Beyond Low Plasma T_3: Local Thyroid Hormone Metabolism during Inflammation and Infection

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Decreased serum thyroid hormone concentrations in severely ill patients were first reported in the 1970s, but the functional meaning of the observed changes in thyroid hormone levels, together known as nonthyroidal illness syndrome (NTIS), remains enigmatic. Although the common view was that NTIS results in overall down-regulation of metabolism in order to save energy, recent work has shown a more complex picture. NTIS comprises marked variation in transcriptional and translational activity of genes involved in thyroid hormone metabolism, ranging from inhibition to activation, dependent on the organ or tissue studied. Illness-induced changes in each of these organs appear to be very different during acute or chronic inflammation, adding an additional level of complexity. Organ- and timing-specific changes in the activity of thyroid hormone deiodinating enzymes (deiodinase types 1, 2, and 3) highlight deiodinases as proactive players in the response to illness, whereas the granulocyte is a novel and potentially important cell type involved in NTIS during bacterial infection. Although acute NTIS can be seen as an adaptive response to support the immune response, NTIS may turn disadvantageous when critical illness enters a chronic phase necessitating prolonged life support. For instance, changes in thyroid hormone metabolism in muscle during critical illness may be relevant for the pathogenesis of myopathy associated with prolonged ventilator dependence. This review focuses on NTIS as a timing-related and organ-specific response to illness, occurring independently from the decrease in serum thyroid hormone levels and potentially relevant for disease progression. (***Endocrine Reviews*** **32: 670–693, 2011)**

I. Introduction

It has been known for many years that profound changes in thyroid hormone metabolism occur during illness, collectively known as the nonthyroidal illness syndrome

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Abbreviations: AP-1, Activator protein-1; D1, deiodinase type 1; D2, deiodinase type 2; D3, deiodinase type 3; D3KO, D3 knockout; GHRP, GH-releasing peptide; GSH, glutathione; HIF, hypoxia inducible factor; HPT, hypothalamus pituitary thyroid; ICU, intensive care unit; IFN, infundibular nucleus; JNK, Jun N-terminal kinase; LPS, lipopolysaccharide; ME, malic enzyme; MPO, myeloperoxidase; NFκB, nuclear factor κB; NTIS, nonthyroidal illness syndrome; PMN, polymorphonuclear leukocyte; PVN, paraventricular nucleus; RXR, retinoid X receptor; Se, selenium; SRC-1, steroid receptor coactivator-1; T_2, 3,3′-diiodothyronine; TG, triglyceride; TR, thyroid hormone receptor; TSHR, TSH receptor; UCP, uncoupling protein; WT, wild-type.

(NTIS), the sick euthyroid syndrome, or the low-T_3 syndrome. This condition is characterized by decreased serum T_3 and, in severe illness, decreased serum T_4, increased serum rT_3, and no increase in serum TSH, reflecting a major change in negative feedback regulation (1). Although there was some doubt initially whether the changes in total thyroid hormone levels during NTIS reflect alterations in free thyroid hormone concentrations (1, 2), it is generally accepted now that free T_4 and free T_3 levels decrease during severe illness (3, 4).

NTIS can be seen as a useful adaptation of the body to counteract excessive catabolism observed during illness and may be viewed as part of the acute phase response (5), which is one of the major defense mechanisms of the body predominantly mediated by cytokines. Proinflammatory cytokines, especially IL-1β, IL-6, TNFα, and interferon γ, inhibit several genes involved in thyroid hormone metabolism *in vitro* (6–8). In an attempt to induce NTIS, several experimental studies have reported the effects of administration of cytokines *in vivo* in both humans and experimental animals (9–11), resulting in altered thyroid hormone metabolism exhibiting some, but not all, features of disease-related NTIS (Table 1). In these studies, cytokine administration induced a flu-like illness. Therefore, it cannot be excluded that the resulting illness, rather than the cytokines *per se*, accounts for the observed changes in thyroid hormone metabolism (9–12). At this stage, a variety of mechanisms including altered thyroid hormone secretion, transport, and clearance, are known to contribute to NTIS (13).

Because the combination of reduced serum T_3 and T_4 levels indicates poor prognosis in severely ill patients, several investigators have focused on the changes in plasma thyroid hormone levels. However, clinical studies aimed at restoring plasma T_3 by T_3 and/or T_4 treatment were generally not beneficial, and were sometimes even harmful (1), indicating that restoring plasma T_3 levels does not equal improved tissue and organ function.

Recent evidence has made it clear that thyroid hormone action at the tissue level during illness is not a simple reflection of serum thyroid hormone concentrations. Instead, NTIS has differential effects on local thyroid hormone metabolism in various organs that appear to occur quite independently from decreased serum T_3 and T_4 concentrations. The net effect of these differential changes is probably a major determinant of thyroid hormone availability and therefore of thyroid hormone action at the tissue level. A better understanding of the mechanisms underlying altered thyroid hormone metabolism in specific target tissues during illness is mandatory to renew our appreciation of NTIS, with the ultimate aim of improving disease outcome in critically ill patients.

TABLE 1. Illness-induced alterations in serum T_3, T_4, rT_3, and TSH in various species

Species	T_3	T_4	rT_3	TSH	Refs.
Human	↓ ↓	N or ↓	↑	N or ↓	1
Mouse	↓	↓ ↓	N or ↓	N	11, 32, 145
Rat	↓	↓	–	↓	31, 58
Rabbit	↓	↓	↓	N	174, 183

↓, Decreased; ↑, increased; N, not affected; –, not reported.

II. The Central Component of the HPT Axis

The combination of low serum thyroid hormone and TSH levels suggests central down-regulation of the hypothalamus pituitary thyroid (HPT) axis, which was supported by the observation in human autopsy samples that TRH gene expression is decreased in the hypothalamic paraventricular nucleus (PVN) of patients with NTIS. Furthermore, the decrease in postmortem TRH mRNA expression in the PVN showed a positive correlation with premortem serum TSH, in keeping with hypothalamic down-regulation of the HPT axis setpoint in severe illness (14). Clinical studies showed that decreased TSH release as well as serum T_3 and T_4 in critically ill patients could be restored to a large extent by the administration of exogenous TRH in combination with GH-releasing peptide (GHRP)-2. This neuroendocrine effect coincided with decreased serum markers of catabolism and increased markers of anabolism (15), suggesting that hypothalamic down-regulation of the HPT axis in patients with prolonged critical illness is an unfavorable condition amenable to treatment. However, the experimental induction of NTIS in rodents resulted in simultaneous changes in hypothalamic, pituitary, and peripheral thyroid hormone metabolism (16), arguing against down-regulation of the HPT axis at the central level as the primary and main determinant of the complex picture of NTIS in the whole organism.

A. Hypothalamic response during illness

1. Thyroid hormone transport

Cellular entry of thyroid hormone is necessary for intracellular conversion of thyroid hormone and for its binding to the nuclear thyroid hormone receptor (TR). Two categories of thyroid hormone transporters have been described, the organic anion transporters and the amino acid transporters. The organic anion-transporting polypeptide family consists of a variety of homologous proteins, of which OATP1C1 is expressed in brain capillaries involved in the uptake of T_4 across the blood-brain barrier. The expression levels of OATP14 mRNA and protein are inversely related to thyroid hormone availability (17).

The thyroid hormone transporter MCT8, belonging to the family of the amino acid transporters, transports T_4 and T_3 and is expressed in neuron populations in many brain areas, including cortical regions, striatum, cerebellum, and hypothalamus (18, 19). Thyroid hormones are probably not involved in the regulation of MCT8 in these neurons because hypothalamic MCT8 expression is not altered by hypothyroidism (18).

MCT10, another member of the amino acid transporter family, preferentially transports T_3 instead of T_4. MCT10 is expressed in kidney, liver, and muscle (20). Recent studies reported MCT10 mRNA expression in primary cultures of mouse astrocytes (21) and the human hypothalamus (22), but its functional role in the central nervous system is still unclear.

Only very sparse data are available on the effects of NTIS on thyroid hormone transporters in the hypothalamus. A study by Mebis *et al.* (23) in a rabbit model of critical illness reported unaltered hypothalamic MCT8 mRNA expression and clearly increased MCT10 and OATP1C1 expression. Because these changes in transporter expression were not related to increased local tissue T_3 concentrations, their functional consequences remain unclear at present (23).

2. Deiodinase expression

One of the major routes for metabolism of thyroid hormones is by deiodination through the iodothyronine deiodinases, together representing a selenocysteine-containing enzyme family consisting of three deiodinases, types 1 (D1), 2 (D2), and 3 (D3) (24). Both the inner (phenolic) ring and the outer (tyrosyl) ring of T_4 can be deiodinated by the deiodinases, ultimately leading to the formation of 3,3′-diiodothyronine (T_2). D1 is localized in the plasma membrane and is able to deiodinate both the inner and the outer ring of T_4. D1 expression is positively regulated by T_3 and expressed in liver, kidney, thyroid, and pituitary (25, 26). D2 is localized in the endoplasmic reticulum and deiodinates T_4 into the biologically active T_3. It is negatively regulated by thyroid hormone both pre- and post-transcriptionally because T_3 down-regulates D2 mRNA expression (27), whereas T_4 as well as rT_3 (both substrates for D2) increase D2 ubiquitination and subsequent proteasomal degradation, resulting in decreased D2 activity (28). D3 is localized in the plasma membrane and can be viewed as the major thyroid hormone-inactivating enzyme because it catalyzes inner-ring deiodination of T_4 and T_3 exclusively, yielding the biologically inactive rT_3 and rT_2 (29).

Deiodinases are differentially expressed in several organs, including the brain, and their expression changes dramatically during NTIS (16). Both D2 and D3 are expressed in the human hypothalamus. D2 is mainly expressed in glial cells of the infundibular nucleus (IFN) and median eminence and in cells lining the third ventricle, whereas D3 expression is predominant in neurons of the PVN, supraoptic nucleus, and IFN (19). Studies in rodents have shown that hypothalamic D2 is a determinant of hypothalamic T_3 production (30). In rodents, the administration of bacterial endotoxin [or lipopolysaccharide (LPS)], which is an established experimental model of acute NTIS, increases D2 mRNA expression (16) and enzyme activity in the hypothalamus, specifically in tanycytes lining the third ventricle (31). Increased D2 expression is not specific for the acute phase of illness because chronic inflammation in mice induces a short-lived increase of hypothalamic D2 expression as well (32), and hypothalamic D2 activity is up-regulated in mice with bacterial sepsis (33) and in rabbits with critical illness (23).

