

The interactions between selenium and iodine deficiencies in man and animals

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Abstract

Up to one billion people live in areas where they may be at risk from I deficiency. Many of the debilitating effects of the deficiency may be irreversible, consequently it is essential to understand the mechanisms whereby lack of I can cause disease through decreased thyroxine and 3,3',5-triiodothyronine (T₃) synthesis. Since Se has an essential role in thyroid hormone metabolism, it has the potential to play a major part in the outcome of I deficiency. These effects of Se derive from two aspects of its biological function. First, three Se-containing deiodinases regulate the synthesis and degradation of the biologically active thyroid hormone, T₃. Second, selenoperoxidases and possibly thioredoxin reductase (EC 1.6.4.5) protect the thyroid gland from H₂O₂ produced during the synthesis of thyroid hormones. The mechanisms whereby Se deficiency exacerbates the hypothyroidism due to I deficiency have been elucidated in animals. In contrast to these adverse effects, concurrent Se deficiency may also cause changes in deiodinase activities which can protect the brain from low T₃ concentrations in I deficiency. Animals with Se and I deficiency have changes in serum thyroid hormone concentrations that are similar to those observed in patients with I deficiency disease. However such animal models show no thyroid involution, a feature which is characteristic of myxoedematous cretinism in man. These observations imply that if Se deficiency is involved in the outcome of I deficiency in human populations it is likely that other interacting factors such as goitrogens are also implicated. Nevertheless the protection of the thyroid gland from H₂O₂ and the regulation of tissue T₃ levels are the functions of Se that are most likely to underlie the interactions of Se and I.

Iodine: Selenium: Nutrient deficiency: Deiodinase: Cretinism

Abbreviations: BAT, brown adipose tissue; cGSHPx, eGSHPx, phGSHPx, cytosolic, extracellular and phospholipid hydroperoxide glutathione peroxidase respectively; ID-I, ID-II, ID-III, iodothyronine deiodinase types I, II and III respectively; T₃, triiodothyronine; T₄, thyroxine; TR, thioredoxin reductase; TSH, thyrotropin.

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Introduction

Consequences of iodine and selenium deficiency in man

I deficiency in man is a major health problem. Over one billion people live in areas which have I-deficient soil, approximately 200 million people have goitre, whilst about 20 million have brain damage. I is essential for thyroid hormone synthesis, indeed this is its only known role. The clinical manifestations of I deficiency are thus considered to arise from impaired thyroid hormone production. The dietary allowance for I is approximately 150–200 $\mu\text{g}/\text{d}$. Deficiency of the trace element gives rise to a number of clinical manifestations, which vary with age. The fetus is most affected by I deficiency with abortion, still birth and endemic cretinism being commonly found in areas of severe I deficiency. Goitre, hypothyroidism and impaired mental and physical development are found in the infant, child and adolescent. In the adult, goitre, mental retardation, decreased fertility and hypothyroidism may occur. Goitre may also arise from ingestion of goitrogens such as cassava, an important food grown in tropical areas. Consumption of cassava can give rise to increased levels of thiocyanate in the thyroid that impairs gland function. In areas where cassava is eaten the balance of the intake of I and thiocyanate appears to be critical in determining if goitre will develop (Delange & Ermans, 1996). Thus, the manifestations of I deficiency range from mild hypothyroidism to the severest forms of cretinism. These latter diseases involve irreversible neurological damage and impaired development, highlighting the importance of recognizing and preventing endemic I deficiency.

Se deficiency in human populations is not as widespread and probably less well recognized than I deficiency. However, in China inadequate intake of Se has been associated with Keshan disease, a cardiomyopathy which occurs mainly in growing children and pregnant females (Ge & Yang, 1993). A similar low Se status in Zaire in Africa does not cause cardiomyopathy, implying that additional factors are also involved in the pathogenesis of Keshan disease (Vanderpas *et al.* 1990). In particular, viral infection may be 'a trigger factor' which precipitates cardiomyopathy in Se-deficient Chinese subjects (Beck *et al.* 1995; Levander & Beck, 1996, 1997). What is clear is that Se supplementation has almost entirely eliminated Keshan disease (Ge & Yang, 1993). Nevertheless, there are concerns that marginal Se deficiency still causes cardiac problems in the Chinese population (Wu *et al.* 1997; Gu *et al.* 1998).

