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Physiological role and regulation of iodothyronine deiodinases: a 2011 update

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Abstract

Thyroxine (T4) is a prohormone secreted by the thyroid. T4 has a long half life in circulation and it is tightly regulated to remain constant in a variety of circumstances. However, the availability of iodothyronine selenodeiodinases allow both the initiation or the cessation of thyroid hormone action and can result in surprisingly acute changes in the intracellular concentration of the active hormone T3, in a tissue-specific and chronologically-determined fashion, in spite of the constant circulating levels of the prohormone. This fine-tuning of thyroid hormone signaling is becoming widely appreciated in the context of situations where the rapid modifications in intracellular T3 concentrations are necessary for developmental changes or tissue repair. Given the increasing availability of genetic models of deiodinase deficiency, new insights into the role of these important enzymes are being recognized. In this review, we have incorporated new information regarding the special role played by these enzymes into our current knowledge of thyroid physiology, emphasizing the clinical significance of these new insights.

Introduction

The primary secretory product of the human thyroid gland is 3,5,3',5' tetraiodothyronine (thyroxine or T4) which must be monodeiodinated to 3,5,3' triiodothyronine (T3) to produce most of its effects. Triiodothyronine is required for normal growth and cognitive development in human infants and regulates metabolism in adults. In order to produce these effects, T3 must enter the nucleus and bind to specific thyroid hormone receptors which in turn are bound to thyroid hormone-responsive genes and regulate their transcription. Thyroid hormone can increase or decrease the expression of specific genes to produce its global effects. The reason T4 must be deiodinated to T3 to produce these effects is that the T4 molecule binds with about 10- to 15-fold less affinity than T3 to the thyroid hormone nuclear receptors.

The abbreviation "T4" reflects the fact that the iodothyronine molecule contains 4 atoms of iodine bound to the thyronine nucleus. One of these is removed to form T3 (Fig.1). Three enzymes, termed the iodothyronine deiodinases, have evolved to activate or inactivate thyroid hormone. Deiodination activates T4 by removing a single outer ring iodine to form T3 or can inactivate T4 and T3 by removal of a second iodine from the inner ring of the iodothyronine molecule (Fig. 1). Activation of T4 is catalyzed by the types 1 and 2 iodothyronine deiodinases (referred to as D1 and D2) while the inactivation of T4 and T3 is the function of a third deiodinase, the type 3 deiodinase (D3). The deiodinases belong to a specialized group of enzymes which contain the rare amino acid selenocysteine (Sec). This

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is located in their active center, which makes enzymes extremely effective in oxidation/reduction reactions, such as deiodination. Replacement of the Sec by Cys results in a 100-300 fold decrease in the V_{max} of D1 and D2 (1). The process of selenoprotein synthesis is complex because the codon for selenocysteine is UGA, which also serves as a translation STOP codon in about 50% of human mRNAs. Overcoming this stop codon function requires a series of proteins dedicated to the synthesis of the selenoproteins and a special tRNA upon which the selenocysteine molecule is synthesized (2, 3) (Fig. 2). Furthermore, eukaryotic selenoprotein mRNAs contain a hairpin loop structure in their 3' untranslated region, the SECIS element, which allows the ribosome to override the STOP codon function of the UGA (2, 3).

In humans, approximately 80% of the T3 produced daily derives from monodeiodination of T4 by D1 and D2. The remaining T3 comes directly from the thyroid itself. Both T3 and T4 are deiodinated in the inner (tyrosyl) ring by D3, which terminates the action of T3 by converting it to 3,3' diiodothyronine and also prevents activation of T4 by converting it to 3,3', 5' triiodothyronine, or reverse T3 (Fig.1).

The role of D1 is primarily to provide T3 for the circulation and it is highly expressed in the liver, kidney, and thyroid. Its action is inhibited by the thiourea drug propylthiouracil, and it is stimulated at transcriptional level by thyroid hormone. On the other hand, D2 primarily provides T3 for the cell interior. D2 is expressed in many tissues, including the central nervous system, pituitary, skin, retina, brown adipose tissue, and skeletal muscle. Cells expressing D2 generally obtain a significant fraction of their nuclear receptor bound T3 from the intracellular conversion of T4 into T3(4). D3 is extensively expressed in fetal epithelial cells and the uteroplacental unit, while in adults is also found in the skin, retina, CNS, pituitary, and adrenal under basal conditions, and can be "reactivated" in liver and skeletal muscle during critical illness or expressed in neoplastic or injured regenerating tissues (Table 1).

The presence of the deiodinases provides the potential for local adjustments of active thyroid hormone in specific tissues at defined times. This is especially important when rapid changes in intracellular T3 concentrations are required for developmental changes or tissue repair. We are only now beginning to identify the factors which regulate these important enzymes in different situations.

The aim of this review is to provide an overview of recent data relevant to the role of deiodinases from a clinical perspective and incorporate new insights into the etiology of various clinical events. For information on the molecular and cellular biology of these enzymes, please see recent reviews (4, 5).

