Mechanisms behind the non-thyroidal illness syndrome: an update

Maria H Warner and Geoffrey J Beckett
Clinical Biochemistry, University of Edinburgh, The Royal Infirmary of Edinburgh, 51 Little France Crescent, Edinburgh EH16 4SA, UK
(Correspondence should be addressed to G J Beckett; Email: g.j.beckett@ed.ac.uk)

Abstract
The mechanisms behind the changes in serum triiodothyronine (T3), thyroxine (T4) and TSH that occur in the non-thyroidal illness syndrome (NTIS) are becoming clearer. Induction of a central hypothyroidism occurs due to a diminution in hypothalamic thyrotropin-releasing hormone. This can be signalled by a decrease in leptin caused by malnutrition and possibly a localised increase in hypothalamic T3 catalyzed by altered expression of hypothalamic iodothyronine deiodinases D2 and D3. Data from D1 and D2 knockout mice suggest that these enzymes may have little contribution to the low serum T3 found in acute illness. The decline in serum T3 and T4 in models of acute illness precedes the fall in hepatic D1, suggesting that much of the initial fall in these hormones may be attributable to an acute phase response giving rise to a reduction in the thyroid hormone binding capacity of plasma. When measured by reliable methods, changes in serum free T4 and free T3 are modest in comparison to the fall seen in total thyroid hormone. Thyroid hormone transporter expression is up-regulated in many models of the NTIS, thus if diminished tissue uptake of hormone occurs in vivo, it is likely to be the result of impaired transporter function caused by diminished intracellular ATP or plasma inhibitors of transporter action. In man, chronic illness leads to an upregulation of thyroid hormone receptor (THR) expression at least in liver and renal failure. In contrast, human and animal models of sepsis and trauma indicate that expression of THRs and their coactivators are decreased in acute illness.


Introduction
Illness may induce profound changes in a number of neuroendocrine systems. The activation of the pituitary–adrenal axis is common, and plasma cortisol concentrations rise rapidly as a result of the acute stress response. The extent of this rise is related to the severity of the illness and is critical for survival (Vermes & Beishuizen 2001). Changes within the hypothalamic–pituitary–thyroid (HPT) axis also occur in illness and are typically associated with low levels of total triiodothyronine (T3), and this has given rise to the term ‘low T3 syndrome’. Sick patients with low serum T3 are often regarded as being clinically euthyroid, and as a consequence, the alternative term ‘Euthyroid sick syndrome’ was widely used in the past. ‘Non-thyroidal illness syndrome (NTIS)’ is now more commonly used to describe the typical changes in thyroid-related hormone concentrations that can arise in the serum following any acute or chronic illness that is not caused by an intrinsic abnormality in thyroid function. It has been much debated whether these changes in the HPT axis during illness are representative of an associated pathology requiring thyroid hormone replacement therapy or are indeed an adaptive response to ‘stress’ to decrease metabolic rate, which in turn may be beneficial to the sick patient.

A wide range of mechanisms give rise to the hormonal changes seen in the NTIS; these include modifications to the hypothalamic–pituitary axis, altered binding of thyroid hormone to circulating binding proteins, modified entry of thyroid hormone into tissue, changes in thyroid hormone metabolism due to modified expression of the intracellular iodothyronine deiodinases and changes in thyroid hormone receptor (THR) expression or function. In this review, the current state of knowledge regarding these processes will be described together with the current consensus view on the need for clinical intervention with thyroid hormone replacement in such sick patients.

Typical changes in thyroid function tests in illness
Mild thyroid disease is very common and may present with only vague non-specific symptoms, particularly in the elderly. Such patients can be readily identified by using ‘thyroid function tests’, measurement of serum thyrotrophin (TSH), thyroxine (T4) and T3. Thyroid function testing becomes problematic when the tests are performed in patients who have any significant co-existing illness (organic or
The non-thyroidal illness syndrome

Changes to the hypothalamic–pituitary axis in organic illness

In illness and central hypothyroidism, serum TSH is often normal but may be suppressed despite the fact that circulating T3 may be low. Both central hypothyroidism and critical illness result in a similar decline in the usual nocturnal surge and pulse amplitude of TSH, and in addition, TSH with impaired biological action may be produced in both conditions (Adriaanse et al. 1993, Bartalena et al. 1993, Fliers et al. 1997, 2006).

Using sensitive TSH assays, it has become apparent that whilst almost all hyperthyroid patients have TSH values <0.01 mU/l, most patients with low TSH due to the NTIS have serum TSH concentrations, which are >0.01 mU/l (Spencer 1988). This is despite a low serum T3 and a reported 50% reduction in overall hypothalamic and pituitary T3 levels in illness, which under normal circumstances should lead to an increase in thyrotropin-releasing hormone (TRH) and TSH secretion.

It seems that the low TSH associated with critical illness (or failure of TSH to rise in the presence of a low T3 and T4) arises from a central hypothyroidism caused by alterations in the set point of the HPT axis. Specific groups of TRH neurons situated in the paraventricular nucleus (PVN) of the hypothalamus are required to promote TSH synthesis in the pituitary and regulate thyroid hormone synthesis, and such neurons appear to be the focus of the set point in the HPT axis (Fliers et al. 2001, 2006). A loss of TRH gene expression occurs in PVN samples taken at post-mortem from patients with prolonged illness who died with serum biochemistry typical of the NTIS (Fliers et al. 1997). Furthermore, administering TRH and GH secretogogues to patients with prolonged critical illness at least partially restores serum T3, TSH and T4 (van der Heyden et al. 1986, Van den Berghe et al. 1998, 1999, 2002).

