

Review

On the importance of selenium and iodine metabolism for thyroid hormone biosynthesis and human health

Lutz Schomburg and Josef Köhrle

Institut für Experimentelle Endokrinologie und Endokrinologisches Forschungs-Centrum der Charité EnForCé, Charité Campus Virchow-Klinikum, Charité Universitätsmedizin, Berlin, Humboldt-Universität zu Berlin, Berlin, Germany

The trace elements iodine and selenium (Se) are essential for thyroid gland functioning and thyroid hormone biosynthesis and metabolism. While iodine is needed as the eponymous constituent of the two major thyroid hormones triiodo-L-thyronine (T3), and tetraiodo-L-thyronine (T4), Se is essential for the biosynthesis and function of a small number of selenocysteine (Sec)-containing selenoproteins implicated in thyroid hormone metabolism and gland function. The Se-dependent iodothyronine deiodinases control thyroid hormone turnover, while both intracellular and secreted Se-dependent glutathione peroxidases are implicated in gland protection. Recently, a number of clinical supplementation trials have indicated positive effects of increasing the Se status of the participants in a variety of pathologies. These findings enforce the notion that many people might profit from improving their Se status, both as a means to reduce the individual health risk as well as to balance a Se deficiency which often develops during the course of illness. Even though the underlying mechanisms are still largely uncharacterised, the effects of Se appear to be exerted *via* multiple different mechanisms that impact most pronounced on the endocrine and the immune systems.

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1 Selenium (Se) intake, Se availability and Se metabolism

The distribution of selenium (Se) varies widely in the environment and in different countries. While the soils of central Asian regions and most European countries are generally low in both iodine and Se concentrations, no such deficiencies are observed in other parts of the world including

Correspondence: Professor Josef Köhrle, Institut für Experimentelle Endokrinologie und Endokrinologisches Forschungs-Centrum der Charité EnForCé, Charité Campus Virchow-Klinikum, Charité Universitätsmedizin Berlin, Humboldt-Universität zu Berlin, Augustenburger Platz 1, D-13353 Berlin, Germany
E-mail: josef.koehrle@charite.de
Fax: +49-30-450-524922

Abbreviations: AITD, autoimmune thyroid disease; DIT, diiodotyrosine; Duox, dual oxidase; GPx, glutathione peroxidase; MIT, monoiodotyrosine; NIS, sodium-iodide symporter; Sec, selenocysteine; SeMet, selenomethionine; SePP, selenoprotein P; Tg, thyroglobulin; TPO, thyroperoxidase; TPOab, TPO autoantibodies; T3, triiodo-L-thyronine; T4, tetraiodo-L-thyronine; TSH, thyroid stimulating hormone; TxnRd, thioredoxin reductase

most regions of the USA or Japan. The Se concentrations in plants and farm animals largely depend on these local availabilities [1]. During the biosynthesis of the amino acids methionine and cysteine, the plants make use of both sulphur (S)- and Se-containing substrates. Depending on the plant, large amounts of Se can thereby enter the food chain [2]. Depending on the type of plant and relative concentration of Se over S in the soil, considerable quantities of selenomethionine (SeMet) and also variable amounts of S-methylselenocysteine, selenocystathionine, selenocysteine (Sec) and γ -glutamyl-Se-methylcysteine are synthesised by plants and taken up through the diet by animals and humans. To avoid adverse health effects because of Se deficiency, the feed of farm animals is often supplemented with adequate Se sources [3]. This supplementation strategy is believed to be responsible for improved fertility, immune system functioning and meat quality in comparison to non-supplemented animals.

In higher mammals, two interconnected cycles of Se-containing proteins are found. SeMet is taken up mainly from plants, *i. e.* vegetables, fruits or cereals, and enters all proteins in response to AUG codons, albeit at a marginal

rate which reflects the relative amount of SeMet over Met in the diet. Depending on this relation, the fraction of SeMet residues compared to Met in, *e.g.* human albumin has been determined to range in the order of 1:8000–1:2800, indicating that only around 1 in 1000 albumin molecules harbours a single SeMet in its primary sequence [4].

In contrast, biosynthesis of the Sec-containing selenoproteins represents a stringently regulated, mRNA-encoded and highly specific process [5]. Biostatistical algorithms have been developed and used to predict that the human genome contains only very few genes that specify Sec-insertion at precisely predefined positions [6]. Labelling experiments with ^{75}Se in rats have indicated the presence of about 50 differently-sized Se-containing proteins [7], consistent with the number of 25 human or 24 rodent genes identified by these elegant *in silico* analyses. Albeit, not all of these proteins are synthesised at constant rates; it appears as if very essential selenoproteins implicated in vital functions of the cell or the organism become preferentially synthesised, even in times of Se-shortage. These proteins include the isozymes of the thioredoxin reductase (TxnRd) family, *i.e.* TxnRd-1 and -2, and the phospholipid-hydroperoxide-specific glutathione peroxidase (PH-GPx), *i.e.* GPx-4. These selenoproteins have been demonstrated to be essential for the development and survival, for their genetic inactivation in mice proved to be lethal [8]. Some of the other selenoproteins appear to represent less vital components of the proteome, including the ubiquitously expressed GPx-1 or the extracellular mainly kidney-derived plasma isozyme, *i.e.* GPx-3. Surprisingly, the recent characterisation of the first identified human patients suffering from a general deficit of selenoprotein biosynthesis because of inherited mutations in the Sec-Insertion Sequence Binding Protein 2 (SBP2) gene displayed a defective thyroid hormone feedback axis [9]. Both the analysis of mRNA and protein concentrations from these patients and the consecutive *in vitro* characterisation of the mutant proteins indicated that the deiodinase isozymes belong to the less-well supplied selenoproteins in times of insufficient biosynthetic capacity.