Deiodinases in thyroid physiology

The critical role of D2 in feedback regulation of thyroid secretion

In propylthiouracil (PTU)-treated rats (in which D1 was inhibited), both T4 and T3 act rapidly to suppress TSH, indicating this was not mediated by D1(6). After injection of ^{125}I -labelled T4 to hypothyroid, PTU-blocked rats, there is an equally rapid (within 30 minutes) increase in ^{125}I T3 in pituitary nuclei which cannot be accounted for by circulating ^{125}I T3 produced in the rest of the animal (7). The generation of pituitary nuclear T3 and the acute decrease in TSH are both blocked by iopanoic acid (a competitive inhibitor of D2) (8). While the circulating levels of TSH falls, thyrotroph TSH synthesis continues resulting in an increase in pituitary TSH, at least transiently (9). Homeostatic feedback regulation of TSH release in rodents and humans depends on a combination of T4 and T3. This was shown using chronic infusions of T3 versus T4 in thyroidectomized rats. In the absence of T4,

normalization of TSH required a circulating T3 concentration nearly twice circulating amounts (10).

Studies using two murine-derived thyrotroph cells, TtT-97 and TalphaT1, demonstrate high expression of D2 in thyrotrophs (11). D2 produces T3 from circulating T4 allowing the pituitary and hypothalamus to monitor both serum T3 and T4 independently (7). We refer to the T3 generated by D2 inside the cell as T3(T4) to distinguish it from the T3 originating from plasma, defined as T3(T3) (12). The relevance of D2 in pituitary feedback has been supported by studies in D2KO mice. Baseline serum T4 and TSH are significantly higher than WT littermates, and TSH is not suppressed by the administration of T4, but only by T3. Thus, the absence of D2 in the thyrotrophic and hypothalamus cell in D2KO mice makes the feedback mechanism insensitive to T4 (13).

Subsequent studies have shown that increased hypothalamic TRH secretion also plays a role in the feedback of TSH release in hypothyroidism. ProTRH mRNA is elevated in hypothyroid rats, but only in the (TRH) neurons of the rat paraventricular nucleus (PVN) (14). Furthermore, as with TSH, normalizing TRH mRNA by constant T3 infusion in a T4 deficient rat required maintaining plasma T3 1.7 times normal (15). This establish a role for T3(T4) in TRH regulation as well. The origin of the T3(T4) which regulates TRH synthesis in the PVN is not certain since there is no D2 present in that location (16). The accepted hypothesis is that it originates from D2-containing tanycytes in inferior portions of the third ventricle- the mediobasal hypothalamus (MBH) (16, 17).

The deiodinases in T3 homeostasis, including hypothyroidism and iodine deficiency

In humans, a relatively small fraction (approximately 10-20%) of the circulating T3 is released directly from the thyroid as a result of thyroglobulin hydrolysis (18) and intrathyroidal deiodination (19, 20) in subjects with adequate iodine intake. The bulk of T3 production is a consequence of peripheral 5'-deiodination due to D1 and D2 as suggested by the observation of nearly normal T3 levels in athyreotic patients receiving L-thyroxine therapy in physiological amounts (4). The relative contribution of the two enzymes is still a matter of debate, as suggested by multicompartmental analysis (21). In patients with hypothyroidism receiving fixed replacement doses of L-thyroxine, PTU administration (which blocks D1) resulted in a 20-30% decrease in serum T3, suggesting D1-catalyzed T3 production is not the only contributor to extrathyroidal T3 production (22). Later studies have further suggested that D2 may contribute significantly to plasma T3 in humans (23). What is clear is that there is a significant modification of deiodinase activity according to the general thyroid status. In the thyrotoxic state, the contribution of D1 is significantly higher (see below). During hypothyroidism or iodine deficiency, on the other hand, the fractional T4-to-T3 conversion increases, as a result of the higher D2 activity associated with low T4 (24). This is due to a longer half-life of the D2-protein as a consequence of a reduction in the degradation rate of D2 by the ubiquitin-proteasome pathway due to reduced substrate exposure (25, 26). This is particularly important in tissues of the central nervous system or pituitary. In hypothyroidism also there is a modest increase in the *Dio2* gene transcription (27). In the pituitary, the rise in TSH during hypothyroxinemia (such as in the early stage of Hashimoto's thyroiditis or during iodine deficiency) is a typical example of the extreme sensitivity of the hypothalamic-pituitary thyroid axis which anticipates a problem with thyroid hormone supply by monitoring the concentration of T4. In the hypothyroid condition, D3-mediated inactivation of T3 and T4 is reduced, helping to compensate for the reduction of T4 (28, 29).

To further illustrate the potency of the homeostatic system preserving tissue T3 levels, mice lacking D1 or D2 (D1/D2 knock out mice) surprisingly, have normal circulating T3 levels due to TSH-induced T3 secretion from the thyroid, without a change in D3 mediated

degradation, confirming the capacity of the hypothalamic-pituitary-thyroid axis to compensate for the lack of peripheral T4-to-T3 conversion (30).

