The molecular basis of the non-thyroidal illness syndrome

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Abstract
The ‘sick euthyroid syndrome’ or ‘non-thyroidal illness syndrome’ (NTIS) occurs in a large proportion of hospitalized patients and comprises a variety of alterations in the hypothalamus–pituitary–thyroid (HPT) axis that are observed during illness. One of the hallmarks of NTIS is decreased thyroid hormone (TH) serum concentrations, often viewed as an adaptive mechanism to save energy. Downregulation of hypophysiotropic TRH neurons in the paraventricular nucleus of the hypothalamus and of TSH production in the pituitary gland points to disturbed negative feedback regulation during illness. In addition to these alterations in the central component of the HPT axis, changes in TH metabolism occur in a variety of TH target tissues during NTIS, dependent on the timing, nature and severity of the illness. Cytokines, released during illness, are known to affect a variety of genes involved in TH metabolism and are therefore considered a major determinant of NTIS. The availability of in vivo and in vitro models for NTIS has elucidated part of the mechanisms involved in the sometimes paradoxical changes in the HPT axis and TH responsive tissues. However, the pathogenesis of NTIS is still incompletely understood. This review focuses on the molecular mechanisms involved in the tissue changes in TH metabolism and discusses the gaps that still require further research.

Introduction
Illness results in profound changes in thyroid hormone (TH) metabolism called the ‘sick euthyroid syndrome’ or ‘nonthyroidal illness syndrome’ (NTIS). NTIS is characterized by decreased serum triiodothyronine (T₃) and thyroxine (T₄) concentrations, increased serum reverse T₃ (rT₃) concentrations and unaltered or inappropriately low serum thyroid-stimulating hormone (TSH), indicating profoundly altered negative feedback in the pituitary and hypothalamus (Docter et al. 1993). The alterations in the central part of the hypothalamus–pituitary–thyroid (HPT) axis are combined with reduced production of T₃ and impaired clearance of rT₃ by the liver, and with specific changes in peripheral TH metabolism in major T₃ target organs. Muscle and adipose tissue show additional and differential changes in TH metabolism. The role of the thyroid gland has been largely neglected with regard to illness induced alterations in TH metabolism for many years, but in vitro studies showed that genes involved in the production and release of T₄ and T₃ are severely affected by high concentrations of pro-inflammatory cytokines (Bartalena et al. 1998). In addition, acute inflammation in mice reduced thyroidal TSH-receptor expression preceded by an acute increase in interleukin 1 beta (IL1β) expression (Boelen et al. 2004a). Thus, the
thyroid gland itself is clearly involved in the pathogenesis of NTIS. The ultimate effects of the observed changes in local TH metabolism on tissue function during illness are currently unknown. However, the common view is that while changes observed during the acute phase of illness are beneficial they may become deleterious during prolonged critical illness, making the stage and severity of illness a major determinant of NTIS.

Cytokines have been implicated in the development of NTIS for more than two decades. IL6 was found to be negatively correlated with serum T₃ concentrations in hospitalized patients (Boelen et al. 1993). In mice, administration of bacterial endotoxin or lipopolysaccharide (LPS) results in an acute increase of serum IL6 and tumor necrosis factor alpha (TNFα) concentrations (Boelen et al. 1995). A causal role for IL6 in the development of NTIS in mice was shown since IL6 knock out mice show a less pronounced drop in serum T₃ during illness (Boelen et al. 1996). However, acute injection of cytokines failed to induce NTIS like features, except for interferon gamma (IFNγ) which reduces serum T₃ and T₄ (Boelen et al. 1995). Chronic infusions with IL1 and IL6 on the other hand mimic certain symptoms such as decreased serum T₃ and T₄ and decreased thyrotropin-releasing hormone (TRH) expression in the hypothalamus in mice (van Haasteren et al. 1994), while chronic IL1β infusions mimicks certain aspects of NTIS in the rat (Hermus et al. 1992).

Mechanisms involved in the pathogenesis of NTIS have predominantly been studied by using a variety of in vivo and in vitro models. Several NTIS rodent models have been described i.e. acute illness, induced by administration of a sublethal dose of LPS (Boelen et al. 2004a, Fekete et al. 2004); chronic inflammation, induced by injection of turpentine in the hind limb ultimately resulting in the formation of an abscess (Chopra et al. 1987, Boelen et al. 2006); bacterial sepsis, induced by inoculation of Streptococcus pneumoniae or i.p. injection with Escherichia coli (Knapp et al. 2003, Boelen et al. 2008) and prolonged critical illness in rabbits induced by burn injury (Weekers et al. 2002). The similarity between these models is the decrease in serum TH concentrations, although the time course, severity and inflammatory response are variable. The availability of transgenic mice provided the possibility to study the specific contribution of genes involved in TH metabolism in the context of NTIS. The role of specific molecular factors involved in the altered TH metabolism has been studied extensively in vitro by using a variety of cell lines that were stimulated with LPS or pro-inflammatory cytokines with and without specific inhibitors. In this review, we discuss changes in TH regulation and metabolism during NTIS, and the molecular mechanisms involved.