In healthy individuals, supplementation with different Se-forms yields a complex picture of effects on the Se status (Fig. 1). SeMet can directly be funnelled into all proteins at Met-specific positions in direct relation to its relative abundance. Thus, enriching one's diet with large amounts of SeMet over regular Met intake leads to an increasing value for total blood Se concentration because of increased fractions of SeMet-containing serum proteins [10]. The overall effect, *i.e.* an increased serum or plasma Se concentration, is largely independent from the original Se status of the individual. In contrast, the effect of increased intake of inorganic Se sources like sodium-selenite or -selenate differs decisively between well-supplied and marginally-supplied individuals. In general, inorganic Se sources can only be used for the biosynthesis of Sec-containing but not of

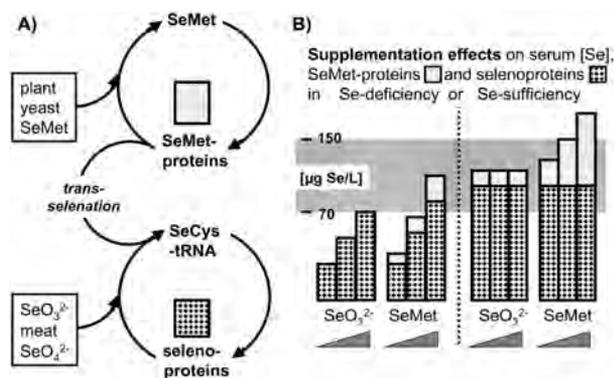


Figure 1. (A) Schematic illustration of Se metabolism, and effects of Se-based supplementations on blood Se status, inspired by references [1–4, 11]. Se is contained in different supplements either as organic SeMet or other Se-containing amino acid derivatives (*e.g.* in plants or Se-enriched yeast, far left) or as purely inorganic selenite (SeO_3^{2-}) or selenate (SeO_4^{2-}). SeMet is directly used for biosynthesis of SeMet-containing proteins (left, top, dotted bars) while inorganic Se can only be used for SeCys-dependent biosynthesis of real selenoproteins (left, bottom, chequered bars). A transselenation pathway can funnel Se from SeMet protein turnover into the SeCys-tRNA-dependent pathway of selenoprotein biosynthesis (left, centre).

(B) Both kinds of Se-based supplements are effective to increase biosynthesis of selenoproteins in marginally-supplied individuals (initial serum Se concentrations below 70 $\mu\text{g/L}$, left), but inorganic Se supplements are with little effect on serum Se concentrations in well-supplied people who display serum Se concentrations in the range of 70–150 $\mu\text{g/L}$ (right). In contrast, SeMet-containing supplements increase serum Se concentrations and the SeMet-containing protein fractions in all users irrespective of initial Se status and cause concerns for long-term side effects (far right). The triangles indicate increasing dosages or periods of time in which the respective supplements are taken. Typical dosages range from 50 to 200 $\mu\text{g Se/day}$ over periods of 6 months to several years.

SeMet-containing selenoproteins. This fact represents the fundamental difference between these two supplementation forms that are sometimes used without detailed description and further discrimination in human, experimental animal or *in vitro* studies. Only the use of SeMet will always result in increased serum Se concentrations. In serum or plasma, there are two soluble selenoproteins that account for the largest fraction of blood Se, *i.e.* GPx-3 mainly from kidney and selenoprotein P (SePP) mainly from liver. Biosynthesis and circulating levels of these two selenoproteins can apparently be saturated, albeit at slightly different Se intake levels [10]. SePP responds over a larger range of supply and is therefore considered to represent the better and more reliable indicator of Se status and Se intake [11]. Nevertheless, once saturated levels of both proteins have been reached, which appears to need less than 80–100 $\mu\text{g Se/day}$ in humans or 50–100 nM Se in cell culture, no further increase in SePP or GPx-3 concentrations is usually observed.

However when SeMet is chosen as Se source for supplementation, total Se concentration in blood increases even in well-supplied individuals [10]. No mechanism is known to limit this gradual increase in the biosynthesis of SeMet-containing proteins. This lack of control has given rise to concern and questioned the choice of SeMet as a suitable supplementation form in human trials. In view of the aforementioned marginal fraction of actually affected proteins such effects appear negligible and have generally not been observed in the respective human studies. Still, the recent re-analysis of the large NPC trial in which more than 1000 participants received either 200 µg SeMet-containing yeast *per* day or placebo over several years has revitalised the fear of some adverse side effects by the chronic use of this organic Se-containing supplement in already well-supplied individuals [12]. This notion has been strengthened by a cross-sectional analysis from participants in the NHANES III survey but the effects were relatively small and limited to the highest quintile of Se status [13]. Whether SeMet-containing proteins show different properties such as higher sensitivity towards spontaneous oxidation compared to their normal sulphur versions remains an open issue.

Inorganic Se sources like sodium-selenate or -selenite are not linearly used for selenoprotein biosynthesis. Their metabolism strongly depends on the Se status of the consumer; a marginally supplied individual will take full advantage of the offered Se-source and steadily increase selenoprotein biosynthesis according to the dietary supply [11]. This is in contrast to individuals who are already Se-replete and present with saturated concentrations of plasma GPx-3 and SePP. Here, the surplus Se is not taken up for protein biosynthesis but rather secreted in the form of Se-containing sugar derivatives, or once a presumably toxic level has been passed, such excessive Se can be exhaled as dimethyl-selenide or lost as trimethylselenonium in urine [14]. In how far healthy and diseased individuals, young and older people, or male and female patients differ in their metabolic routes of dietary-derived Se is currently a matter of intensive research and scientific discussion [15]. Unfortunately, large-scale and long-time supplementation studies with inorganic Se sources are missing, therefore direct comparisons of both the beneficial and potentially adverse effects appear impossible at present [16].