Deiodinases and development

The importance of thyroid hormones for normal development is clearly illustrated by the severe neurological impairment and mental retardation associated with endemic (iodine deficiency) cretinism, the most common preventable cause of mental retardation in the world today (31). Thyroid hormone regulates many processes such as myelination, neuronal migration, glial differentiation and neurogenesis (32). The concentration of the active hormone T3 in CNS is controlled by two local mechanisms: the uptake of circulating plasma T4 and T3 mediated by transporters located in the blood-brain barrier and the plasma membrane of neural cells, such as MCT8 or OATP1C1 (33) or local production of T3 through the D2-mediated deiodination of T4. The local activation of T4 at a tissue level is more important in providing T3 to the neurons than is plasma (17, 34, 35). A gene expression study recently conducted in mice with no D2 or MCT8 (double KO mice), showed that MCT8 has little effect on the expression of multiple genes regulated by the thyroid hormone due to a compensatory increase in D2 activity in discrete areas of mouse cerebrum. On the other hand, *Dio2* disruption alone did not affect the expression of positively regulated genes, but, as in hypothyroidism, it allowed the increased expression of genes negatively regulated by T3 (36). In contrast, in D3KO mice, despite normal or low serum thyroid hormone concentrations, the absence of D3 activity in the brain results in excessive T3 effects in multiple brain regions, causing altered neuronal function (37). A paracrine paradigm for T3 supply is thought to occur in the central nervous system: D2 is highly expressed in glial cells providing T3 for neurons, which have higher D3 than D2 activity (34). This paracrine response is present in vivo as well, since the systemic administration of LPS, which induces a rapid increase of D2 in the tanycytes of the mediobasal hypothalamus and a consequent suppression of TRH expression (38), is absent in D2KO mice (39).

Spatio-temporal regulation of T3 by the coordinated pattern of action of D2 and D3-deiodinases in discrete areas of the brain has been observed in several species. During development, there is a synergism between D3 and D2. During the proliferative phase of some cell type precursors, D3 expression minimizes intracellular T3. Subsequently, D3 decreases and D2 is expressed inducing cell type specific differentiation. The development of the auditory system provides a clear example of necessity for deiodinases to provide the appropriate amount of T3 in a temporal fashion by specific coordination of the expression of the two enzymes. Early in development, D3 is expressed in the immature cochlea. If D3 is absent, such as in D3KO mice, the premature exposure to inappropriate T3 levels accelerates cochlear differentiation resulting in an auditory deficit (40). Additionally, in early postnatal life, a sharp focal burst of D2 activity in the cochlear precursors is also required to provide the proper amount of T3 for the final development of normal hearing (41). This spike is missing in D2KO mice leading again to severe hearing impairment, and this deficit can be prevented by the properly timed administration of T3 to the mother (42). Deiodinases also play a role in retinal maturation. The development of cones, the photoreceptors for daylight and color vision, requires protection from thyroid hormone by D3. Either excessive thyroid hormone given to wild-type pups or the elimination of D3 by gene targeting abolishes approximately 80% of the cones (mostly those expressing opsin photopigments) while the rod photoreceptors are spared. Type 3 deiodinase thus limits the exposure of the cones to T3, permitting both cone survival and the patterning of opsin that is required for cone function (43).

In the tibial growth plate of embryonic chickens, Sonic Hedgehog induces WSB-1, a SOCS-box-containing WD-40 protein, which is part of the E3 ubiquitin ligase for type 2

iodothyronine deiodinase. Hedgehog-stimulated D2 ubiquitination via WSB-1 reduces intracellular T3 production in chondrocytes which, in turn, results in parathyroid hormone-related peptide (PTHrP) synthesis to induce chondrocyte differentiation. Thus, there is a pre-programmed local modulation of the action of thyroid hormones through the posttranslational regulation mediated by WSB-1(44).

Deiodinases and metabolic control

D2 is essential in mature brown adipocytes for mediating the full thermogenic response of BAT to cold-exposure. Cold induces the activation of the sympathetic nervous system leading to increases in cyclic AMP in the brown adipocytes. This, in turn, increases *Dio2* gene expression and local T4 to T3 conversion within this tissue, and a consequent induction of T3 responsive genes, including uncoupling protein 1 (UCP1), important for adaptive thermogenesis (45, 46). Despite the acute increase in D2 activity and T3 in BAT during cold exposure, the serum T3 remains unchanged. Cold-exposed D2KO mice are deficient in brown adipocyte T3 production resulting in reduced lipolysis and lipogenesis, leading to an impairment of BAT thermogenesis. D2KO mice survive cold exposure by a compensatory increase in shivering, which requires a marked increase in total body fuel consumption (47, 48).

D2 has also plays a role in another BAT function, diet-induced thermogenesis. Thus, mice fed bile acids in conjunction with a high fat diet (HFD) are resistant to diet-induced obesity, but D2KO mice are not. This appears to be dependent upon increased D2 expression induced by bile acids in BAT during a high fat diet, although D2 in other tissues may have a role in this as well (49). A potential metabolic role for D2 has also been suggested in human studies in patients receiving replacement doses of T4; in these subjects basal metabolic rate correlates directly with free T4 and inversely with serum TSH but not with free T3 (50). This implies a role for T4, presumably due to its D2-mediated conversion to T3 in BAT (or other tissues), in the maintenance of normal resting energy expenditure.

