REVIEW

Mechanisms behind the non-thyroidal illness syndrome: an update

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Abstract

The mechanisms behind the changes in serum triiodothyronine (T_3), thyroxine (T_4) 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 T_3 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 T_3 found in acute illness. The decline in serum T_3 and T_4 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 T_4 and free T_3 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.

Journal of Endocrinology (2010) **205**, 1–13

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 (Vermees & Beishuizen 2001). Changes within the hypothalamic–pituitary–thyroid (HPT) axis also occur in illness and are typically associated with low levels of total triiodothyronine (T_3), and this has given rise to the term ‘low T_3 syndrome’. Sick patients with low serum T_3 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 (T_4) and T_3 . Thyroid function testing becomes problematic when the tests are performed in patients who have any significant co-existing illness (organic or

Figure 1 Schema showing the general changes that occur in serum thyroid-related hormones following illness of varying severity. The yellow area represents the reference range. The changes for free T_4 and free T_3 represent those found using either ultrafiltration or equilibrium dialysis of serum with low sample dilution. Publications that use methods for free hormone measurement that are unreliable in situations of low serum hormone binding capacity tend to follow the same profile as for total T_3 and total T_4 (see text for full explanation).

psychiatric), since the illness *per se* may give rise to changes in thyroid function tests that mimic a thyroid disorder (Fig. 1).

A decrease in T_3 is the most common finding in 'euthyroid' sick patients, occurring in even the mildest forms of non-thyroidal illness (NTI). Patients with moderate to severe illness may also show abnormalities in TSH and T_4 . Whilst serum TSH is regarded clinically as being the most sensitive and specific thyroid function test, in the acute hospital setting, as many as 3% of patients may exhibit TSH concentrations that are <0.1 mU/l (typical TSH reference range 0.3–4.0 mU/l), and in around 75% of these patients, the low TSH can be attributed to the NTIS or the use of glucocorticoid or dopamine medication causing TSH suppression (Spencer *et al.* 1987). During recovery from illness, TSH may increase transiently above the reference range (Spencer *et al.* 1987). Similarly, a high, normal or low T_4 may also occur in sick patients. In the intensive care unit, the prevalence of abnormal thyroid function tests is extremely high with more than 70% of patients showing low total T_3 and around 50% have low total T_4 (Ray *et al.* 2002).

The abnormalities in thyroid function tests are not limited to organic illness but also manifest themselves in acute psychiatric admissions (Hein & Jackson 1990, Arem & Cusi 1997, Premachandra *et al.* 2006, Sabeen *et al.* 2009). In such patients, a raised T_4 is frequently found with either a normal or high TSH, suggesting central activation of the HPT axis. In most instances, the thyroid function tests normalise within 2 weeks of admission (Hein & Jackson 1990). Suppressed TSH levels, usually associated with a normal free T_4 (FT $_4$), have also been described in such psychiatric patients.

With regard to serum FT $_4$ and free T_3 (FT $_3$), the picture is complicated further by the fact that the changes in free

hormone concentration observed in illness vary according to the analytical method (Beckett 2006). Reference methods often show an increase in FT $_4$ with illness of a moderate severity, whilst less robust assays that are commonly used for routine diagnostic purposes often show subnormal results in the same samples (Beckett *et al.* 1991). Similarly, the prevalence of a low FT $_3$ in illness is dependent on the methodology (Faber *et al.* 1987, Faber & Siersbaek-Nielsen 1996, Beckett 2006). These analytical problems are discussed later.

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 T_3 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 T_3 and a reported 50% reduction in overall hypothalamic and pituitary T_3 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 T_3 and T_4) 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 secretagogues to patients with prolonged critical illness at least partially restores serum T_3 , TSH and T_4 (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 FT $_4$ 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

A number of factors mediate the effects of physiological stimuli on the TRH neurons of the PVN (Fliers *et al.* 2001, 2006, Lechan & Fekete 2004, Fekete & Lechan 2007, Lechan 2008, Chiamolera & Wondisford 2009, Mebis *et al.* 2009b and references therein).