2 Se status, Se-dependent diseases and supplementation studies

The relative amount of SeMet-containing and 'real' selenoproteins, and the metabolism of dietary-derived Se is not constant but depends largely on the actual nutritional and health status of the individual. This complex system of interactions is responsible for the lack of unanimous consensus on the recommended Se intake *per* day or the optimal Se concentration in blood [17]. Current assessments

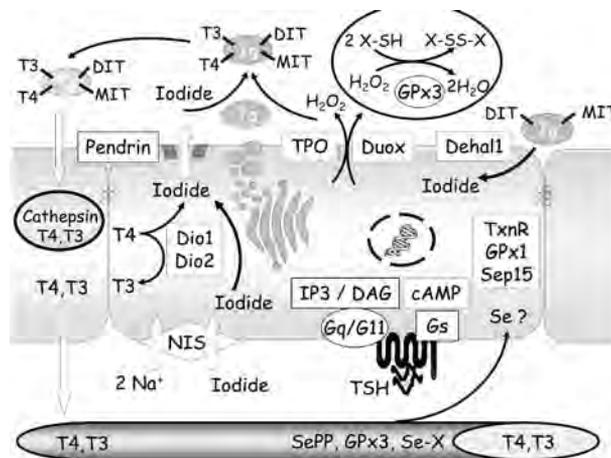


Figure 2. Schematic presentation of thyroid hormone biosynthesis by thyroid follicular cells and potential function of GPx3 in removal of excess H₂O₂.

The NIS accumulates iodide at the basolateral thyrocyte membrane. Iodide is exported across the apical membrane into the colloid lumen by the anion transporter pendrin. Duox generates H₂O₂ at the apical luminal surface, which is utilised by TPO to iodinate Tg secreted into the colloidal lumen, yielding mono- (MIT) and di-iodotyrosine (DIT) residues in the Tg protein chain. TPO also catalyses coupling of MIT and DIT residues to yield the iodothyronine side chains as precursors of T4 and T3. Hormone-containing Tg is internalised by micropinocytosis at the apical membranes. T4 and T3 are liberated by lysosomal cathepsin catalysed proteolysis of Tg and secreted into the blood stream. Iodide is liberated from MIT and DIT residues by dehalogenase (Dehal1) and recycled for Tg iodination. The apically secreted selenoprotein GPx3 degrades excess H₂O₂ not utilised for iodination and coupling. Intracellular Se-dependent deiodinase isozymes form T3 and liberate iodide from T4. Additional selenoproteins including TrxRd, GPx1 and selenoprotein P15 (Sep15) are involved in intracellular redox control and antioxidant defense. The pituitary hormone TSH is the key regulator of thyroid hormone biosynthesis, storage and secretion and acts *via* the G-protein (Gq/G11, Gs) coupled TSH receptor. The major uptake mechanism for the circulating Se-containing compounds (Se-X) is currently unresolved.

have mainly relied on the average Se concentrations found in areas where no deficiency symptoms are observed, the amount needed to prevent the development of Se deficiency symptoms, or the amount needed to saturate circulating levels of GPx-1 in erythrocytes, GPx-3 or SePP in plasma or serum. Some consensus is reached in suggesting that a daily intake of around 1 µg Se/kg body weight is definitely safe to prevent symptoms related to Se deficiency without carrying the apprehension of any adverse side effects.

Most inhabitants of the USA do easily reach or even exceed this recommended intake while many Europeans and people from Central China are usually slightly or considerably below this value [18]. Still, this suboptimal intake cannot easily be made responsible for obvious health issues

in those marginally supplied areas. On the contrary, a daily supplementation of 200 µg Se proved of chemopreventive potency in the large aforementioned NPC trial and appears thus advisable to even the well-supplied North Americans that participated in this study and displayed Se blood concentrations largely exceeding average European values [19]. These effects are unlikely to be mediated by those selenoproteins that we can measure easily from serum or plasma for they have likely already been saturated at the beginning of the supplementation period in both verum- and placebo-treated individuals.

The question whether there were further bioactive Se-containing metabolites present in the yeast preparations chosen, or whether such metabolites have been generated *in vivo* in the participants, or whether those selenoproteins that we can not readily measure from human blood do need a higher Se supply than GPx-1, GPx-3 or SePP to become saturated can not be answered, yet. Thus, with our knowledge on the common final pathway of inorganic and organic Se sources in marginally supplied individuals, *i. e.* the improved biosynthesis of Sec-containing selenoproteins, we can easily and confidently recommend how to avoid or treat conditions of Se deficiency – but we have not yet reached solid scientific, experimental or empirical ground to finally decide on the optimal intake level of Se or the most suitable form to be used [20].

Yet, some very recent clinical trials have given further support to the well-appreciated notion that most people should benefit from increased Se intake. The follow-up analyses from the NPC trial convincingly verified the chemopreventive potential of 200 µg Se-enriched yeast *per day* to decrease, *e. g.* prostate cancer risk especially for participants that resided in the lowest tertile of baseline Se concentrations at the beginning of the study [21]. In Europe, a recently published case-control study indicated a pronounced inverse association of bladder cancer risk and serum Se concentrations [22]. Besides cancer, cardiovascular events represent the other major mortality reason in humans. An inverse association of Se blood levels with systolic and diastolic blood pressure has been reported in marginally supplied Europeans [23]. Interestingly, this effect was only observed in men but not in women [24]. In addition, activity of GPx-1 from erythrocytes was found strongly associated with cardiovascular event risk in patients with coronary artery disease [25]. Even though we do not yet understand the underlying mechanisms or whether GPx-1 was actively involved in the physiological effects or rather represented a convenient surrogate marker from blood to estimate the Se status, these clinical findings also support the claim for an increased daily Se intake.