D2 has been identified in the skeletal muscle of humans and in mice (51, 52). Since muscle is an important site of thermogenesis and insulin-mediated glucose disposal, local control of thyroid hormones may be important. Moreover, active brown adipose tissue, previously thought to be important only in human newborns, has been found in adult humans by PET/CT (53). In an intriguing case report, a patient with extreme insulin resistance due to an insulin receptor mutation showed a dramatic improvement in glucose homeostasis after initiation of levothyroxine therapy for hypothyroidism. Subcutaneous adipose tissue around the neck demonstrated molecular features of BAT including increased uncoupling protein-1 and type 2 deiodinase expression. Discontinuation of levothyroxine resulted in decreased FDG uptake in the fatty tissue and a diminished volume of BAT depots accompanied by worsening of diabetic control, suggesting a positive thyroid hormone effect on BAT activity and glucose homeostasis (54).

The classical theory of thyroid hormone regulation of metabolism is that T4 is activated in the peripheral tissues such as muscle or liver, thus stimulating energy expenditure through mitochondrial uncoupling or acceleration of the turnover of ATP-utilizing enzymes (55). A recent study suggests that there may be a direct effect of T3 to inhibit AMP kinase in the ventro-medial hypothalamus leading to upregulation of de novo lipogenesis in the hypothalamus. This is proposed to activate BAT via the sympathetic nervous system, leading to increased thermogenesis and weight loss in rats (56). This proposal argues that the clinical manifestations of hypo- and hyperthyroidism are due to the amounts of stimutable brown adipose tissue in each individual. In this very dynamic field, further investigations are still required to identify the precise role of deiodinases in metabolic regulation in humans and rodents.

Deiodinases and bone

Hypothyroidism reduces and hyperthyroidism increases bone turnover, both leading to an increase in fracture risk. In the osteoblast, D2 is upregulated during hypothyroidism but is reduced in hyperthyroidism. D2-mediated T4-to-T3 conversion mitigates the adverse effects of T3 deficiency on bone mineralization, whereas the reduced D2 activity in osteoblasts limits the detrimental effects of thyroid hormone excess. D2KO mice have low bone turnover and impaired osteoblast activity with a reduced mineralized surface, resulting in bones which are brittle and have reduced fracture resistance (57). The authors speculate that the absence of D2 in these mice results in intracellular hypothyroidism in the osteoblast despite a normal circulating T3. Thus the absence of D2 would preclude compensation in overt hypo or hyperthyroidism, resulting in increased susceptibility to fracture in both conditions. From a clinical point of view, in a recent study, patients carrying a missense polymorphism in the human DIO2 gene (Thr92Ala), which is associated with lower D2 expression in several tissues (58), display reduced bone mineral density in association with alterations in markers of bone turnover and remodeling (59).

Deiodinases and skeletal muscle

Muscle is a major target of thyroid hormone action (60) and expresses D2 (51, 61, 62). In mouse skeletal muscle, D2 is high in early postnatal life then decreases rapidly in postnatal life. Recent modifications of the methodology for D2 measurements have shown a fiber-specificity of D2 expression (higher in slow-twitch-oxidative vs. fast-twitch-glycolytic muscles) and an increase in D2 activity in hypothyroidism due to a post-translational upregulation (52).

Studies in D2KO mice have shown that D2 regulates the transcription of many physiologically relevant genes in muscle including MyoD, the master regulator of the myogenic developmental and regeneration program (63). Dio2 mRNA and activity are present in satellite cells (the muscle stem cell equivalent), under the control of Forkhead box O3 (FoxO3). D2 expression increases along with muscle differentiation. Blocking D2 activity inhibits the differentiation of myoblasts into mature myotubes, which are kept in active proliferation phase. In vivo experiments mirror this requirement for an increase in intracellular T3 since the expression of T3-dependent genes is reduced in the muscle of D2KO mice, which also have an inadequate MyoD response to injury and a marked delay in muscle regeneration (64). These observations are very exciting, considering that the capacity to modulate the thyroid status of muscle precursors could be a valuable tool in designing cell-based therapies for patients with muscle disease.

Deiodinases in tissue injury and inflammation

Several studies have shown that the deiodinases, under control of various transcription factors, contribute to inflammation and to the local response to tissue damage. Examples are the induction of D3 after myocardial infarction (65, 66) in right ventricular failure (67), and in peripheral nerves after injury (68). Hypoxia is a potent stimulus for D3 through the activation of the HIF (hypoxia-induced factor) expression pathway common to many of these conditions (69). Whether the D3-induced local hypothyroidism is adaptive (reducing energy turnover in ischemic/hypoxic conditions) or maladaptive (hampering the TH-dependent revascularization process) or merely an epiphenomenon of progression of the disease (67) is still a matter of debate.

In acute inflammation, as in E.Coli-induced peritonitis or in S.Pneumonia-induced pulmonitis, or in a chronic inflammation, as in turpentine-induced muscle abscess, D3 is expressed in tissue infiltrating polymorphonuclear granulocytes under the control of cytokine network (70, 71). Since the response to inflammation is, to some extent, impaired

in D3KO mice, D3 may have a protective role against acute bacterial infection by enhancing the microbial killing capacity of neutrophils by increasing intracellular iodine (72).

