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THYROID HORMONES PARADOX, OXIDATIVE STRESS, AND SELENIUM

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Reactive oxygen species (ROS) play an important role in physiological processes, but - when being in excess - ROS cause oxidative damage to molecules. Under physiological conditions, the production and detoxification of ROS are more-or-less balanced. Also in the thyroid, ROS and free radicals participate in physiological and pathological processes in the gland. For example, hydrogen peroxide (H_2O_2) is crucial for thyroid hormone biosynthesis, acting at different steps of the process. Additionally, H_2O_2 is believed to participate in the Wolff-Chaikoff's effect, undergoing in conditions of iodide excess in the thyroid. It is the purpose of this review to attempt a synthesis of what we currently know of thyroid hormones production and their relation to oxidative stress and selenium, a trace element.
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Key words: autoimmune disease, lipid peroxides, selenium-glutathion peroxidase

INTRODUCTION

Oxidative stress is a general term used to describe a state of damage caused by reactive oxygen species (ROS). This damage can affect a specific molecule or the entire organism. Reactive oxygen species, such as free radicals and peroxides, represent a class of molecules that are derived from the metabolism of oxygen and exist in all aerobic organisms . Much of the reactive oxygen species production occurs in mitochondria, via oxidative phosphorylation. Because the mitochondria contain specific receptors for the thyroid hormones, being one of the "favorite" target for them, the concept about a possible relationship between reactive oxygen species production and thyroid pathology has increasing importance.

Here, we highlight oxidative stress and selenium involvement in thyroid gland pathophysiology at experimental and clinical conditions.

THYROID HORMONES SYNTHESIS: OXIDATIVE STRESS IN PHYSIOLOGICAL CONDITIONS

The thyroid contains two hormones, L-thyroxine (tetraiodothyronine, T_4) and L-triiodothyronine (T_3) (Fig. 1). Iodine is an indispensable component of the thyroid hormones, comprising 65% of T_4's weight, and 58% of T_3's. The thyroid hormones are the only iodine-containing compounds with established physiologic significance in vertebrates. Ingested iodine is absorbed through the small intestine and transported

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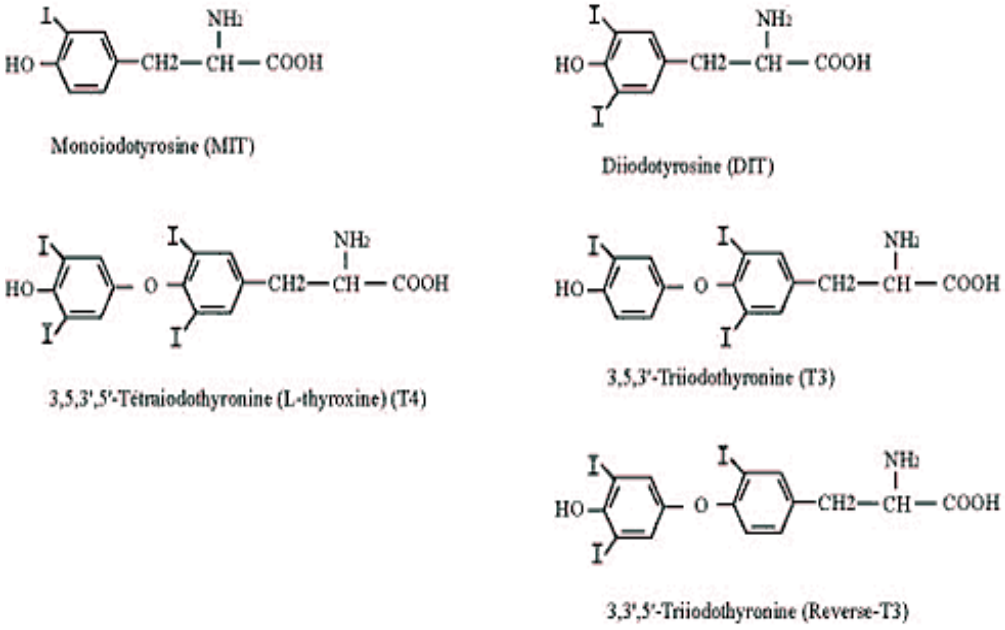

Figure 1. *Structural formula of thyroid hormones and precursor compounds. From www.THYROIDMANAGER.ORG)*

in the plasma to the thyroid, where it is concentrated, oxidized, and then incorporated into thyroglobulin (Tg) to form monoiodotyrosine (MIT) and diiodotyrosine (DIT) and later T_4 and T_3 (Figure 2). After a variable period of storage in thyroid follicles, Tg is subjected to proteolysis and the released hormones are secreted into the circulation, where specific binding proteins carry them to target tissues.

Thyroperoxidase (TPO)

After concentrating iodide, the thyroid rapidly oxidizes it and binds it to tyrosyl residues in Tg, followed by coupling of iodotyrosines to form T_4 and T_3. The process requires the presence of iodide, a peroxidase (TPO), a supply of H_2O_2, and an iodine acceptor protein (Tg). Thyroperoxidase oxidizes iodide in the presence of H_2O_2. The enzyme activity is dependent on the association with a heme, the ferriprotoporphyrin IX or a closely related porphyrin (1,2). Chemical removal of the prosthetic group inactivates the enzyme, and recombination with the heme protein restores activity (3). The apoprotein from human thyroid is not always fully saturated with its prosthetic group (4). Some congenitally goitrous children have poor peroxidase function because the apoprotein has weak binding for the heme group (4).