**Illness induced alterations in hypothalamic TRH expression**

The combination of low serum TH and an inappropriately low TSH response suggests central down-regulation of the HPT axis. This is supported by the observation that TRH gene expression in the paraventricular nucleus (PVN) of the hypothalamus was decreased in post mortem hypothalamic tissue of patients who died after prolonged illness as compared with patients with acute cardiac arrest. Moreover, TRH mRNA expression in the PVN correlates positively with pre-mortem serum TSH and T₃ levels (Fliers et al. 1997). In addition, several animal studies show that hypothalamic TRH expression also decreases after acute inflammation (Kakućska et al. 1994), chronic inflammation (Boelen et al. 2006) and prolonged critical illness in rabbits (Mebis et al. 2009). However, the underlying mechanisms are incompletely understood. Local T₃ bioavailability in the hypothalamus might play a role as TRβ⁻/⁻ mice display an impaired illness induced TRH decrease and TRβ signalling is important for the feedback regulation of T₃ on TRH neurons. However, the role of circulating TH is probably limited as alterations in the hypothalamus that are supposed to be involved in the illness induced TRH decrease precede the decrease in circulating TH levels (Fekete et al. 2005). A striking observation that has been linked to the illness induced TRH decrease is a marked increase in type 2 deiodinase (D2/Dio2) mRNA expression both in tanycytes, specialised cells lining the wall of the third ventricle (de Vries et al. 2014a) and in the hypothalamus of a variety of rodent and rabbit NTIS models (Boelen et al. 2004a, 2006, Fekete et al. 2004, Mebis et al. 2009). D2 is the main T₃ producing enzyme in the brain and involved in the regulation of local TH availability. In an in vitro coculture system, increased D2 expression in glial cells results in an increase of T₃ responsive gene expression in cocultured neurons, indicating that increased T₃ production by D2 in tanycytes could influence adjacent neurons in a paracrine fashion (Freitas et al. 2010). The observation that TRβ⁻/⁻ mice do not show a hypothalamic TRH decrease supports the role for local T₃ in the suppression of TRH secretion (Boelen et al. 2009a). The mechanisms involved in the illness induced increase in Dio2 mRNA expression are discussed in the following section. In addition, both IL1β and corticosterone are known to affect Trh expression directly.
and could also contribute to the TRH decrease upon inflammation (Kakucska et al. 1994, 1995). TRH is also decreased during starvation suggesting that part of the observed TRH decrease during illness might be a result of the diminished food intake associated with illness. However, in a mouse model for chronic inflammation, diminished food intake does not play a role in the observed Trh decrease (Boelen et al. 2006).

Illness induced alterations in pituitary TSHβ expression

One of the characteristics of NTIS is the absence of an appropriate TSH response in the face of low serum T₄ and T₃ concentrations. Low TSH serum concentrations and decreased Tshα mRNA expression in the pituitary have been described in a wide variety of animal models (Boelen et al. 2004a, 2006, Fekete et al. 2004, Mebis et al. 2009). The mechanism involved is still unclear although we showed that the illness induced decrease of Tshβ expression depends on functional thyroid hormone receptor (TR) signaling, since TRβ₁⁰/¹⁰ mice show a blunted Tshβ decrease upon LPS stimulation compared to their WT counterparts (Boelen et al. 2009a). The diminished food intake that is associated with chronic inflammation is only partly responsible for the observed Tshβ decrease (Boelen et al. 2006).

The pituitary expresses both D₁/Dio1 and D₂ (Alkemade et al. 2006). The D₂ mediated conversion of T₄ to T₃ has been thought to be important for the feedback of TH on TSH, since D₂ knock out mice show a disturbed negative feedback (Schneider et al. 2001). As the LPS induced suppression of TRH in the hypothalamic PVN is associated with an increase of Dio2 expression in the mediobasal hypothalamus (Boelen et al. 2004a, Fekete et al. 2004), it was speculated that the LPS induced decrease in Tshβ expression might also be dependent on increased D₂ activity in the pituitary. Surprisingly, the response of Dio2 expression in the pituitary after LPS appeared to be dependent on the species, strain and type of illness studied; both increased and decreased Dio2 expression have been observed (Boelen et al. 2004a, 2006, 2009a, Fekete et al. 2004). Furthermore, administration of LPS to rats results in an increased pituitary D₂ activity after 12 and 24 h, which is dependent on the fall in TH concentrations, in contrast to the hypothalamic D₂ increase (Fekete et al. 2005). Further studies using pituitary specific D₂ knock out mice are necessary to investigate the exact role of D₂ in the LPS induced alterations in TH metabolism in the pituitary.