Deiodinases and hyperthyroidism

Although thyroidal T3 contributes 20% or less of total T3 production in normal iodine-sufficient humans, this is much higher in patients with a hyperactive thyroid, increasing up to 2-fold (73). The major part is produced from T4 deiodination in the thyroid, either by D1 or D2. In patients with Graves' disease the hyperthyroidism can be exasperated either by an increase of D1 mRNA and activity due to the T3-responsiveness of the *Dio1* promoter and by a direct stimulation of D1 activity by TRAb IgG (74-76). In these patients, one day treatment with a combination of iodide and PTU resulted in 50% greater decrease in plasma T3 than treatment with iodide plus methimazole. In euthyroid subjects, high doses of PTU cause a decrease in serum T3 ranging from 0 to 25% (22, 77). Thyroidal D2 is also increased in Graves' thyroid tissues or in other forms of hyperthyroidism, despite elevated circulating levels of thyroid hormones, leading to an increase of intrathyroidal T4-to-T3 conversion (19). The relative contribution of the two enzymes was the subject of a study of a consecutive series of patients with hyperthyroidism caused by Graves' disease or by multinodular toxic goiter who were randomly treated with high-dose propylthiouracil (PTU) to block D1 or PTU plus sodium ipodate to additionally block D2. Independently of the genesis of the hyperthyroidism, PTU resulted in a reduction of the T4-to-T3 conversion to 47% of the initial value, while adding sodium ipodate caused a further decrease in the ratio to 34 % of the initial value. These results suggest that the major source of the excess T3 was D1-mediated T4 monodeiodination (50%), with a minor role for D2 (15-20%) (78, 79).

Consumptive hypothyroidism in patients with hepatic hemangiomas and other forms of neoplasia

There are some unusual circumstances in which an excess of inactivating D3 activity leads to abnormal thyroid function. A summary of these conditions is listed in Table 2. Infantile liver hemangiomas, the most common tumors in infancy (prevalence of 5-10% among one year olds) express high levels of D3. Depending on the size of the tumor mass, D3 activity may reach levels sufficiently high that they induce a syndrome we term "consumptive hypothyroidism". This results when the rate of D3-mediated inactivation of T4 and T3 overcomes the capacity of the thyroid to synthesize it (4, 80). Typically, there are low levels of T4 and T3, a high TSH and rT3 (due to rapid inner ring deiodination of T4) and a high thyroglobulin. Normalization of thyroid hormone levels requires high doses of either levothyroxine or liothyronine (T3) orally or by intravenous infusion. It typically occurs in infants, but has also been found in adults with huge vascular tumors (81, 82). Needless to say, in newborns, given the age of the patients and the importance of thyroid hormones for normal CNS development, misdiagnosis or inadequate treatment of these conditions can lead to permanent neurological abnormalities.

Overexpression of other deiodinases can also have an impact on peripheral thyroid hormone concentrations. In some patients with metastatic follicular thyroid carcinomas, an increased ratio of serum T3 to T4 in the absence of autonomous production of T3 by the tumor has been described due to overexpression of type 2 deiodinase in tumoral tissue (83). In patients with McCune-Albright syndrome mutations in adenylate cyclase-stimulating G alpha protein induces constitutive cAMP signaling and multiple endocrine dysfunctions, including an increase of the T3/T4 ratio in part secondary to a cAMP-mediated intrathyroidal activation of D2 and an elevated D1 activity (84).

Deiodinases and non-thyroidal illness

In response to fasting or a broad range of illnesses (trauma, surgery, tissue transplantation) there are significant changes in circulating thyroid hormone concentrations known as the non-thyroidal illness syndrome (NTIS), or the euthyroid sick syndrome (ESS). The extent of the changes in thyroid hormones are related to the duration of the disease (acute vs. chronic), as well to the severity of the illness (Fig.3). During mild illnesses there is a reduction in the serum T3 concentration and an increase in reverse T3 (rT3), with normal T4 and TSH. In more severe illness, the decrease in T3 and the increase in rT3 become more evident, together with a reduced serum T4 and an inappropriately normal or, in the most severe illnesses, a low TSH (4). Whether these changes are teleologically beneficial (for example, to reduce the metabolic rate during stressful circumstances) or a maladaptive process is still a matter of debate. However, the degree of reduction in thyroid hormone levels in these conditions does correlate with their mortality (85, 86). The mechanisms underlying these multifactorial endocrinological alterations are not completely defined. The deiodinases, which activate and inactivate thyroid hormones, play an important role, either in the periphery or at a central level (Fig. 3). Peripherally, the decrease in circulating T3 levels, the most common abnormality in patients with NTIS, is mainly due to a reduced activation of T4 to T3 by D1 and D2 (87) and accelerated inactivation of T3 and T4 by D3. Sick patients often show both a robust reactivation of D3 in liver and skeletal muscle and a decreased liver D1 activity (86, 88).

Since a decrease in D1 (either due to a reduction in cofactor or in the D1 protein per se) or an increase in D3 will both result in a decrease in T3 and increase in rT3, it seems likely that both are playing a role in these changes, with a decrease in D2 also contributing to the low T3. Moreover, since D1 is a T3-responsive gene, a reduction in this enzyme is further accentuated as the T3 falls. The role of D2 in the whole picture is poorly defined, its activity being decreased (86), unaltered (89) or increased (90) in skeletal muscle of severely ill patients. Furthermore, a potential contribution of a reduction in thiol cofactor of D1 or D2, due to an increase in the intracellular reactive oxygen species, is likely but not yet documented.