Interest in interactions between Se and I deficiency first arose from the discovery that Se is essential for normal thyroid hormone metabolism. This connection was first recognized as an elevated plasma thyroxine (T_4) concentration and a decreased plasma 3,3',5 triiodothyronine (T_3) concentration in Se-deficient animals (Arthur *et al.* 1987; Beckett *et al.* 1987). These changes were shown not to be secondary consequences of the decreased food intake that sometimes occurs with prolonged Se deficiency. The acute and specific effects of Se deficiency on thyroid hormone metabolism were therefore hypothesized to be inhibition of hepatic iodothyronine deiodinase (*EC* 3.8.1.4) (ID-I) which converts T_4 to T_3 by 5' monodeiodination. Subsequently, ID-I was shown to be a Se-containing protein (Arthur *et al.* 1990a; Behne *et al.* 1990). Cloning of ID-I showed that it contained a selenocysteine at the active site coded for by a stop codon (TGA) (Berry *et al.* 1991a,b). Conversion of the active site selenocysteine to cysteine greatly decreased the activity of the cloned enzyme demonstrating how effective a catalyst the ionized selenocysteine group is at physiological pH (Berry *et al.* 1991c). Se deficiency in animals causes rapid and specific decreases in hepatic and renal ID-I activities (Beckett *et al.* 1987; Arthur *et al.* 1990a), emphasizing the essentiality of Se for maintaining thyroid hormone metabolism. The effects of Se deficiency on thyroid hormone metabolism are complex and are discussed in more detail later in this review.

As well as ID-I, there are type II and type III deiodinases (ID-II, ID-III) also essential for interconversion of the biologically active and inactive forms of thyroid hormone (Arthur & Beckett, 1994; Beckett & Arthur, 1994; Kohrle, 1994; St Germain & Galton, 1997; Richard *et al.* 1998). ID-II which occurs in brain and central nervous system tissue of animals and also in brown adipose tissue of some species converts T_4 to T_3 within these tissues. This enzyme provides an important regulatory function in such tissues since circulating T_3 does not readily gain access to the intracellular nuclear receptors and thus exert biological activity (Safran *et al.* 1991). The development and function of the central nervous system is particularly sensitive to thyroid hormone supply and hence to the expression of deiodinase enzymes. Some tissues express ID-III, which converts T_4 to the metabolically inactive reverse T_3 , thus bypassing the production of biologically active T_3 . ID-III also converts T_3 to diiodothyronine, again helping to regulate precise tissue levels of T_3 (Karmarkar *et al.* 1993; KoopdonkKool *et al.* 1996). During development clear changes occur in the ontogeny of the deiodinases suggesting that these enzymes may have important roles regulating thyroid hormone supply and thus the timing of switching on and off thyroid-responsive genes (Richard *et al.* 1998). Initial studies on the effect of Se deficiency on ID-II and ID-III provided equivocal results that could be interpreted as the enzymes being Se-containing or Se-independent (Chanoine *et al.* 1992; Meinhold *et al.* 1993). However cloning of ID-II and ID-III showed that they both contained 'in frame' TGA codons which specify selenocysteine at the active site of the enzyme (Croteau *et al.* 1995, 1996; Salvatore *et al.* 1995; Larsen, 1996). The maintenance of ID-II and ID-III activities, even in severe Se deficiency is indicative of the importance of these enzymes for normal thyroid hormone metabolism (Beckett *et al.* 1989; Meinhold *et al.* 1993; Mitchell *et al.* 1997).