H_2O_2 generating system

By definition, a peroxidase requires H_2O_2 for its oxidative function. H_2O_2 is produced at the apical plasma membrane by an enzyme that requires both calcium and NADPH (5,6,7). The enzyme named thyroid NADPH oxidase, probably composed of several molecular species, has not yet been completely characterized. Complementary DNAs corresponding to two members of the NADPH oxidase family of 1,551 (ThOX1) and 1,548 (ThOX2) amino acids have been cloned. The two proteins share 83% homology; in their N-terminal part, they present 43% similarity with TPO. The C-terminal part is clearly related to leukocyte $gp91^{phox}$. Both ThOX proteins contain intracellular consensus sequence for FAD and NADPH binding sites and two EF-hand motives as calcium binding sites (8,9). The current model assigns seven transmembrane domains to ThOX1 and ThOX2. The two proteins are glycoproteins, their apparent molecular mass ranges from 170-180 kDa. Immunolabeling experiments revealed the presence of ThOX inside the cells and at the apical plasma membrane (8,10). ThOX proteins are components of the H_2O_2 generating system, but additional polypeptide chains are required to get the complete thyroid H_2O_2 generating system (11). One scheme for H_2O_2 generation by NADPH oxidase proposes the reduction of O_2 into superoxide ion (O_2^-) and the conversion to H_2O_2 by superoxide dismutase (12). Another scheme involves the direct formation of H_2O_2 from O_2 (13). TSH, via the cAMP cascade, activates the expression of ThOX1 and ThOX2 (14). TSH

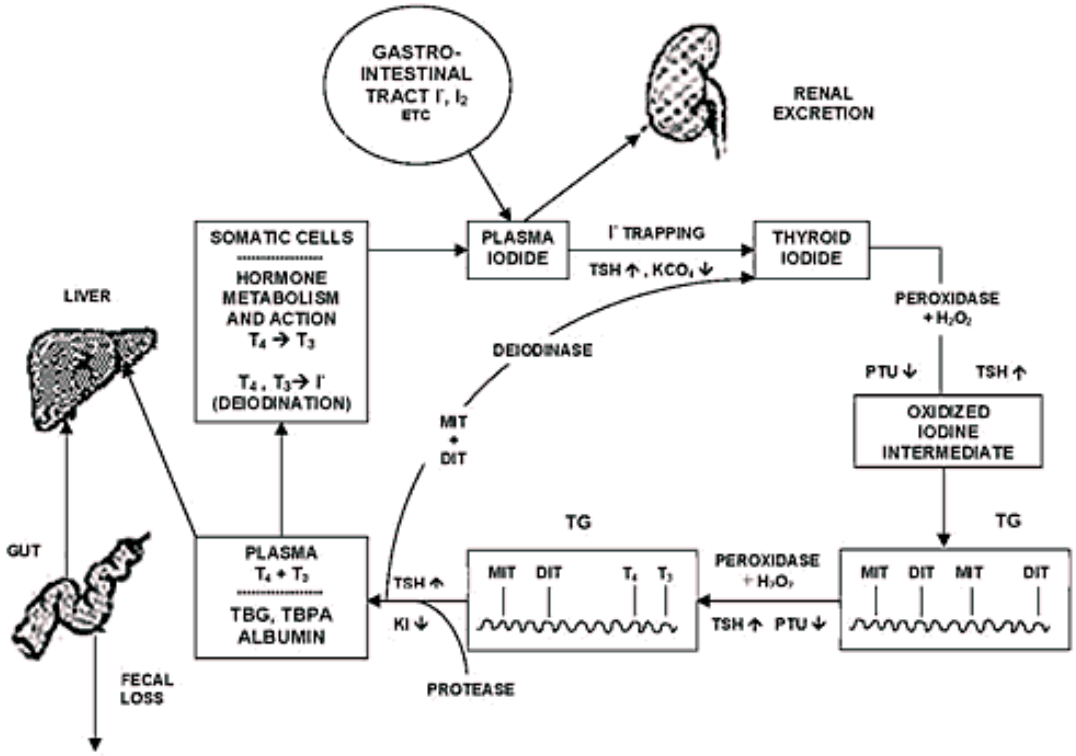

Figure 2. *The iodide cycle. Ingested iodide is trapped in the thyroid, oxidized, and bound to tyrosine to form iodotyrosines in thyroglobulin (TG); coupling of iodotyrosyl residues forms T_4 and T_3. Hormone secreted by the gland is transported in serum. Some T_4 is deiodinated to T_3. The hormone exerts its metabolic effect on the cell and is ultimately deiodinated; the iodide is reused or excreted in the kidney. A second cycle goes on inside the thyroid gland, with deiodination of iodotyrosines generating iodide, some of which is reused without leaving the thyroid. From www.THYROIDMANAGER.ORG*