Central hypothyroidism is yet another component of NTIS, usually manifested in prolonged or severe illnesses. Several studies have suggested a role of increased intracellular D2-mediated T3 production in suppression of hypothalamic-pituitary-thyroid axis (4). Bacterial lipopolysaccharide (LPS) infusion in rats causes changes in thyroid hormones analogous to NTIS: in these circumstances, D2 activity is induced in tanycytes of mediobasal hypothalamus, leading to a local thyrotoxicosis and consequent TSH suppression, similar to what is observed in NTIS (38, 91) not initiated but sustained by TNF α , an inflammatory cytokine increased in NTIS (91, 92).

Studies of the etiology of the changes in NTIS have been hampered by differences between rodents and humans in the role of deiodinases. For example, in mice with D1 and D2 deficiency, the fact that serum T3 remains normal despite the absence of both the deiodinases, is related to the fact that the thyroid, rather than T4, is a much more important source of plasma T3 in rodents (30). Furthermore, hypothalamic-pituitary suppression occurs much sooner during illness or fasting in rodents than in humans, making extrapolation to the human situation even more difficult. The deiodinases are certainly the cause of the changes in peripheral hormone levels in humans, but what is not clear is whether these are physiologically beneficial, harmful or neutral. Treatment of sick patients with thyroid hormones does not seem to alter the outcome of the illness in most studies (85, 93).

The overall picture is also complicated by modification in thyroid hormone—binding proteins, tissue uptake via thyroid hormone transporters and expression of thyroid hormone nuclear receptors during prolonged disease, emphasizing the necessity of further studies in order to clarify the pathophysiological origins of these changes.

Deiodinases and maternal-fetal physiology

While lack of thyroid hormones, such as occurs in iodine deficiency, has a detrimental impact on mammalian neurological development, exposure of the fetus to maternal levels of thyroid hormone is dangerous as well (31). Since the human thyroid does not fully develop until 10-12 week of gestation and the feedback regulatory process does not mature until 18-20 weeks, most of the fetal thyroid hormones in the first half of human gestation derive from the maternal circulation. These are modified by deiodinases expressed in the fetus as well as in surrounding tissues: D3 is expressed in the placenta, uterus, and in all of the epithelial surfaces of the human fetus (skin, respiratory epithelium, gastrointestinal tract and urinary epithelium) (94-96). This barrier controls the rate of transfer of maternal thyroid hormone to the fetus, allowing the fetal hypothalamic-pituitary-thyroid axis to be autonomous with respect to the mother (97). Despite this barrier, there is a narrow maternal-fetal gradient that allows maternal T4 to enter the fetal circulation providing the requisite prohormone for early embryological development. This is progressively replaced by fetal thyroid hormone after midgestation. Neonates with congenital hypothyroidism have detectable levels of T4 in cord blood (30% to 50% of normal values) which is of maternal origin and drops sharply after birth (98). D2 is also present in the decidual membranes, and increases during pregnancy (99), and is a potential source of T3 to the embryo.

Since hypothyroidism is much more common in women than men, and since autoimmune thyroiditis is an extremely common disease, a significant fraction (perhaps 5%) of women are receiving T4 treatment for primary hypothyroidism when they become pregnant. The requirements for thyroid hormone increase about 20-40% in many pregnant women and this may well due to a mild form of consumptive hypothyroidism due to the widespread expression of D3 in the maternal-fetal unit. In addition, the large increase in estrogen levels will induce D3 expression in all tissues, since the *Dio3* promoter is responsive to estrogens (100). This is also likely to play a role in the increase of T4 requirement of post-menopausal women receiving estrogen replacement therapy (101).

The physiological relevance of this protective mechanism is strengthened by the defective development of the D3KO mouse, which is exposed to supraphysiological amounts of thyroid hormones in utero and perinatal life, causing a significant perinatal mortality, a transient thyrotoxicosis which leads to a profound suppression of the hypothalamus-pituitary-thyroid axis (102, 103), similar to newborns from mothers with incomplete control of hyperthyroidism during pregnancy (104).

Deiodinases and cell proliferation

Effects of thyroid hormones on cellular differentiation and proliferation have been recognized for many years, based on studies of amphibian metamorphosis (see above). During this process, thyroid hormones orchestrate a programmed sequence of cell death and proliferation during which many organs are remodeled or replaced. In this situation, T3 deficiency seems to promote proliferation of undifferentiated cells, whereas a decrease in D3 and an increase in D2 activity lead to differentiation (64). These changes in deiodinases are controlled by various developmental transcription factors that have just begun to be identified.

D3 expression has been further identified in several tumors such as astrocytomas, gliomas, pituitary tumors and colon carcinomas or tumoral cell lines, such as CaCO2 (105). In particular, D3, expressed in developing organs and mostly absent in adult tissues, is re-activated in some tumor samples, causing intracellular hypothyroidism thus overcoming the differentiating effects of T3. This can result in a proliferative advantage for the malignant cells. In human and mouse basal cell carcinomas (BCC), the morphogen Sonic Hedgehog (SHH) induces D3 in proliferating keratinocytes (106). Provision of excess T3 or inactivation of D3 slows tumor growth both in vitro and in vivo (106).