Fasting

Fasting leads to a diminution in steady state T_3 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 T_3 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 ghrelin 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 T_3 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 or 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) T_3 produced by iodothyronine 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 T_3 from T_4 . Tanycyte processes may extract T_4 from portal capillaries, blood vessels in the arcuate nucleus or the CSF (in the third ventricle). The T_3 can then be released back into the CSF or the blood stream. TRH neurons may take up T_3 via diffusion from the CSF, by axonal terminals of the TRH neurons present in the median eminence, or the release of T_3 into the arcuate nucleus may influence the activity of arcuate neurons that project into the PVN (Lechan & Fekete 2005).

Sepsis and trauma

T_3 has an important feedback inhibitory action on TRH production in the PVN but TRH neurons lack the ability to produce T_3 from T_4 and as such appear unable to directly sense circulating T_4 . 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 T_3 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 T_3 from T_4 . Tanycyte processes may extract T_4 from portal capillaries, blood vessels in the arcuate nucleus or the CSF (in the third ventricle). The T_3 can then be released back into the CSF or the blood stream. TRH neurons may take up T_3 via diffusion from the CSF, by axonal terminals of the TRH neurons present in the median eminence, or the release of T_3 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 lipopolysaccharide (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 T_3 . Inflammation induced by turpentine also decreases the expression of D3 in the hypothalamus (D3 metabolises T_3 to inactive T_2) accompanied by a lowering of hypothalamic TRH (Boelen *et al.* 2006). Thus, infection may initiate D2-mediated increased conversion of T_4 to T_3 and diminished D3-mediated catabolism of T_3 (Fig. 3) leading to a local tissue hyperthyroidism, which in turn exerts a negative feedback control on TRH synthesis in hypophyseotropic 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 T_3 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 regulation 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 T_4 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 T_4 to the biologically active T_3 , or bio-inactive reverse T_3 (rT_3) and T_2 . 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 T_4 in significantly larger quantities than the biologically active T_3 . Although released from the thyroid in a ratio of $\sim 17:1$ ($T_4:T_3$; Pilo *et al.* 1990), the circulating levels of each hormone are also determined by extra-thyroidal conversion of T_4 to T_3 , which in healthy humans accounts for more than 80% of T_3 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 D1, D2 and D3 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 T_4 by removing an iodine atom from the outer ring (5' deiodination), forming T_3 . On the other hand, D3 inactivates both T_3 and T_4 by removing an iodine atom from the inner ring (5-deiodination) generating T_2 and rT_3 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 T_3 produced from D2 thus potentially has ready access to the nucleus due to its close proximity (Zeold *et al.* 2006a,b), whilst T_3 produced by D1 is more readily exported into plasma. These differences in intracellular location may explain why D2 appears particularly adept at supplying T_3 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 T_3 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 T_3 in humans. Although initially D1 was thought to provide the majority of plasma T_3 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 T_3 than D1. For example, treatment with the D1 inhibitor PTU only leads to an ~30% decrease in serum T_3 in patients with primary hypothyroidism receiving fixed doses of exogenous T_4 , suggesting an important potential role for D2 in generation of plasma T_3 (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 T_3 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 T_4 without the generation of T_3 ; 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 T_3 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 T_3 respectively, it seems that at least in rodents, deiodinase action is not critical for maintenance of plasma T_3 concentrations (Galton *et al.* 2009, St Germain *et al.* 2009). D1 and D2 knockout mice maintain normal plasma T_3 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 T_3 content of brain is decreased in these animals despite brain T_4 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 T_3 .

Combined D1/D2 knockout mice have plasma T_4 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 rT_3 showed the most dramatic differences between genetic strains with the D2 knockout having no effect on rT_3 , whilst D1 and combined D1/D2 knockout had an increase in serum rT_3 of two- and sixfold respectively. The increase in rT_3 seen in the D1/D2 knockout may be due to impaired catabolism of rT_3 through diminished 5'-deiodination and/or enhanced rT_3 production from T_4 catalyzed by D3. It is clear that D3 has an important role in the production of rT_3 , since rT_3 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 T_3 in the D1/D2 knockout animals is that enhanced thyroidal production of T_3 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 T_3 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 T_4 to T_3 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 rT_3 from T_4 and increased catabolism of T_3 to produce T_2 (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 T_3 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 T_3 and T_4 , 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 T_3 and T_4 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 T_3 and total T_4 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 T_3 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-1 β release (Kwakkel *et al.* 2009).