In addition to Dio2, the expression of Dio1 in the pituitary is increased during inflammation (Boelen et al. 2004a, 2009a). This change is mediated by the increase in cytokines during the acute phase response, since animals deficient for IL12 and IL18 do not show the LPS induced increase (Boelen et al. 2004b,c). If the D₁ increase in the pituitary gland serves a purpose with regard to the Tshβ decrease is unknown at present. Alternatively, the Tshβ decrease might be dependent on the changes in Dio2 expression in the mediobasal hypothalamus, which could affect the pituitary either via suppression in Dio2 expression or theoretically via an increase in T₃ transported from the hypothalamus to the pituitary via the portal capillaries (Fekete & Lechan 2007).

In vitro studies using primary cultures of pituitary cells show that cytokines have a pronounced effect on pituitary release of TSHβ. Both IL1β and TNFα decrease basal TSHβ release independently of T₃ uptake and action in the pituitary cells (Harel et al. 1995, Wassen et al. 1996). Interestingly, acute energy deprivation has no effect on TSHβ release from pituitary cells in culture, consistent with the in vivo studies discussed above showing that the TSHβ decrease during chronic inflammation is only partly explained by decreased food intake (Boelen et al. 2006).

The effect of cytokines on TH synthesis

Several components of the TH synthesis pathway are downregulated by cytokines directly on the level of the thyrocyte, ultimately leading to decreased secretion of T₄ and T₃ (Bartalena et al. 1998).

Supraphysiological concentrations of the pro-inflammatory cytokines IL₁α and IL₁β inhibit the TSH-induced thyroglobulin (Tg) mRNA expression and Tg release in human cultured thyrocytes via suppression of cAMP (Rasmussen et al. 1988, 1994, Yamashita et al. 1989). IL₁α and IL₁β also decrease ¹²⁵I incorporation and T₄ and T₃ secretion from human thyrocytes in the presence of TSH (Sato et al. 1990). Thyroid peroxidase (TPO) mRNA expression and protein content, important for the oxidation of iodide to iodine, is also directly affected by IL₁ in human thyrocytes and rat thyroid FTRL-5 cells (Asakawa et al. 1996, Gerard et al. 2006). Moreover, IL₁β impairs basal and TSH-stimulated uptake of iodide by the natrium/iodide symporter (NIS) in porcine thyroid follicles (Nolte et al. 1994). The role of IL6 is less well established: one study showed that IL6 inhibits the TSH- and cAMP-induced increase in TPO mRNA expression and T₃ secretion in thyrocytes obtained from Graves’ disease patients (Tominaga et al. 1991), while IL6 has
only a minor effect on cultured human thyroid cells (Rasmussen et al. 1991).

IFNγ is a cytokine that is mainly involved in anti-viral and anti-bacterial responses and is produced by natural killer and T-cells. IFNγ has a variety of effects on human thyrocytes in culture; it inhibits TSH-induced TH and Tg secretion (Nagayama et al. 1987, Kung et al. 1992) as well as Tg mRNA expression (Sato et al. 1990), TSH-induced TPO expression (Ashizawa et al. 1989) and the TSH- and cAMP-induced upregulation of TSH receptors on the thyrocyte (Nishikawa et al. 1993). IFNγ also inhibits the TSH-induced increase in NIS expression in rat FRTL-5 cells resulting in diminished iodide uptake (Ajjan et al. 1998). Interestingly, overexpression of IFNγ in thyroid cells in a transgenic mouse leads to primary hypothyroidism mainly due to a big decrease in NIS mRNA and protein expression (Caturegli et al. 2000).

TNFα plays an important role in the acute phase response and is known to inhibit the TSH-induced cAMP response and Tg production (Deus et al. 1992) and release (Poth et al. 1991, Rasmussen et al. 1994) in cultured thyrocytes. TNFα also inhibits NIS expression in rat FRTL-5 cells (Ajjan et al. 1998).

Finally, cytokines are able to inhibit D1 expression and activity in the rat thyrocyte and FRTL-5 cells (Pekary et al. 1994, Hashimoto et al. 1995, Tang et al. 1995). Taken together, these studies clearly show that cytokines, either alone or synergistically, are able to downregulate various components of the TH synthesis pathway in the thyroid, ultimately leading to decreased secretion of T4 and T3 (Fig. 1).