Since the only known biological role for I in mammals is as a component of the thyroid hormones, the major basis of Se and I interactions is the synthesis and subsequent utilization of these thyroid hormones. Se however, has many biological functions and the thyroid hormone deiodinases are just three of at least thirty mammalian selenoproteins. These proteins have been identified either by purification and cloning or by *in vivo* labelling with ^{75}Se (Behne *et al.* 1988, 1996; Evenson & Sunde, 1988; Wu *et al.* 1995). The additional Se-containing proteins include four glutathione peroxidases, which provide intracellular and extracellular protection against the potentially injurious effects of H_2O_2 and lipid hydroperoxides (Sunde, 1990; Arthur *et al.* 1996). Additionally, the Se-containing flavoenzyme, thioredoxin reductase (EC 1.6.4.5; TR), is essential for redox regulation of certain proteins and it may also contribute to cell antioxidant systems (Gladyshev *et al.* 1996; Howie *et al.* 1998). Further extracellular antioxidant capacity may be provided by selenoprotein P. This protein contains ten selenocysteine residues, twenty-three histidine residues and seventeen cysteine residues, thus indicating a large capacity to bind transition metals, which would normally initiate free radical activity (Hill *et al.* 1991; Burk & Hill, 1992; Burk *et al.* 1995). The relevance of the latter selenoproteins to I metabolism becomes evident on consideration of the involvement of H_2O_2 in the process of thyroid hormone synthesis. Other selenoproteins such as selenoprotein W are not discussed in the present paper but have been reviewed elsewhere (Sunde, 1990; Arthur *et al.* 1996).

Thyroid hormone synthesis

Fig. 1 shows the pathway for thyroid hormone production. The functional unit for thyroid hormone synthesis is the follicle, a structure made up of clusters of thyrocytes. The lumen of the follicle is filled with colloid, which is mainly composed of thyroglobulin. This is a high molecular mass protein synthesized by thyrocytes, which is then exported and stored in the follicular lumen. Synthesis of thyroid hormones requires iodination of tyrosyl residues on

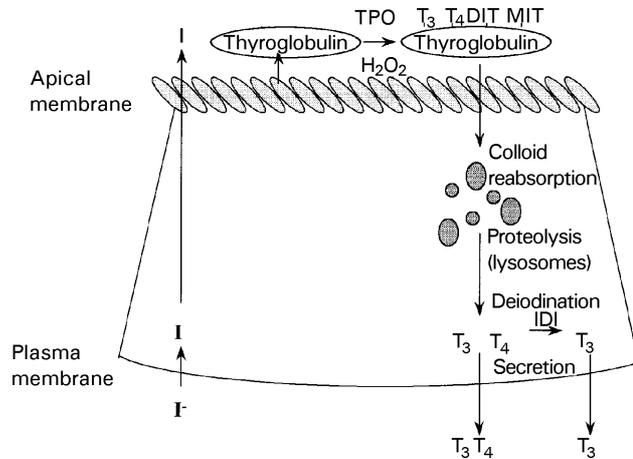


Fig. 1. The mechanism of synthesis of thyroid hormones. Iodine is transported through the thyroid cell to the follicular lumen, where in the presence of hydrogen peroxide and thyroid peroxidase (TPO) it is incorporated into tyrosyl residues on thyroglobulin. The colloid is then reabsorbed into the thyrocyte from where thyroid hormones are released into the circulation after proteolytic release from thyroglobulin. DIT, diiodotyrosine; IDI, iodothyronine deiodinase; MIT, moniodotyrosine; T₃, triiodothyronine; T₄, thyroxine.

thyroglobulin followed by coupling of these iodinated derivatives. These reactions take place within the follicular lumen at the surface of the apical membrane. The steps required for thyroid hormone synthesis can be summarized as follows and are reviewed in detail by Taurog (1996).

Active transport of iodide

I is usually presented to the thyroid as the inorganic form iodide. Iodide is transported into the thyrocyte and thus 'trapped' by a Na⁺/iodide symporter with the ion gradient being generated by a Na⁺, K⁺-ATPase (*EC* 3.6.1.37) (Dai *et al.* 1996). Thyrotrophin (TSH) stimulates iodide uptake probably acting through adenylate cyclase (*EC* 4.6.1.1). Following uptake, iodide is transported across the thyrocyte into the follicular lumen.