A decrease in T_3 may also be a consequence of increased catabolism of T_3 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 r T_3 in serum and negatively correlated with serum T_3 , which raises the question of whether D3 expression in liver and skeletal tissue may be important contributors to the low T_3 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 T_3 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 T_3 seen in acute illness is more likely to be due to either impaired thyroidal production of T_3 (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 T_3 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 T_3 is well maintained due to a switch to thyroidal production, whilst total T_4 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 T_4/T_3 -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 THRs to confer biological action. Thus, the concentration of total T_3 and total T_4 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 T_3 and total T_4 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 T_4 and T_3 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 T_3 and total T_4 .

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 FT_4 in their serum and FT_4 is rarely low (Fig. 1). In contrast, the use of significant sample dilution prior to equilibrium dialysis results in a fall in FT_4 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, FT_4 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 FT_4 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 FT_3 in serum has been even more problematic than for FT_4 yet the measurement of the 'true' FT_3 concentration in illness is fundamental to the perception of the 'thyroid status' of patients with NTI. The literature is quite clear that serum total T_3 falls in illness, and the degree of this fall is often proportional to the severity of illness. Most routine methods for FT_3 show that these concentrations tend to parallel the changes in serum total T_3 . However, results from two studies that have utilised a 'reference' ultrafiltration method for FT_3 analysis have concluded that illness results in only a modest fall in serum FT_3 (mean FT_3 concentrations in NTIS group being only

10% lower than the healthy control group; Faber *et al.* 1987, Faber & Siersbaek-Nielsen 1996). Using a commercial FT_3 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 FT_3 below the reference range was in the order of 27%, whilst 70–80% of patients had low total T_3 . In the same study, using a reliable assay for FT_4 , we also found that whilst around 50% of patients had subnormal total T_4 only around 2% had low FT_4 (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 T_3 and total T_4 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 TT_4 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 T_4 and Free T_3 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 G J 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 T_3 , particularly that which interacts with the THR α s in the nucleus. Intracellular T_3 and T_4 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 T_4 cannot take place, giving rise to diminished peripheral production of T_3 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 monocarboxylate 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 both a high affinity and a high specificity for thyroid hormone have been identified. OATP1C1 is localised in the brain capillaries and transports T_4 and rT_3 . 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 T_4 and T_3 .

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 T_3 , decreased T_4 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 T_3 nor the entry of T_4 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 T_3 levels are translated into high intracellular T_3 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 T_3 into the brain since in the knockout model, T_3 uptake into the brain was almost absent. T_4 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 T_4 (Trajkovic *et al.* 2007). It has been suggested that impaired T_3 uptake in the brain may be the primary event in the generation of the high T_3 levels, reduced uptake preventing significant T_3 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 TT_3 and TT_4 . 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 T_3 and T_4 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 the 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 T_4 into cultured hepatocytes (Hennemann *et al.* 2001).

Expression of THR α s in illness

Thyroid hormone action is largely dependent upon binding to THR α s, which are ligand-regulated transcription factors that bind to thyroid hormone response elements (TREs) in target genes. THR α s 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). THR α s 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 THR α s are encoded by two genes, THRA and THRB, which give rise to several major THR isoforms, THR α 1 (THR α 2 does not bind TH), THR β 1 and THR β 2, 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, THR α s 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 THR α s in models of the NTIS, with somewhat conflicting findings. Early work in patients with liver failure or chronic renal failure showed increased THR α and THR β 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 THR α and THR β 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 *Thr β 1* mRNA after LPS administration in an animal model. A lowering of *THR β 1* 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, THR α s 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 THR β 1 was seen after 5 days but with no associated change in THR α 1, 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, *RXR α* mRNA increased, *RXR β* mRNA was unchanged and *RXR γ* decreased.

THR β 1 is regarded as the main regulator of D1 expression in the liver (Ammal *et al.* 2001), with increased T₃ levels acting via THR β 1, to induce hepatic D1. In the absence of THR β 1, liver *D1* mRNA expression is decreased, although THR α 1 can partly take over the role of THR β 1 to induce D1 by T₃ (Ammal *et al.* 2001). As such, changes in the expression of THR β 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 THR β −/− 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 THR β −/− 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 THR β 1.

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

Figure 5 Summary of the mechanisms that give rise to the serum thyroid hormone changes in the non-thyroidal illness syndrome.

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 homeostasis 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 T_3 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 s 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

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

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

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Received in final form 9 December 2009

Accepted 16 December 2009

Made available online as an Accepted Preprint
16 December 2009