**Illness induced alteration in TH transport**

Cellular entry of TH is necessary before intracellular conversion of TH by deiodinating enzymes and binding to the nuclear TR can take place. Two categories of TH transporters have been described i.e. 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 and in astrocytes where it is involved in the uptake of T4 across the blood–brain barrier (Sugiyama et al. 2003). Well-known amino acid transporters of solute carrier (SLC) group are MCT8 and MCT10. MCT8 transports both T4 and T3 and is expressed in many tissues including liver, kidney and in various brain areas including cortical regions, striatum, cerebellum and hypothalamus (Alkemade et al. 2005, Heuer et al. 2005, Visser et al. 2011). MCT10 preferentially transports T3 instead of T4 and is expressed in kidney, liver and muscle (Visser et al. 2011). Once transported into the cell, THs can be metabolized by outer or inner ring deiodination through the iodothyronine deiodinases.

In a rabbit model for prolonged critical illness, hypothalamic Oatp1c1 and Mct10 expression was upregulated, while Mct8 expression was unaltered (Mebis et al. 2009). The functional consequences of these changes were unclear. In mice that received a turpentine injection in the hindlimb, leading to the formation of a sterile abscess, Mct8 as well as D3 (discussed in the following section) was found to be expressed in infiltrating neutrophils (Boelen et al. 2005). The rabbit and mouse studies show that MCT8 does respond to a variety of illnesses, but more extensive studies, including functional studies, will be needed to address this topic in more detail.

**TH production and degradation by deiodinases**

THs can be produced and degraded by iodothyronine deiodinating enzymes, so-called deiodinases. These enzymes belong to a selenocysteine containing enzyme family and comprise three types: D1, D2 and D3 (Kohrle 2000). D1 and D2 are T3 producing enzymes while D3 inactivates T4 and T3. The expression and activity levels of all three deiodinases are affected during illness. The basic expression levels of the different deiodinases differ; some organs express predominantly D2 and D3 while other organs showed a limited expression of D2 or D3 but do express D1. The combination of the deiodinases expressed in a cell together or in the same tissue determine the availability of T3 and thereby cellular and tissue function.

**Type 1 deiodinase**

The role of D1 in the pathogenesis of NTIS has been extensively studied as D1 is thought to be involved in the production of serum T3 (decreased during illness) via outer ring deiodination and in the clearance of rT3 (rT3 concentrations are increased during illness in humans) via inner ring deiodination. D1 is localized in the plasma membrane, and expressed in liver, kidney, thyroid and pituitary. It is positively regulated by T3 (Toyoda et al. 1995, Jakobs et al. 1997). Illness induces a marked decrease in liver D1 mRNA expression and activity in critically ill patients (Peeters et al. 2003, 2005) and in a variety of NTIS animal models (Boelen et al. 1995, 2004a, 2005, 2008, Debaveye et al. 2005).
The Dio1 gene is activated via a TR/RXR heterodimer which indicates that decreased Dio1 expression may result from reduced TR or/and reduced RXR expression. T3 positively regulates the expression of Dio1 via TRβ mediated binding to TH responsive elements in the Dio1 promoter and functional TRβ signaling is therefore essential for basal expression of Dio1 (Amma et al. 2001).

Liver TRβ expression is downregulated during acute inflammation in mice (Beigneux et al. 2003, Boelen et al. 2004a). In vitro studies suggest a major role for cytokines, as IL1β decreases TRβ mRNA expression in a human hepatoma cell line (HepG2; Kwakkel et al. 2006). Furthermore, TNFα, IL1 and IL6 decrease the binding capacity of T3 to the TR (Jakobs et al. 1997). An important intracellular signalling pathway for cytokines is the nuclear factor-kappa B (NF-κB) pathway. Nagaya et al. (2000) show that TNFα impairs the T3 dependent induction of Dio1 expression in HepG2 cells via interference of NF-κB with TR function. There is, however, no evidence for a direct interaction between the TR and NF-κB which suggests a common cofactor by NF-κB and the TR to play a role (Nagaya et al. 2000).

Although it was assumed that mainly the TRβ was involved in the illness induced D1 repression in the liver, studies in TRβ0/0 and TRα0/0 mice show that while the LPS induced D1 decrease is still present in the TRβ0/0 mice, this response is attenuated in the TRα0/0 mice (Kwakkel et al. 2008, 2010). In addition, the IL1β induced decrease in TRβ mRNA expression in HepG2 cells is solely dependent on NF-κB signaling, while the decreases in Dio1 and TRα are dependent on both NF-κB and activator protein-1 (AP-1) signaling (Kwakkel et al. 2006, 2007). This suggests that...
diminished expression of the TRβ by itself is not the only factor in the illness induced liver Dio1 decrease.