It is likely that there may be multiple causes for the loss of TRH in hypothalamic neurons in the NTIS including prolonged diminished calorie intake and release of inflammatory cytokines. In some sick patients, an increase in serum FT3 occurs (when measured by a reference method), which potentially may feedback on the hypothalamus/pituitary to moderate TRH/TSH release (Beckett et al. 1991).
Mechanisms regulating hypothalamic TRH production in illness and fasting


Fasting

Fasting leads to a diminution in steady state T3 levels and lowers the set point of the HPT axis. Decreased TRH in the PVN occurs in fasting and is thought to be brought about through decreases in leptin. This action of leptin appears to involve two principal classes of neuroendocrine cells in the arcuate nucleus (Fig. 2). The neurons of the PVN that secrete TRH are innervated by neurons from the arcuate nucleus that contain α-melanocyte-stimulating hormone (α-MSH or MC1R), neuropeptide Y (NPY), agouti-related protein (AGRP) and the inhibitory neurotransmitter GABA. Both NPY and AGRP stimulate food intake. Recent work indicates that hypothalamic T3 production, catalyzed by D2, triggers the production of mitochondrial uncoupling protein 2, which is critical for the appropriate activation of NPY/AgRP neurons in the arcuate nucleus during fasting (Coppola et al. 2007). Both NPY and AGRP inhibit TRH gene expression, an action accentuated by gherelin and prevented by leptin. In contrast, α-MSH stimulates TRH gene expression in the cells of the PVN, and this effect is enhanced by leptin (Fig. 2). It is presumed that the inhibitory effect of AGRP on TRH gene expression is due to it antagonising the effects of α-MSH, whereas the inhibitory effect of NPY occurs by reducing cAMP. During fasting, when leptin decreases, the inhibition of α-MSH production and the concurrent increase in AGRP and NPY production reduce CREB phosphorylation in TRH neurons, thereby reducing the set point for feedback inhibition of the TRH gene by thyroid hormone. Animals deprived of food show a decline in T3 and TRH in the PVN similar to the changes found in NTIS, and such changes can be reversed by giving leptin or introducing lesions into the hypothalamic arcuate nucleus. Although the above observations relating to fasting may in part explain the changes in PVN TRH found in illness, other factors that are disease specific may also operate. For example, in infection, α-MSH gene expression is increased rather than decreased, which in turn should promote TRH production (Sergeyev et al. 2001). However, given that malnutrition is a component of many acute and chronic illnesses, it is impossible to separate the effects of starvation from systemic illness in an individual patient.

Figure 2 Proposed mechanisms behind the central hypothyroidism (low hypothalamic TRH) induced by the NTIS and fasting. (Left panel) The neurons of the paraventricular nucleus (PVN) that secrete TRH are innervated by neurons from the arcuate nucleus (ARC) that contain melanocyte-stimulating hormone (α-MSH), neuropeptide Y (NPY), agouti-related protein (AGRP), and the inhibitory neurotransmitter GABA. Both NPY and AGRP inhibit TRH gene expression, an action prevented by leptin. During fasting, when leptin decreases, the inhibitory actions of NPY of AGRP can prevail leading to diminished TRH. The expression of TRH in the PVN is stimulated by MSH, and this effect is enhanced by leptin. Thus, in fasting (low leptin), the stimulatory action of MSH on TRH expression in the PVN is diminished. (Right panel) T3 produced by iodothyroinine deiodinase D2 in tanycytes has important feedback inhibitory actions on TRH production in the PVN. During sepsis and trauma, there is an increase in tanycyte D2, which is postulated to lead to an increased generation of T3 from T4. Tanycyte processes may extract T4 from portal capillaries, blood vessels in the arcuate nucleus or the CSF (in the third ventricle). The T3 can then be released back into the CSF or the blood stream. TRH neurons may take up T3 via diffusion from the CSF, by axonal terminals of the TRH neurons present in the median eminence, or the release of T3 into the arcuate nucleus may influence the activity of arcuate neurons that project into the PVN (Lechan & Fekete 2005).
Sepsis and trauma

T3 has an important feedback inhibitory action on TRH production in the PVN but TRH neurons lack the ability to produce T3 from T4 and as such appear unable to directly sense circulating T4. The tanycyte is a unique glial cell type that lines the floor of the third ventricle having processes that extend deep into the hypothalamus. These cells in the mediobasal hypothalamus may be an important source of T3 to provide localised feedback regulation on the TRH neuron situated in the PVN (Fliers et al. 2001, 2006, Lechan 2008). During sepsis and trauma, there is an increase in tanycyte D2, which is postulated to lead to an increased generation of T3 from T4. Tanycyte processes may extract T4 from portal capillaries, blood vessels in the arcuate nucleus or the CSF (in the third ventricle). The T3 can then be released back into the CSF or the blood stream. TRH neurons may take up T3 via diffusion from the CSF by axonal terminals of the TRH neurons present in the median eminence, or the release of T3 into the arcuate nucleus may influence the activity of arcuate neurons that project into the PVN (Fig. 3; Lechan & Fekete 2005).

Recent studies in rodents highlight the potential importance of the tanycyte in sepsis for eliciting a downregulation of TRH production and modifying the set point of the HPT axis to produce central hypothyroidism. Administration of bacterial lipopolysaccaride (LPS) to rats produces a fourfold increase in D2 in the hypothalamus (Fekete et al. 2004, 2005), and a similar effect is seen in LPS-treated mice or mice subjected to chronic inflammation induced by turpentine (Boelen et al. 2006). LPS treatment produces little effect on D2 expression in the anterior pituitary of these animals suggesting a cell-specific effect on hypothalamic D2 in sepsis, which is independent of a decrease in circulating T3. Inflammation induced by turpentine also decreases the expression of D3 in the hypothalamus (D3 metabolises T3 to inactive T2) accompanied by a lowering of hypothalamic TRH (Boelen et al. 2006). Thus, infection may initiate D2-mediated increased conversion of T4 to T3 and diminished D3-mediated catabolism of T3 (Fig. 3) leading to a local tissue hyperthyroidism, which in turn exerts a negative feedback control on TRH synthesis in hypophysiotropic neurons (Fig. 2). It is not yet known whether these or similar mechanisms operate in humans, but in a rabbit model of prolonged critical illness (7 days following infliction of full thickness burn injury) TRH in the PVN was decreased accompanied by an increase in hypothalamic D2 expression. Although hypothalamic T3 levels were unchanged, this does not preclude localised changes in the environment of the PVN and arcuate nucleus (Mebis et al. 2009b).