Interestingly, since tissue regeneration is dependent on cellular proliferation and is associated with activation of fetal genes, it is not surprising that D3 expression has been induced in liver after partial hepatectomy. The degree of increase in D3 was correlated with an increased cellular proliferation and decreased serum and liver T3 and T4 levels, illustrating the importance of thyroid hormone in determining the balance between cellular proliferation and differentiation (107).

Deiodinases and pharmacological agents

Amiodarone is a potent cardiac antiarrhythmic drug that shares some structural similarity to thyroid hormones and has high iodine content. Patients receiving amiodarone typically have a decrease in T4 to T3 conversion due to an inhibition of D1 and D2 (108-110). Patients receiving levothyroxine for hypothyroidism often require an increase in their dosage with amiodarone treatment. The hypothalamic-pituitary-axis responds appropriately to this by increasing TSH and thereby raising T4 and rT3 (111, 112).

Therapy with tyrosine kinase inhibitors in patients with cancer is associated with modification of thyroid hormone profile, ranging from hypothyroidism (transient or persistent) or thyrotoxicosis. Since elevation of serum TSH has been documented in previously thyroidectomized patients taking imatinib, motesunib or sorafenib leading to an increase L-thyroxine replacement therapy, this implies a potential effect of these agents on thyroid hormone metabolism. In a recent study, in patients with progressive thyroid carcinoma treated with sorafenib for 26 weeks, serum fT4 and fT3 decreased, with an increase in rT3 and TSH (113). These results suggest the presence of increased D3 activity in patients taking sorafenib, and potentially a mild form of consumptive hypothyroidism. Since these agents may also cause a destructive thyroiditis, it is important to monitor the thyroid status of these patients at 4-6 week intervals to maintain a euthyroid status.

Deiodinase deficiency due to inhibition of selenoprotein synthesis

Deiodinases are selenoproteins containing the rare aminoacid selenocysteine (Sec) in their active center (Figure 2). Selenocysteine is encoded by UGA, which normally acts as stop codon mediated by a multiprotein complex that includes Sec insertion sequence binding protein 2 (SECISBP2, also known as SBP2) (2). Failure of this mechanism can result in miscoding of the UGA codon to be read as a stop codon resulting in an untruncated inactive deiodinase protein. The important role of deiodinases in thyroid economy has been recently emphasized by the discovery of several families with a genetic mutation in SBP2 that is either homozygous or compound heterozygous (114-116). Patients harboring these mutations present an abnormal thyroid profile characterized by low T3, high T4 and high rT3 concentrations and high normal TSH in serum. This is associated with a reduction in D2 activity in fibroblasts from affected individuals. SBP2 mutations also impair the synthesis of other selenoproteins including glutathione peroxidase, thioredoxin reductase and selenoprotein P and N, resulting in variable phenotypes including growth retardation, azoospermia, myopathy, skin photosensitivity, abnormal immune cell function and enhanced insulin sensitivity. The complexity of the phenotype and the lack of correlation between

genotype and phenotype are likely to be due to the variable affinity of SBP2 with the SECIS element in different genes, which contributes to a hierarchical differential reduction of translational efficiencies of the different selenoproteins (117). Further studies are needed to clarify this issue, as well as determining if T3 treatment would be beneficial (118).

Gene polymorphisms

Several single nucleotide polymorphisms (SNP) in the deiodinase gene have been described, but their functional relevance still needs to be clarified.

The best studied is the sequence change in D2 Thr92Ala, a polymorphism common in various ethnic groups, i.e. Pima Indians (119) (Table 3). This change has been associated with decreased enzyme velocity in human thyroid and skeletal muscle (58), although it is not certain that the coding mutation per se is the cause of the reduction in D2 action. The reduced activity mentioned above may be due to another abnormality in the gene since studies with the mutated D2 protein have not revealed any abnormal function in vitro.

Data reporting an association between this change and the risk of hypertension (120, 121) or insulin resistance/diabetes are conflicting (58, 122-124). In a recent large case-controlled study of 1057 diabetics and 516 controls, the frequency of Thr92Ala was higher in the diabetic group. This was confirmed by a subsequent meta-analysis including over 11,000 individuals (125).

Moreover, the possibility of a functional linkage of the Thr92ALA polymorphism with Pro121Ala in PPAR γ 2 needs to be considered, since the combination of the two SNPs can modulate insulin-resistance, hypertension and other features of metabolic syndrome (126).

The same polymorphism is in linkage disequilibrium with the susceptibility to generalized osteoarthritis (127), decreased femoral neck bone mineral density and higher bone turnover independently of serum thyroid hormones (59). In addition, this SNP is associated with higher serum TSH but not thyroid hormone levels in healthy individuals (128) and with lower T3 release after TRH infusion (129). However, this change was not correlated with differences in general well-being, cognitive function or the response to therapy with T3+T4 in hypothyroid patients in same study, but not in others (130, 131).