In contrast to the fasting-induced up-regulation of hypothalamic D2, which is related to the interplay between decreased serum leptin and increased serum corticosterone (34), the mechanism behind the induction of hypothalamic D2 during illness is still unclear. Remarkably, it appears to be independent of decreased serum thyroid hormone and increased corticosterone concentrations (35, 36). The dissociation between decreased serum thyroid hormone concentrations on the one hand and molecular changes in thyroid hormone metabolism at the tissue level on the other hand has been observed in many NTIS conditions (see *Sections III, IV and V*). Regarding the mechanism of hypothalamic D2 induction, activation of the nuclear factor κB (NFκB) pathway may be involved because the D2 promoter contains NFκB-responsive elements (31, 37). However, hypothalamic D2 activation precedes the hypothalamic up-regulation of NFκB, arguing against this notion (38). Moreover, inhibition of TNFα does not affect the LPS-induced stimulation of hypothalamic D2 despite reduced IL-6 levels, ruling out a major role for IL-6 or TNFα. A recent study showed that blocking TNFα does reduce the LPS-induced D2 response in a subpopulation of tanycytes located in the midportion of the third ventricle wall called α-tanycytes. In these cells, LPS induces the expression of Iκ-Bα (inhibitor of NF-κB α), which is a sensitive marker for cytokine signaling through the NFκB pathway (38).

In contrast to hypothalamic D2, the expression of D3 decreases during both acute (39) and chronic inflammation (32), whereas hypothalamic D3 activity is unaltered, with a tendency to higher levels in rabbits with prolonged critical illness (23). Although T_3 positively regulates D3 activity in brain (29), a causal role for decreased serum T_3 in lowering hypothalamic D3 during inflammation can be excluded because serum T_3 levels are similarly low in pair-

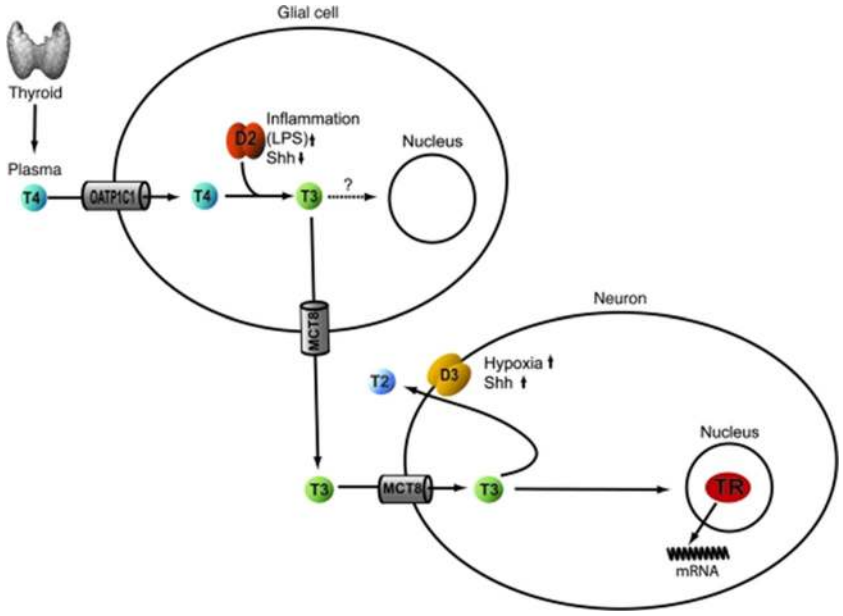

Fig. 1. Proposed model of thyroid hormone signaling in the brain during inflammation and hypoxic conditions. Three steps characterize this paracrine mechanism. First, the prohormone T_4 enters the glial cells (astrocytes and tanycytes) via the thyroid hormone transporter OATP1C1. Subsequently, T_4 is activated via D2, and the resultant T_3 exits the glial cell compartment and enters adjacent neurons via the thyroid hormone transporter MCT8. Finally, T_3 binds to neuronal TR and regulates transcriptional activity. LPS activates D2 transcription, and sonic hedgehog (Shh) promotes D2 inactivation via WSB-1–mediated ubiquitination; both hypoxia and Shh activate D3 gene transcription. [Adapted from B. C. Freitas *et al.*, Paracrine signaling by glial cell-derived triiodothyronine activates neuronal gene expression in the rodent brain and human cells. *J Clin Invest* 120:2206–2217, 2010 (50), with permission. © 2010, American Society for Clinical Investigation.]

fed control mice that do not display decreased hypothalamic D3 mRNA expression (32).

3. The role of the TR

T_3 is bound to the TR expressed in target tissues. TR are members of the nuclear receptor family and are encoded by two genes, TRα and TRβ. Due to alternative splicing and alternative promoter usage, the TRα gene may give rise to six isoforms: TRα1, TRα2, TR$\Delta\alpha$1, TR$\Delta\alpha$2, p43, and p28 (40). The TRα1 isoform is the only *bona fide* TRα because it has both a ligand binding and a DNA binding domain and modulates gene transcription (41–43).

The TRβ gene encodes the TRβ1 and TRβ2 isoforms. In contrast to the TRα, TRβ isoforms arise by alternative promoter usage exclusively, thereby differing in the N-terminal region (44). Both TRβ1 and TRβ2 bind T_3 and are able to modulate gene transcription (40). TR are differentially expressed in various organs. TRα1 is abundantly expressed in the brain (45), whereas the distribution of TRβ in the brain is probably more restricted. TRβ2 is expressed in a number of hypothalamic nuclei, including the human and rat PVN (46), and is assumed to be the predominant receptor involved in negative HPT axis feedback regulation (47).

Hypothalamic TR mRNA expression does not show dramatic changes during NTIS (23), but changes in specific TR isoform expression in PVN neurons have not been carefully evaluated during NTIS. This could be of interest because the LPS-induced increase in hypothalamic D2 and decrease in hypothalamic D3 are exaggerated in TR$\beta^{-/-}$ mice compared with wild-type (WT) mice (39), indicating that TRβ modulates the hypothalamic deiodinase responses to acute inflammation. In line, TR$\alpha^{0/0}$ mice do not display prominent changes in hypothalamic D2 and D3 responses to LPS (48).

4. Thyroid hormone action

Hypothalamic up-regulation of D2 and down-regulation of D3 during illness probably results in net increased local T_3 production, which may explain the decreased TRH mRNA expression in the PVN as observed during illness both in animals and in humans (32, 46, 49). Recently, Bianco and colleagues (50) used an *in vitro* coculture system, confirming that glial D2 modulates T_3 concentrations as well as neuronal D3 expression. The modulating role of LPS in these experiments reinforces the concept of increased hypothalamic T_3 during NTIS (schematically depicted in Fig. 1). Experiments in mice lacking TRβ showed a less pronounced illness-induced decrease of hypothalamic TRH compared with WT, again supporting a role for T_3 and TRβ in TRH suppression during NTIS (39).

Very few studies have addressed hypothalamic T_3 concentrations during NTIS directly. Mebis *et al.* (23) showed that prolonged illness in rabbits does not increase hypothalamic T_3 tissue concentrations despite increased D2 expression, perhaps related to difficulties in selectively harvesting IFN and PVN samples for the assessment of T_3 concentrations. In postmortem human hypothalamic specimens, however, T_3 concentrations were even lower in NTIS patients compared with patients with acute death from trauma (51).

An additional factor in the pathogenesis of the illness-induced down-regulation of hypothalamic hypophysiotropic TRH neurons may be regulation by afferent pathways projecting from brainstem nuclei to the PVN. LPS is known to modulate autonomic centers in the lower brainstem, in turn activating hypophysiotropic CRH neurons in the PVN (52, 53). Like CRH neurons, hypophysiotropic TRH neurons receive neural input from the brainstem. However, because transsection of these ascending pathways did not affect the LPS-induced decrease of TRH ex-

pression in the PVN, neural input from the brainstem appears to be relatively unimportant in this respect (54).

B. Pituitary response during illness

1. Thyroid hormone transport

Few data have been reported on the expression and function of thyroid hormone transporters in the pituitary. A study in human anterior pituitary showed marked MCT8 immunostaining in the folliculostellate cells (55). Animal studies reported that the pituitary of MCT8-null mice is unable to sense adequately high serum T_3, although the pituitary of these mice seems to be euthyroid (56). The effect of NTIS on the pituitary expression of thyroid hormone transporters has not been reported yet.

2. Deiodinase expression

Both D1 and D2 are expressed in the rodent anterior pituitary, whereas the human pituitary expresses D2 and D3 (55). Although this may represent a species difference, another potentially important factor is that human studies rely on postmortem investigations. By definition, this implies some degree of NTIS, as well as a postmortem delay of at least a few hours. By contrast, the animal studies are performed in tissues harvested and processed immediately after acute death. The current view of negative feedback of thyroid hormone on pituitary TSH involves local D2-mediated conversion of T_4 into T_3, which is subsequently bound by TR, finally resulting in repression of the TSHβ gene (55). The crucial role of pituitary D2 in TSH regulation is supported by impaired thyroid hormone feedback on TSH in D2 knockout mice (57). It is tempting to speculate that the mechanism for down-regulation of pituitary TSHβ during illness is similar to the repression of hypothalamic TRH during illness, *i.e.*, increased D2 expression with subsequent increased T_3 production. In line with this assumption, proinflammatory cytokines increase D2 mRNA expression and activity in reaggregates of rat anterior pituitaries via activation of NFκB (5, 58). However, whereas hypothalamic D2 increases in various NTIS models studied so far, the pituitary D2 response to LPS shows a marked variation, depending on genetic background, species, and type of illness (58–60). Moreover, the LPS-induced D2 increase in the rat hypothalamus is independent of circulating thyroid hormone levels, whereas the increase in D2 activity in the anterior pituitary is absent when thyroid hormone levels are normal (35).

LPS treatment decreases pituitary D1. This response depends on proinflammatory cytokines because it is completely abolished in mice devoid of IL-12, IL-18, and the interferon γ receptor (59, 60), but not in TR$\beta^{-/-}$ mice (39). The LPS-induced decrease of pituitary D1 is difficult to reconcile with T_3-induced repression of the TSHβ gene.