stimulates NADPH oxidase activity and H_2O_2 production. This effect has been reported to be mediated through cAMP in dog thyrocytes (15,16) but by the phospholipase C/calcium cascade in FRTL-5 cells (17) and porcine thyrocytes (16). H_2O_2 generation in *in vitro* systems appears to be regulated by iodide (18). At low concentrations and short-term incubation, iodide increases H_2O_2 production (19) whereas, at high iodide concentrations, H_2O_2 generation is inhibited (20). The H2O2 generating system and TPO are closely associated.The interaction between Duox and TPO was studied using incubated follicles prepared from patients undergoing thyroidectomy, and COS-7 cells transiently transfected.: Duox and TPO from membranes are coprecipitated, This association is up-regulated through the Gq-phospholipase C-Ca(2+)-protein kinase C pathwa and down-regulated through the Gs-cAMP-protein kinase A pathway, H(2)O(2) increases the association of Duox1 and Duox2 to TPO in cells and in membranes. Coimmunoprecipitations show that Duox and TPO locate closely in the plasma membranes of human thyrocytes, and this association can be modulated by H(2)O(2), optimizing working efficiency and minimizing H(2)O(2) spillage (21)

Thyroglobulin iodination and hormone synthesis

Thyroglobulin is the most abundant protein in the thyroid gland; its concentration within the follicular lumen can reach 200-300 g/L. Its main function is to provide the polypeptide backbone for synthesis and storage of thyroid hormones (22). It also offers a convenient depot for iodine storage and retrieval when external iodine availability is scarce or uneven. Neosynthesised Tg polypeptide chains entering the lumen of the rough endoplasmic reticulum (RER) are subjected to core glycosylation, dimerises and are transferred to the Golgi where they undergo terminal glycosylation (Fig.3.). Iodination and hormone formation of Tg occur at the apical plasma membrane-lumen boundary and the mature hormone-containing molecules are stored in the follicular lumen, where they make up the bulk of the thyroid follicle colloid content.

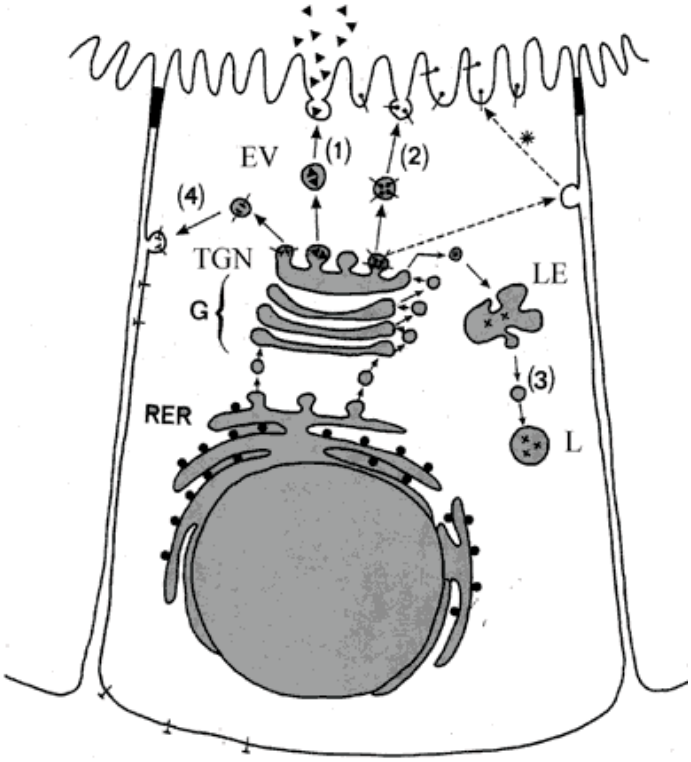

Figure 3. *A polarized thyroid epithelial cell synthesizing soluble proteins, Tg (▲) and lysosomal enzymes (X) and membrane proteins, NIS (⊥) and TPO (°). The polypeptide chain(s) generated by RER membrane-bound polysomes, enter the lumen of RER for the former and remain inserted into the RER membrane for the latter. Inside the lumen of RER, newly-synthesized proteins undergo core glycosylation and by interacting with chaperones acquire their conformation. Proteins are then transported to the Golgi apparatus (G), where terminal glycosylation and other post-translational reactions take place. In the Trans-Golgi network (TGN), mature proteins undergo sorting processes and are packed into transport vesicles. The vesicles carrying soluble proteins (inside the vesicle) and membrane proteins (as integral vesicle membrane protein) deliver them at the appropriate plasma membrane domain: the apical domain (1) and (2) or the basolateral domain (4). Vesicles carrying lysosomal enzymes (3) conveyed their content to prelysosomes or late endosomes (LE) and lysosomes (L). Apical plasma membrane proteins may reach their final destination by an alternative route involving a transient transfer to and then a retrieval and transport (∗) from the basolateral membrane domain to the apical domain. From www.THYROIDMANAGER.ORG*

