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Defending plasma T3 is a biological priority

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Summary

Triiodothyronine (T3), the active form of thyroid hormone is produced predominantly outside the thyroid parenchyma secondary to peripheral tissue deiodination of thyroxine (T4), with <20% being secreted directly from the thyroid. In healthy individuals, plasma T3 is regulated by the negative feedback loop of the hypothalamus–pituitary–thyroid axis and by homoeostatic changes in deiodinase expression. Therefore, with the exception of a minimal circadian rhythmicity, serum T3 levels are stable over long periods of time. Studies in rodents indicate that different levels of genetic disruption of the feedback mechanism and deiodinase system are met with increase in serum T4 and thyroid-stimulating hormone (TSH) levels, while serum T3 levels remain stable. These findings have focused attention on serum T3 levels in patients with thyroid disease, with important clinical implications affecting therapeutic goals and choice of therapy for patients with hypothyroidism. Although monotherapy with levothyroxine is the standard of care for hypothyroidism, not all patients normalize serum T3 levels with many advocating for combination therapy with levothyroxine and liothyronine. The latter could be relevant for a significant number of patients that remain symptomatic on monotherapy with levothyroxine, despite normalization of serum TSH levels.

Introduction

The thyroid gland takes up iodide and produces iodinated molecules that have pleiotropic effects in vertebrates.[1] The two main iodinated molecules secreted by the thyroid gland are thyroxine (T4) and triiodothyronine (T3). Even though both molecules can trigger biological effects, T3 is considered the biologically active thyroid hormone that binds to thyroid hormone receptors (TR), while T4 is a prohormone that must be converted to T3 in order to initiate signalling and gain biological activity. A corollary is that the level of T3 inside the cells defines how much T3 is bound to TR and hence the intensity of signalling and T3-dependent biological effects, aka ‘thyroid status’. In an organism, thyroid status can be considered the sum of all T3-dependent signalling events and depends on (i) circulating T3 levels and (ii) tissue/cell-specific factors influencing the intracellular concentration of T3. An organism is known to exhibit *thyrotoxicosis* when the intracellular levels of T3 are increased, whereas *hypothyroidism* results from thyroid hormone deficiency. In addition, individual tissues could be said to have specific thyroid status, that is, thyrotoxic or

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hypothyroid, relatively independent of serum thyroid hormone levels; this is because of tissue/cell-specific factors such as deiodinase activities and/or transport mechanisms.[2] In this regard, the type 2 deiodinase (D2) catalyses T4 to T3 conversion and increases intracellular levels of T3 potentially leading to local thyrotoxicosis. The opposite is observed in cells expressing the type 3 deiodinase (D3), which depletes the cell of T3 by deiodination to T2 and can cause local hypothyroidism. Understanding these basic mechanisms that regulate thyroid hormone metabolism and action has clinical implications and might affect the choice of therapy for hypothyroid patients, a highly controversial area in the thyroid field.

What is the biologically active thyroid hormone?

Thyroid hormone signalling is initiated by binding of T3 to one of its TR isoforms, TRα or TRβ, thus affecting the expression of thyroid hormone-dependent genes.[1] This is also known as the genomic effect of thyroid hormone and explains the biological actions of thyroid hormone in the various organs/systems, that is, development, growth, metabolic control and cognition. The fact that the plasma concentration (and presumably intracellular concentration) of free T4 and free T3 are similar (5–10 pM) and that the TR affinity for T3 is approximately 10-fold greater than T4, makes T3 the most potent TH. In fact, acknowledging that T4 is a prohormone indicates that conversion to the more active T3 molecule is required for biological activity. Notably, at higher concentrations, T4 can also bind to TR, modify gene transcription and trigger biological effects; however, this is considered minimal at physiological levels.[1] In addition, T4 also exhibits significant nongenomic effects such as acceleration of the type 2 iodothyronine deiodinase (D2) inactivation via ubiquitination.[3]