3. The role of the TR

TRβ2 protein is present in TSH-producing cells of the rat anterior pituitary (61, 62) and mediates the negative regulation of TSHβ mRNA expression by thyroid hormone (47). TRβ2 is also implicated in the regulation of pituitary D1 by thyroid hormone (63). Acute inflammation decreases TRβ2 mRNA expression in the pituitary (16). Because acute inflammation decreases pituitary D1 mRNA to the same extent in TR$\beta^{-/-}$ and WT mice (39), a role for TRβ2 in the down-regulation of pituitary D1 during acute inflammation is unlikely. In line, the illness-induced decrease in pituitary D1 mRNA expression is abolished in mice lacking IL-12, IL-18, and interferon γ. Finally, pituitary D1 expression decreases to a larger extent in TR$\alpha^{0/0}$ mice upon LPS administration despite a similar acute phase response, pointing to a possible role for TRα in this respect (48).

4. Thyroid hormone action

One of the hallmarks of NTIS is the unresponsiveness of the pituitary to the low serum thyroid hormone levels, which implies that the physiological negative feedback mechanism of the HPT axis is altered in a major way during illness (13). Increased pituitary D2 mRNA expression may contribute to locally increased T_3 concentrations, finally resulting in decreased pituitary TSHβ mRNA expression as has been observed in rodents. Alternatively, high T_3 concentrations in the pituitary may result indirectly from increased D2 activity in hypothalamic tanycytes, increasing T_3 concentrations in the portal capillary system, which may then suppress TSHβ mRNA expression in the anterior pituitary via the bloodstream (64). The assumption of elevated pituitary T_3 concentrations during NTIS is, however, not in line with observations in human pituitary specimens showing low pituitary T_3 concentrations in patients with NTIS compared with control patients who died from sudden trauma (51).

A role for the TR, and thus for T_3, is strongly supported by the observation that lacking the TRβ gene prevents the LPS-induced decrease of pituitary TSHβ mRNA (39). A role for D2 in altered TSH expression during illness is questionable, however, because LPS decreases TSHβ mRNA expression in the pituitary whereas D2 expression may decrease (16) or increase (39).

The administration of exogenous TSH to mice during LPS-induced NTIS attenuates the decline in serum T_4 (65), strengthening the concept that central down-regulation of the HPT axis is one of the determinants of the decrease in serum thyroid hormone levels.

Recently, the existence of two novel glycoprotein subunits, α2 (GPA2) and β5 (GPB5), was reported, assumed to heterodimerize into a glycoprotein hormone coined

"thyrostimulin." Thyrostimulin activates the TSH receptor (TSHR) both *in vitro* and *in vivo* (66). Because GPA2 and GPB5 colocalize in the rat and human anterior pituitary where the TSHR is expressed, a paracrine role for thyrostimulin was suggested (67, 68). However, the putative biological role for the heterodimer *in vivo* is still a matter of debate (69–71). Because proinflammatory cytokines (TNFα and IL-1β) regulate transcription of the GPB5 subunit via activation of NFκB in a murine pituitary corticotroph cell line, Suzuki *et al.* (72) postulated that thyrostimulin may play a role in the pathogenesis of NTIS. Subsequent *in vivo* studies in mice showed LPS-induced upregulation of GPB5—but not GPA2—transcription in the pituitary and hypothalamus, whereas lacking the GPB5 subunit reduced LPS-induced suppression of TSHR mRNA in the same tissues. Although these observations confirmed a role for GPB5 in the regulation of TSHR expression during acute illness, this was not reflected in illness-induced alterations of serum thyroid hormone levels (73), again pointing to a dissociation between low serum thyroid hormone levels during NTIS and local thyroid hormone metabolism at the organ level.

III. Differential Effects of Acute Inflammation on Thyroid Hormone Metabolism in Metabolic Organs

Although there is no question that illness profoundly affects the central part of the HPT axis, it is still unclear whether these hypothalamic and pituitary changes suffice to induce the marked decrease in serum thyroid hormone levels observed during NTIS. Interestingly, recent experimental studies have shown that down-regulation of the central part of the HPT axis observed during inflammation does not necessarily induce decreased thyroid hormone metabolism in key metabolic organs such as liver, muscle, and adipose tissue.

Local thyroid hormone metabolism during acute inflammation has not been studied extensively in humans. The effects of major surgery on the HPT axis have been investigated as a model for acute NTIS because major surgery induces a rapid inflammatory response characterized by activation of neutrophils and the release of a variety of proinflammatory cytokines (74–76). For example, patients undergoing cardiac surgery show significant alterations in serum T_3, T_4, and rT_3 concentrations and in T_3/rT_3 and T_3/T_4 ratios, suggestive of impaired thyroid hormone conversion (77). Nevertheless, animal studies are still needed to determine the effects of acute inflammation on thyroid hormone metabolism within metabolic organs. For this purpose, LPS can be used as an established animal model of acute NTIS. LPS elicits a strong inflammatory response characterized by the production of a variety of cytokines such as TNFα, IL-1, and IL-6 via the activation of signaling pathways like NFκB and activator protein-1 (AP-1) (78).

A. Hepatic thyroid hormone metabolism during acute illness

One of the key target organs of thyroid hormone is the liver, where it acts as a determinant of hepatic glucose and lipid metabolism. In liver, TRβ1, TRα1, and TRα2 isoforms are abundantly expressed, and approximately 60% of liver T_3-regulated genes are TRβ-dependent. Furthermore, both D1 and D3 are expressed in liver, although D3 is expressed only at very low levels in a healthy liver. For many years, liver D1 was thought to be the major source of plasma T_3 resulting from deiodination of T_4 (1). Because liver D1 expression decreases during acute inflammation in rodents, this was assumed to contribute significantly to the low serum T_3 levels observed during illness (16). Liver D1 regulation is primarily driven via the TRβ (79, 80), but only sparse *in vitro* work has been performed on the effects of cytokines on liver TR expression. TR binding capacity is decreased in the human hepatoma cell line HepG2 after stimulation with TNFα, IL-1, and IL-6 (81), whereas TRβ and TRα mRNA expression decrease in HepG2 cells upon stimulation with IL-1β. The IL-1β-induced decrease of TRβ is exclusively mediated by the NFκB pathway, whereas the IL-1β-induced decrease of TRα mRNA is abolished only by simultaneous inhibition of NFκB and AP-1 (82, 83). *In silico* analysis (84) revealed the presence of three NFκB-responsive elements in the TRβ promoter, which may explain the NFκB-dependent repression of the TRβ gene. The IL-1β-induced decrease of TRα1 and TRα2 expression is a direct effect of decreased promoter activity (83). The exact mechanism remains unknown, but may involve phosphorylation-dependent repression of the TRα promoter. In animal studies, liver TRα and TRβ1 expression decreases after LPS administration (16, 85).

Because the D1 gene is activated via a TR/retinoid X receptor (RXR) heterodimer, decreased D1 expression during acute inflammation may result from reduced TR expression. Alternatively, the decrease in nuclear RXRα protein may play a role because this protein decreases after LPS administration due to rapid nuclear export via Jun N-terminal kinase (JNK) phosphorylation and subsequent proteasomal degradation (86). Because the IL-1β-induced decrease of D1 mRNA is not prevented by inhibition of JNK alone (82), it is unlikely that decreased RXR expression is the only factor responsible for the decrease in D1 mRNA observed during acute illness. In addition, because lacking TRβ does not prevent the illness-induced decrease in liver D1 mRNA either, a key role of TRβ in this respect

is unlikely as well (87). Surprisingly, experiments in TR$\alpha^{0/0}$ mice showed that the down-regulation of liver D1 is partly mediated via the TRα (88), probably resulting from diminished D1 promoter activity due to the effect of cytokines (89, 90).

An alternative mechanism for TRβ-mediated repression of liver D1 during acute inflammation has been proposed by Yu *et al.* (91, 92), who showed both *in vivo* and *in vitro* that adding exogenous coactivator steroid receptor coactivator-1 (SRC-1) attenuates the illness-induced liver D1 decrease. These studies indicate that competition for limiting amounts of SRC-1, which is a shared coactivator for TR and inflammatory signaling pathways, is one of the mechanisms involved in the illness-induced D1 decrease (Fig. 2). Interestingly, restoration of liver D1 expression by exogenous SRC-1 in the study by Yu *et al.* (91) prevented the fall in serum thyroid hormone levels after LPS. Deiodinating enzymes require an endogenous intracellular thiol cofactor for catalytic activity. Glutathione (GSH) has been proposed to be one of these factors necessary for D1 activity *in vivo*. As a consequence, changes in the intracellular concentrations of GSH may play a role in the regulation of D1 during NTIS. A recent study showed T_3 production by D1 in intact cells to be suppressed by IL-6. The addition of N-acetyl-cysteine, an antioxidant that restores intracellular GSH levels, prevented the IL-6-induced inhibitory effect on D1-mediated T_3 production. This suggests that the effect of IL-6 on D1 activity is mediated by intracellular GSH (93).

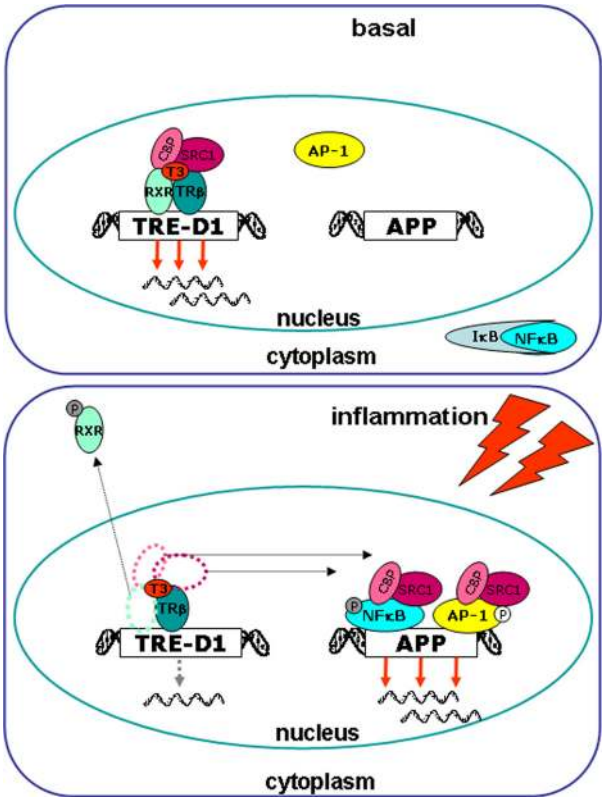

Fig. 2. Schematic model of the proposed mechanisms involved in illness-induced down-regulation of liver D1 expression. In this model, limited amounts of SRC-1, a coactivator for both NFκB and TRβ1, decrease liver D1 mRNA expression as a result of competition between cytokine-induced NFκB and the TRβ1/D1 complex for SRC-1. Note the role of decreased nuclear RXRα protein by LPS-induced rapid nuclear export via JNK-phosphorylation and subsequent proteasomal degradation. APP, Acute phase protein; TRE, thyroid hormone responsive element.