A third major health risk is often encountered when by chance or surgery the immune system becomes activated beyond its regular extent. Infection, inflammation or traumas can induce vivid acute phase responses that reduce serum Se concentrations depending on the strength and the

duration of the stimuli [26]. In sepsis patients, a clear correlation of mortality risk and blood Se concentrations has been established [27]. In a recent randomised, double-blind multiple-centre study, Se supplementation after the onset of severe sepsis has proven effective to improve health and reduce mortality [28]. Even when at present some similar trials failed to confirm the beneficial outcome upon acute supplementation with high Se dosages, there is consensus that patients with low Se concentrations have a poorer prognosis compared to well-supplied individuals. Neither the mechanisms by which Se concentrations decline during the acute phase response, nor the fate of the Se that disappears from blood are currently unequivocally resolved. Nevertheless, the clinical observations still argue for a preventive upregulation of the personal Se stores to be well prepared to survive such accidental or surgery-related challenges.

A last example for the potentially beneficial effects of increased Se supply to combat a premature mortality risk is given by recent results from a clinical trial on patients with HIV infections. In general, AIDS patients tend to develop Se deficiency during the course of the disease, and Se blood concentrations negatively correlate to AIDS mortality. Outcome from a respective double-blinded Se supplementation trial with AIDS patients has just been published [29]. Strong concerns had been raised against such trials before based on the identification of a Se-containing isozyme from the GPx family in the viral genome [30]. It was argued that not only the immune system of the patient but also proliferation rate and agility of the virus might become strengthened by Se supplementation. Fortunately, these fears have not come true, and both CD4-positive cell counts increased and viral RNA titres decreased in response to Se supplementation [29]. Further trials are needed to work out the impact of antiviral therapy on these effects. Still, increasing the Se status might represent a new promising adjuvant therapy option especially in Se deficient AIDS patients.

But as mentioned above, the long-term results from the NPC trial with those participants from the highest tertile of baseline plasma selenium level that appeared to carry an increased diabetes risk indicate that an upper limit of daily Se intake should not be exceeded [12]. Yet, most patients in hospital or under chronic ambulant therapy are unlikely to reside among the best supplied individuals and this risk can almost be completely excluded for people living in marginally supplied areas, *i. e.* large parts of Europe, Asia or Africa.

Taken together, it is obvious that disease impacts strongly on the Se metabolism in both men and women and their resulting Se status. Especially those patients with an activated immune response are in danger of developing a Se deficiency and might profit over-proportionally from Se supplementation. Still, cautious use of Se-containing supplements is indicated, especially if already a high Se status has been reached. The potential interaction of Se supple-

mentation with the regular medications has often not been characterised, as mentioned above for AIDS and antiviral therapy. Moreover, potential interference with other widespread used pharmaceuticals like statins [31] or aminoglycoside antibiotics [32] have been described *in vitro* but are at present of unknown importance for Se metabolism in humans.

3 Thyroid gland functioning and thyroid hormone biosynthesis

Adequate function of the thyroid hormone axis critically depends on the essential trace elements iodine, Se and iron. The thyroid gland contains the highest iodine and Se concentration among the human tissues [33]. Biosynthesis of thyroid hormones, which are key regulators of brain development, body growth and intermediary metabolism, utilises iodine as building block for iodinated tyrosine residues of thyroglobulin (Tg). Iodide uptake is mediated against a concentration gradient via the sodium–iodide symporter (NIS), which is located in the basolateral membrane of thyrocytes (Fig. 2). NIS expression and activity are under thyroid stimulating hormone (TSH, thyrotropin) control and the energy required for this import is provided by the activity of the Na-K-ATPase [34]. The organification of iodide is catalysed by the hemoprotein thyroperoxidase (TPO), which uses H₂O₂ as cosubstrate [35]. TPO catalyses both the iodination of tyrosyl residues of Tg and the H₂O₂-dependent coupling of iodinated tyrosyl residues to generate the iodothyronines, which exhibit the typical diphenylether ring structure of thyroid hormones. Thyrooxidase 1 and 2 (Thox) also named dual oxidase 1 and 2 (Duox), the enzymes generating the cytotoxic H₂O₂ molecules, have recently been cloned and functionally characterised [35]. Iodinated Tg which contains monoiodotyrosine (MIT), diiodotyrosine (DIT), triiodo-L-thyronine (T3) and tetraiodo-L-thyronine (T4, L-thyroxine) residues as constituents of its polypeptide backbone, becomes generated, deposited and stored in the colloid lumen of the thyroid follicles, until it is used for thyroid hormone liberation. Iodinated Tg is reabsorbed by the apical plasma membrane of thyrocytes by micropinocytosis. Intracellular fusion of these vesicles with lysosomal vesicles activates proteases from the cathepsin family of hydrolytic enzymes [36] in secondary lysosomes, which completely degrade iodinated Tg to generate MIT, DIT, T3 and T4. MIT and DIT are locally dehalogenated by the flavoprotein dehalogenase (DEHAL) [37], which generates iodide for reutilisation by TPO. In contrast, T3 and T4 are liberated into the vascular bed of the circulation, where they bind with high affinity to three plasma distributor proteins for thyroid hormone transport, *i.e.* thyroxine binding globulin (TBG), transthyretin (TTR) and albumin [38]. These proteins prevent the hydrophobic thyroid hormones T4 and T3 from intrusion into

lipid membranes, keep a very low free hormone concentration, distribute the hormones to their target tissues, prevent rapid renal elimination and maintain a significant serum pool of thyroid hormones. The high fraction of T4 that is bound to proteins in serum results in a biological half-life of 1 wk, the longest among all hormones known. The hydrophobic but charged thyroid hormones reach their intracellular targets, *i.e.* T3-specific receptors (TR) that belong to the superfamily of intracellular DNA-binding ligand modulated nuclear receptors, by energy-dependent membrane transporters (*e.g.* MCT8, OATP14), which are selective for the specific thyroid hormones [39]. Degradation and inactivation of thyroid hormones occurs via deiodination, liberating again iodide for reutilisation, conjugation at the 4'-phenolate position to yield sulphates or glucuronides, or to a minor extent by metabolism of their alanine side chain or oxidative cleavage of the diphenylether bridge [33].