Measuring plasma T3 levels

Similarly to T4, most T3 in human serum is bound to carrier proteins, namely thyroid-binding globulin (TBG), transthyretin and albumin, while <0·4% of T3 is free.[4] Given its important biological activity, knowing the serum total T3 (TT3) levels is important to understand systemic thyroid hormone status. Ideally, one would prefer assessing circulating free T3 (FT3) levels as they provide information about the level of T3 that is biologically available to enter cells and initiate thyroid hormone action. Of course, this is not the only factor defining thyroid hormone action, also defined by cellular transporters that facilitate passage across the cell membrane, deiodinases that can activate or inactivate thyroid hormone as well as TRs and its co-regulators.[5]

Measuring serum FT3 levels is labour intensive and requires equipment not readily available in most clinical reference laboratories. Typically, the first step to measure serum FT3 is to physically separate the free T3 molecules from the T3 molecules that are bound to serum proteins, without disturbing the endogenous equilibrium between fractions. This is achievable by equilibrium dialysis or ultrafiltration; the second step is to measure T3 in the dialysate or ultrafiltrate, respectively. Ultrafiltration is far less labour intensive and therefore is the method used in many reference laboratories.[6–8] Following equilibrium dialysis and ultrafiltration, a variety of detection techniques are available to measure free hormone concentration, including immunoassays and tandem mass spectrometry (MS/MS).

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The majority of all commercially available assays utilize an alternative approach to analyse FT3 concentrations directly in the presence of proteins (unfractionated samples of serum). This is referred to as the direct FT3 method that unfortunately is subject to interferences and lack of specificity, exhibits inconsistent analytical performance and has poor correlation with equilibrium dialysis/ultrafiltration LC/MS/MS methods, currently regarded as gold standards.[7] The main factors known to interfere with the direct FT3 methods originate in the sample and include abnormal binding proteins, heterophilic antibodies, autoantibodies and possibly free fatty acids.[7]

Thus, in the absence of equilibrium dialysis/ultrafiltration LC/MS/MS methods, it is not clear that using a direct FT3 assay offers any clinical advantage over measuring serum TT3 levels. In this case, indirect information about free T3 levels can be derived from the resin T3 uptake, which quantifies the empty T3 binding sites available in the serum carrier proteins.[9]

Where is T3 produced? How much T3 is produced? Via which pathway?

Most T3 is produced outside the thyroid gland via deiodination of T4, with <20% being secreted directly from the thyroid.[10] Thyroidal T3 derives from intracellular thyroglobulin digestion that is supposedly enriched via intrathyroidal deiodination of T4 to T3, although it is uncertain how much thyrocyte deiodination contributes to the daily thyroidal T3 production. The molar ratio of T4 to T3 in the human thyroglobulin is 15:1, and some estimates put the thyroidal secretion as containing a molar ratio of 11:1, which is supportive of thyroidal deiodination of T4.[11–13] Furthermore, this ratio might decrease even further in situations such as iodine deficiency or thyroid disease through mechanisms that include changes in thyroglobulin iodination and possibly intrathyroidal deiodination.[11,12]

It is estimated that healthy adult subjects produce about 30 μg T3/day, of which about 5 μg are secreted directly from the thyroid and the rest is produced outside of the thyroid parenchyma via T4 deiodination.[10] Deiodination is catalysed by a group of enzymes known as iodothyronine deiodinases that mediate (i) activation of T4 into T3 or (ii) inactivation of T4 into rT3 and of T3 into 3,3′-T2. All deiodinases are dimeric integral membrane thioredoxin-fold like containing selenoenzymes that are expressed in multiple tissues and cells, including the thyroid gland.[2,14,15] There are two deiodinase pathways that lead to extrathyroidal T3 production, the type 1 deiodinase (D1) and D2 pathways. In turn, the third deiodinase pathway (type 3 deiodinase, aka D3) is involved in catabolism of both T4 and T3, terminating thyroid hormone action.