However, a dominant role for impaired type 1 deiodination in lowering serum T_3 levels is not supported by the observation that illness-induced alterations in serum T_3 and T_4 levels are similar between WT and TR$\beta^{-/-}$ mice despite marked differences in liver D1 mRNA expression and activity (87).

Similar to hypothalamic D3, liver D3 decreases during acute inflammation (94). This may be mediated via TRβ because the LPS-induced decrease in hepatic D3 is abolished in TR$\beta^{-/-}$ mice and aggravated in TR$\alpha^{0/0}$ mice (88). These studies suggest a dominant role of TRβ in the illness-induced regulation of liver D3 because TR$\alpha^{0/0}$ mice have more sensitive TRβ signaling (80). Involvement of TRβ in the regulation of hepatic D3 suggests a role for T_3. However, the time course of the changes in serum T_3 and liver D3 expression after LPS administration does not support this (94).

To date, no *in vitro* studies reporting decreased D3 expression upon cytokine administration have been published. By contrast, TGF-β stimulates D3 expression in primary human fibroblasts, and this effect is abolished by inhibition of the MAPK ERK and p38 (95).

The decrease in liver D3 does not appear to contribute to changes in serum thyroid hormone levels because illness-induced alterations in serum T_3 and T_4 are similar in TR$\beta^{-/-}$ and WT mice despite differences in liver D3 expression (87). This was recently supported by a similar decrease of serum thyroid hormone levels in *Streptococcus pneumoniae*-infected D3 knockout (D3KO) mice compared with WT mice (33).

Liver MCT8 and MCT10 transporter expression has not been studied extensively. Mebis *et al.* (96) studied MCT8 and MCT10 expression in postmortem liver biopsy samples of prolonged critically ill patients compared with patients after elective abdominal surgery. They observed lower liver MCT8 mRNA expression during acute surgical stress compared with prolonged illness, whereas liver MCT10 expression did not differ between the groups. For obvious reasons, it was not possible to compare hepatic transporter expression in both groups to healthy controls, which makes the study difficult to interpret (96).

The combination of low liver D1 and low liver D3 should theoretically result in only moderate changes in liver T_3 content. It is tempting to speculate that low liver

D3 activity during the acute state of illness compensates for reduced production of T_3 by D1 and thereby helps to maintain organ function. To our knowledge, no human or animal studies have been performed reporting liver thyroid hormone concentrations during the acute state of inflammation. As an indirect measure of local T_3 availability, the determination of T_3-regulated gene expression in liver during acute illness is an interesting option because it reflects local thyroid status. T_3 is known to stimulate a number of genes, *e.g.*, liver malic enzyme (ME) (97), Spot 14 (98), and cholesterol 7α-hydroxylase (Cyp7a) (99). It has been shown that LPS results in a rapid decrease of liver Cyp7α mRNA expression in Syrian hamsters (100) and liver ME and Spot 14 mRNA expression in mice (85). These observations are in keeping with reduced T_3 action in the liver during acute inflammation, possibly reflecting decreased T_3 liver content. However, several caveats should be taken into account: liver TR expression decreases during the acute phase of illness (16, 85), making it impossible with this approach to discriminate between reduced T_3 action and reduced T_3 content. Furthermore, the kinetics of the observed decreases in T_3-regulated gene expression are in disagreement with low liver deiodinase activity levels, making a key role for diminished T_3 availability less likely. Future studies reporting liver T_3 content during acute inflammation are needed.

B. Muscle thyroid hormone metabolism during acute illness

Thyroid hormones are key players in skeletal muscle function because a variety of genes expressed in muscle are positively or negatively regulated by T_3. Skeletal muscles consist of a mixture of muscle fiber types. The type 1 fibers are innervated by slow motor neurons and found in low-twitch muscles. These fibers are rich in mitochondria and rely on oxidative phosphorylation for ATP generation. Lipids are their preferred substrate, whereas glycogen content is low. By contrast, type II fibers are active during high-frequency contraction and in need of high ATP turnover resulting from glycolysis (type IIA) or anaerobic glycolysis yielding lactate (type IIB). Most skeletal muscles contain both type I and II fibers. Muscle activation starts with the recruitment of type I fibers, which is followed by the activation of type II fibers. Type I fibers are highly dependent on thyroid hormone status, whereas type II fibers act relatively independent of thyroid hormones (101). The molecular machinery expressed in muscle required for thyroid action includes D2, D3, TRα1 [the major TR isoform in muscle (102)], TRβ1, three RXR isoforms, and the thyroid hormone transporters MCT8 and MCT10 (96, 103). Human skeletal muscle D2 was reported to be involved not only in local T_3 production but also in peripheral production of T_3 in the basal condition (104). However, this concept was recently challenged because D2 is expressed only at very low levels under euthyroid conditions in healthy persons (105). Interestingly, muscle D2 mRNA expression is modulated by fasting and by insulin serum levels (106), suggesting that muscle D2 is involved in energy metabolism. Several recent studies have reported alterations in muscle thyroid hormone metabolism during NTIS, both in acute and in more chronic models.

Acute inflammation in rodents increases D2 mRNA expression in skeletal muscle, whereas the effects on D2 activity are unknown at this stage (87). The increase in muscle D2 transcription is mirrored by a decrease in D3 mRNA and TRα1 mRNA expression (88). TR expression has also been studied in the diaphragm, which is a ventilatory muscle with high energy demands, relying on lipid oxidation. A sublethal dose of LPS to mice profoundly decreases TRα1 and TRβ1 mRNA expression in the diaphragm in association with decreased expression of coactivators involved in nuclear receptor activation (107).

The role of TR isoforms in altered muscle D2 and D3 expression has been studied in TR knockout mice. The LPS-induced decrease of muscle D3 is attenuated in TR$\alpha^{0/0}$ mice, suggesting the involvement of TRα. The regulation of muscle D3 via TRα is supported by lower basal muscle D3 levels in TR$\alpha^{0/0}$ mice, but difficult to reconcile with the observation that LPS decreases TRα expression in forelimb but not in hindlimb muscle, whereas D3 is affected in both limbs. The LPS-induced increase of muscle D2 is more pronounced in TR$\beta^{-/-}$ mice, suggesting that TRβ and/or T_3 partly suppress D2 induction during illness (80).

Comparison of MCT8 and MCT10 expression in muscle tissue of prolonged critically ill patients with patients after elective abdominal surgery showed remarkably low MCT8 mRNA expression during acute surgical stress compared with prolonged illness. By contrast, MCT10 expression was similar between the groups. Acute surgical stress patients displayed somewhat lower serum T_3 levels compared with reference values. However, because MCT8 and MCT10 expression levels are unknown in muscle of healthy persons, no conclusion can be drawn at this stage about the possible role of these thyroid hormone transporters in altered serum concentrations of thyroid hormone during NTIS (96). The same authors also studied D2 mRNA expression and activity in skeletal muscle tissue of patients with acute surgical stress compared with a healthy control group. A significant decrease in serum T_3 levels was observed during the surgical procedure, but D2 mRNA expression and activity in muscle were unaltered compared with controls (108).

In summary, D2 increases whereas D3 decreases in skeletal muscle during acute inflammation. The changes in

deiodinase expression coincide with decreased TR expression. The combination of increased D2 and decreased D3 is likely to result in increased local T_3 availability. Two important questions that should be addressed are whether this assumption is supported by any significant changes in T_3 target gene expression in muscle and whether the observed changes in thyroid hormone metabolism in muscle are clinically relevant.

Few data have been reported on expression levels of T_3 target genes in muscle tissue during acute inflammation. Muscle uncoupling protein (UCP) 3, a T_3 target gene, has been studied in a rat model of sepsis involving cecal ligation and puncture, which is an acute sepsis model characterized by a systemic inflammatory response. Both UCP3 mRNA and protein expression increased in skeletal muscles of rats 16 h after cecal ligation and puncture (109). Likewise, LPS administration to mice resulted in increased UCP3 mRNA expression in skeletal muscle, preceded by a rapid increase of the T_3-responsive transcription factor PGC-1 (110). Similarly, a study in humans showed increased UCP3 expression in skeletal muscle of NTIS patients, coinciding with increased D2 mRNA expression, although a variety of other T_3 target genes (SERCA, MHC, and GLUT4) were unaltered (111). In contrast, GLUT4 expression decreased after LPS administration in the diaphragm of mice (107).

Taken together, these results suggest a selective increase in T_3 signaling in skeletal muscle tissue during acute inflammation. A complicating factor is simultaneous downregulation of TRα1 and TRβ1 mRNA expression in muscle during acute inflammation, which is contradictory at first sight. However, whereas serum T_3 contributes approximately 50% to TR occupancy in most tissues, TR saturation in D2-expressing tissues is much higher due to local generation of T_3 (29). The TR heterodimer associates, in the absence of T_3, with corepressor proteins, which results in the repression of basal transcriptional activity (aporeceptor function) (112). Thus, decreased TR expression combined with increased production of local T_3 by D2 is likely to result in near maximal occupancy of the TR. The resulting maximal activation of T_3 target genes supports local activation of T_3-regulated genes.

The clinical relevance of increased muscle T_3 signaling is unclear at present. Thyroid hormone promotes the shift from slow to fast muscle fiber types and regulates contractibility (101). T_3 also increases the glycolytic and oxidative capacities of skeletal muscle and activates the insulin-activated glucose transporter GLUT4. An association between a mutation in the D2 gene (*Dio2*Thr92Ala) and insulin resistance has been established in healthy volunteers (113). This polymorphism is associated with diminished muscle D2 activity, suggesting that reduced local T_3 availability might be involved in insulin resistance (114). Of note, a recent case-control study and meta-analysis showed an association between the Thr92Ala polymorphism in the *Dio2* gene and type 2 diabetes, confirming earlier reports of an association with insulin resistance (115). Because acute inflammation results in hyperinsulinemia in rodents (116) and activation of the TLR4 by LPS in skeletal muscle induces a shift in substrate handling from lipids (preferred substrate type 1 fibers) to glycogen (preferred substrate type 2 fibers), a functional role for altered T_3 availability in the adaptation of muscle metabolism and function during NTIS seems likely (107, 117).