Locally produced cytokines may exert an important negative feedback control on TSH release by the thyrotroph in the pituitary (Prummel et al. 2004). Pro-inflammatory cytokines produced peripherally by patients with sepsis, trauma and autoimmune disease may thus also act directly on the pituitary thyrotroph to impair TSH release. Interleukin (IL)-6 appears particularly potent at suppressing TSH in plasma, but other cytokines, including TNF-α and interferon-γ, may have a similar effect (Boelen et al. 1993, 1995, 1996, 2004a,c).

Stress and a number of drugs such as glucocorticoids and dopaminergic drugs also suppress TSH (Fliers et al. 1997, Bartalena et al. 1998, Van den Berghe et al. 1998).

Thyroid hormone metabolism in health and illness

Whilst the thyroid gland provides the only source of T4 for peripheral tissues, a fine regulation of the thyroid hormone environment in extra-thyroidal tissues is made possible by differential tissue expression of the iodothyronine deiodinases. These enzymes are capable of metabolising T4 to the biologically active T3, or bio-inactive reverse T3 (rT3) and T2. It is now clear that the expression of these deiodinases is modified by illness and such modifications can be highly organ specific resulting in tissue-specific modifications to thyroid status in illness.

Health

The thyroid produces T4 in significantly larger quantities than the biologically active T3. Although released from the thyroid in a ratio of ~17:1 (T4:T3; Pilo et al. 1990), the circulating levels of each hormone are also determined by extra-thyroidal conversion of T4 to T3, which in healthy humans accounts for more than 80% of T3 production. Activation and inactivation of thyroid hormone are carried out by a group of three iodothyronine deiodinases, each of which is a selenoprotein encoded by a separate gene. The deiodinases 1, 2 and 3 have distinct tissue distributions, substrate affinities and physiological roles (Fig. 3; Bianco & Kim 2006, Gereben et al. 2008a,b). All deiodinases are integral membrane proteins, and although their cellular localisation varies, all their catalytic domains reside within the cell cytosol (Toyoda et al. 1995, Baqui et al. 2000, Friesema et al. 2006). D1 and D2 activate T4 by removing an iodine atom from its outer ring (5’ deiodination), forming T3. On the other hand, D3 inactivates both T3 and T4 by removing an iodine atom from the inner ring (5-deiodination) generating T2 and rT3 respectively, a reaction that can also be catalyzed by D1 inactivates both T3 and T4 by removing an iodine atom from the inner ring (5-deiodination) generating T2 and rT3 respectively, a reaction that can also be catalyzed by D1.

Figure 3 Deiodination reactions catalyzed by the iodothyronine deiodinase isoenzymes D1, D2 and D3.

(Fig. 3). The D1 and D3 isoenzymes are located in the plasma membrane, whilst D2 is retained in the endoplasmic reticulum. The T₃ produced from D2 thus potentially has ready access to the nucleus due to its close proximity (Zeold et al. 2006a,b), whilst T₃ produced by D1 is more readily exported into plasma. These differences in intracellular location may explain why D2 appears particularly adept at supplying T₃ for local use in the nucleus of the cell particularly in tissues such as brain, pituitary and brown adipose tissue (Bianco et al. 2002, Galton et al. 2009), whilst tissues that express high levels of D1 such as liver and kidney export into plasma much of the T₃ they produce (Chanoine et al. 1993, Bianco et al. 2002, Bianco & Kim 2006).

Debate has continued for many years regarding the predominant source of plasma T₃ in humans. Although initially D1 was thought to provide the majority of plasma T₃ in humans, as is the case in rodents, further investigations provided evidence to suggest that D2 may quantitatively be a more important source of plasma T₃ than D1. For example, treatment with the D1 inhibitor PTU only leads to an ~30% decrease in serum T₃ in patients with primary hypothyroidism receiving fixed doses of exogenous T₄, suggesting an important potential role for D2 in generation of plasma T₃ (Geffner et al. 1975, Saberi et al. 1975, LoPresti et al. 1989).

Whilst initial studies suggested that in humans as much as two-thirds of plasma T₃ may be generated by D2 from skeletal muscle (Maia et al. 2005), it has become clear that D2 activity in skeletal muscle may have been markedly overestimated in these early studies. The assay for deiodinase activity involves assessing the release of iodine from iodinated iodothyronines. It seems that in muscle homogenates, iodide can be released from T₄ without the generation of T₃; thus providing an important tool for assessing the release of iodine from iodinated iodothyronines. In skeletal muscle (Maia et al. 2005), it has become clear that D2 activity in skeletal muscle may have been markedly overestimated in these early studies. The assay for deiodinase activity involves assessing the release of iodine from iodinated iodothyronines. It seems that in muscle homogenates, iodide can be released from T₄ without the generation of T₃; thus providing an overestimate of D2 activity in this tissue (Larsen 2009). It now appears that D2 activity in human skeletal muscle is in fact very low (Heemstra et al. 2009). Thus, whilst D2 may still contribute to circulating T₃ in humans, it appears that skeletal muscle is not an important tissue source.