In a healthy adult individual, D2 is thought to mediate the bulk of T3 production, approximately 20 μg/day, with a minor contribution provided by D1 (5 μg/day).[10] Relatively high D2-specific activity can be found in the brain, pituitary gland, brown adipose tissue (BAT) and heart.[10,16] D2 is present in many other tissues at lower specific activity, including skin, skeletal muscle, skeleton, vascular smooth muscle and testis.[17–21] In contrast, D1 expression is restricted to the liver and kidney where it also processes conjugated T3, clearing these metabolites from the circulation.[22] For example, T3 is a poor D1 substrate but once it is sulphated in its phenolic ring, T3S gains water solubility and is

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rapidly metabolized via D1, conceivably to conserve iodide before the molecule is eliminated in the bile.[22,23] T3S has no biological activity but sulfatases present in tissues and the intestinal microflora can convert T3S back to T3. It is unclear at the moment how much these pathways play a role in the daily T3 economy in humans.

Given the widespread D2 expression throughout the body, it is likely that multiple organs/tissues collectively contribute to the daily T3 production via the D2 pathway. Brain also expresses relatively high levels of D3, the inactivating deiodinase, minimizing its potential contribution as an organ to plasma T3. Brown adipose tissue, on the other hand, has no D3 activity, and thus it is potentially an important source of circulating T3. This has previously been shown in rodents,[24] and the more recent finding of D2-containing brown adipose tissue in humans[25] also highlights its relevance as a source of T3. Less is known about the role played by tissues that express D2 at low specific activity such as skeletal muscle, vascular smooth muscle or skin, but, given the size of these organs/tissues, their contribution is probably relevant as well.

What are the cellular bases of deiodinase-mediated T3 production/catabolism?

T3 production via deiodination is an intracellular event. T4 enters cells via specific transmembrane transporters and can then be converted to T3.[26] D1 is located in the plasma membrane and thus T3 produced via the D1 pathway exits the cells promptly and rapidly equilibrates with the plasma pool of T3.[27,28] In fact, kinetics studies indicate that T3 molecules generated via the D1 pathway stay inside the cell for just about 30 min before exiting to plasma. In contrast, D2 is an endoplasmic reticulum (ER)-resident protein, and thus, T4 to T3 conversion through this pathway occurs in a cellular compartment that is physically associated with the cell nucleus, where the TRs are located.[27,29] Thus, D2-originated T3 also diffuses to the nucleus and perhaps for this reason remains in the target cell for a longer period time, approximately 8 h.[28,30] The significance of these observations is that D2-generated T3 also interacts with the local TRs and triggers biological effects in the same cell where it was produced. Eventually, D2-generated T3 exits the cell and equilibrates with the circulating pool of T3.

D3 is located in the plasma membrane and thus has easy access to incoming thyroid hormone molecules.[31,32] Furthermore, under hypoxic or ischaemic conditions, D3 is preferentially inserted into the nuclear membrane where it inactivates thyroid hormone, thereby decreasing thyroid hormone signalling.[33] T3 is inactivated by D3 via conversion to T2, a pathway that is present in the brain, skin and placenta.[34,35] The latter explains in part the need for increased dose of thyroid hormone replacement to hypothyroid patients during pregnancy.[36] Furthermore, D3 expression can be enhanced in multiple other tissues in disease states, including the liver, lungs, heart and brain, particularly during ischaemic or hypoxic states. In rare cases, D3 is greatly expressed in infantile hemangiomas to such a degree that inactivates T3 at a faster rate than it can be produced.[37] Similarly, the use of the tyrosine kinase inhibitors imatinib and sunitinib in patients with cancer is also associated with hypothyroidism, which seems to be the result of marked D3 overexpression within the tumours.[38] In fact, the use of these drugs commonly results in hypothyroidism when used

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for the treatment of a range of other tumours including differentiated thyroid cancer. The latter routinely requires an increase in their levothyroxine suppressive therapy when treated with tyrosine kinase inhibitors. The cause of hypothyroidism in these situations is not fully understood, and it could be multifactorial, including enhanced D3 expression in other tissues. All of these conditions characterize the syndrome of consumptive hypothyroidism, a state of systemic hypothyroidism caused by excessive degradation of T3 and T4 via abnormally high expression of D3.