Another potentially relevant aspect is the capacity of both T_3 and T_2 to increase mitochondrial activity via cytochrome c oxidase (118, 119). In addition, T_3 is able to induce transcription of the mitochondrial genome via p43, a truncated isoform of the TRα, which is located in the mitochondrial matrix. Finally, stimulation of mitochondrial biogenesis probably involves genomic action of T_3 via TRα1 or TRβ1 (120). This complex interaction between thyroid hormone and mitochondrial function makes it very difficult to define a role for T_3 in skeletal muscle mitochondria during acute illness, without the availability of experimental data to discriminate between short-term and long-term effects, and between nongenomic and genomic effects. However, it is tempting to speculate at this stage that T_3 is beneficial in improving muscle function in the acute state of illness by affecting a variety of target genes relevant for muscle metabolism and mechanical function.

C. Thyroid hormone metabolism in adipose tissue during acute illness

Adipose tissue is a key metabolic organ that stores energy-rich fuels mainly as triglycerides (TG). In times of energy shortage, lipolysis is stimulated both via the central and autonomic nervous system (adrenaline and noradrenaline) and via endocrine routes. Acute inflammation stimulates lipolysis in adipose tissue while decreasing TG synthesis, explaining in part the increased serum free fatty acid levels observed during acute illness (121).

Thyroid hormone is one of the major determinants of lipid metabolism. For instance, thyrotoxicosis increases TG-derived fatty acid uptake in muscle and heart, whereas hypothyroidism increases TG-derived fatty acid uptake in white adipose tissue in association with increased lipoprotein lipase activity (122). White adipose tissue expresses multiple genes involved in thyroid hormone transport and metabolism, such as MCT8, D1, D3, TRα1, TRβ1, RXRγ, and a variety of coactivators in-

volved in TR signaling (121), making a metabolic role for local thyroid hormone metabolism likely.

The effect of acute inflammation on thyroid hormone metabolism in adipose tissue has not been extensively studied. No data are available on illness-induced alterations in deiodinase expression. As for effects on TR, LPS administration to mice suppresses the mRNA expression in white adipose tissue of a variety of nuclear hormone receptors, including TRα1 and TRβ1. This is associated with a decrease in coactivators and reduced expression of ME and Spot 14, both T_3 target genes (121). In addition, LPS markedly decreases GLUT4 and phosphoenolpyruvate carboxykinase expression, reducing glycerol-3-phosphate production in adipose tissue, which is supposed to limit TG synthesis. Limiting TG synthesis in white adipose tissue may help to shuttle free fatty acids to the liver, promoting TG synthesis and very low-density lipoprotein production in liver necessary for an adequate acute phase response (123). Against this background, reduced T_3 action in white adipose tissue during acute illness might be a beneficial adaptation. Although one study suggests a link between altered thyroid hormone and lipid metabolism in adipose tissue during acute illness (121), the precise role of thyroid hormone has yet to be established.

IV. Differential Effects of Chronic Inflammation and Sepsis on Thyroid Hormone Action in Metabolic Organs

Chronic inflammation and sepsis are associated with markedly decreased plasma thyroid hormone levels. The severity of illness is reflected in the magnitude of the decrease in serum T_3. In critical illness, defined as a clinical condition requiring prolonged support for failing vital organs (mechanical ventilation, need for inotropics), serum T_3 may become very low or even undetectable. In severely ill patients, serum T_4 decreases as well and is inversely correlated with mortality (1). Human studies in the intensive care unit (ICU) setting have shown that decreased serum thyroid hormone levels are associated with changes in deiodinase expression in liver and muscle. From recent animal experiments, the mechanism of these changes in muscle and liver appears to be very different from the changes in acute inflammation. Studying chronic inflammation in experimental animals requires a rigorous methodology because reduced food intake during illness interferes in a major way with thyroid hormone metabolism at the organ level (106, 124, 125). Interestingly, the molecular effects of chronic inflammation and sepsis on thyroid hormone metabolism appear to depend on the organ studied.

A. Hepatic thyroid hormone metabolism during chronic inflammation

The effects of chronic inflammation on hepatic thyroid hormone metabolism have been studied in rodents by inducing a sterile abscess with turpentine inoculation in a hindlimb. This experimental model resembles local tissue injury and inflammation and leads to serial activation of specific proinflammatory cytokines that are associated with decreased serum thyroid hormone levels and reduced food intake (32, 94, 126). In contrast to LPS-induced acute inflammation, liver D1 mRNA expression is unaffected in mice with chronic inflammation, whereas liver D3 mRNA expression and activity decrease in both models (94). A role for cytokines in decreasing liver D3 expression is unlikely because the decrease in liver D3 mRNA precedes the increase in liver IL-1β mRNA. Corticosteroids may be involved because dexamethasone acutely decreases D3 expression in chicken liver (127), whereas both acute and chronic inflammation increase serum corticosterone (39, 128). The role of diminished food intake on the observed alteration in liver deiodinase expression is unknown at present but may certainly interfere because it has been shown that food restriction in chickens increases liver D3, whereas liver D1 is not affected (129). The role of T_3 can be excluded because serum T_3 decreases to a similar extent in mice with chronic inflammation and pair-fed controls, whereas serum T_4 and rT_3 levels are lower during inflammation than in pair-fed controls (32). Apart from the effect on deiodinases in liver, no consistent changes in liver TRα1, TRα2, and TRβ1 mRNA expression are reported in turpentine-treated rats (130).

In the clinical setting, Chamba *et al.* (131) studied hepatic TRα1, TRα2, and TRβ1 and T_3 target gene expression in patients with a variety of chronic liver diseases and found no changes in TR or T_3 target gene expression despite reduced serum thyroid hormone levels, indicating a euthyroid condition in the liver of these patients. These findings are in line with the chronic inflammation mouse and rat models published to date and represent another example of a dissociation between serum thyroid hormone levels and thyroid hormone action at the organ level during NTIS.

B. Muscle thyroid hormone metabolism during chronic inflammation

Although the animal model of chronic inflammation needs inoculation of turpentine in the hindlimb, which results in an abscess located between muscle and subcutis, the inflammatory response is not restricted to the injected muscle and can be characterized as a systemic acute phase response. In these mice, D2 mRNA expression and activity increase in the (noninjected) forelimb muscle. This phenomenon is unrelated to serum thyroid hormone levels,

proinflammatory cytokines, and activation of inflammatory pathways or D2-ubiquitinating factors. However, activation of the cAMP pathway does coincide with the simultaneous induction of D2 in muscle tissue of chronically inflamed mice (132), pointing to a role of cAMP in D2 induction as has been reported in brown adipose tissue (133). Unexpectedly, D3 mRNA expression and activity increase simultaneously with D2 (132). Dentice *et al.* (134) studied the effects of local muscle injury induced by a snake toxin on D2 mRNA expression and activity. Without any effect on serum thyroid hormone levels, muscle D2 appeared to increase within 1 wk and was followed by regeneration of muscle tissue. Interestingly, muscle regeneration was significantly impaired in Dio2$^{-/-}$ mice, although no morphological differences were observed early after injury between Dio2$^{-/-}$ and WT mice. Thus, injury-induced D2 induction in muscle is likely to be an adaptive response. Forkhead O3 (FoxO3), a forkhead box transcription factor involved in the regulation of a variety of cellular functions such as differentiation and proliferation, is probably important for D2 induction during differentiation (134). It is unknown at present whether FoxO3 also plays a role in turpentine-induced D2 induction in muscle. Recent studies reported D2 up-regulation in skeletal muscle by bile acids via the G protein-coupled receptor TGR5, followed by an increase in mitochondrial activity (135). In addition, activation of the peroxisome-proliferator activated receptor γ, which is an important metabolic regulator, increases D2 expression in skeletal muscle cells as well (136). The role of bile acids during chronic inflammation in muscle is not completely understood at present.

Muscle thyroid hormone metabolism has also been studied in patients with noninflammatory NTIS and in patients undergoing hip or knee replacement. Skeletal muscle biopsies in the NTIS group were taken within the first 48 h after ICU admission and in the control group during hip or knee surgery. Thus, the design of this study does not allow for a classification as either chronic or prolonged illness. In the NTIS patients, serum T_3 was lower, whereas serum rT_3, TNFα, IL-6, IL-8, and IL-2R were higher despite similar NFκB activation. Skeletal muscle displayed lower TRα1, TRβ1, RXRγ, and D2 mRNA expression compared with controls, whereas MCT8 expression did not differ (111), suggestive of decreased thyroid hormone action in this model.

In contrast to acute illness that leads to increased D2 and decreased D3 expression, chronic inflammation results in simultaneously increased D2 and D3 activity. This will inevitably result in a coordinated breakdown of both T_4 and T_3, finally leading to increased intracellular T_2 levels as depicted in Fig. 3 (137). Both T_3 and T_2 are known

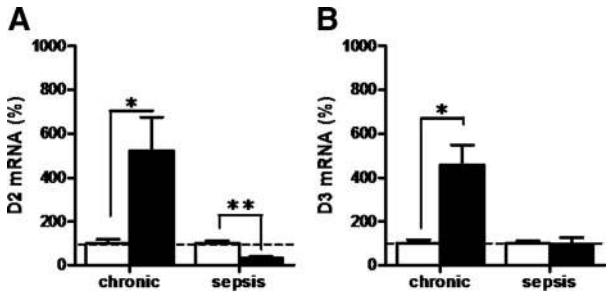

Fig. 3. Muscle D2 (A) and D3 (B) mRNA expression during chronic inflammation (turpentine injection in the hindlimb) and sepsis (intranasal inoculation of *S. pneumonia*). Control mice are pair-fed. *Dark bars* represent inflammation at 48 h, and *open bars* represent saline-treated controls. *Bars* represent mean values (n = 6) and SEM of mRNA expression relative to HPRT, which is a housekeeping gene. The *dotted line* indicates expression in the control group set at 100%. Differences between specific groups are indicated by *, $P < 0.05$; and **, $P < 0.01$. (Data summarized from Ref. 132.)

to regulate mitochondrial activity in skeletal muscle (118, 119), and the question is whether a shift toward local T_2 and away from local T_3 production might be relevant.

We have proposed that increased muscle T_3 concentration might be beneficial during acute illness by supporting the illness-induced shift in substrate handling from lipids to glucose (107, 117) and by increasing mitochondrial activity (118, 119). Transition from the acute to chronic state might be accompanied by alterations in the metabolic state of skeletal muscles. The IL-1-induced increase in plasma lactate and lactate content in skeletal muscle probably lowers local pH (138). T_2 activates cytochrome c oxidase most efficiently at pH 6.4, whereas for T_3 the optimal pH is 7.4 (139). Thus, increased muscle T_2 production during chronic inflammation may occur as an adaptation to altered muscle metabolism.