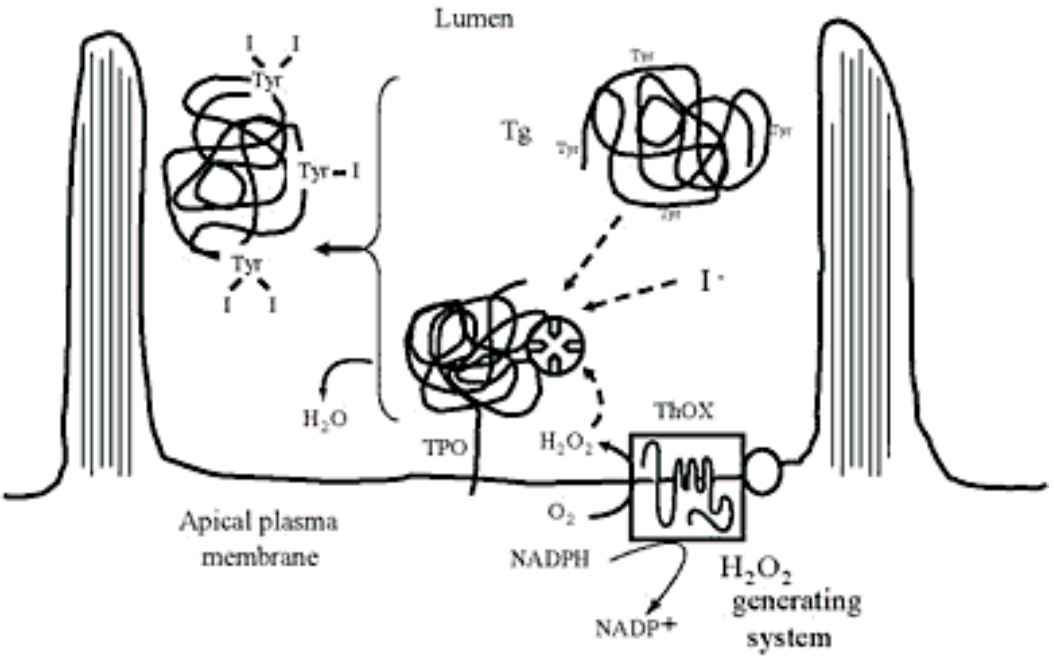

Figure 4. *Iodination of Tg at the apical plasma membrane-follicle lumen boundary From www.THYROIDMANAGER.ORG*

The step preliminary to thyroid hormone formation is the attachment of iodine to tyrosyl residues in Tg to produce MIT and DIT. This process occurs at the apical plasma membrane-follicle lumen boundary and involves H_2O_2, iodide, TPO, and glycosylated Tg. All rendezvous at the apical membrane to achieve Tg iodination. First, iodide must be oxidized to an iodinating form. An extensive literature has sought to identify the iodinating species, but the issue is still not resolved (23). One scheme proposes that oxidation produces free radicals of iodine and tyrosine, while both are bound to TPO to form MIT which then separates from the enzyme . Further reaction between free radicals of iodine and MIT gives DIT. Experimental studies by Taurog (23) and others suggest that the TPO reduction occurs directly in a two electron reaction. A second proposal, based on studies of rapid spectral absorption changes (24), is that TPO-I^+ is the iodination intermediate and that the preferred route is oxidation of TPO by H_2O_2 followed by two electron oxidation of I^- to I^+, which then reacts within a tyrosine. As a third possibility, Taurog (23) proposed a reaction between oxidized TPO and I^- to produce hypoiodite (OI^-), which also involves a two electron reaction. Whatever the precise nature of the iodinating species, it is clear that iodide is oxidized by H_2O_2 and TPO, and transferred to the tyrosyl groups of Tg. All tyrosine residues of Tg are not equally accessible to iodination. The molecule has about 132 tyrosyl residues among its two identical chains; at most, only about 1/3 of the tyrosyls are iodinated. As isolated from the thyroid, Tg rarely contains more than 1% iodine or about 52 iodine atoms. The final step in hormone synthesis is the coupling of two consenting iodotyrosyl residues to form iodothyronine (Fig. 5). Two DIT form T4; one DIT and one MIT form T3. Coupling takes place while both acceptor and donor iodotyrosyl are in peptide linkage within the Tg molecule.The reaction is catalyzed by TPO, required H_2O_2 (25, 26, 27, 28) and is stringently dependent on Tg structure (29).The generation of the iodothyronine residue involves the formation of an ether bond between the iodophenol part of a donor tyrosyl and the hydroxyl group of the acceptor tyrosyl. After the cleavage reaction that gives the iodophenol, the alanine side chain of the donor tyrosyl remains in the Tg polypeptide chain as dehydroalanine (30, 31, 32). Observations both in vivo and in vitro show an appreciable delay in coupling after initial formation of iodotyrosines. A typical distribution for a Tg containing 0.5% iodine (a normal amount for iodine-sufficient individuals) is 5 residues MIT, 5 of DIT, 2.5 of T4 and 0.7 of T3 (22). More iodine increases the ratios of DIT/MIT and T4/T3, while iodine deficiency decreases them. In addition to its role as component of the iodoamino acids, iodine is associated with cleavage of peptide bonds of Tg, at least in vitro (22). This has been attributed to generation of free radicals during oxidation (33). Exposure of Tg to reducing agents yields an N-terminal peptide of about 20-26kDa, depending