Whilst under normal circumstances D1 and D2 appear to provide important systemic and local sources of T₃ respectively, it seems that at least in rodents, deiodinase action is not critical for maintenance of plasma T₃ concentrations (Galton et al. 2009, St Germain et al. 2009). D1 and D2 knockout mice maintain normal plasma T₃ concentrations, and their general health and reproductive capacity appear to be unimpaired. However, D2 knockout animals do show deficits in TSH regulation and thermogenesis, and the T₃ content of brain is decreased in these animals despite brain T₄ being increased. These changes in brain thyroid hormone content are associated with only minimal changes in expression of thyroid hormone-responsive genes, motor function and learning ability. These findings support the view that 5’ deiodinase action (D1 or D2) is not essential for general health or maintenance of plasma T₃.

Combined D1/D2 knockout mice have plasma T₄ almost twice that found in wild-type animals and a plasma TSH of 2-6 times the level found in wild type or D1 knockout. The levels of plasma rT₃ showed the most dramatic differences between genetic strains with the D2 knockout having no effect on rT₃, whilst D1 and combined D1/D2 knockout had an increase in serum rT₃ of two- and sixfold respectively. The increase in rT₃ seen in the D1/D2 knockout may be due to impaired catabolism of rT₃ through diminished 5’-deiodination and/or enhanced rT₃ production from T₄ catalyzed by D3. It is clear that D3 has an important role in the production of rT₃, since rT₃ is undetectable in the triple D1/D2/D3 knockout mouse (Galton et al. 2009).

These largely unexpected observations in the D1/D2 knockout mice raise major questions regarding the role of the deiodinases in maintaining thyroid status and also the role that these deiodinases may play in the NTIS (see below). The most likely explanation for the maintenance of plasma T₃ in the D1/D2 knockout animals is that enhanced thyroidal production of T₃ takes place, a process driven by the increase in TSH concentration that occurs in these animals. This explanation is also consistent with the observation that selenium-deficient rats that express very low levels of renal and hepatic D1 largely maintain plasma T₃ through an increased thyroidal production of the hormone (Beckett & Arthur 2005).

**Illness**

Data concerning the potential role of the deiodinases in the pathogenesis of NTIS is conflicting. The generally accepted view was that extra-thyroidal conversion of T₄ to T₃ is diminished in illness due to a diminution in both hepatic/renal D1 activity and skeletal muscle D2 activity. In addition to these changes, there may be an increase in hepatic and skeletal muscle D3 activity, which leads to increased production of rT₃ from T₄ and increased catabolism of T₃ to produce T₂ (Chopra 1997, Peeters et al. 2005, De Groot 2006). It was argued that together these modifications to deiodinase expression could be the major contributors to the low T₃ concentrations associated with the NTIS. The trigger for these changes in deiodinase expression has been attributed to an increase in serum glucocorticoids and pro-inflammatory cytokines that often occurs in NTIS (Boelen et al. 1995, 2005, Hosoi et al. 1999, Jakobs et al. 2002, Kwakkel et al. 2007, 2009).

The validity of these conclusions has, however, now been challenged, and some have argued that the modifications to deiodinase expression in the NTIS may be a consequence of the changes that occur in T₃ and T₄, rather than the cause of these hormonal changes (O’Mara et al. 1993, Debaveye et al. 2008). This hypothesis is supported by studies on both D3 knockout mice (Boelen et al. 2009) and D1/D2 knockout mice (St Germain et al. 2009) subjected to treatment with LPS. In these mice, the changes in T₃ and T₄ that occurred in response to LPS were essentially no different from the changes seen in wild-type animals.

Furthermore, in wild-type mice injected with LPS, the decrease in plasma total T₃ and total T₄ precedes the fall in hepatic D1 with D2 expression in muscle being increased by
such treatment (Kwakkel et al. 2008). In humans, it seems that skeletal muscle D2 does not contribute to the low T₃ syndrome in either prolonged or acute illness, and indeed in prolonged critical illness a two- to threefold increase in muscle D2 expression occurs (Mebis et al. 2007).

In mice, inflammation induced by turpentine increases D2 in skeletal muscle but in contrast infection with *Streptococcus pneumoniae* leads to a decrease in muscle D2 (Kwakkel et al. 2009). These seemingly paradoxical D2 responses may be related to the different inflammatory signalling cascades that occur in the two models of illness. The D2 increase following turpentine administration results from activation of the cAMP pathway, whilst in severe bacterial infection D2 is down-regulated as a response to diminished food intake and IL-1β release (Kwakkel et al. 2009).

A decrease in T₃ may also be a consequence of increased catabolism of T₃ by D3. In critically ill patients, D3 expression occurs in skeletal muscle and liver, tissues which usually do not express the deiodinase in the adult (Peeters et al. 2005). The expression of D3 was found to be positively correlated with rT₃ in serum and negatively correlated with serum T₃, which raises the question of whether D3 expression in liver and skeletal tissue may be important contributors to the low T₃ in chronic NTIS in humans.

The above-mentioned observations thus question the role of the D1 and D2 deiodinases in the pathogenesis of the low plasma T₃ seen in NTIS at least in the acute situation (Boelen et al. 2008, 2009, Debaveye et al. 2008, St Germain et al. 2009). The results suggest that the rapid fall in T₃ seen in acute illness is more likely to be due to either impaired thyroidal production of T₃ (due to central hypothyroidism) and/or the result of the acute phase response leading to a diminution in serum thyroid hormone-binding proteins (discussed later).

An important role of thyroidal potassium channels for the maintenance of adequate thyroid hormone production has very recently been described (Roepke et al. 2009). No data currently exist regarding the influence of illness on the activity of these potassium channels.