An very elegant alternative mechanism for TRβ-mediated repression of liver D1 during acute inflammation has been proposed by Yu et al., who showed both in vivo and in vitro that adding exogenous co-activator steroid receptor co-activator-1 (SRC-1) attenuates the illness induced liver D1 decrease (Yu & Koenig 2000, 2006). 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. Indeed, restoration of liver Dio1 expression by exogenous SRC-1 prevents the fall in serum TH levels after LPS (Yu & Koenig 2000, 2006).

After LPS administration, hepatic RXRα protein rapidly migrates to the cytoplasm where it can be degraded. This process is mediated by the inflammatory pathway JNK (Beigneux et al. 2000). However, the IL1β induced decrease of liver Dio1 mRNA is not prevented by inhibition of JNK alone (Kwakkel et al. 2006), which makes it unlikely that RXR is solely responsible for the illness induced decrease in liver Dio1.

An additional possibility is that decreased amounts of a specific co-factor glutathione (GSH), required for D1 catalytic activity (Goswami & Rosenberg 1987) may play a role in the illness induced decrease of liver Dio1 activity. D1 activity in intact liver cells can be suppressed by IL6 and the addition of N-acetyl-cysteine, an antioxidant that restores intracellular GSH levels, prevents the IL6-induced suppression of D1 (Wajner et al. 2011, Fig. 2).

Although these studies provide mechanisms behind the illness induced D1 decrease in the liver, the importance of this decrease for the development of NTIS is questioned by studies in D1/D2 knock out mice, showing similar responses to LPS administration with regard to changes in serum T4 and T3 compared to WT littermates (St Germain et al. 2009). Although it is unknown at present whether a lack of D1 affects the illness induced liver T3 concentrations, it has been shown in critically ill rabbits that the suppression of liver D1 activity was correlated with decreased hepatic T3 concentrations (Debaveye et al. 2008).

**Type 2 deiodinase**

D2 is localized in the endoplasmic reticulum and deiodinates T4 into the biologically active T3. D2 is the main enzyme involved in the production of tissue T3 and is therefore heavily involved in local TH metabolism. D2 is negatively regulated by TH, both pre- and post-transcriptionally, as T3 down regulates Dio2 mRNA expression (Burmeister et al. 1997), while T4 as well as rT3 (both substrates for D2) affect D2 activity via increasing D2 ubiquitination and subsequent proteasomal degradation (Sagar et al. 2007).

Many studies have focused on the role of D2 in the central part of HPT axis as the setpoint of the central HPT axis that is altered during illness. The unresponsiveness of the HPT axis to low serum TH levels has been suggested to be mediated by increased production of T3 via elevated D2 activity in tanycytes (Fekete et al. 2004) as mice lacking the TRβ do not show an illness induced hypothalamic Trh decrease (Boelen et al. 2009a). In addition, global D2 knock out mice do not show a suppression of Trh upon LPS stimulation (Freitas et al. 2010).

The inflammation induced D2 upregulation in the hypothalamus was found to be independent of the fall in serum TH concentrations, in contrast to D2 expression in other brain areas like the cortex and in the pituitary (Fekete et al. 2005). A role for inflammatory cytokines was suggested as LPS administration results in a rapid increase of pro-inflammatory cytokines including TNFα, IL1 and IL6. The Dio2 promoter contains NF-κB responsive elements and is thus sensitive to inflammatory signal transduction pathways (Fekete et al. 2004, Zeold et al. 2006). NF-κB is therefore highlighted as a possible mediator of the inflammation induced increase in Dio2 expression in the hypothalamus.

In vitro, NF-κB is able to induce Dio2 expression in mesothelioma cells endogenously expressing D2 (Zeold et al. 2006). In a primary culture of rat astrocytes and human glioma cells, LPS induces Dio2 mRNA expression via the NF-κB and MAPK pathways (Lamirand et al. 2011). However, the significance of these findings with regard to NTIS are debatable, since the changes in Dio2 expression occur relatively late in astrocytes compared to tanycytes. Stimulation of primary tanycytes with LPS also results in an increase of Dio2 expression. This effect can be completely blocked when the transcriptional activity of the NF-κB pathway is inhibited (de Vries et al. 2014a) indicating an important role for NF-κB in the relevant cell type. These results will have to be replicated in vivo, since a study by Sanchez et al. (2010) showed that 1α-25, a marker for NF-κB activation, is expressed secondary to the rise in Dio2 in tanycytes after LPS administration in rats.

During fasting, a rise in D2 activity in the hypothalamus is also observed, however the magnitude of this response is marginal compared to the increase in D2 activity during inflammation. Furthermore, the
mechanisms behind the fasting induced increase in D2 are different and dependent on leptin, corticosterone and changes in neuropeptide expression (Diano et al. 1998).

