friesema et al. 2008). Although

![Figure 3](http://joe.endocrinology-journals.org)

**Figure 3**

Relative mRNA expression of thyroid hormone transporters $Mct8$ and $Mct10$ (upper panel) and the thyroid hormone receptors $Tra1$, $Tra2$, and $Trb1$ (lower panel) in the fasting (circles) and restriction (squares) experiment. Open circles and squares represent ad libitum fed control rats, closed circles the 36-h fasted rats, and closed squares the rats that received 50% of their baseline food intake for 21 days. Mean values ± S.E.M. are shown. Symbols indicate differences between fed vs fasted/restricted groups (** *P* ≤ 0.001) as evaluated by Student’s t-tests.
by shuttling it into the liver, where it can be degraded by D3 to prevent a local hyperthyroid state. This concept is supported by the decreased hepatic T₃ concentrations we observed after 36 h fasting. During 3-week food restriction, liver Mct10 expression did not change while liver Dio3 expression increased. The combination of these changes might play a role in the decrease in hepatic T₃ in the absence of a decrease in serum T₃.

Interestingly, we found a differential regulation of Car mRNA expression between the two models. Car expression was induced after 36 h fasting, while it was unchanged after 3 weeks of 50% food restriction. The induction of Car was associated with an increased expression of Ugt1a1, which indicates a role for glucuronidation in the clearance of T₃ and T₄ from the circulation during fasting. Car has been shown before to be important for the fasting-induced changes in serum TH levels (Maglich et al. 2004). Sulfotransferase expression did not increase and Sult1b1 was even lower after 36 h of fasting, which has been found before in rats (de Jong et al. 1992, Kester et al. 2003). This indicates that increased sulfation does not play a major role in the decreased clearance of TH during fasting.

In the food restriction experiment, Sult1b1 decreased while Ugt1a1 increased slightly without a change in Car expression, which points to possible involvement of other nuclear receptors such as PXR (Chai et al. 2013).

The key metabolic T₃-regulated enzymes Spot14, FAS, and PEPCK were differentially regulated in the two-caloric restriction models. In the fasting model, metabolic requirements clearly overrule the regulation of Pepck, as this gene is upregulated while Fas and Spot14 are downregulated. In the food restriction model, none of these genes were affected despite lower hepatic T₃ concentrations. Although the expression of TRs did not change, this effect might be explained by a decreased binding capacity of TRs as has been observed in a hepatoma cell line upon stimulation with cytokines (Wolf et al. 1994).

In summary, food restriction results in a local decrease in TH concentrations in the liver, which is not always reflected in the expression of three key T₃-regulated genes. The observed alterations are different from the changes observed in experimental inflammation models where food intake was also reduced. Acute inflammation, chronic inflammation, and bacterial sepsis in rodents resulted in decreased activity of liver D1 and D3 concentrations (Boelen et al. 2004, 2005, 2008, 2009). However, in a rabbit model of prolonged critical illness, hepatic D3 was shown to increase and this was reversed when parenteral feeding was applied during the period of illness (Debaveye et al. 2005, Mebis et al. 2012).
The observed differences in D3 expression and activity may be due to differences in serum leptin concentrations. While prolonged critical illness decreases serum leptin levels, acute and chronic inflammation increase serum leptin via IL-1β (Faggioni et al. 1998). Critically ill patients showed a minor increase in liver D3 activity, while sulfotransferase activity did not change (Peeters et al. 2003, 2005). A role of diminished food intake cannot be excluded in this setting. Analysis of post mortem liver tissue of critically ill patients showed reduced TH concentrations compared with acute death patients (Arem et al. 1993), which might be a result of decreased D1 and increased D3 activity. This would fit with our findings of reduced hepatic T3 and T4 concentrations after 3-week caloric restriction. Liver TH transporter expression appeared to be differentially regulated during fasting/food restriction and illness: we observed a marked increase in liver Mct10 mRNA expression during fasting, while in critically ill patients and in critically ill rabbits, the expression of Mct8 was increased and Mct10 expression did not change (Mebis et al. 2009). In the latter studies, it was difficult to discriminate between feeding-related changes per se and inflammation-related changes. It is likely that the observed decrease in serum T3 during illness depends predominantly on diminished food intake as observed before (Boelen et al. 2006), although it remains speculative whether liver D3 is involved.