**Selenium status, deiodination and the NTIS**

As plasma selenium levels are often low in sick patients, especially those with severe illness and sepsis (Maehira et al. 2002), it has been suggested that the expression of the selenoenzymes D1, D2 and D3 may be limited by the low selenium supply in these patients, and that this represents a mechanism for the pathogenesis of the low T₃ seen in the NTIS. There is little evidence to support this view, indeed the typical plasma thyroid hormone profile seen in selenium deficiency is that plasma T₃ is well maintained due to a switch to thyroidal production, whilst total T₄ rises in plasma (Beckett & Arthur 2005, Gartner 2009). It seems more likely that the low selenium status seen in acute and chronically ill patients is a result of diminished concentrations of selenoproteins in plasma as a consequence of the acute phase response (Sammalkorpi et al. 1988, Maehira et al. 2002, Renko et al. 2009).

**Effects of illness on plasma thyroid hormones and thyroid hormone-binding proteins**

Thyroid hormones are bound, reversibly, to thyroxine-binding globulin (TBG), transthyretin (previously known as thyroxine-binding pre-albumin) and albumin. Transthyretin is the major T₄/T₃-binding protein in the plasma of rodents, whilst in humans TBG transports most of thyroid hormone in blood. Transthyretin and TBG are acute phase proteins and their concentration can fall markedly in a wide range of illnesses.

Under normal circumstance <0.05% of the hormone circulates unbound (free) in plasma. The ‘free hormone hypothesis’ assumes that it is only this small ‘free’ hormone fraction that is able to enter the cell and interact with the nuclear THR to confer biological action. Thus, the concentration of total T₃ and total T₄ in plasma is heavily dependent on the concentration of these binding proteins, whilst the free hormone concentrations should be largely independent of binding protein concentrations.

In severe NTI, the concentration of the thyroid hormone-binding proteins often decreases as a consequence of the ‘acute phase response’; this arises from impaired synthesis, rapid breakdown and movement out of the plasma space (Jirasakuldech et al. 2000). For example, following bypass surgery, TBG levels may fall as much as 60% in 12 h (Afandi et al. 2000). The acute fall in these plasma binding proteins may thus account for much of the changes in plasma total T₃ and total T₄ seen in acute illness. In some, but not all patients with chronic illness, a desialylated form of TBG is synthesised by the liver, and this protein appears to have an affinity for thyroid hormone of approximately one-tenth of that of normal TBG (also known as slow TBG because of altered electrophoretic mobility); this also gives rise to a fall in the circulating levels of total thyroid hormone as a consequence of the diminished thyroid hormone binding capacity (Reilly & Wellby 1983, Costante et al. 1985). In rodents, inflammation and fasting leads to a marked decrease in transthyretin, the major plasma thyroid hormone-binding protein in this species (Dickson et al. 1982, Wade et al. 1988).

Early literature suggested that substances, similar to non-esterified fatty acids (NEFA), may accumulate in plasma in the NTIS and inhibit the binding of T₄ and T₃ to their binding proteins, again lowering the thyroid hormone binding capacity of plasma. Much doubt has been cast on the role of NEFA as endogenously produced binding inhibitors for thyroid hormones (Mendel et al. 1991), since the concentration of NEFA in plasma rarely reaches that required to inhibit thyroid hormone binding *in vitro*. However, the serum binding capacity for thyroid hormone is significantly

decreased in sick patients, and it is likely that many substances that compete with thyroid hormone binding to plasma proteins accumulate in the plasma of patients with hepatic and renal failure. Drugs such as furosemide, fenclofenac, carbamazepine and salicylate compete with the binding of thyroid hormone to plasma proteins at therapeutic concentrations; this also leads to a decrease in total T₃ and total T₄.

Free thyroid hormone measurements in NTIS

Equilibrium dialysis or ultrafiltration methods are widely regarded as reference methods for the measurement of free thyroid hormones in serum, although neither is completely satisfactory unless the measurement system has been well characterised (Fritz et al. 2007a,b). When using these dialysis methods, it is essential to minimise disruption of the original equilibrium by keeping both sample dilution and the ratio of the volumes of dialysing buffer and sample compartments as low as practically possible. If these conditions are met, samples from patients with NTI often show normal or raised FT₄ in their serum and FT₄ is rarely low (Fig. 1). In contrast, the use of significant sample dilution prior to equilibrium dialysis results in a fall in FT₄ measured using these ‘reference’ methods (Beckett et al. 1991, Christofides et al. 1999a,b, Beckett 2006).

Many routine assays for the measurement of free thyroid hormone in serum are prone to artefacts that tend to underestimate the ‘true’ free hormone concentration that is in the serum of patients (and animals) with NTIS. Samples with the lowest serum binding capacity for thyroid hormones (NTIS samples) are particularly prone to this effect (Christofides et al. 1999a,b). Unfortunately, most published work regarding the effects of NTIS on free thyroid hormones have used inappropriate methodology, and these methodological artefacts have resulted in a mass of literature that gives a misleading picture of the effects of illness on free thyroid hormone concentrations in blood. Indeed, FT₄ may appear to be low, normal or raised even in the same sample depending on the assay method (Beckett et al. 1991, Christofides et al. 1999a,b, Beckett 2006). In preterm infants, serum FT₄ measured by equilibrium dialysis shows a much lower frequency of subnormal results than when the same samples are measured by routine diagnostic methods (Deming et al. 2007).