An analogous situation is observed in patients treated with anti-epileptic drugs such as diphenylhydantonin or carbamazepine. There is accelerated biliary elimination and reduction in serum thyroid hormone levels due to enhanced conjugation to glucuronic acid.[39] Notably, such patients do not exhibit an increase in serum thyroid-stimulating hormone (TSH) but warrant close monitoring as they may need treatment and/or adjustment of thyroid hormone replacement dose.

The corollary of these studies is that D1- and D2-expressing tissues produce a ‘positive’ flow of T3 that exits the cells, entering the circulation. In contrast, D3-expressing tissues function as a sink for TH, whereby there is a ‘negative’ flow of both T4 and T3 that enter the cells not to return to the circulation.[2,40]

Plasma T3 levels are relatively stable over time

Serum TT3 and FT3 exhibit minimal circadian rhythmicity that is due to a nocturnal increase in TSH secretion.[41,42] Otherwise, serum T3 is remarkably stable over periods of days, weeks or months in healthy adult individuals, despite a relatively short half-life (approximately 12–18 h). This is likely the result of combined homoeostatic mechanisms involving (i) the hypothalamus–pituitary–thyroid axis as well as (ii) the group of deiodinases (Fig. 1).

It is well accepted that TSH secretion is negatively regulated by T4 and T3, although the hypothalamus–pituitary axis seems to be particularly sensitive to circulating T4 levels. In fact, few examples exist in which serum T3 is low in the face of normal serum T4 and TSH levels. In most such examples, there is nonthyroidal illness that results in central hypothyroidism and inappropriately low/normal serum TSH levels. A unique experimental setup illustrating that serum T3 *per se* is an important determinant of TSH secretion is the acute administration of large doses of PTU to thyroidectomized individuals kept on levothyroxine replacement therapy.[43] The approximately 20% drop in serum T3 as a result of D1 inhibition was sufficient to double serum TSH even as serum T4 levels remained stable.[43] It is unknown whether this TSH response is originated at the hypothalamus, pituitary or both levels.

The deiodinase group has the potential to defend serum T3 levels given the inverse relationship between D2 and D3 observed during hypo- or hyperthyroidism.[2,35,44] Whereas Dio2 is negatively regulated by T3, the opposite is observed for Dio3. As a result, during hypothyroidism, there is increase in the fractional conversion of T4 to T3 that reflects the higher D2 activity and decreased clearance of T3 that reflects lower D3 activity, both presumably helping maintain serum T3 levels within the normal range.[45–47]

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Perhaps the most dramatic deiodinase-mediated homoeostatic mechanism is the post-translational loss of D2 activity induced by interaction with T4 via conjugation to ubiquitin.[3,48] D2 is a type I endoplasmic reticulum (ER)-resident D2 with a variable short half-life that depends on whether its natural substrate T4 is available. In the presence of T4, D2 is inactivated with an approximately 20 min half-life, whereas in the absence of T4, its half-life is prolonged to hours.[49] This provides a mechanism through which the production of T3 can be regulated according to the availability of T4.

D2 ubiquitination is the molecular mechanism underlying these changes in D2 half-life, that is, the covalent attachment of multiple ubiquitin molecules to D2, which both inactivates the enzyme and targets it to degradation in the proteasomes.[48] Ubiquitination is thought to inactivate D2 by disrupting the conformation of the D2:D2 dimer, critical for enzyme activity.[3,50] A unique 18-amino acid loop confers intrinsic metabolic instability to D2, facilitating binding to proteins involved in the ubiquitination process.[51,52]

The ubiquitin-activating enzymes UBC6 and UBC7 are critical in the process of D2 ubiquitination,[53,54] as well as two ubiquitin ligases, the hedgehog-inducible WSB-1, and TEB4, a ligase involved in the degradation of proteins in the endoplasmic reticulum.[52,55] D2 is structured as an homodimer, D2:D2, and monomers are inactive.[15] Ubiquitinated D2 (UbD2) is not immediately taken up by the proteasomes. Instead, UbD2 can be reactivated by deubiquitination, a process catalysed by two USP-class D2-interacting de-ubiquitinases (DUBs), USP-20 and USP-33.[56] The other two deiodinases, D1 and D3, are not known to be ubiquitinated or undergo post-translational modifications.