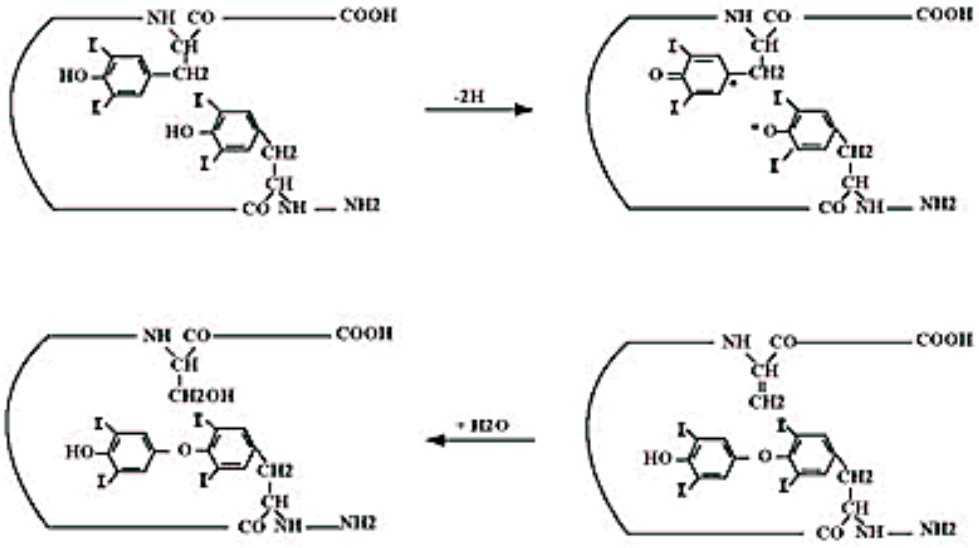

Figure 5. *Possible coupling reaction sequence. Oxidation of iodotyrosines may produce iodotyrosyl radicals. The free radicals could combine to generate the iodothyronine residue (at the tyrosine acceptor site) and a dehydroalanine residue (at the tyrosine donor site), which in the presence of H2O converts into a serine. From www.THYROIDMANAGER.ORG*

on the animal species, that contains the major hormonogenic site of Tg (34). This peptide appears in parallel with iodination or may slightly precede it (35). Further addition of iodine cleaves the 26kDa further, to produce an 18kDa (on human Tg), an event that also occurs with TSH stimulation (35). Thus, iodination-associated cleavage appears to be part of the maturation of the Tg molecule. The amount of iodine has important effects on thyroid hormone production (36). The initial reaction between TPO and H2O2 produces the so-called "compound I", which oxidizes iodide and iodinates Tg. Next, the two reactants form compound II, which is necessary for the coupling reaction to make thyroid hormones. However, if excessive iodine is present, conversion to compound II does not take place, and hormone synthesis is impaired. Other iodinated compounds occasionally inhabit the thyroid. Thyroalbumin excited considerable interest several decades ago. This is an iodinated albumin, recently shown to be serum albumin that is iodinated in the thyroid (37). Occasionally, large amounts are found in certain thyroid diseases, including Hashimoto's thyroiditis (38), congenital metabolic defects (39), thyrotoxicosis (40) and thyroid carcinoma (41). In all these cases, there are abnormalities in thyroid structure which might explain the access of serum albumin to intrathyroidal iodination sites. However, in physiological conditions, serum albumin can reach thyroid follicle lumina by transcytosis i.e. basolateral endocytosis and vesicular transport to the apical plasma membrane (42). The thyroid also iodinates lipids and many different iodolipids have been described after high doses of iodide in vitro (43. 44). Of particular interest is 2-iodohexadecanal (45, 46). It occurs in the thyroid of several species following administration of KI, and its amount increases linearly with additional iodine, in contrast to iodination of Tg which eventually is inhibited by excess iodide. This compound inhibits the action of NADPH oxidase, which is responsible for H2O2 production (47). These findings have led to the suggestion that iodination of lipids impairs H2O2 production and, therefore, decreases further Tg iodination. This is the most probable mechanism for the Wolff-Chaikoff effect (18).

THYROID HORMONES PARADOX

Thyroid hormones themselves also pose a paradox. This `thyroid hormone paradox' is that they both promote damage by ROS, by stimulating aerobic metabolic activity, but they also act as antioxidants themselves, as well as influencing other antioxidant defences (48). Hyperthyroidism increases lipid peroxidation in young, old and very old rats and hypothyroidism results in lower levels of lipid peroxidation in young rats (49). One study reports that hypothyroidism in rats significantly decreases lipid peroxidation in skeletal muscle but not thymus, spleen or lymph nodes (50), whilst another reports that it results in no reduction in lipid peroxidation in liver, cardiac or skeletal muscle (51). In rats, hyperthyroidism results in increased levels of lipid peroxidation products in liver, heart, soleus muscle and lymph nodes (51, 52). Other experimental stuy on adult, white, Wistar rat reports a significant increase in lipid peroxidation and proteins carbonyl in the main thyroid hormones target tissues: thyroid gland, heart, liver, skeletal muscle, brain (53, 54, 55, 56). Higher levels of mitochondrial enzymes and increased ROS production by submitochondrial particles has also been reported for hyperthyroid rats (57,58).