The hormonal control of thyroid hormone biosynthesis, their storage and release is mainly exerted by TSH, a glycosylated proteohormone produced and secreted by the thyrotrope cells of the anterior pituitary and acting *via* the TSH receptor located in the basolateral membranes of thyrocytes. Hypothalamic thyroliberin (TRH, TSH-releasing hormone) regulates TSH production and secretion, and both are under negative feedback control of systemic and local thyroid hormones T4 and T3. The exact physiological role of thyrostimulin, another pituitary glycosylated proteohormone produced by the corticotrope cells and also addressing the TSH receptor of thyrocytes, remains to be established [40].

The G-protein coupled TSH receptor signals *via* the adenylate cyclase – cAMP – protein kinase A cascade and *via* Gq/G11 coupled stimulation of phospholipase C. While most of the thyrocyte-specific processes such as iodide uptake and expression of genes relevant for thyrocyte-specific functions are under control of the cAMP pathway, albeit with several species differences, recent data generated in transgenic and knockout mouse models suggest that the reactions relevant for iodine organification and thyroid hormone secretion are depending on the Gq/G11-signalling cascade [41]. This indicates that TSH-dependent proliferation and adaptive growth in response to goitrogen exposure are dependent on phosphoinositol, diacylglycerol and Ca²⁺ signalling. This recent information also suggests that at least some of the Se-dependent reactions of thyrocytes might be controlled by the Gq/G11-pathway.

4 The role of Se in thyroid hormone metabolism

Iodide accumulation and thyroid hormone biosynthesis commence at the end of the first trimester in the foetal thyroid gland and continue throughout the whole life. This

implies life-long TSH-regulated generation of H_2O_2 inside of the thyroid follicular lumen. As H_2O_2 represents a highly reactive cytotoxic metabolite, its generation would be difficult to control and to tailor to the particular demand of thyroid hormone biosynthesis if it was generated within the cytosol of thyrocytes. Therefore, the unique follicular organisation of the monolayer of epithelial thyrocytes that forms the extracellular colloid luminal space as a well-confined biological containment, and the dense microvascular supply with iodide, nutrients and blood on the basolateral side provide a logistic masterpiece of evolution. This unique compartmentation enables the use of a rather strong chemical, *i. e.* H_2O_2 , for iodination and coupling, which can now proceed in a radical-mediated reaction mode [33]. In contrast to some outdated textbook statements, the production of H_2O_2 by Duox, its utilisation by TPO for iodination of tyrosyl residues and the coupling of iodinated tyrosyl side chains to iodothyronines in the Tg protein occur extracellularly at the surface of the apical plasma membrane facing the colloid space. In this 'thyroxisome' organisation [35], the extracellular active site of Duox delivers H_2O_2 to the extracellular active site of TPO, which uses both extracellular H_2O_2 and extracellular Tg as its two substrates. Reaction control is exerted by recently identified intracellular Duox activator proteins [42]. Still, additional precautions are required to protect thyrocytes from any excess of extracellularly-generated H_2O_2 . This task cannot be fulfilled by the intracellular catalase alone, which displays a K_m value for H_2O_2 that is too high to be efficient in its degradation. Here, members of the family of Se-dependent GPx display the appropriate K_m values, and particular isozymes react efficiently with H_2O_2 to generate H_2O , *e. g.* the secreted isozyme GPx-3. High expression of GPx-3 transcripts in the thyroid gland, production and secretion of functional GPx enzyme by thyrocytes and thyroid cell lines and noncovalent attachment of GPx-3 to Tg globules in the colloid has recently been demonstrated [43]. Previous studies indicated that production and secretion of GPx-3 by thyrocytes is under negative control of Ca^{2+} signalling pathways [44]. This regulation is compatible with the above mentioned Gq/G11-mediated control of iodination and thyroid hormone secretion pathways. Otherwise, a concomitant parallel generation and degradation of H_2O_2 would not allow for optimal control of efficient thyroid hormone biosynthesis, and the maintenance of the integrity of thyrocytes and their follicular organisation would be at risk.

The high expression of the selenoprotein GPx-3 in thyrocytes and its secretion into the closed and protected colloid lumen (Fig. 2) might be one explanation for the high Se content of the thyroid gland observed in humans and many other species. Apart from GPx-3, several other members of the selenoprotein family are expressed in thyrocytes, *e. g.* TxnRd1 and TxnRd2, additional GPx family members (GPx-1, GPx-4), both 5'-deiodinase isozymes (Dio1, Dio2), Sep15, SePP, selenoproteins M and S [43].

To what extent these selenoproteins contribute to the Se content of the thyroid gland and whether they are also involved in antioxidant defence and redox control of thyrocytes remains to be studied. However, even if secreted GPx-3 was capable of degrading excess H_2O_2 efficiently, additional precautions are required to protect both the cell membrane and the intracellular compartments from any H_2O_2 diffusing into the thyrocytes. These functions might be elicited by both the GPx-1 and GPx-4 isozymes, the Se-dependent TxnRd enzymes and peroxiredoxins in addition to intracellular catalase.