D2 is expressed in skeletal muscle (Bianco & Kim 2006) and is thought to be involved in the peripheral production of T3 under basal circumstances (Maia et al. 2005). Dio2 expression in skeletal muscle increases in intensive-care unit patients (Mebis et al. 2007), in several NTIS animal models of acute (Kwakkel et al. 2009) and chronic inflammation (Kwakkel et al. 2009), while in septic patients and mice muscle Dio2 expression decreases (Rodriguez-Perez et al. 2008, Kwakkel et al. 2009). The increased Dio2 expression during chronic inflammation is likely due to enhanced CREB signalling (Kwakkel et al. 2009), while the decrease during sepsis might be mediated by decreased food intake since 62 h of fasting decreased muscle Dio2 expression in healthy volunteers (Heemstra et al. 2009) (see Fig. 3).

Like inflammation, hypothyroidism increases D2 activity in the hindlimb muscle of mice (Marsili et al. 2010). The increased production of T3 in muscle by D2 plays an important role during myogenesis, muscle regeneration and differentiation, and is mediated via the forkhead box O3 (FoxO3) pathway (Dentice et al. 2010, Marsili et al. 2011). Fox transcription factors are a class of transcription factors that bind to forkhead regulatory elements in the DNA and regulate a variety of cell functions (Accili & Arden 2004). Whether the FoxO3 pathway also plays a role in the inflammation induced D2 increase remains questionable at this stage.

D2 is also expressed in the lung, although the significance in the healthy adult lung is unknown (Escobar-Morreale et al. 1997, Ohba et al. 2001). During LPS induced lung injury (caused by intranasal LPS administration) and ventilator-induced lung injury (VILI) in mice, the expression of D2 in the lung is increased (Ma et al. 2011). In addition, D2 protein expression is increased in human microvascular endothelial cells that are subjected to cyclic stretch (Ma et al. 2011). The increase in D2 expression and the subsequent rise in local T3 concentrations might be an adaptive and protective mechanisms of the lung to prevent lung damage during inflammation, since knocking down D2 in vivo aggravates lung injury after VILI.
VILI leads to local increases of TNFα, IL6 and IL1β, again suggesting the involvement of cytokines in the upregulation of Dio2 (Barca-Mayo et al. 2011) via NF-κB activation (Lentsch et al. 1998, Leeper-Woodford & Detmer 1999). Indeed, inhibiting NF-κB activation protects mice from LPS induced acute lung injury (Wang et al. 2013).

Interestingly, TH metabolism is also tightly linked with the innate immune system. Increased expression of Dio2 is found in resident macrophages in the liver upon chronic and acute inflammation. However, this is not mediated by NF-κB and the mechanisms involved are unknown to date (Kwakkel et al. 2014).

D2 is homeostatically regulated by a post transcriptional mechanism involving ubiquitination mediated conformational changes and subsequent proteosomal degradation, explaining its short half-life compared to the other deiodinases. This mechanism was first described by Gereben et al. (2000) and was shown to be induced by the major substrate of D2, T4. Ubiquitination of D2 by the ubiquitin conjugating enzymes UBC6 and UBC7 is mediated via WSB-1 (a D2 specific E3 ubiquitin ligase adaptor subunit) which changes the conformation of the D2 dimer and thus its catalytic activity (Sagar et al. 2007). D2 can be reactivated by de-ubiquitination by the de-ubiquitinating enzyme USP-33 (Curcio-Morelli et al. 2003). In tanycytes, both WSB-1 and USP-33 are co-expressed with D2 (Fekete et al. 2007). Recent studies, however, show that T4 induced ubiquitination in tanycytes is minimal, probably to ensure the sensitivity of the TRH producing neurons in the PVN to fluctuations in serum TH concentrations (Werneck de Castro et al. 2015).

In line with these findings, there is no evidence that ubiquitination is involved in the regulation of D2 during inflammation. Also in muscle, where D2 expression and activity is increased upon inflammation, WSB-1 and
USP-33 expression is not correlated with the increased D2 activity during chronic inflammation and sepsis (Kwakkel et al. 2009).

**Type 3 deiodinase**

D3 is localized in the plasma membrane and can be viewed as the major TH inactivating enzyme, as it catalyzes inner-ring deiodination of both T4 and T3, exclusively resulting in the production of biologically inactive rT3 and rT2 (Kohrle 2000). D3 is highly expressed in the placenta during fetal development, thereby protecting the fetus from an overexposure of T3 (Darras et al. 1999). In the adult organism, D3 is expressed in neurons in the brain, the liver and in parts of the innate immune system, although physiological levels are very low (Gereben et al. 2008).

Illness influences D3 expression and activity in the liver, but the results from animal studies vary. While during acute and chronic inflammation and during sepsis liver Dio3 mRNA expression and activity levels are decreased (Boelen et al. 2005, 2008), hepatic D3 expression and activity are increased in rabbits with prolonged critical illness (Debaveye et al. 2005). Slightly increased D3 activity is also observed in the livers of severely ill patients (Peeters et al. 2003).