The reliable measurement of FT₃ in serum has been even more problematic than for FT₄ yet the measurement of the ‘true’ FT₃ concentration in illness is fundamental to the perception of the ‘thyroid status’ of patients with NTI. The literature is quite clear that serum total T₃ falls in illness, and the degree of this fall is often proportional to the severity of illness. Most routine methods for FT₃ show that these concentrations tend to parallel the changes in serum total T₃. However, results from two studies that have utilised a ‘reference’ ultrafiltration method for FT₃ analysis have concluded that illness results in only a modest fall in serum FT₃ (mean FT₃ concentrations in NTIS group being only 10% lower than the healthy control group; Faber et al. 1987, Faber & Siersbaek-Nielsen 1996). Using a commercial FT₃ method that shows good agreement with the ultrafiltration reference method, we have found that the percentage of patients in an intensive care unit who had a FT₃ below the reference range was in the order of 27%, whilst 70–80% of patients had low total T₃. In the same study, using a reliable assay for FT₄, we also found that whilst around 50% of patients had subnormal total T₄ only around 2% had low FT₄ (Fig. 4; Ray et al. 2002).

These observations support the view that the marked changes that occur in total thyroid hormone concentration during acute NTIS are largely due to changes in the serum binding capacity for thyroid hormones as a result of the acute phase response. Whilst free hormones do change in illness, it would appear that such changes are of a more modest nature than that of total thyroid hormone. It should be remembered that most published studies of the NTIS in small animals have measured only total thyroid hormone concentrations. Since the effect of the acute phase response on serum total thyroid hormone-binding proteins may be marked, it may mask more subtle changes that could be occurring in free hormone concentrations in the various animal models. Indeed, the importance of the acute phase response at influencing total T₃ and total T₄ may explain why these hormones fall in a similar fashion in wild-type and D1, D2 and D3 knockout mice after exposure to LPS.

It should be noted that in diseases that give rise to hepatic inflammation, plasma TBG concentrations (and thus the thyroid hormone binding capacity) may transiently increase as a consequence of increased release from the liver; this produces an increase in TT₄ during the acute phase of such inflammatory conditions (Gardner et al. 1982).

Figure 4 The percentage of patients admitted to an intensive care unit who had serum thyroid hormone and TSH concentrations below the lower reference limit. Data for the first 3 days of admission are shown in both survivors and non-survivors, and significant differences between these two groups are shown by (*). Free T₄ and Free T₃ were measured by Amerlite MAB methodology (Ortho Clinical Diagnostics, Amersham), which show good correlation with equilibrium dialysis and ultrafiltration respectively (see text for details). Data taken from Table 4, Ray D C, Macduff A, Drummond G B, Wilkinson E, Adams B & Beckett GJ 2002 Endocrine measurements in survivors and non-survivors from critical illness. Intensive Care Medicine 28 1301–1308 with kind permission from Springer Science & Business Media.
Thyroid hormone uptake by tissue in NTIS; the role of thyroid hormone transporters

Although changes in the circulating concentrations of thyroid hormones are commonly discussed in reference to NTIS, tissue thyroid status is governed by intracellular T₄, particularly that which interacts with the THRs in the nucleus. Intracellular T₃ and T₄ is dependent not only on the local variation in the activity of D₁, D₂ and D₃, but also on the ability of the cell to transport thyroid hormone. Clearly, if the cellular uptake of thyroid hormone is impaired, then intracellular deiodination of T₄ cannot take place, giving rise to diminished peripheral production of T₃ and also potentially local tissue hypothyroidism.

The cellular uptake of thyroid hormones is not simply the result of passive diffusion across the lipid bilayer, but involves ATP-dependent transport processes. Thyroid hormone transporters are required for both entry and exit of thyroid hormone from the cell, and these transporters have different tissue distributions and ligand affinities. Thyroid hormone transport proteins include Na-taurocholate cotransporting polypeptide, fatty acid translocase, multidrug resistance-associated proteins, amino acid transporters, and members of the organic anion transporter polypeptide (OATP) and monocarboxylase transporter (MCT) families (discussed below).

The majority of the thyroid hormone transporters demonstrate a low specificity and an apparently low affinity for thyroid hormone. As yet, only three key transporters with a high affinity and a high specificity for thyroid hormone have been identified. OATP1C1 is localised in the brain capillaries and transports T₄ and rT₃. MCT8, localised in the brain, hypothalamus, pituitary gland, liver, heart and placenta, skeletal muscle, kidney and MCT10, found in the intestines, liver, kidney, skeletal muscle and placenta (Visser 2007, Visser et al. 2008, Dayan & Panicker 2009) both transport T₄ and T₃.

The physiological importance of thyroid hormone transporters is exemplified by mutations in the MCT8 gene. Mutations in this transporter have been associated with a form of X-linked mental retardation in about 20 different families. In addition to the psychomotor effects, these patients present with abnormal serum thyroid hormones, highly elevated T₃, decreased T₄ and normal TSH. In the MCT8 knockout mouse, these same abnormalities in serum thyroid hormones are seen but without evidence of neurological impairment (Dumitrescu et al. 2006, Trajkovic et al. 2007). Importantly, the impact of MCT8 deletion was tissue specific. Neither the entry of T₃ nor the entry of T₄ into the liver was found to be affected, perhaps not surprising given the multiple thyroid hormone transporters that are expressed here. As hepatic thyroid hormone uptake is not impeded, the high serum T₃ levels are translated into high intracellular T₃ levels, as demonstrated by an increased expression of hepatic D1. Indeed, both groups believe that these livers may be in a state of hyperthyroidism (as SHBG and other markers of hyperthyroidism are elevated). Although not crucial to thyroid hormone transport in the liver, it appears that MCT8 has a pivotal role for transportation of T₃ into the brain since in the knockout model, T₃ uptake into the brain was almost absent. T₄ was not affected, which has been attributed to the presence of other TH transporters such as OATP1C1 at the blood–brain barrier that exhibit a restricted specificity towards T₄ (Trajkovic et al. 2007). It has been suggested that impaired T₃ uptake in the brain may be the primary event in the generation of the high T₃ levels, reduced uptake preventing significant T₃ inactivation by neuronally localised D3 (Trajkovic et al. 2007).