Thyroid-induced increases in lipid peroxidation are not restricted to enhanced mitochondrial activity. For example, in rats T3 increases superoxide production, NADPH oxidase activity and lipid peroxidation in hepatic microsomes (57). Polymorphonuclear leucocytes from hyperthyroid rats have an enhanced capacity to produce superoxide when stimulated *in vitro* and this seems primarily related to thyroid-hormone-enhanced NADPH oxidase activity. A similar effect has been observed in phagocytes from hyperthyroid humans (58). Both T4 and T3 can also stimulate, *in vitro*, the activity of myeloperoxidase isolated from human leukocytes (59).

Low-density lipoproteins isolated from hyperthyroid humans also show an elevated lipid peroxidation compared to those from euthyroid individuals, intriguingly as do those from hypothyroid individuals (60). These results suggest that, at least in humans, the euthyroid condition represents that which results in the minimal peroxidative damage to low-density lipoproteins. Whether this also applies to other molecules capable of oxidative damage is not known.

Antioxidant enzymes include superoxide dismutase (SOD), glutathione peroxidases and catalases (61). The influence of thyroid status on antioxidant defences is mixed and there appears to be no consistent pattern. Some studies report that hyperthyroidism increases vitamin E content of cardiac muscle (51,62) whilst others show no effect (52). Hyperthyroidism increases glutathione levels in erythrocytes and serum (63,64) but decreases glutathione levels in rat liver (63). During hyperthyroidism, glutathione peroxidase is elevated in liver, erythrocytes, spleen and skeletal muscle (63,64) diminished in thymus, heart and skeletal muscle

(52,63,64) and unaffected in liver and heart (57). Whilst in hypothyroidism it has been reported as both increased in cardiac and skeletal muscle (57,62) and reduced in thymus and skeletal muscle (51,52). Glutathione reductase activity appears relatively unaffected by thyroid status (51) although it has been reported to be elevated in liver during hyperthyroidism (63). Catalase activity is reported both to increase in skeletal muscle (50) and decrease in thymus, spleen, cardiac and skeletal muscle (50,52,64) during hyperthyroidism. The activity of Cu2+,Zn2+ -, SOD is also reported to be increased in thymus, leucocytes and skeletal muscle (50) but decreased in cardiac and skeletal muscle (52) whilst Mn2+ - SOD is reported to be increased in leucocytes, thymus, spleen, cardiac and skeletal muscle (50,52) during hyperthyroidism. No general pattern can be discerned from the diverse responses of antioxidant defences to hyperthyroidism. Most studies involving hypothyroidism report no significant influence on levels of either antioxidant scavengers or enzymes.

The question as to whether thyroid themselves can act as antioxidant compounds was first raised after De Caro (65) observed that T4 can decrease oxygen uptake by solutions of unsaturated fatty acids. It was later shown that T4 could also prevent lipid peroxidation in isolated erythrocytes and liver homogenates (66), and that T4, T3 and 3,5-T2 (but not the non-iodinated thyronine) could inhibit lipid peroxidation in isolated rat liver mitochondria as well as dramatically inhibiting carbon tetrachloride accelerated lipid peroxidation (67). As with many early studies, the T4 concentrations used in these experiments were high and non-physiological. Wynn (68) used an *in vitro* system to show that Fe2+ could activate lipid peroxidation of rat liver microsomes and that T4 could terminate such lipid peroxidation by acting as an antioxidant. The lowest T4 concentration examined was 100 nm, and at this concentration, T4, rT3 and T3 all showed antioxidant activity (in decreasing intensity) and their antioxidant abilities were greater than that of either vitamin E or ascorbic acid in the same system. He also showed that pharmacological doses of T4 could reduce *in vivo* the peroxide content of epididymal fat pads in the rat (68). Because of its relative metabolic inertness such a tissue is unlikely to have had significant thyroid-hormone-induced peroxidation.

In these lipid peroxidation experiments, T4 was degraded when exerting its antioxidant effects. This was consistent with the finding that the *in vivo* rate of T4 degradation seemed to be related to its clinical effect (69). Lipid peroxidation can also be catalyzed *in vitro* by hemoglobin, and iodothyronines inhibit such peroxidation, and are more potent antioxidants in these situations than vitamin E, glutathione or ascorbic acid (70). The physiological importance of the antioxidant properties of thyroid hormones was questioned when the antioxidant capacities of both T3 and T4 in retarding the auto-oxidation of rat brain homogenate and free-radical-mediated oxidation of rat erythrocyte membranes was evaluated and found to be very low (1±2%) at hormonal concentrations of 50 nm but substantial at micromolar hormonal levels (71).