During prolonged critical illness, decreased food intake might be an important factor in regulating liver deiodinases. Fasting for 36 h or a 50% reduction in food intake for 3 weeks results in pronounced increase of D3 expression and activity levels are decreased (Boelen et al. 2005, 2008), hepatic D3 expression and activity are increased in rabbits with prolonged critical illness (Debaveye et al. 2005). Slightly increased D3 activity is also observed in the livers of severely ill patients (Peeters et al. 2003).

Illness induces changes in muscle D3 expression that depend on the type and timing of the illness. While acute inflammation decreases muscle D3 (Kwakkel et al. 2010), bacterial sepsis does not affect D3 and chronic inflammation even increases D3 expression (Kwakkel et al. 2009). In critically ill patients, muscle D3 is also increased (Peeters et al. 2003). These changes seem to be independent on inflammatory pathways since neither sepsis nor chronic inflammation induces phosphorylation of the NF-κB and ERK pathways (Kwakkel et al. 2009). In proliferating myoblasts, D3 functions as a survival factor by decreasing TH concentrations and suppressing TH induced FoxO3 mediated gene expression (Dentice et al. 2014). In contrast, during muscle cell differentiation, inhibition of D3 gene expression is mediated by histone H3 demethylating enzyme (LSD-1) that relieved activation marks on the D3 promoter and at the same time activates D2 expression by removing the repressive marks on the D2 promoter in a reciprocal fashion (Ambrosio et al. 2013). Whether these epigenetic modifications of deiodinase gene expression are also important during inflammation remains to be investigated.

In cardiomyocytes, D3 expression is low under physiological conditions. Myocardial infarction and pressure induce hypertrophy in rats lead to an upregulation of D3 activity (Wassen et al. 2002, Olivares et al. 2007). It was postulated that the increase in D3 in peripheral organs might be regulated by hypoxia due to decreased tissue perfusion during illness (Peeters et al. 2003), and this assumption is supported by the observation that both D3 and hypoxia-inducible factor 1 alpha (HIF1α) are upregulated in the hypertrophic heart. Furthermore, HIF1α appeared to regulate D3 expression in a variety of cell lines under hypoxic conditions (Simonides et al. 2008). HIF1α activity is regulated by prolyl hydroxylases (PHD’s) that prime HIF1α for degradation (Aragones et al. 2009). Both oxygen and 2-oxoglutarate (2-OG) are necessary cofactors for PHD’s and therefore involved in HIF1α regulation. Under hypoxic conditions, HIF1α stabilizes and translocates to the nucleus, dimerizes with HIF1β and activates Dio3 gene transcription (Simonides et al. 2008). However, decreased concentrations of 2-OG might also play a role in the stabilization of HIF1α as diminished food intake, frequently observed during illness, could result in decreased concentrations of 2-OG due to glucagon and increased gluconeogenic flux (Ochs 1984). In addition, during inflammation NF-κB also directly enhances HIF1α gene transcription, thereby increasing total HIF1α availability (Oliver et al. 2009, Fig. 4).

It was recently shown that the upregulation of D3 during myocardial infarction is also associated with increased expression of a specific set of microRNA’s that might enhance the proliferative capacity of the cardiomyocytes (Janssen et al. 2013).

In addition to the organs and tissues mentioned, D3 is also expressed by infiltrating polymorphonuclear leukocytes upon the induction of a sterile abscess by turpentine injection in the hindlimb (Boelen et al. 2005). In addition,
peritonitis induced by \textit{E. coli} and pneumonia induced by \textit{S. pneumoniae} stimulates D3 expression in infiltrating granulocytes in the liver and lungs respectively (Boelen et al. 2008). Granulocytes are part of the innate immune system and have intracellular bacterial killing mechanisms such as the myeloperoxidase (MPO) system (Klebanoff 2005). It is hypothesized that following bacterial infection, the increased activity of D3 provides the MPO system with I\textsuperscript{K} that is released when the deiodination of T\textsubscript{3} and T\textsubscript{4} takes place to ensure an effective microbial killing machinery (Boelen et al. 2008). However, the mechanisms involved in the increase in D3 activity in activated neutrophils are currently unknown.

Although previously assumed otherwise, increased tissue D3 activity is not involved in the illness induced alterations in serum TH concentrations, since D3 knock out and WT mice showed similarly decreased serum TH concentrations during inflammation (Boelen et al. 2009b).