C. Hepatic thyroid hormone metabolism during bacterial sepsis

Sepsis, a whole-body inflammatory state caused by bacterial infection, is a major complication in critically ill patients associated with poor outcome (140). Bacterial peritonitis and pneumonia are the two most common causes of sepsis (141, 142). It is well known that sepsis is associated with profound alterations in thyroid hormone serum levels in both humans and experimental animal models (143–145), closely resembling those observed in acute inflammation. Among the differences between experimental sepsis and LPS-induced acute inflammation are the magnitude of the inflammatory response and the severity of illness because sepsis is often lethal, whereas LPS-induced acute inflammation is a sublethal model. Sepsis is characterized by activation and deactivation of the systemic inflammatory response. Activation is reflected by the production of proinflammatory cytokines, increased endothelial activation, and activation of immune effector

cells like granulocytes, monocytes, and lymphocytes. The dampening of the inflammatory response, on the other hand, is characterized by the production of antiinflammatory cytokines like IL-10, IL-1 receptor antagonist, and by impaired up-regulation of granulocytes in response to proinflammatory signals. Uncontrolled activation of both pro- and antiinflammatory responses can lead to cell exhaustion, organ dysfunction, and death (146).

Liver thyroid hormone metabolism has not been studied extensively in sepsis patients. One study in critically ill children with sepsis or septic shock showed the expected profound alterations in serum thyroid hormone concentrations. Based on these changes, the authors suggested altered peripheral deiodination including an induction of liver D3 and inhibition of liver D1 as an explanation (147). However, this pattern was not confirmed by animal experimental studies performed in well-characterized sepsis models. Experimental sepsis can be induced by inoculation with *S. pneumoniae*, which is an important pathogen in a majority of clinical pneumonia cases (148). *S. pneumoniae* administration to mice induces severe bronchopneumonia and stimulates the production of TNFα, IL-1, IL-6, IL-10, and the chemokines (cytokine-induced neutrophilic chemoattractant) and macrophage inflammatory protein-2 (14, 149). In this sepsis model, serum thyroid hormone levels and TSH decrease in association with decreased expression of both liver D1 and D3 (33). Liver D3 mRNA and activity have also been studied in abdominal sepsis model induced by *Escherichia coli*, an enteric Gram-negative bacterium (150). Again, in this model liver D3 expression was decreased (151).

A requirement for appropriate D1 synthesis is the availability of selenium (Se). This trace element is critical to the function of D1, although studies have shown that Se deficiency only affects liver D1 activity and not thyroidal and pituitary D1. Despite the fact that plasma Se levels are decreased in severe illness, Se substitution to patients with severe sepsis failed to restore serum thyroid hormone levels (152).

The putative role of deiodinating enzymes in the observed alterations in serum thyroid hormone concentrations has been studied in D3 null (D3KO) mice. These mice display similar changes in thyroid hormone concentrations during sepsis as WT mice, which is a strong argument against a pivotal role for D3 in the pathogenesis of low serum thyroid hormone levels during sepsis. In addition, basal liver D1 activity is relatively low in D3KO mice and not responsive to sepsis, suggesting that changes in liver D1 are not critical either for the decrease in serum thyroid hormone levels (33).

In summary, liver thyroid hormone metabolism during experimental sepsis is characterized by decreased D1 and D3 activity and accompanied by unaltered TRβ1 expression. Because no information is present about liver T_3 content during sepsis, it is still puzzling whether the observed changes in liver deiodinase expression decrease local T_3 availability. Local T_3 availability may be determined both by serum thyroid hormone levels and by tissue deiodinase activities. The inhibition of hepatic D3 during sepsis might additionally affect local tissue T_3 concentrations (29), but it is unknown at present whether T_3-regulated gene expression is affected in liver during sepsis.

D. Muscle thyroid hormone metabolism during bacterial sepsis

In patients with septic shock, muscle thyroid hormone metabolism was compared with patients undergoing knee or hip replacement, representing acute surgical stress. Muscle D2 mRNA expression decreased during sepsis, whereas D2 activity was hardly detectable and did not differ from the control subjects undergoing surgery. By contrast, muscle D3 activity increased in septic patients compared with controls, possibly enhancing T_3 clearance in muscle. Furthermore, TRβ1 mRNA as well as RXRγ mRNA decreased during sepsis, whereas MCT8 expression did not change (103).

In animal models, muscle D2 expression decreased during *S. pneumoniae* infection, corresponding to the decrease reported in muscle tissue of septic patients (103). An effect of fasting in decreased D2 expression during sepsis cannot be excluded at this stage because D2 expression decreases by fasting and sepsis is associated with severely diminished food intake (106). During *S. pneumoniae* infection D3 mRNA expression was unchanged, whereas D3 activity tended to increase (132). These changes in skeletal muscles of septic mice were not related to serum T_3 levels and correlated negatively with muscle IL-1β mRNA expression. The signaling pathways of CREB, NFκB and ERK1/2 were not altered in muscles of septic mice compared with controls (132), implying involvement of noninflammatory mechanisms.

The combination of decreased D2 expression and unaltered or increased D3, observed in both human and experimental models for sepsis, may result in decreased T_3 bioavailability in muscle tissue. However, muscle UCP3 mRNA expression, which is a T_3 target gene, is unaltered in muscle tissue of septic patients compared with control patients (103). Thus, down-regulation of TR expression might be an adaptation to restore TR occupancy.

A shortage of T_3 may result in mitochondrial dysfunction, which is thought to play a role in the pathogenesis of sepsis (153, 154). Adding exogenous cytochrome c oxidase improves cardiomyocyte mitochondrial function in an animal model of sepsis (155). Furthermore, be-

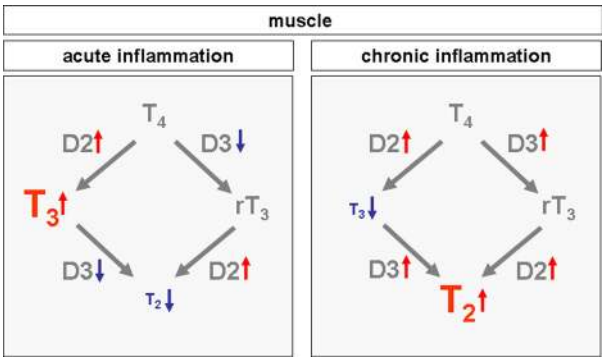

Fig. 4. Schematic representation of the alterations in muscle deiodinase expression in acute inflammation (*left*, LPS administration) and chronic inflammation (*right*, turpentine-induced abscess) and the theoretical net result on local T_3 and T_2 concentrations. Note the increase in T_3 in acute inflammation, and the increase in T_2 in chronic inflammation.

cause thyroid hormone is an important regulator of muscle fiber type and contractibility (101), changes in muscle deiodinase expression and, by inference, in local thyroid hormone availability might contribute to critical illness myopathy, which is frequently observed in ICU patients (156).

The complex discrepancies in muscle deiodinase expression between acute inflammation, chronic inflammation, and sepsis (Fig. 4) may relate to the severity of illness and support the concept that the net result of altered muscle deiodinase expression determines muscle function during specific stages of illness.

E. Thyroid hormone metabolism in adipose tissue during bacterial sepsis

Thyroid hormone metabolism has been studied in sc white adipose tissue of NTIS patients with septic shock within 5 d after admission to the ICU and compared with patients with acute surgical stress. White adipose tissue expresses MCT8, TRβ1, TRα1, RXRα, RXRβ, and RXRγ and also D1, D2, and D3 (103). Sepsis appeared to be associated with moderately decreased MCT8, TRβ1, TRα1, and RXRγ mRNA expression, whereas no differences were present in D1 and D2 mRNA expression. D1 and D3 activities were measured in adipose tissue samples and showed no differences (103). Thus, septic shock seems to be associated with decreased T_3 uptake and TR expression in adipose tissue. Surprisingly, UCP3, a T_3 target gene, increased in adipose tissue during sepsis (103) suggesting increased T_3 signaling, again showing the dissociation between T_3 uptake and TR expression on one hand and T_3 action on the other. Alternatively, T_3 target gene expression might be affected by other genes. Because increased T_3 signaling in adipose tissue during sepsis is opposite to decreased T_3 signaling observed during nonlethal acute inflammation, the question remains whether the observed alterations are an active response to the septic state or rather represent a derangement of the tissue-specific response during inflammation, characteristic for sepsis.

F. Deiodinase expression in granulocytes

In addition to complex changes in deiodinase expression in muscle during illness, we recently reported strong D3 expression in infiltrating leukocytes during chemical and bacterial inflammation (94, 151). In this setting, D3 is highly expressed in polymorphonuclear leukocytes (PMN) infiltrating the abscess or the infected organ (Fig. 5). PMN are an important cell population involved in the innate immune response induced by bacterial pathogens because PMN are essential for adherence to and subsequent phagocytosis of bacteria. For this purpose, PMN contain bactericidal and tissue-toxic mechanisms such as the myeloperoxidase (MPO) system. During phagocytosis, invagination of the cell membrane occurs, which finally results in the incorporation of bacteria into the intracellular phagosome. Subsequently, an oxygen burst occurs together with a discharge of the content of cytoplasmic granules. MPO release into the phagosome then forms an effective antimicrobial system together with H_2O_2 and iodide, chloride, bromide, or thiocyanate ions by oxidizing these ions (157). Oxidation of iodide results finally in the formation of hypoiodite, and the subsequent iodination of bacteria by hypoiodite promotes killing and degradation of the ingested bacteria. A clear relationship was observed between iodination of the

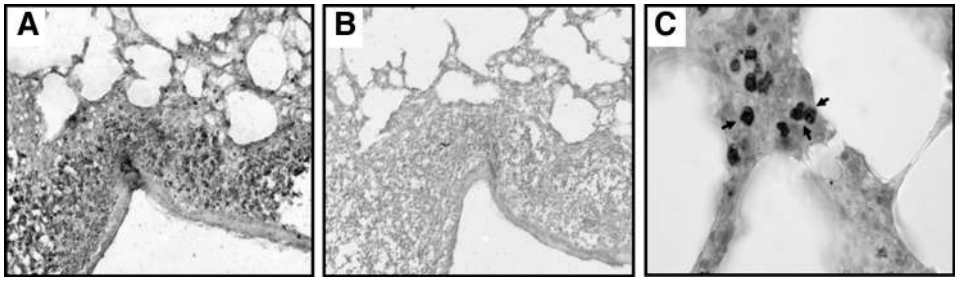

Fig. 5. An overview of a lung section showing *S. pneumoniae*-induced infiltrate after 48 h stained both with anti-D3 antibody (A) and preimmune serum (B). Note clearly stained D3-positive granulocytes in the infiltrated area that are shown in more detail in panel C (indicated by *arrows*). [Adapted from A. Boelen *et al.*, Type 3 deiodinase is highly expressed in infiltrating neutrophilic granulocytes in response to acute bacterial infection. *Thyroid* 18:1095–1103, 2008 (151), with permission. © 2008, Mary Ann Liebert Inc.]

ingested bacteria and the bacterial killing capacity of PMN, suggesting that iodination is essential for their bacterial killing capacity (158).