Recently, a polymorphism in the *DIO3* gene, has been shown to be protective against osteoarthritis (especially knee and hip) in a large European metanalysis: modifications of local thyroid status in the growth plate during the endochondral ossification process may thus be another example of the relevance of the modulation of tissue-specific thyroid status independently from the general circulation (132).

Conclusions

Recent observations have pointed out the advantages of dual level of monitoring of thyroid hormone activity: control both in the circulation as well as control of intracellular availability in target tissues independent of circulating levels. Deiodinases are critical enzymes in both circumstances. New scenarios where this is important are now being recognized, given the discovery of a critical role for deiodinases in regeneration, cancer and metabolic diseases. The availability of conditional tissue-specific knock out mice will contribute to studies further dissecting out the contributions of these enzymes, acting alone or in concert with other genetic variants, in a multitude of physiological situations. The opportunity to manipulate intracellular T3 concentrations by pharmacological tools through deiodinases modification presents a potential therapeutic opportunity to modify a variety of

clinical disorders, based on our increasing knowledge of the ubiquitous functions of the deiodinase enzymes.

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Figure 1. Schematic representation of the deiodinase-mediated activation or inactivation of thyroxine and triiodothyronine

Figure 2. Mechanism for the incorporation of selenocysteine at the UGA Sec codon of selenoprotein mRNAs

When the ribosome encounters the in frame UGA codon its dissociation from the mRNA is inhibited and read through facilitated by a complex containing the specific selenoprotein charged tRNA, the selenoprotein specific elongation factor (EFsec) and the SECIS Binding Protein 2 (SBP2), all bound to the selenocysteine insertion-sequence (SECIS) element found in the 3' untranslated region of all eukaryotic selenoprotein mRNAs. Modified from ref. (1)

Figure 3. Pathophysiology of the changes in the thyroid system during illness
Schematic representation of changes in circulating hormones with progressively more severe illness (A) and the effect on the deiodinases and TSH (B).

Table 1

Characteristics of iodothyronine deiodinases

		D1	D2	D3
substrates		rT3(5'); T4S(5)	T4>rT3	T3>T4
Km (range)		mM (with DTT); nM (with GSH)	nM	nM
protein half life		~12 hours	~ 20 minutes	~12 hours
molecular weigh (kDa)		29	30	32
location		plasma membrane	endoplasmic reticulum	plasma membrane /early endosomes
active center		cytosol	cytosol	cell surface?
susceptibility to inhibitors				
	propylthiouracil	very high	very low	very low
	iopanoic acid	high	high	high
tissue expression		liver	CNS (mainly glia)	CNS (mainly neurons)
		kidney	pituitary	placenta
		thyroid	brown adipose tissue	uterus
			thyroid	fetus
			skeletal muscle	skin
			bone	regenerating tissues
			cochlea	
			retina	
change with thyroid status	hypothyroidism	decrease (kidney, liver), increase (thyroid)	increase	decrease (brain)
	hyperthyroidism	increase	decrease (except in the thyroid)	increase (brain)
physiological regulation	induction	T3	sympathetic nervous system	T3
			high fat diet	tissue injury
			bile acids	TGFβ & other growth factors
			cAMP	hypoxia (Hif 1)
			FOXO3	regeneration
			LPS/NFκB	sonic hedgehog
			cellular differentiation	cellular proliferation
				oxidative stress?
	repression	fasting	T4 (post-translational)	growth hormone
		illness	T3 (transcriptional)	glucocorticoids
			ubiquitination	cellular differentiation
			sonic hedgehog	
			oxidative stress?	
physiological role		extracellular T3	intracellular T3	inactivation of T3 and T4
		clearance of rT3 and T3S	hypothalamic-pituitary feedback	cellular proliferation
			thermogenesis in BAT	fetal control
			source of plasma T3 in hypothyroidism or iodine deficiency	tissue repair

	D1	D2	D3
		muscle regeneration	
		bone formation	
pathophysiological role	primary source of plasma T3 especially in hyperthyroidism	increase T4>T3 conversion in some metastatic thyroid cancer and McCune-Albright syndrome	consumptive hypothyroidism (refer to table 2)

Table 2

Clinical manifestations of excessive type 3 deiodinase function.

Clinical features	Mechanism
1) Consumptive hypothyroidism in patients with hemangiomas	D3 overexpression in neoplastic vascular tissue
2) Increased requirements for levothyroxine during pregnancy	a) High D3 levels in the utero-placental unit b) Stimulation of the <i>Dio3</i> gene by estradiol
3) The “low T3-high reverse T3 syndrome” during illness	“Reactivation” of D3 expression in liver and skeletal muscle
4) Increased requirements for levothyroxine in patients receiving tyrosine kinase inhibitors	Induction of D3?
5) Enhanced proliferation of cancerous tissues such as basal cell carcinoma cells (in mouse models) or gliomas	D3 overexpression in neoplastic tissue

Table 3

Evidence for association of the human *Dio2* polymorphism Thr92Ala with certain clinical manifestations (see text for references).

Clinical features	Association
insulin resistance/type 2 diabetes	conflicting
hypertension	conflicting
development of Graves' disease	yes
generalized osteoarthritis	yes
decreased bone density	yes
higher serum TSH	yes
lower acute TRH-stimulated T3 release	yes
well being and cognitive function	conflicting