**TH production and degradation by alternative pathways**

TH are also metabolized in peripheral tissues via alternative pathways. Many of these processes take place in the liver. T\textsubscript{3} and T\textsubscript{4} can be conjugated to a sulphate group at the phenolic hydroxyl group, producing sulphated T\textsubscript{3} (T\textsubscript{3}S) and sulphated T\textsubscript{4} (T\textsubscript{4}S). T\textsubscript{3}S has no affinity for the TR, while sulphated TH is prone to degradation by D1 (Mol & Visser 1985, Visser et al. 1998). Sulfation is mediated by sulfotransferases (Sults), a family of enzymes that sulfate both endogenous and exogenous substances, and is also dependent of the availability of specific cofactors (3’-phosphoadenosine-5’-phosphosulfate) and the availability of inorganic sulfate (Kaptein et al. 1997). Not much is known about the activity of Sults during illness. In serum of patients who died in the ICU, the T\textsubscript{4}S concentrations are significantly elevated, but this is due to a decrease in clearance by D1 and not to increased Sult activity (Peeters et al. 2005). Furthermore, changes in sulfate availability during illness and diminished food intake could also play a role. In addition to sulfation, TH can be glucuronidated by UDP-glucuronosyltransferases (UGTs; Taurog et al. 1952). T\textsubscript{4}, and to a lesser extent T\textsubscript{3}, are substrates of a variety of UGT iso-enzymes. Glucuronidation facilitates the excretion of TH via the bile and feces (Tukey & Strassburg 2001). No alterations have been described in glucuronidation during critical illness per se, but methodological issues include increased glucuronidation due to the administration of drugs (Visser 1994) which will further decrease T\textsubscript{4} concentrations in ill patients.

A way of TH metabolism that is less well studied is ether link cleavage (ECL). This involves the breaking of the ether bridge in between the two tyrosines, yielding diiodotyrosine as a main product. This reaction is catalyzed by peroxidases, such as MPO that is present in leukocytes. In vitro, exposure to zymosan (a compound of yeast that induces phagocytosis) increased breakdown

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**Figure 4**
Schematic representation of mechanisms supposed to be involved in D3 regulation during illness. HIF1\textalpha\, is a protein that is regulated post-transcriptionally by proteasomal degradation. When oxygen and 2-oxoglutarate (2-OG) are present, HIF1\textalpha\, is hydroxylated by a class of enzymes called prolyl hydroxylases (PHD’s) which makes HIF1\textalpha\, prone to degradation. When oxygen or 2-OG are low during illness (due to decreased tissue perfusion or reduced food intake respectively), PHD’s do not hydroxylate HIF1\textalpha\,, resulting in its stabilization and translocation to the nucleus where it dimerizes with constitutively expressed HIF1\textbeta\, and activates gene transcription. Upon inflammation, NF-kB is also able to induce HIF1\textalpha\, transcription which could contribute to D3 regulation.
of T₄ and T₃ by ECL in leukocytes (Burger et al. 1983), indicating that this mechanism, besides deiodination, might be important for the bacterial killing machinery in the leukocyte. The role of ECL in TH metabolism under physiological conditions is thought to be limited, since only 5% of total body clearance of TH is mediated via ELC (Faber et al. 1989). However, it has been suggested that the serum concentration of DIT, a product of ELC, increase after surgery and during sepsis (Gramm et al. 1989, Meinhold et al. 1991), indicating that in specific pathophysiological conditions, ECL might attribute to TH clearance.

Concluding remarks

Several mechanisms are known to be involved in the illness induced alterations in the HPT axis and TH target tissues:

i) The illness induced suppression of TRH in the PVN is hypothesized to be mediated by increased T₃ production via increased D2 expression in tanyocytes. Studies using specific inhibitors reported a causal role for NF-kB in the upregulation of D2. However, no conclusive data is available whether the induction of D2 observed in illness results in increased local T₃ concentrations.

ii) Whether the decreased thyroidal secretion during illness is due to central suppression of the HPT axis or to a direct inhibitory effect of cytokines on the thyroid gland is still unclear. In vitro studies showed that a variety of pro-inflammatory cytokines are able to inhibit crucial steps involved in TH production, from iodide uptake to TH secretion.

iii) The D1 decrease in liver during illness is likely due to suppressed TR signalling, possibly mediated by NF-kB, AP-1 and competition for common cofactors. Whether the suppression of liver D1 is causal for the illness induced decrease in serum T₃ is uncertain.

iv) Changes in D2 and D3 are observed in muscle, innate immune cells, adipose tissue and lung (D2) during illness. Inflammatory pathways might play a role, although NF-kB is not involved in the D2 increase in muscle and macrophages. Activation of the CREB pathway may be involved in the regulation of D2 in muscle.

More studies will be necessary to further define the underlying mechanisms and more importantly, to investigate the functional consequences of the changes in TH metabolism for cellular function. Ultimately, thorough knowledge of the pathogenesis and role of NTIS in critical illness may help to improve clinical outcome through targeted interventions in TH metabolism.

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