Thyroid hormone metabolism in leukocytes was studied decades ago, when it appeared that leukocytes can take up and deiodinate T_4, thereby generating inorganic iodide and small quantities of T_3 (159). Experimental induction of phagocytosis by leukocytes markedly enhanced T_4 deiodination (160), and clinical observations of pulmonary uptake of ^{131}I-labeled T_4 during pneumonia (161) suggested binding of the isotope in the area of inflammation. If deiodination of thyroid hormone takes place at a higher rate in areas of bacterial infection, peripheral disposal of thyroid hormones should be accelerated. Indeed, an increased disposal rate of both T_4 and T_3 has been shown in patients with acute pulmonary bacterial infections (144, 162, 163).

We hypothesized that the induction of D3 in PMN during inflammation should not only inactivate T_4 and T_3 but also yield substantial amounts of iodide within the leukocyte. Therefore, D3 may contribute to the microbial killing capacity of the cell via MPO, finally resulting in the iodination of ingested pathogens. Our group then showed that D3KO mice have a much higher bacterial load in blood, lung, and spleen compared with WT upon infection with *S. pneumoniae* (Fig. 6) (33). Thus, the lack of D3 severely impaired the bacterial clearance capacity of the host, suggesting a completely novel and protective role for D3 in the defense against acute bacterial infection via iodine production (Fig. 7). D3-expressing granulocytes have subsequently been found in infected lungs, liver (151), and spinal cord (164), indicating that this phenomenon is highly characteristic of the innate immune response in general and is not limited to *S. pneumoniae*-induced sepsis.

V. Thyroid Hormone Metabolism in Metabolic Organs during Prolonged Critical Illness

Although during acute inflammation NTIS may represent an adaptive response, partly driven by the hypothalamus and aimed to support the immune response, NTIS might turn disadvantageous when critical illness enters a chronic phase necessitating prolonged mechanical ventilation, dialysis, and inotropic support. This perception of the neuroendocrine response to illness as a dynamic process with distinct features in the acute and chronic phase of critical illness was originally formulated by Van den Berghe (165). A relatively small number of studies have addressed changes in local thyroid hormone metabolism in patients with prolonged critical illness. It should be mentioned that some studies were based on samples obtained from critically ill patients shortly after death, introducing some degree of postmortem delay. To circumvent this bias and to be able to manipulate various confounding factors in a systematic way, a rabbit model of prolonged critical illness was developed in Leuven, displaying profound alterations in thyroid hormone metabolism while the serum thyroid hormone changes closely mimic those in critically ill ICU patients (166).

A. Hepatic thyroid hormone metabolism during prolonged illness

Peeters *et al.* (167) studied thyroid hormone metabolism in deceased critically ill patients who had participated in a large, randomized, controlled study on intensive insulin treatment in the ICU setting. Liver biopsies were obtained within minutes after death. As expected in these patients, low serum T_3, T_4, and TSH and increased serum rT_3 levels were observed. In the liver biopsies, D1 activity was low and related to the cause of death: the lowest D1 activities were observed in patients who had died of cardiovascular collapse, and the highest D1 activities were observed in patients who had died of severe brain damage (167). Liver D1 activity was negatively correlated to serum T_4 sulfate concentrations that were clearly increased during prolonged illness. This suggested that D1 plays a role in the illness-induced increase in serum T_4 sulfate because liver sulfotransferase, which is an enzyme that catalyzes thyroid hormone sulfation, was unaltered (168). In con-

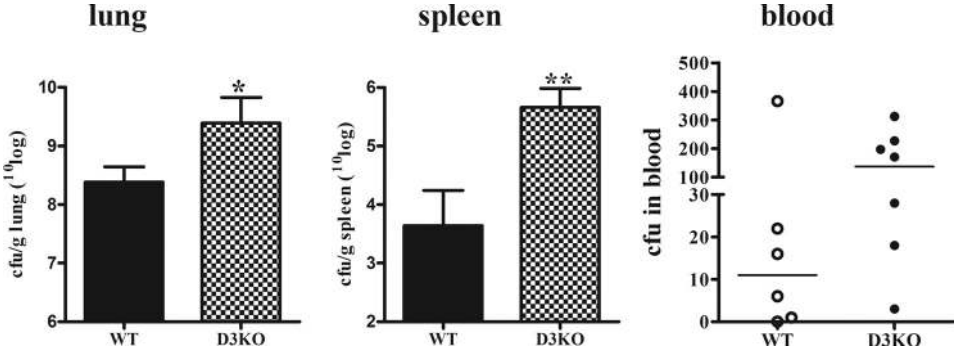

Fig. 6. Bacterial load in blood, lung, and spleen of WT mice (*black bars*) and D3KO mice (*checkered bars*) after *S. pneumoniae* infection. For lung and spleen, mean ^{10}log values of colony-forming units (cfu)/g ± SEM are depicted. Differences between groups are indicated: *, $P = 0.05$; **, $P = 0.01$. For blood, the number of cfu for each sample is depicted. [Adapted from A. Boelen *et al.*, Impaired bacterial clearance in type 3 deiodinase-deficient mice infected with *Streptococcus pneumoniae*. *Endocrinology* 150:1984–1990, 2009 (33), with permission. © 2009, The Endocrine Society.]

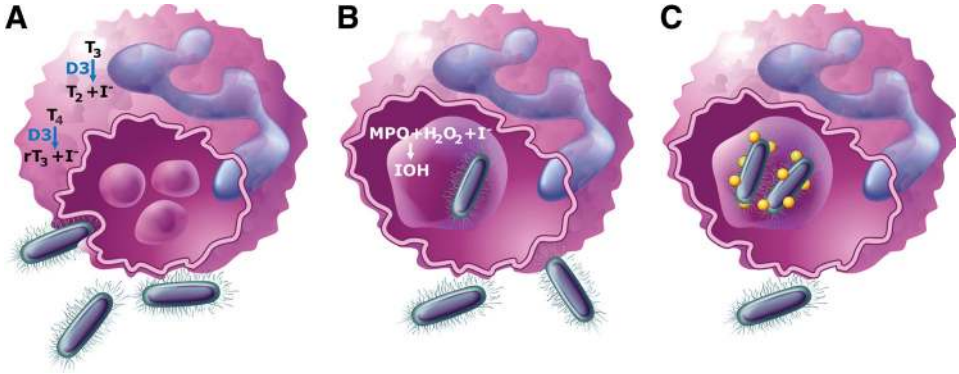

Fig. 7. Schematic overview of the proposed antibacterial action of type 3 deiodinase expressed in granulocytes. A, Deiodination of T_4 and T_3 at the cell surface and phagocytosis of bacteria. B, Formation of a phagocytome by invagination of the cell membrane and generation of oxidized iodine compounds. C, Iodination of bacteria in the phagocytome, followed by microbial killing.

trast to low liver D1, D3 was induced in liver of severely ill patients although no correlation was observed between activity levels and cause of death (167). Furthermore, liver D3 activity was positively correlated to serum rT_3 concentrations and negatively to serum T_3 levels (169).

Thyroid hormone transporter expression was also studied in these patients in comparison with liver biopsies obtained from living patients undergoing acute surgical stress. Liver MCT8 but not MCT10 mRNA expression increased during prolonged illness compared with acute surgical stress (96). Determination of TRα and TRβ mRNA expression in liver biopsies of patients with chronic liver disease before liver transplantation showed increased mRNA expression of both isoforms associated with low serum T_3 and T_4 levels (170). In postmortem liver biopsies from ICU patients, the TRα1/TRα2 ratio increased with the severity of illness, whereas no relation was observed between liver D1 mRNA and the TR isoforms (171).

Because these studies are not suitable for dynamic observations, a rabbit model for prolonged critical illness was developed displaying severe, disease-induced, and feeding-resistant wasting; high mortality rate; limited spontaneous recovery; and a biphasic neuroendocrine response pattern. In addition, this model showed clinical, biochemical, and endocrine features resembling those observed in ICU patients (166, 172). Thyroid hormone levels in these rabbits change profoundly, with a persistent decrease in serum T_3 and T_4 within 3 d after injury without an increase in TSH (173). Liver D1 activity is clearly decreased in these rabbits and positively correlated with serum T_3 as well as with T_3/rT_3 ratio. Liver D3 activity tends to be up-regulated and to correlate negatively with serum T_3 and the T_3/rT_3 ratio (174). Liver MCT8 mRNA increases. The administration of exogenous thyroid hormones reduces MCT8 expression combined with an increase in serum thyroid hormone levels, suggesting thyroid hormone-dependent regulation of MCT8 during illness (96).

In summary, prolonged illness results in profound alterations in liver thyroid hormone metabolism compared with acute and chronic inflammation. Thyroid hormone transport into the liver increases, whereas liver D1 decreases and liver D3 increases, the latter change perhaps resulting from tissue hypoxia (167). This might be mediated via the transcription factor hypoxia inducible factor (HIF)-1α because an *in vitro* study showed that HIF-1α interacts with the *DIO3* promoter, increasing D3 expression upon HIF-1α activation. Using a rat model of cardiac failure, the simultaneous induction of HIF-1α and D3 in the same myocardial area has been observed (175). This mechanism may be relevant for the ICU setting because tissue ischemia is frequently observed in critically ill patients.

Arem *et al.* (51) analyzed liver samples of NTIS patients and healthy controls and reported lower liver T_3 and T_4 concentrations in NTIS, indicating that the liver might be deficient in thyroid hormones during prolonged critical illness. This observation has been confirmed in an animal model because liver T_3 levels were decreased in critically ill rabbits (176). Not much is known about T_3-regulated gene expression in liver during prolonged illness, but considering the fact that liver T_3 levels are low, T_3 target gene expression is probably reduced.