In NTIS, transport of thyroid hormone into tissues is diminished but this does not appear to be due to a downregulation of the expression of thyroid hormone transporters; indeed, the expression of these transporters appears to be increased in both chronic and acute illness. Mebis et al. (2009a) found that an increase in MCT8 but not MCT10 gene expression occurs in the liver and skeletal muscle of patients in an intensive care unit with prolonged critical illness. The expression of the MCT8 gene demonstrated a strong inverse correlation with circulating TT₃ and TT₄. Using a rabbit model of prolonged critical illness (full thickness burn injury), this group observed an increase in hepatic MCT8, an induction of MCT10 in skeletal muscle and an increase in MCT10 and OATP1C1 in the hypothalamus (Mebis et al. 2007, 2009a). In this rabbit model, treatment with T₃ and T₄ produced a subsequent downregulation of MCT8 expression in liver and MCT10 in muscle; again transporter expression correlated inversely with circulating thyroid hormone concentrations. These observations suggest that changes in the expression of thyroid transporters are a consequence rather than a cause of changes in the concentration of plasma thyroid hormone found in NTI. In contrast to the observations of Mebis et al., Rodriguez-Perez et al. (2008) observed that MCT8 expression was unchanged in skeletal muscle of patients with septic shock but declined in subcutaneous adipose tissue; liver was not investigated.

Current evidence thus suggests that downregulation of thyroid hormone transporters does not occur in the NTIS, and other mechanisms must be responsible for the impaired uptake of thyroid hormone that is manifest in illness. Such mechanisms may include depletion of hepatic ATP or the presence in plasma of substances that impair hepatic uptake of thyroid hormone. NEFA and numerous substances that accumulate in the plasma of patients with renal or liver dysfunction inhibit cellular transport of T₄ into cultured hepatocytes (Hennemann et al. 2001).

Expression of THR in illness

Thyroid hormone action is largely dependent upon binding to THR, which are ligand-regulated transcription factors that bind to thyroid hormone response elements (TREs) in target genes. THR reside in both the nucleus and cytoplasm.
of the cell, and there is shuttling of these receptors between the two compartments (Davis et al. 2008). THRs in the nucleus form heterodimers with retinoid X receptors (RXR) which are another member of the nuclear receptor superfamily. These THR/RXR dimers bind to the target DNA sequences of the TREs to form a complex, which leads to the recruitment of transcriptional coactivators and corepressors.

The THRs are encoded by two genes, THRA and THRb, which give rise to several major THR isoforms, THRα2 (THRα2 does not bind TH), THRβ1 and THRβ2, which bind T3 with similar affinity and have similar transcriptional activity (for a review of thyroid hormone action see Oetting & Yen (2007)). This transcriptional activity is modulated by corepressors and coactivators. In the absence of T3, THRs bind to TREs and repress basal transcription of positively regulated target genes through association with a variety of corepressors, which include nuclear receptor corepressor (NCOR1), and silencing mediator of retinoic acid receptor and THR. Coactivators include steroid receptor coactivator (SRC-1) complex, which is one of at least two major complexes involved in ligand-dependent transcriptional activation of the nuclear hormone receptors.

A number of studies have examined the changes in the expression of THRs in models of the NTIS, with somewhat conflicting findings. Early work in patients with liver failure or chronic renal failure showed increased THRα and THRβ mRNA expression in peripheral mononuclear cells and liver tissue taken from biopsy (Williams et al. 1989). More recently, data from models of acute illness have been published, which have included mice treated with LPS or cytokines (Beigneux et al. 2003, Boelen et al. 2004b, Feingold et al. 2004) and patients with septic shock (Rodriguez-Perez et al. 2008) and in each model, THR expression is diminished. In the mouse LPS-treated model, a rapid and marked decrease in THRα and THRβ occurs accompanied by a decrease in the expression of the RXR isoforms, SRC-1 and other coactivators in the heart (Feingold et al. 2004). In agreement with these studies, but in contrast to the findings of Williams et al. 1989, Boelen et al. (2004b) reported a rapid decrease in liver Thrβ1 mRNA after LPS administration in an animal model. A lowering of THRβ1 mRNA in response to inflammatory cytokines also occurs in vitro using a hepatoma cell line (Yu & Koenig 2000). In the rabbit model of prolonged (7 days) burn injury, THRs were unchanged (Mebis et al. 2009b). Thus, in general, it appears that in chronic illness, THR expression is increased, whilst in acute illness THR expression is downregulated.

In humans with septic shock, a diminished expression of THRβ1 was seen after 5 days but with no associated change in THRα1, whereas decreases were seen in both receptors in subcutaneous adipose tissue. No differences were found in NCOR1 or SRC-1 in either tissue. The RXR showed both isoform and tissue-specific changes in expression in these patients. For example, in smooth muscle, RXRα mRNA increased, RXRβ mRNA was unchanged and RXRγ decreased.

THRβ1 is regarded as the main regulator of D1 expression in the liver (Amma et al. 2001), with increased T3 levels acting via THRβ1, to induce hepatic D1. In the absence of TRβ1, liver D1 mRNA expression is decreased, although THRα1 can partly take over the role of TRβ1 to induce D1 by T3 (Amma et al. 2001). As such, changes in the expression of THRβ would be expected to have profound effects on the expression of D1 in the liver and perhaps also the circulating concentrations of T3. This hypothesis has recently been challenged in light of results obtained using an LPS model of acute illness in knockout THRβ−/− mice (Kwakkel et al. 2008). In a 24-h period after LPS treatment, similar relative decreases were found in T₃, T₄ and liver D1 in both wild-type and THRβ−/− knockout mice. These observations have led to the conclusion that the decrease in liver D1 mRNA and activity observed in illness is not mediated via THRβ1.