D2 ubiquitination occurs via K48-linked ubiquitin chains, and exposure to its natural substrate, T4, accelerates UbD2 formation.[57] UbD2 is taken up by proteasomes located in the cytoplasm. D2 retrotranslocation to the cytoplasm occurs via interaction with the p97–ATPase complex. D2 retrotranslocation also includes deubiquitination by the p97-associated DUB Ataxin-3. Once in the cytosol, D2 is delivered to the proteasomes as evidenced by coprecipitation with 19S proteasome subunit S5a and increased co-localization with the 20S proteasome.[57]

Therefore, a number of transcriptional and post-transcriptional mechanisms underlie the homoeostatic role played by the deiodinase system in defending serum T3 levels. In combination with the hypothalamus–pituitary–thyroid axis, both central and peripheral pathways explain the stability of serum T3 levels.

The hypothalamus–pituitary–thyroid axis is wired to preserve serum T3

Based on the above discussion and on the fact that the deiodinases contribute with approximately 60% of circulating T3 in rodents (and about 80% in humans), one would expect major interference in serum T3 homoeostasis upon disruption of the deiodinase system. However, serum T3 levels are normal in mice with single or combined targeted inactivation of both Dio1 and Dio2 genes.[58–61] In these animal models, there is resetting in the hypothalamus–pituitary–thyroid axis that includes increase in serum T4 and/or serum TSH, leading to increased thyroidal T3 secretion that makes up for the lack of extrathyroidal

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T3 production. Even if the disruption in Dio2 is restricted to the TSH-producing cells, serum TSH and T4 are increased while preserving serum T3 levels.[62]

A response mediated by the hypothalamus–pituitary–thyroid axis also seems to be involved in the maintenance of serum T3 levels during iodine deficiency or mild hypothyroidism.[63,64] In both cases, there is an increase in serum TSH levels due to the drop in serum T4 while serum T3 remains within normal range.

That the level of serum T3 is a main target around which serum T4 and TSH are adjusted constitutes a shift in the paradigm traditionally accepted for the function of the hypothalamus–pituitary–thyroid axis. For example, it is unexpected that the hypothalamus–pituitary–thyroid axis would tolerate an increased serum T4 in order to preserve serum T3.[60–62] This observation challenges the dogma that locally produced T3 via D2 mixes at a prereceptor level with incoming plasma T3 in the hypothalamus and pituitary so that both, indistinctively, reduce expression of TRH and TSH. The new evidence obtained in the genetically modified animals suggests the existence of a distinct unknown mechanism within the hypothalamus–pituitary axis that selectively detects plasma T3.

Of course there are a number of physiological conditions in which the hypothalamus–pituitary–thyroid axis does adjust in response to environment cues such as food availability, temperature, breeding season or pregnancy, all of which reflect in adaptive changes in serum T3. In addition, there are a number of pathophysiological conditions in which the hypothalamus–pituitary–thyroid axis does not defend serum T3, which are let drop below the reference range. These are largely centrally mediated and include fasting/caloric restriction, nonthyroidal illness, hypothalamic hyperthyroidism due to Dio3 inactivation, or targeted inactivation of TRH gene.[65,66] In fact, the finding of low serum T3 in the presence of normal serum T4 and TSH levels is a prevalent event as nonthyroidal illness is common. In addition, certain species exhibit significant seasonal variation in thyroid hormone levels that is mediated through adjustments in the hypothalamus–pituitary–thyroid axis.