B. Muscle thyroid hormone metabolism during prolonged illness

Prolonged critically ill patients often develop muscle dysfunction that is probably due to decreased mitochondrial activity and content (177). Muscle dysfunction is also associated with lower concentrations of energy-rich substrates and higher lactate content (153). Because thyroid hormones are involved in mitochondrial function and biogenesis, muscle thyroid hormone metabolism has been

studied during prolonged illness in sample biopsies of deceased critically ill patients. Muscle biopsies were taken within minutes after death and showed significant D3 activity levels, whereas D2 activity was undetectable. Muscle D3 activity was related to serum rT_3 concentrations (169), and the serum rT_3/T_4 ratio was higher in patients treated with inotropes (167). Mebis *et al.* (108) did report increased D2 activity in muscle tissue of prolonged critically ill patients compared with patients with acute surgical stress and to healthy volunteers. The samples were taken from the same cohort as reported by Peeters *et al.* (167), but the techniques and conditions were different (108). In contrast, muscle D2 and D3 activity were both undetectable in tissue of critically ill rabbits (174).

Muscle MCT8 mRNA expression increases significantly during prolonged illness compared with acute surgical stress, whereas MCT10 mRNA expression does not change. MCT8 expression correlates negatively to serum T_3 and T_4 concentrations (96). Muscle MCT8 and MCT10 mRNA expression was also studied in an animal model of prolonged illness, and the findings markedly differ from the human samples because MCT8 mRNA expression did not change, whereas MCT10 markedly increased. Treatment with thyroid hormones restored the illness-induced increase in MCT10 expression, suggesting that thyroid hormone transporter status is regulated by thyroid hormone levels during prolonged illness (96).

Of interest is whether high D2, high D3, and increased thyroid hormone transport in muscle of patients with prolonged critical illness results in substantially altered tissue thyroid hormone concentrations. Only one study has reported muscle T_3 concentrations in postmortem tissue of patients with prolonged illness showing marked interindividual variation ranging from very low to extremely high values (51). No information is available at present about T_3-regulated gene expression in muscle tissue during prolonged illness. However, because muscle T_3 content is supposed to be a determinant of muscle function, which may be severely impaired during prolonged illness, T_3 concentrations may be assumed to be low. However, future studies will be needed to confirm or refute this assumption.

VI. Endocrine Interventions in NTIS: Areas of Uncertainty

A. Endocrine interventions

Because administration of thyroid hormone in various clinical settings associated with NTIS did not improve clinical outcome or organ function (178), whereas at the same time carrying the risk of overdosing, an interesting novel approach in light of the hypothalamic alterations in TRH expression is administration of TRH in combination with other hypothalamic peptides. Van den Berghe *et al.* (179) showed that infusion of TRH alone or in combination with GH secretagogues to critically ill patients augmented the pulsatile TSH release that was associated with an increase in serum T_4 and T_3 levels. Prolonged administration of TRH and GHRP-2 to critically ill patients also showed that protein degradation was reduced in association with restoration of plasma thyroid hormone levels and reactivation of the blunted TSH secretion (15). Likewise, in the animal model of protracted critical illness, the administration of TRH in combination with GHRP-2 restored the illness-induced down-regulation of TSH shortly after administration of the hypothalamic peptides (166). Interestingly, infusion of TRH alone or in combination with GHRP-2 also restored the decrease in liver D1 decrease in relation to alterations in serum T_3 and T_4 (174). In mice, the administration of TRH during LPS-induced acute illness attenuated the decrease in serum T_4 (65), confirming that stimulation of the pituitary during NTIS may partly restore the illness-induced decrease of serum thyroid hormone levels. These studies provide convincing evidence that the central part of the HPT axis is important in the pathogenesis of NTIS, while representing a possible treatment target. Treatment of NTIS with thyroid hormones in patients with prolonged illness has not been the focus of many recent clinical trials. Pingitore *et al.* (180) reported the effects of T_3 infusion in NTIS patients with ischemic or nonischemic dilated cardiomyopathy and showed that short-term T_3 therapy improved the neuroendocrine profile as well as ventricular performance. A recent systemic review evaluated the effects and risks of postoperative T_3 treatment, analyzing 14 randomized clinical trials involving T_3 administration in variable doses and durations, predominantly during cardiac surgery. Short duration postoperative T_3 treatment appeared to increase cardiac index. However, mortality was not affected (181). The effects of two-dose regimens of thyroid hormone treatment have also been studied in prolonged critically ill rabbits. The low dose or substitution dose did not affect the illness-induced serum T_3 decrease, whereas serum T_4 was even further decreased upon treatment. Only a supraphysiological dose of thyroid hormone, aimed to target serum thyroid hormone levels within the range obtained earlier by TRH infusion, increased serum T_3 and T_4 levels and liver D1 activity within the normal range (176), which implicates enhanced degradation and/or excretion of thyroid hormone during prolonged illness. An additional study by the same authors showed that circulating T_3 concentrations are causally related to liver D1 activity during prolonged illness, indicating that the illness-induced decrease of liver D1 is secondary to low serum thyroid hormone levels (182) in this particular model. Thyroid hormone treatment also restored illness-induced alterations

in liver MCT8 and muscle MCT10 mRNA expression. It is, however, unknown at present whether the various treatment strategies are beneficial in terms of clinical outcome (96).

B. Areas of uncertainty

In summary, illness results in profound alterations in local thyroid hormone metabolism that are only partly related to changes in serum concentrations. Generally, acute illness is associated with reduced T_3 action in liver and adipose tissue and increased T_3 action in skeletal muscle. In contrast, chronic inflammation appears to be associated with unchanged T_3 action in liver, whereas both D2 and D3 increase in skeletal muscle theoretically leading to increased T_2 concentrations. Sepsis, a whole-body inflammatory state caused by bacterial infection and characterized by uncontrolled activation of the pro- and antiinflammatory responses, is associated with decreased muscle D2 activity and increased T_3 action in adipose tissue. These differential changes are schematically presented in Fig. 8.

Experimental animal models are frequently used to study illness-induced alterations at the tissue level, and these studies have generally revealed similar changes in tissue thyroid hormone metabolism between humans and rodents. However, the serum characteristics of NTIS are not identical between humans and rodents (Table 1). This is a complicating factor in attempts to extrapolate data obtained in experimental animal studies to the human situation. In addition, the role of reduced food intake as a result of illness affects thyroid hormone metabolism. Therefore, the use of pair-fed controls is essential for an unbiased interpretation of animal experiments on NTIS. Furthermore, posttranscriptional regulation of deiodinase expression has been reported, resulting in a potentially uncertain relationship between deiodinase mRNA expression and activity. However, in most studies on NTIS, the illness-induced alterations in deiodinase mRNA expression and enzyme activity are strictly correlated.

A number of key issues remain unsolved at present:

- Thyroid hormone availability should be assessed at the tissue level to establish the net result of the observed alterations in local deiodinase activities. The further development of liquid chromatography-mass spectrometry as a tool to assess various molecules in one tissue sample will probably facilitate these studies both in human and rodent tissue samples.
- The functional consequences of altered thyroid hormone metabolism in terms of organ or cellular function are largely unknown at present. Important and clinically relevant areas of NTIS research during sepsis and prolonged illness include mitochondrial function in skeletal muscle, bacterial killing capacity of infiltrating granulocytes during the innate immune response, and lipid metabolism in adipose tissue. We can only speculate at this stage that these future studies bear clinical relevance.
- Small but promising endocrine intervention studies in patients with protracted critical illness, involving treatment with neuropeptides and resulting in largely restored plasma thyroid hormone levels as well as improved metabolic parameters, should be expanded to larger studies involving more patients and longer treatment periods. These studies will help to establish whether or not such interventions are meaningful in terms of clinical outcome.

VII. Conclusion

Current knowledge has completely altered the concept of NTIS. In the classic view, NTIS is a syndrome with lower plasma thyroid hormone concentration of unknown sig-

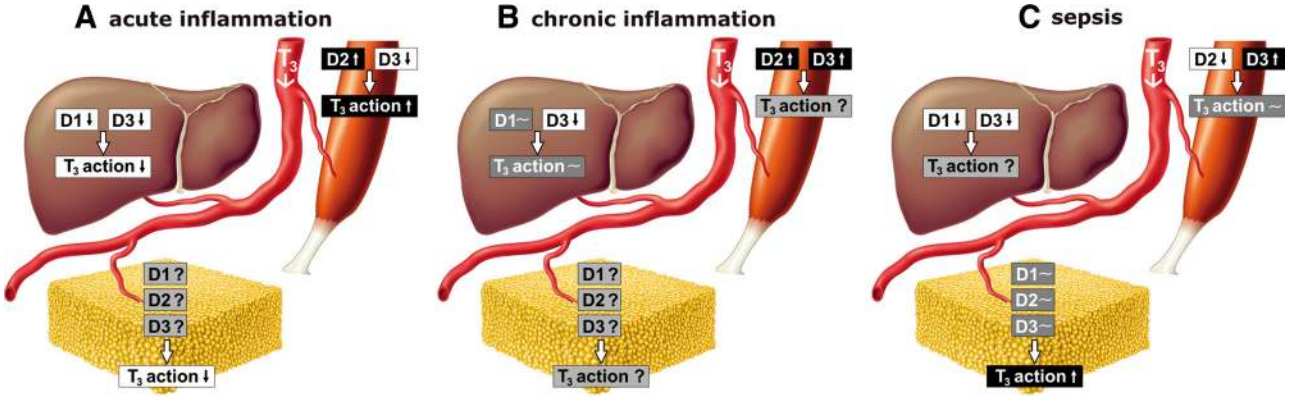

Fig. 8. Schematic representation of the differential expression of deiodinating enzymes and the resulting change in thyroid action in T_3 target tissues during acute inflammation (A), chronic inflammation (B), and sepsis (C). The scheme summarizes experimental and human studies. *Black boxes* represent an increase, *white boxes* a decrease, and *gray boxes/white font* no change. *Gray boxes/black font* symbolize a lack of data.

nificance as its key phenomenon. Recent studies, however, have clearly shown that NTIS represents a profound and differential change in thyroid hormone physiology, both at the level of the HPT axis in terms of setpoint regulation and at the organ level in terms of local thyroid hormone metabolism. It should be interpreted in the context of type of illness and of the organ/tissue studied. Organ- and timing-specific changes in D1–D3 highlight deiodinases as proactive players in the response to illness. Furthermore, the granulocyte is proposed as a novel and important cell type involved in NTIS during bacterial infection. Finally, changes in thyroid hormone metabolism in muscle during critical illness may be relevant for the pathogenesis of respiratory failure.

Acknowledgments

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