That thyroid hormones possibly have antioxidant activity is also illustrated by the finding that although resting neutrophils exhibit negligible deiodination of both T4 and T3, when they are stimulated to phagocytose (and ROS production is consequently activated), the deiodination of both T4 and T3 dramatically increases severalfold. Neutrophils from individuals with chronic granulomatous disease, which are deficient in the capacity to produce ROS, degrade T4 and T3 poorly during phagocytosis (72). Examination of the potential role of thyroid hormones as potent natural membrane antioxidants also deserves attention in light of other observations. A strong correlation has been observed in rats between the phospholipid content of tissues and their T4 content. Analysis of this relationship, assuming that all T4 is associated with membranes, suggests a molar ratio of T4:phospholipid of approximately 1:1000. This is similar to the molar ratio of vitamin E: lipid observed in biological membranes which is of the order of 1:2000±3000 (73). Whilst obviously not all thyroid hormone molecules are associated with membranes, measurements in human erythrocytes suggest that approximately half of cellular thyroid hormone molecules (34% of T4 and 60% of T3) are associated with membranes (74). As the human erythrocytes lack intracellular membranes and in this particular study they were washed several times before measurement, these are likely to be minimum value Another reason why this area is worthy of careful examination is that the resting oxygen consumption of mammals is proportional to approximately the 0.75 power of body mass (75). This is the same allometric exponent observed for whole – mammal ethane exhalation rate, which is indicative of lipid peroxidation (76) and for the degradation rates of T4 and T3 in mammals (77). These findings of similar allometric exponents for these three processes means that, in mammals, irrespective of metabolic activity, there is a relatively constant molar ratio between oxygen consumption, phospholipid peroxidation and deiodination of T4 and T3.

SELENIUM AND THE THYROID GLAND

The thyroid contains more Se per gram of tissue than any other organ (78) and Se, like iodine, is essential for normal thyroid function and thyroid hormone homeostasis.

Selenium as an antioxidant in the thyroid

During thyroid hormones synthesis, the thyrocyte is continually exposed to potentially toxic concentrations of H_2O_2 and lipid hydroperoxides. The cytotoxic effects of H_2O_2 on thyroid cells include caspase-3-dependent apoptosis that occurs at H_2O_2 concentrations that are insuffcient to induce necrosis. In Se deficiency the apoptotic response to H_2O_2 is increased (79). When Se intake is adequate the intracellular glutathione peroxidase (GPX) and thioredoxin reductases (TR) systems protect the thyrocyte from these peroxides. Furthermore, in iodine deficiency or Grave's disease, where hyperstimulation of the TSH receptor signals increased H_2O_2 production, activation of the calcium-phosphoinositol cascade stimulates GPX1 production and particularly TR1 (80) thus providing an up-regulation of antioxidant protection.

Thyroid hormone deiodinases and selenium

Three iodothyroinine deiodinases (D1, D2 and D3) have been identified. Each has a selenocysteine residue at the active centre that confers the high catalytic activity of the enzymes. The deiodinases have differing substrate specificities and tissue distribution (81). The enzymes can catalyse the removal of iodine from the 5 or 5' positions of iodothyronine substrates and in doing so have an important regulatory role in the activation and inactivation of the thyroid hormones in all tissues. Recently details of the protein structure of the deiodinases has become available. The extra-membrane portion of the deiodinases belongs to the thioredoxin-fold superfamily, a superfamily that also includes the GPXs. Furthermore, a large deiodinase region embedded in the thioredoxin fold shares strong similarities with the active site of iduronidase, a member of the clan GH-A-fold glycoside hydrolases. The substrates for the deiodinases (iodothyronines such as thyroxine (T4), reverse tri-iodothyronine (rT3) and 3,5,3'-tri-iodothyronine (T3) and substrates for the iduronidase (sulphated α-L-iduronic acid) are structurally similar, having O-linked hexagonal rings substituted with bulky groups lying *ortho* to the linker. It would thus appear that the deiodinases have iduronidase-like sequences embedded in the selenocyteine-containing thioredoxin fold that are critical for iodothyronine binding. The predicted protein structure of the deiodinases together with site-directed mutagenesis experiments have allowed the elucidation of some of the critical amino acids that are responsible for the differences in substrate specificity and enzyme kinetics observed between D1, D2 and D3 (82). The deiodinases show marked tissue- and time-specific expression during the foetal period and may be important regulators of this maturation process by modifying the supply of T3 to T3-responsive genes (83, 84). The deiodinase D1 is the major isoform in liver, kidney, thyroid and pituitary. It can catalyse 5 or 5'-monodeiodination and thus can convert T4 to the inactive metabolite rT3 or the active isomer T3. The important physiological roles of D1 include providing an important source of plasma T3 and degrading rT3 and T3 sulphate.

There are species-specific differences in the expression of D2. In rats, D2 is predominantly expressed in brain, brown adipose tissue and pituitary with little or no expression being found in thyroid, skeletal muscle or heart. In humans, Northern blotting or activity measurements suggest that D2 expression occurs in thyroid, heart, brain, spinal cord, skeletal muscle, placenta, pituitary and keratinocytes and to some extent in kidney and pancreas. D2 can only perform 5'-deiodination reactions and the enzyme has a short half-life (<1 h), which is controlled by ubiquitination. Physiologically, D2 provides an intracellular source of T3 to specific tissues and, particularly in humans, it also appears to provide a significant source of plasma T3. Among its other physiological roles, D2 is critical for regulating brain development, TSH secretion in the pituitary and adaptive thermogenesis in brown adipose tissue. D3 is found in the plasma membrane of brain, placenta and foetal liver and performs only 5-monodeiodination (85).