Iodination of tyrosyl residues on thyroglobulin

This reaction takes place in the follicular lumen and leads to the formation of mono-iodo and di-iodo tyrosine residues on thyroglobulin. The reaction requires the generation of H₂O₂ in high concentrations and also the action of thyroid peroxidase (*EC* 1.11.1.8), an enzyme located on the luminal side of the apical membrane. The generation of H₂O₂ appears to be the rate-limiting step in thyroid hormone synthesis and is regulated by a complex network of interacting second messenger systems. In many species, including man, the production of H₂O₂ is regulated through the Ca-phosphoinositol signalling cascade but in some animals the cAMP pathway may also be involved (Dumont, 1971; Bjorkman & Ekholm, 1992; Corvilain *et al.* 1994). It has been suggested that activation of protein kinase C, elevated free Ca²⁺ concentrations and the phospholipase C/Ca²⁺ cascades may act in concert to promote H₂O₂ production. *In vitro*, the

addition of the Ca ionophore A23187 to thyrocytes or thyroid slices can stimulate H_2O_2 formation. TSH can also promote *in vitro* production of H_2O_2 but only at high non-physiological concentrations. There is disagreement as to the site and mechanism of H_2O_2 generation. It has been suggested that H_2O_2 is generated in the cytoplasm of the thyrocyte through the action of superoxide dismutase (*EC* 1.15.1.1). H_2O_2 can then cross the apical membrane to the follicular lumen where it becomes available for reaction with thyroid peroxidase. An alternative pathway has been proposed whereby H_2O_2 is formed directly on the luminal side of the apical membrane by a flavoprotein-NADPH oxidase (*EC* 1.6.5.3) system. Whilst H_2O_2 is essential for thyroid hormone synthesis it is extremely toxic. Irrespective of the site of generation, the thyrocyte will be exposed to high concentrations of H_2O_2 and toxic lipid hydroperoxides. The selenoenzymes present in the thyrocytes are involved in preventing peroxidative damage and may thus represent a major function for Se in thyroid hormone metabolism.

Coupling of iodinated tyrosyl residues to produce thyroid hormones

The mechanisms by which iodinated tyrosyl residues are coupled to produce T_3 and T_4 are unclear but may involve thyroid peroxidase and H_2O_2 . If two di-iodotyrosyl residues on thyroglobulin are coupled T_4 is formed, whilst coupling of a mono-iodotyrosyl with a di-iodotyrosyl molecule will lead to the formation of T_3 or the biologically inactive isomer reverse T_3 .

Proteolysis and thyroid hormone release

The colloid is absorbed by pinocytosis into the thyrocyte where it undergoes proteolysis in the lysosomes to liberate thyroid hormones. This process appears to be under the influence of TSH. Further metabolism of thyroid hormones can occur by deiodination and the expression of thyroidal ID-I and ID-II selenoenzymes is regulated through the TSH receptor.

Thyroid hormone production: adaptation to iodine deficiency

In chronic I deficiency in animals and man, marked adaptations to the pathways involved in thyroid hormone synthesis take place. The changes act to maintain euthyroid status (Delange & Ermans, 1996) and are triggered by elevated TSH levels and give rise to: increased trapping of iodide; marked increases in each of the steps involved in thyroid hormone production; increase in mono-iodotyrosyl : di-iodotyrosyl ratio on thyroglobulin thus favouring the production of T_3 ; preferential secretion of T_3 ; increased thyroidal mono-deiodination of T_4 to produce T_3 ; altered peripheral conversion of T_4 to T_3 ; development of goitre.

As a result, the characteristic pattern of hormones in plasma in I deficiency is increased TSH and low T_4 . Plasma T_3 may be normal or low depending on the severity of I deficiency. The changes which occur in plasma TSH, T_4 and T_3 in I deficiency in human subjects appear to be age-related, with the highest TSH and lowest T_4 values appearing in young infants. It is thought that goitre may be a side effect of I deficiency rather than a beneficial adaptive response.