Thus, experimental data support the concept that in healthy adult mice the hypothalamus–pituitary–thyroid axis is wired to preserve serum T3,[60–62] except when it deliberately ‘allows’ serum T3 to drop in response to physiological or pathophysiological mechanisms, or its function is disrupted to the point that is no longer capable of reacting adequately to a fall in serum T3.[65,66]

How well defended is serum T3 in the absence of a functional thyroid gland?

Experimental evidence indicates that in the absence of T3-producing deiodinases, the hypothalamus–pituitary–thyroid axis resets and is capable of defending serum T3. Would the deiodinases be able to do the same in the absence of a functional thyroid gland? Studies in thyroidectomized rats indicate that monotherapy with levothyroxine alone does not normalize serum and tissue T3 and T4 simultaneously at normal TSH levels.[67] Only the combination of levothyroxine and liothyronine therapy was able to normalize serum TSH as well as serum and tissue T4 and T3 concentrations simultaneously in all tissues.[68]

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In humans, multiple studies have looked at serum T3 levels in clinically euthyroid athyreotic or hypothyroid patients maintained on levothyroxine replacement therapy. The consensus has been that such patients maintain levels of T3 that are slightly but significantly lower and of T4 that are slightly higher than control individuals.[69] In addition, normal serum T3 levels can be achieved but at the expense of having relatively lower/suppressed serum TSH.[70,71] A large cross-sectional study involving approximately 1800 athyreotic patients clinically euthyroid on levothyroxine monotherapy revealed that the distribution of serum FT3 levels is shifted to the left and that of FT4 levels is shifted to the right when compared to the distribution patterns obtained in approximately 3900 controls.[72] In this study, for every quintile of serum TSH distributed within the normal range, athyreotic patients on levothyroxine monotherapy exhibited significantly lower serum FT3 and higher serum FT4 when compared to the controls on the same serum TSH quintile.

Notably, a study of 50 patients undergoing elective total thyroidectomy indicated that monotherapy with levothyroxine alone was able to bring serum T3 levels back to the same presurgical levels without suppressing serum TSH, although serum FT4 levels were significantly higher after surgery.[9]

Is having a relatively high serum T4 and low serum T3 clinically relevant?

Whereas it has become widely accepted that serum T3 is relatively lower in hypothyroid patients maintained on levothyroxine alone, it is unclear whether this is clinically relevant. In addition, it is equally unclear whether those patients that remain symptomatic despite having normal serum TSH levels are the same patients that cannot maintain serum T3 levels within the normal range. Clinical trials are needed to answer these questions, particularly trials with outcomes including objective biological responses to T3. For example, an acute drop in serum T3 of approximately 20% in thyroidectomized individuals maintained on levothyroxine replacement therapy resulted in doubling of serum TSH,[43] indicating that at least from the thyrotroph's perspective, a small reduction in serum T3 is clinically relevant. The long-term systemic consequences of a relatively low serum T3 are unknown.

Is there evidence-based support for treatment of hypothyroid patients with combination therapy?

The renewed attention to serum T3 and the possibility that its levels might not be fully restored in hypothyroid patients after conventional treatment has led to clinical trials analysing patient satisfaction and quality of life questionnaires in hypothyroid patients kept on monotherapy *vs* combination therapy with levothyroxine and liothyronine. Readers are also referred to recent comprehensive reviews on this highly controversial area.[73–75]

Some studies suggest that patients tend to prefer combination therapy based on patient preference/satisfaction,[76,77] body weight,[76] quality of life, depression, neuropsychological function, mood and anxiety rating scales;[77,78] the majority of the studies indicate that both forms of treatment provide similar clinical outcomes.[79–81] However, a meta-analysis of eleven randomized controlled trials and a total of 1216 participants comparing combination therapy *vs* monotherapy revealed no difference in the effectiveness of combination therapy