The involvement of changes in coactivator and corepressor expression in the NTIS should also be considered. This is exemplified by the effects of IL-1 and IL-6, which impairs the ability of T₃ to induce D1 expression in primary cultures of rat hepatocytes. This effect could be partially overcome by cotransfection of the coactivator SRC-1 (Yu & Koenig 2000). Similarly in a mouse model, the decrease in hepatic D1 and plasma T₃ caused by LPS could be prevented by forced expression of SRC-1 (Yu & Koenig 2006). These observations are consistent with the view that cytokine-induced competition for limiting amounts of coactivators may be one mechanism behind the diminished hepatic D1 expression seen in the NTIS.

Should thyroid hormone replacement be advocated in NTIS?

Controversy surrounds the need for thyroid hormone replacement therapy in the NTIS. There have been very few clinical studies designed to address whether such replacement is advantageous, and if so which preparation (T₃ or T₄) should be used. If as is sometimes argued, the changes represent a physiological adaptation, attempts to restore thyroid hormone levels could even have adverse effects on patient outcome.

In a study of intensive care patients randomly assigned to T₄ or a placebo for 2 weeks, no improvement in survival was seen (Brent & Hershman 1986). The use of T₃ as the principal treatment on burns patients has similarly shown no improvement to outcome (Becker et al. 1982). A more novel approach to replacement in critically ill patients has been suggested by Van den Berghe et al. (1999, 2002). They used a continuous infusion of TRH together with a GH secretagogue and successfully restored both thyroid hormone and TSH concentration, and found improvements in catabolic parameters.

Given that thyroid hormones have significant effects on cardiac function, the consequence of thyroid hormone replacement on cardiac patients has been studied in a number
of settings including surgery, heart failure and transplant. These have been reviewed at length (Farwell 2008). It is suggested that T3 may be beneficial for stabilisation or improvement of cardiac function in donors before cardiac transplantation. Indeed, several consensus conferences in the US and Canada have recommended that T3 be included in a panel of hormones that are aimed at cardiac resuscitation, when the ejection fraction is \( <45\% \). Short-term studies involving T4 and T3 treatment in heart failure patients also show promise, with one of the observed benefits being increased cardiac output. No studies have so far looked at its long-term use in these patients.

Despite some promising work in both human and animal models, there is as yet no persuasive evidence for the use of thyroid hormone replacement in any patient with NTIS with the possible exception of patients with cardiac failure.

Changes in thyroid function tests during recovery from illness

When a patient recovers from illness, abnormalities in serum TSH and thyroid hormone concentrations eventually resolve. In some patients, however, TSH concentrations may rise transiently above the reference range in this recovery phase. In hospitalised patients, an elevated TSH is as likely to be due to recovery from NTIS as primary hypothyroidism (Spencer 1988, Stockigt 1996), and it is essential that clinicians understand this in order to avoid misdiagnosis that could lead to inappropriate lifelong T4 replacement therapy. Prospective studies on critically ill patients with burns, sepsis and acute renal failure showed that the rise in TSH during recovery consistently preceded the rise in T3 and T4 suggesting that this TSH rise is essential in some patients to return thyroid hormone homoeostasis to normal during recovery (Hamblin et al. 1986).

Conclusions

The mechanisms behind the hormonal changes seen in the NTIS are now becoming clearer and are summarised in Fig. 5. Induction of a central hypothyroidism appears to be common in many models of illness. Inadequate calorie intake resulting in decreased leptin can lead to central hypothyroidism due to diminished hypothalamic TRH in the neurons of the PVN. In addition, sepsis or trauma increases the expression of D2 in hypothalamic tanocytes, which may increase the T3 supply to the TRH neurons in the PVN, thus suppressing TRH production. Increased cytokines in sepsis and trauma may also directly inhibit TSH release from the pituitary thyrotroph.

The total concentrations of plasma T3 and T4 fall acutely in illness and precede any decline in hepatic D1, whilst D2 in muscle is increased. It would seem, therefore, that much of the acute fall in circulating thyroid hormones in the NTIS can be attributed to an acute phase response giving rise to loss of plasma TBG or transthyretin and also the accumulation of substances that lower the thyroid hormone binding capacity of plasma. When measured by reliable methods, the fall in FT4 and FT3 may be modest in comparison to the fall seen in total thyroid hormone; unreliable methods for free hormone measurement considerably underestimate the ‘true’ free hormone concentration in the serum from sick patients.

Whilst in vitro cell studies suggest that thyroid hormone uptake by cells may be impaired in the NTIS, this is not the result of a downregulation of thyroid hormone transporters. Indeed, these transporters tend to be up-regulated or remain unchanged in many models of the NTIS. Thus, if diminished hormone uptake does occur in vivo, it is likely to be the result of diminished intracellular ATP or possible accumulation of substances in plasma that compete with thyroid hormones for the thyroid hormone transporters. Evidence from animal
knockout models indicate that changes in D1 and D2 may have only a modest contribution to the low plasma T3 in NTIS, at least in acute illness.

In humans, chronic illness appears to lead to an upregulation of THR expression, at least in liver and renal failure. In contrast, human and animal models of sepsis and trauma indicate that expression of THR and their coactivators are diminished in acute illness.

Despite some promising work in both human and animal models, there is as yet no persuasive evidence for the use of thyroid hormone replacement in any patient with NTIS, with the possible exception of patients with cardiac failure.

Declaration of interest

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