In critically ill patients, illness-related nutrient restriction contributes to decreased TH levels. In these patients, early parenteral nutrition appeared to have a negative effect on clinical outcome compared with late parenteral nutrition (Langouche et al. 2013). Thus, nutrient restriction in critical illness may be a beneficial adaptation. However, it is unknown at present if the low TH concentrations in ICU patients with protracted critical illness represent a useful adaptation or rather a reflection of neuroendocrine failure. In the absence of large randomized clinical trials, there is no convincing evidence at present that treatment of these patients with TH is beneficial in terms of clinical outcome. A number of small clinical trials showed that treatment with combinations of the neuropeptides growth hormone-releasing hormone, growth hormone-releasing peptide 2, and TRH partially restores serum TH concentrations, while in addition some anabolic parameters improve in ICU patients with protracted critical illness (Van den Berghe et al. 1998, 1999). However, at this stage it is unknown if this approach improves inflammatory parameters and restores TH metabolism at the tissue level.

In conclusion, long-term caloric restriction may overrule the inflammatory mediated decrease in liver D3 activity that is seen in several inflammation models and likely contributes to the illness-induced changes in hepatic TH metabolism observed during prolonged illness. However, both during fasting and caloric restriction, the changes in hepatic TH metabolism are not mediating the serum decreases in TH (Kwakkel et al. 2008, 2010, Mebis et al. 2012, Galton et al. 2014).

Figure 5
Relative mRNA expression of the metabolic genes phosphoenolpyruvate carboxykinase (Pepck), fatty acid synthase (Fas), and Spot14 in the fasting (circles) and restriction (squares) experiment. Open circles and squares represent ad libitum fed control rats, closed circles the 36-h fasted rats, and closed squares the rats that received 50% of their baseline food intake for 21 days. Mean values ± S.E.M. are shown. Symbols indicate differences between fed vs fasted/restricted groups (***P ≤ 0.01, **P ≤ 0.001) as evaluated by Student’s t-tests.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Acknowledgements
The authors thank the staff of the Laboratory of Endocrinology for measuring serum thyroid hormones and L Eggels for her expert technical assistance with the animal experiments.

References
van Beeren HC, Kwakkel J, Ackermans MT, Wiersinga WM, Fliers E & Boelen A 2012 Action of specific thyroid hormone receptor α(1) and β(1) antagonists in the central and peripheral regulation of thyroid hormone metabolism in the rat. Thyroid 22 1275–1282. (doi:10.1089/thy.2012.0135)
Boelen A, Maas MA, Lowik CW, Platvoet MC & Wiersinga WM 1996 Induced illness in interleukin-6 (IL-6) knock-out mice: a causal role of IL-6 in the development of the low 3,5,3'-triodothyronine syndrome. Endocrinology 137 5250–5254. (doi:10.1210/endo.137.12.8940342)
Galton VA, Hernandez A & St Germain DL 2014 The 5'-deiodinases are not essential for the fasting-induced decrease in circulating thyroid hormone levels in male mice: possible roles for the type 3 deiodinase and tissue sequestration of hormone. Endocrinology 155 3172–3181. (doi:10.1210/en.2013-1884)
Kwakkel J, Chassande O, Van Beeren HC, Wiersinga WM & Boelen A 2008 Lacking thyroid hormone receptor β gene does not influence

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-14-0533
© 2015 Society for Endocrinology
Printed in Great Britain
Published by Bioscientifica Ltd.
Fasting and hepatic thyroid hormone metabolism

E M De Vries and others