In Se-suffcient rats, hepatic D1 provides an important source of circulating T3 yet in Se-deficient animals, when hepatic D1 expression falls to approximately 10% of that in Se-adequate animals, plasma T3 concentrations are largely maintained. The maintenance of plasma T3 in these Se-deficient animals arises from an adaptive response driven by a rise in TSH that in turn signals increased *de novo* synthesis of T3 on thyroglobulin and also increased expression of thyroidal D1 that promotes high rates of T4-to-T3 conversion (65, 86). In humans, thyroidal D2 may also contibute to maintaining plasma T3 in Se deficiency. The paradoxical increase in thyroidal D1 found in Se-deficient rats is made possible because the gland retains adequate amounts of the trace element in dietary Se deficiency (87). Not all animal species express thyroidal D1 and theoretically those lacking the enzyme may be less able to maintain plasma T3 concentrations in Se de-

ficiency (88). Since D2 expression and T3 production are vital for regulating thermogenesis in brown adipose tissue, Se-deficient animals may show impaired production of D2 and uncoupling protein, with poor survival when subjected to a cold stress (86).

Selenium and iodine deficiency

In humans, attention has focused on how Se status may modify the effects of iodine deficiency and the pathogenesis of endemic myxoedematous cretinism (89,86,90), a condition associated with severe hypothyroidism, thyroid involution and stunted growth. Some epidemiological studies have suggested that the increased generation of H_2O_2 caused by the high TSH associated with iodine deficiency, together with a loss of thyroidal selenoperoxidase activity due to concurrent Se deficiency, produces the marked thyroid atrophy found in myxoedematous cretinism. In contrast, if Se supply is adequate thyroid destruction may be prevented due to the maintenance of thyroidal GPX and TR. The importance of Se in protecting the thyroid from oxidative damage is supported by rodent experiments (91). These animal studies suggest also that myxoedematous cretinism may also result from a Se-deficiency-induced disturbance in the inflammatory response (92). More recent reports have failed to provide convincing support for this hypothesis and the possible roles of other additional factors such as dietary thiocyanates must again be considered (93).

Thyroid hormones, selenium and iodide excess

Environmental iodine deficiency continues to be a significant public health problem worldwide. On the other hand, iodide excess results principally from the use of iodine-containing medicinal preparations or radiographic contrast media. Iodide excess impairment on prooxidant/antioxidant balance of the thyroid gland, hepatic tissue and in blood and the effect of selenium administration on oxidative stress markers under the same circumstances were explored using an experimental model. Iodide excess had prooxidant effects, leading to an increased lipid peroxides level and catalase activity in target tissues and in blood and to a decreased H+ donor ability of the sera. Selenium supplementation had opposite effects (54).

Selenium and autoimmune thyroid disease

The links between Se deficiency, altered immune function and inflammation have prompted studies in humans to examine if Se supplementation can modify auto-antibody production in patients with chronic autoimmune thyroiditis. Double-blind, randomized, placebo-controlled trials using daily Se supplements of 200 μg selenite produced a significant decline in TPO antibody (TPOAb) concentration accompanied in some patients by an improved ultrasound echogenicity of the thyroid (94, 95). This effect of Se on TPOAb concentration has been demonstrated both in an area of Germany with marginal dietary iodine and Se intakes (94) and in an area around Athens where iodine and Se intakes were close to requirement (96). In these studies Se supplements had no significant effect on the concentration of thyroglobulin antibodies or the concentration of TSH or thyroid hormone concentrations. The mechanism by which Se exerts effects on TPOAb production is likely to be due to the ability of high doses of Se to modify the inflammatory and immune responses (97). Further work is required to examine what long-term clinical benefits Se supplementation may have when given to patients with autoimmune thryoiditis. It would be important to determine if Se supplementation could modify the course of Graves' disease since there is one report of Se supplements decreasing the concentration of TSH receptor antibodies in these patients (98).

CONCLUSION

Thyroid hormones synthesis requires iodide, thyroglobulin and an oxidation system to oxidize iodide, to iodinate tyrosyl groups in thyroglobulin and couple them into iodothyronines. This oxidation system is constituted of a thyroperoxidase that oxidizes iodide in the presence of hydrogen peroxide and an ill-defined hydrogen peroxide generating system-using NADPH as coenzyme (10). Iodination mechanism consists in several steps, having iodinium (I+) and hypoiodite (IO-) as extremely reactive intermediate products. Thyroid hormones pose a paradox: they both promote damage by ROS, by stimulating aerobic metabolic activity, but they also act as antioxidants themselves, as well as influencing other antioxidant defence. The thyroid gland contains more selenium per gram of tissue than any other organ and selenium, like iodine, is essential for normal thyroid function and thyroid hormone homeostasis.

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