Currently no detailed information is available on the modes of entry of Se compounds into thyrocytes (see below) but all available data hint towards an unparalleled priority of the thyroid gland over the other tissues with respect to Se supply and Se retention. An isolated Se deficiency does not necessarily lead to any obvious destruction of the highly active gland structure or to an increased death of H_2O_2 -producing thyrocytes. In contrast, even elevated thyroid deiodinase expression levels and activities have been reported in Se deficient animal models [45, 46]. Further dedicated transgenic animal models will be needed to elucidate the function of a thyroid gland that has been deprived of specific selenoproteins, and its consequences for thyroid hormone biosynthesis and secretion.

5 Privileged supply of the thyroid gland by Se

Among all the tissues, the thyroid gland contains the highest Se concentration in the human body [33]. This feature is largely independent from the Se status of the organism, *i. e.* even in experimental animal studies where Se supply was strongly reduced by feeding diets that were almost completely deprived of every Se source, the brain and the endocrine glands, especially the thyroid, retained almost normal concentrations of the essential trace elements. Se concentrations and GPx activities in plasma, liver or kidneys become almost undetectable under these conditions [45]. Several recent transgenic mouse models have sharpened our ideas on the molecular metabolism of dietary-derived Se and its transport routes within the organism [47].

Since its first identification, the Se-rich plasma protein SePP has been assumed to be involved in Se transport and Se storage. Indeed, upon cloning of the SePP-encoding mRNA, 10 separate Sec-insertion codons were identified within the ORF, and they are largely conserved between rodents and humans [48]. Se isotopes injected into mammals appear fast in SePP-specific serum fractions and point towards the liver as the likely origin of circulating SePP molecules. This model for SePP biosynthesis and function was verified when transgenic SePP deficient mice were generated and analysed. On regular chow, SePP-KO mice displayed strongly reduced Se concentrations in plasma and almost all the other organs except for liver and thyroid.

Liver obviously still retained its Se uptake mechanisms, but instead of channelling the available Se into SePP biosynthesis and secretion, SePP-KO mice accumulated surplus hepatic Se amounts mainly in the form of GPx-1, the most abundant hepatic selenoprotein. Accordingly, other Se- and SePP-dependent compartments including serum, kidney, brain or testis displayed reduced Se contents [49, 50]. These results demonstrated the importance of Se and SePP for brain function and development for the first time, since purely dietary restrictions had never been successful before to reduce brain Se concentrations [47].

In this model, the impaired Se organification and transport *via* SePP led to Se-dependent neurological deficits including ataxia and seizures. Interestingly, these phenotypes were successfully rescued by increasing the diet with either sodium-selenite or SeMet. Thus, a regular SePP metabolism was important to guarantee privileged brain Se status. It remained to be determined whether hepatically-derived SePP or locally produced brain SePP was responsible for preferential Se supply into the CNS. Inactivation of all hepatic selenoproteins including hepatic SePP revealed that under these conditions of again strongly-reduced plasma Se concentrations, brain Se content was surprisingly only slightly affected [51]. The results indicated that hepatically produced and secreted SePP was not necessary for Se status in brain, in contrast to *e.g.* supply of plasma or the kidney. Locally expressed SePP mRNA and the Se-dependent translation and biosynthesis of mature SePP within the brain appeared indispensable for regular brain Se concentrations [52]. The uptake and turnover in brain may be related to the presence of specific SePP-receptors, as recently identified and described in testis [53]. Interestingly, both under Se-restricted nutrition and upon genetic SePP inactivation, the thyroid gland contained largely unaffected high concentrations of the essential trace element. The direct comparison to other organs places the thyroid gland even on the very top of the hierarchical Se supply among the organs, definitely on top of brain since thyroid Se levels remained unaffected even in the complete absence of SePP biosynthesis [46]. Whether this priority is affected in illness, by medical manipulation of thyroid hormone biosynthesis, or subject to changes with age has not been worked out, yet.

Still, epidemiological analyses have correlated Se status to thyroid volume, *i.e.* goitrous enlargement [54]. As with some phenotypes in the mouse models, the inverse association of thyroid gland size and Se blood concentration in humans displayed sex-specific differences and applied only in females. This finding points again to sexually dimorphic Se and selenoprotein metabolism in humans, which might involve differences in the tightly regulated efficiency of selenoprotein biosynthesis on the posttranscriptional level [55].

6 Inadequate Se supply results in a vulnerable thyroid gland

First evidence for a vital role of Se in thyroid gland function came from observations in animal experiments and epidemiologic studies in some parts of Zaire, where endemic myxedematous cretinism has been observed [56]. This form of cretinism is characterised by mental and developmental retardation (dwarfism), severe hypothyroidism, myxedema but no goitre. Experimental animal, interventional and clinical studies suggest, that combined iodine and Se deficiencies in conjunction with nutritional exposure to goitrogens precipitate this disease, in which an inadequate iodine supply and lack of thyroid hormone biosynthesis lead to enhanced TSH stimulation of the gland *via* an impaired negative hypothalamic-pituitary feedback control [57]. Enhanced TSH receptor activation stimulates among other targets Duox activity and H₂O₂ generation. Due to inadequate iodide availability, H₂O₂ will accumulate and start to damage thyrocytes and follicular integrity and result in enhanced necrosis of epithelial tissue, which is replaced by fibrotic structures. The concomitant Se deficiency might impair the regular degradation of excess H₂O₂ *via* reduced GPx activity resulting in inadequate protection of thyrocytes and follicular structure from radical damage. Additional goitrogen exposure exaggerates this vicious circle by inhibiting NIS-mediated iodide uptake and TPO activity, both well known targets for several goitrogens. Whether inadequate Fe supply additionally impairs synthesis and function of the central hemoprotein TPO has not been studied in detail, yet [58].