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vs monotherapy with regard to bodily pain, depression, fatigue, body weight, total serum cholesterol, triglyceride levels, low-density lipoprotein, high density lipoprotein or adverse events.[82] In addition, a systematic review of ten randomized controlled trials comparing combination therapy *vs* monotherapy found no statistically significant differences in biochemical markers, mood states, adverse effects or drop-out rates, suggesting that combination therapy does not improve well-being, cognitive function, or quality of life as compared to monotherapy.[83]

There is new evidence that patients bearing the Thr92Ala D2 polymorphism might benefit from combination therapy.[84] This genetic polymorphism causes an amino acid change in a critical loop of the D2 molecule that controls its half-life.[52] It is not known how this polymorphism affects D2 function but it could hypothetically disrupt thyroid hormone signalling in D2-expressing tissues, making these cells more susceptible to a reduction in serum T3.

In view of the experimental evidence that (i) serum T3 levels are stable in healthy adult individuals, and (ii) preserving a stable plasma T3 is the default condition after disruption of the hypothalamus–pituitary–thyroid axis, the failure of combination therapy to provide superior clear cut clinical outcome might reside on the fact that current forms of liothyronine replacement, that is, tablets, do not ensure stable serum T3 levels throughout the day. Given the relatively short T3 half-life and the relatively fast absorption of liothyronine, patients receiving a tablet of liothyronine experience a transient increase in serum T3 that subsides during the next few hours.[85] In fact, it has been reported that at least three tablets daily of liothyronine are necessary to avoid peaks of serum T3 that are above the normal range, which makes this approach impractical.[86] Most importantly, these observations put in check the conclusions obtained in clinical trials that used a single dose of liothyronine when comparing monotherapy *vs* combination therapy.

New randomized double-blind placebo controlled trials should be performed once new technology allows for stable deliver of liothyronine, for example slow release formulations. For example, oral administration of 3,5,3′-triiodothyronine sulphate (T3S) that banks on the lack of biological activity of T3S and the endogenous desulphating pathway that slowly produces T3 from T3S. In hypothyroid humans, T3S is absorbed following oral administration and results in steady state serum T3 levels for 48 h.[87]

Conclusion

Disruption of key elements in the hypothalamus–pituitary–thyroid axis as well as the deiodinase system in animals suggests that maintaining a stable serum T3 within normal range is a biological priority. At the same time, an analysis of a large number of hypothyroid patients maintained on levothyroxine replacement therapy indicates that monotherapy restores serum TSH levels without normalizing serum T3 in a portion of patients. The clinical relevance of a relatively lower serum T3 is unknown. Many clinical trials comparing monotherapy *vs* combination therapy might not have been useful, given limitations to normalize serum T3 with a single tablet of liothyronine daily.