Three observations are of particular note in this context. Firstly, mild Se deficiency results in a partial protection of the thyroid hormone axis and thyroid hormone metabolic pathways, because decreased activity of the Se-dependent deiodinase isozymes in Se deficient peripheral tissues reduces turnover of thyroid hormones, prolongs their biological half-lives and enhances enterohepatic recycling of iodothyronines and their conjugates [59]. This leads to an iodine-sparing effect, which relieves the pressure on the thyroid gland to supply more hormones. Evidence for this reasoning came from a supplementation trial in an iodine- and Se deficient and probably also goitrogen-exposed myxedematous cretin in Zaire. In contrast to initial expectations, Se supplementation resulted in disruption of his thyroid hormone status and led to myxedematous coma. Apparently, the chronically damaged thyroid gland of this patient was unable to efficiently utilise the offered iodide anymore that was liberated by the restored activity of the Se-dependent deiodinase enzymes [56]. From this study it was concluded that an adequate iodine status has to be restored first before Se supplementation can be initiated, at least under conditions of combined severe nutritional deficiencies of both trace elements.

Interestingly, myxedematous cretinism has also been observed in other regions than central Zaire such as in some parts of India or Tibet [33]. Though also in these areas, a combination of severe iodine and Se deficiency has been described as an underlying reason, there are no indications that the phenotype is associated with a specific goitrogen. Instead, other precipitating factors such as toxins in grain (aflatoxin) or drinking water (fulvic acid) appear more likely to be involved in disease development. In addition, this type of cretinism seems to be associated with Kashin-Beck disease ('big joint disease'), a severe, irreversible chondro-osteopathy, which apparently can be prevented by adequate Se (and iodine?) supply [60]. In model systems, fulvic acid exposure leads to excessive superoxide formation [61], which might explain a protective effect of adequate Se supply and GPx activity for the degradation of H₂O₂.

In otherwise healthy and well-supplied humans, an isolated mild Se deficiency does not alter measurable thyroid hormone concentrations, and no evidence for an impaired or altered activity level of deiodinase isozymes has been found in regions with moderate or low Se intake [62]. Similar results have been observed in critical care patients who display strongly reduced serum Se levels but failed to respond with altered serum thyroid hormone concentrations upon Se supplementation [63]. These results indicate that the deiodinase isozymes are sufficiently expressed to maintain regular steady-state thyroid hormone concentrations under normal or even moderately Se deficient conditions. Only severe or chronically-reduced Se availability, potentially in combination with unfavourable genetic predisposition, will lead to impaired Dio expression and insufficient function in humans. Such conditions are difficult to be mimicked in cell culture or animal models, but their consequences leading to reduced thyroid hormone metabolism might be of fundamental importance to explain certain pathophysiological settings, *e.g.* the increased frequency of goitres in the EU or the still enigmatic low-T3 syndrome in severe illness [64].

The issue of excessive iodine intake (*e.g.* by nutritional overload, the application of iodinated X-ray contrast agents or the antiarrhythmic drug amiodarone) under conditions of inadequate Se supply is probably even more problematic. Chronic high nutritional intake of iodine in humans (>500 µg/day) leads to a substantial increase in thyroid size and an impaired thyroid hormone biosynthesis. Such situations are not uncommon in *e.g.* northern Japan, where local diet frequently contains more than 1000 µg iodine/day. Several observational and epidemiological studies indicate that successful table salt iodination programmes in regions of iodine deficiency or inadequate iodine supply (which still applies for 2/3 of our populated world!) lead to a significant but transient increase in autoimmune thyroid diseases (AITDs) in the respective population [65]. This effect is accompanied by an altered pattern of thyroid cancer forms

in the long run. Here, a shift from the more problematic follicular to the less aggressive and treatable papillary forms is reported [66]. Successful table salt iodination programmes also reduce the incidence and prevalence of nodular alterations of the thyroid gland and reduce goitre growth, thereby improving the health status in both short and long term perspective. Whether Se-iodine, Fe-iodine, or even Se-Fe-iodine interactions and imbalances contribute to the transient increase in AITD accompanying such successful iodination programmes is still unclear and needs to be taken into account in future programme planning for micronutrient supplementation.

From these considerations, we would like to support the notion that an increased iodine supply to a thyroid gland which has been adapted to mild, moderate or even severe iodine deficiency requires concomitant improvement of its Se (and Fe?) intake to protect the organ from its increased iodising activity. Enhanced Se supply is needed to equip the thyrocytes and colloidal lumen with adequate amounts of functionally active GPx, TxnRd, Dio, Sep15 and other redox-active selenoproteins in order to optimally protect this vulnerable structure from any excessive iodide-derived oxidation products or H₂O₂-derived reactive oxygen species. Support for this hypothesis derives from a number of *in vitro*, experimental animal and human studies. In cells in culture, inadequate Se supply leads to decreased GPx activity and a parallel aberrant intracellular iodination of proteins [67]. Exposure to iodide excess results in severe necrosis and destruction of integral follicular organisation in Se deficient and combined Se- and iodine-deficient animals [57], whether or not concomitant exposure to goitrogens further aggravates this condition. These observations have been interpreted as being due to excessive H₂O₂ and ROS production by thyroid cells which result in, *e.g.* liberation of TGFβ, a strong inductor of fibrosis. Pretreatment with TGFβ-immunoneutralising sera was able to prevent this deleterious condition, and adequate Se supply proved to be beneficial in preventing necrosis and fibrosis, possibly by supporting apoptotic repair events [57]. Whether only GPx or also TxnRd and other selenoproteins and selenoenzymes contribute to thyroid protection from excess iodide, H₂O₂ and oxidative reactive products remains to be studied. So far, no distinctions between different Se species or nutritional forms have been made in their potential to exert these protective actions and a molecular link between Se protective effects and mechanism of fibrotic destruction of thyroid tissue has not been provided, yet.

7 Se deficiency and autoimmune thyroid disease

During the last years, several studies (Table 1) have reported on beneficial or preventive effects of Se supplementation in AITD. AITD is not uncommon in females dur-

Table 1. Clinical studies on adjuvant Se-based treatment of AITD

Treatment group	Study type	Form of Se	Major outcome	Reference
Autoimmune thyroiditis, Hashimoto's thyroiditis	Observational treatment	Selenite	Decreased TPOab titres, improved patient status	[70]
Autoimmune thyroiditis, Hashimoto's thyroiditis; females	Prospective, placebo controlled, double blind, crossover	200 µg Selenite	Decreased TPOab titres, improved patient well being, normalised echogenicity in thyroid ultrasound	[71]
Autoimmune thyroiditis, Hashimoto's thyroiditis	Prospective, placebo controlled, double blind	200 µg SeMet	Decreased TPOab titres, improved patient well being	[72]
Autoimmune thyroiditis, Hashimoto's thyroiditis; females	Prospective, placebo controlled, double blind	100/200 µg SeMet	Decreased TPOab titres	[73]
TPOab positive pregnant women	Prospective, placebo controlled, double blind	200 µg SeMet	Decreased incidence of both postpartum thyroiditis and permanent hypothyroidism	[74]

ing their childbearing years. Pathophysiology of various forms of AITD is still not understood and so far no causative treatment is available for any of the major disease forms or its variants. Graves' disease (m. Basedow) is caused by autoantibodies stimulating the TSH receptor on thyrocytes and retroorbital fibroblasts and leads to severe hyperthyroidism and in most cases endocrine orbitopathy. Antithyroid medication is clinical routine in AITD, and sometimes surgical or radioiodine intervention is necessary and may be accompanied by anti-inflammatory treatments or agents blocking the β -adrenergic receptors. Determination of stimulating TSH receptor autoantibodies (TRAK) is the main diagnostic procedure. M. Hashimoto, a destructive autoimmune disease, leads to hypothyroidism, which requires life-long substitution of L-thyroxine, one of the most often prescribed drugs worldwide. Pathogenesis of m. Hashimoto is still unclear, but TPO and Tg autoantibodies are found and determined in patients. While only limited data have been reported for adjuvant treatment of Graves' disease with Se compounds in combination with 'other antioxidants' [68], remission and outcome has been linked to adequately high serum Se levels ($>120 \mu\text{g/L}$) and low TRAK values [69]. In contrast to Graves' disease, much more information is available on positive effects of Se supplementation in the treatment of m. Hashimoto.

Several observational and three prospective, double-blind controlled studies [70–73] reported on beneficial effects of Se supplementation in patients with m. Hashimoto and autoimmune thyroiditis. The effects after 3–12 month of supplementation were remarkably independent on the Se compound used, *i.e.* selenite, SeMet or Se-enriched yeast. Titres for TPO autoantibodies (TPOab) decreased (not those for Tg), the ultrasound pattern of the thyroid gland improved and in one study a standardised questionnaire on quality of life indicated improved contentedness of Se-treated patients [71]. Since all these Se-containing supplements funnel ultimately into the common final path of selenoprotein biosynthesis, it is conceivable that the beneficial effects are mediated by Se-responsive

selenoenzymes. The nature of these selenoproteins involved in autoimmune disease and the site of effect, *i.e.* within the thyroid gland or *via* the immune system, remain to be established. Unfortunately, no clear evidence has yet been presented for a supplementation-induced conversion of m. Hashimoto or autoimmune thyroiditis to normal euthyroidism, which would not require further concomitant L-thyroxine replacement medication. Thus, Se supplementation can currently only be regarded as an adjuvant option to complement L-thyroxine replacement at best.

A very recent prospective controlled blinded study provided surprising results on positive Se-dependent treatment of postpartum thyroiditis and hypothyroidism, again a very frequent serious complication during pregnancy associated with AITD [74]. TPOab and thyroid inflammatory activity were decreased and the incidence of hypothyroidism in the postpartum period was reduced by the Se supplementation. If this result can be verified and confirmed by further independent studies, a systematic screening for TPOab and the adjuvant treatment to increase the Se status of the women will provide a major progress in preventing and treating the deleterious effects of maternal thyroid disease for both the mother and the developing child.

8 Outlook and perspectives

Currently available data from epidemiological analyses and clinical and experimental animal studies support a health claim for Se supplementation to prevent or to treat a variety of widespread diseases either as isolated supplementation or in an adjuvant mode. Conceivably, there is some risk of side-effects or even intoxication, which rapidly will be detected, if too high amounts are taken for too long, as there is with every other biologically active substance. Yet, from our current knowledge, only very few individuals who already display very high baseline levels may develop side-effects on regular high-dose Se intake. This is very unlikely for most humans, especially when they reside in less well-

supplied areas like everywhere in Europe or in most parts of Asia or Africa. The positive supplementation effects described and verified to date clearly highlight the enormous potential of actively controlling and increasing a person's Se status. Beneficial effects have been observed on the cancer risk of several organ systems, on cardiovascular parameters, on the immune system, the endocrine axes with a special emphasis on thyroid gland function and thyroid hormone metabolism. Epidemiological analyses have indicated that a large fraction of goitres can be prevented in endemic areas by improving the Se status of the inhabitants – provided that adequate iodide supply is established before Se intervention, and recent Se supplementation studies proved effective to treat two forms of AITD. Clearly, more prospective and monitoring studies with larger cohorts of patients and longer durations are needed to confirm the surprisingly efficient health effects. Nevertheless, it appears as if both the molecular research and the clinical trials have finally come of age to be taken serious and to receive the attention and sometimes even the funding that is needed to verify the findings and broaden the promising application fields of this potent trace element.

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