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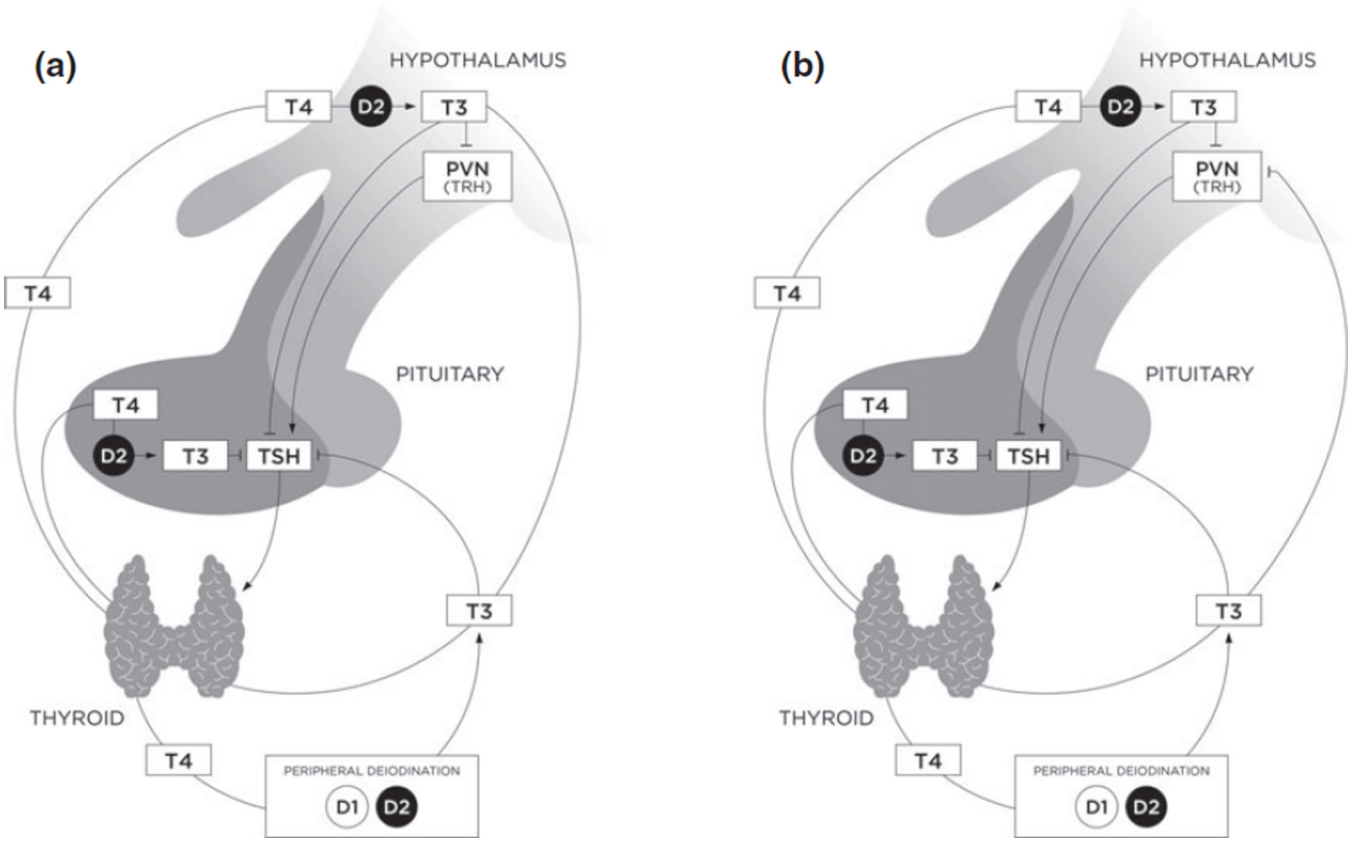

Fig. 1.

Schematic representation of the feedback mechanisms in the hypothalamus–pituitary–thyroid axis. The thyroid gland secretes T4 and T3, the biologically active thyroid hormone; T3 is also produced outside the thyroid gland via deiodination of T4; T3 initiates the negative feedback at the hypothalamus and anterior pituitary gland by suppressing TRH and TSH expression and secretion, respectively; only about 20% of the T3 produced daily is directly secreted by the thyroid gland; most T3 (about 80%) is produced outside of the thyroid gland (periphery) via two deiodination pathways, D1 and D2; T3 from both sources mixes in the plasma, defining the pool of circulating T3; D2-generated T3 also acts locally, before exiting the cell where deiodination of T4 took place; this occurs in the hypothalamus and pituitary gland and explains how circulating T4 can signal the feedback mechanism; in the hypothalamus, D2 is expressed in specialized ependymal cells (tanycytes), whereas in the anterior pituitary gland, D2 is expressed in the TSH-producing cells, thyrotrophs; the current model for the feedback mechanism is depicted in (a) and predicts that plasma T3 mixes with D2-generated T3 both in the hypothalamus (to suppress TRH) and in the pituitary gland (to suppress TSH); the data obtained in the genetically modified mice support a new model (depicted in (b)) in which plasma and D2-generated T3 do not mix in the hypothalamus; consequently, plasma T3 can be monitored independently of circulating T4.