Selenium and iodine deficiency in the thyroid

The thyroid gland produces significant quantities of H_2O_2 for thyroid hormone synthesis. However, this peroxide can also be damaging if produced in excess and allowed to react with

the components of the cell. Any impairment of thyroidal peroxide metabolizing systems by Se deficiency therefore will allow damage to occur in the thyrocyte. The importance of the peroxide-metabolizing enzymes is further increased in I deficiency. As I supplies decrease, levels of T_4 also decrease which in turn leads to an increase in TSH production in the pituitary. This then hyperstimulates the thyroid gland and thus H_2O_2 and lipid hydroperoxide production. When Se is available extracellular glutathione peroxidase (EC 1.11.1.9) (*eGSHPx*), phospholipid hydroperoxide glutathione peroxidase (*phGSHPx*), cytosolic glutathione peroxidase (*cGSHPx*), and TR may all protect against potential oxidative damage (see Howie *et al.* 1998). Provided supplies of Se are adequate, I deficiency results in increased production of enzymes, particularly *cGSHPx*, with resultant enhancement of antioxidant activity (Mitchell *et al.* 1996). The induction of *cGSHPx* in preference to *phGSHPx* suggests that it is more likely that H_2O_2 rather than lipid hydroperoxide has the potential to damage the thyroid during overstimulation; *cGSHPx* can metabolize H_2O_2 much more effectively than *phGSHPx*. Additionally, Se and I deficiencies can change peripheral thyroid hormone metabolism and function through modulation of deiodinase activities in different organs. These latter effects have been elucidated in many animal-model studies, the results of which form the basis of much of our understanding of the combined deficiency in human subjects.

Selenium and iodine deficiency in animals

Iodine deficiency

As discussed earlier, in I deficiency the synthesis of T_4 is inhibited yet, initially, plasma T_3 concentrations are maintained due to the action of TSH on the thyroid gland increasing T_3 synthesis and intrathyroidal deiodination of T_4 to give T_3 . These responses are dependent on Se supply for the synthesis of ID-I, as well as glutathione peroxidase enzymes, which help to protect the thyroid tissue from the toxic effects of H_2O_2 and its highly reactive metabolites. In addition, deiodinase expression in the liver, kidney and central nervous system is modified in I deficiency. These changes are discussed here in the context of combined Se deficiency.

Selenium deficiency

Studies using Se-deficient rats indicated that the brain and endocrine organs are able to retain the element at the expense of other organs such as the liver and muscle (Behne *et al.* 1988). In addition, within a particular organ, there is a hierarchy of Se supply to specific selenoproteins (Bermano *et al.* 1995; Larsen & Berry, 1995). In Se deficiency, this hierarchy helps to maintain plasma T_3 concentrations by decreasing the hepatic catabolism as well as increasing the thyroidal synthesis of T_3 by ID-I. Despite an increase in plasma T_4 concentration in Se deficiency, plasma TSH concentration is unchanged due to an impairment of the normal negative feedback mechanism in the pituitary. However, thyroidal stores of T_4 , T_3 and I are decreased in Se deficiency (Arthur *et al.* 1990c).

Combined selenium and iodine deficiency: 'long term' and 'acute' studies

In several feeding trials, over more than one generation, plasma T_4 concentrations were not significantly different between rats with combined Se and I deficiency and those with I deficiency alone (Beckett *et al.* 1993; Meinhold *et al.* 1993; Hotz *et al.* 1997; Wu *et al.* 1997;

Mitchell *et al.* 1998). In addition, Meinhold *et al.* (1993) studied the effects of Se deficiency over three generations and I deficiency for 2 months on ID-I, ID-II and ID-III activities in the rat and found no interaction between the deficiencies. Data on the acute effects of Se and I deficiency on thyroid hormone metabolism were obtained from male weanling rats fed on diets deficient in Se, I or Se and I for 7 weeks (Beckett *et al.* 1993). Amounts of T₄, T₃ and I in thyroid were far more depleted in combined Se and I deficiency than in Se or I deficiency alone. Furthermore, the Se- and I-deficient rats had larger thyroid glands, higher plasma TSH concentrations and higher cerebral ID-II activities than I-deficient rats. Thus, Se deficiency, at least acutely, can exacerbate some of the effects of I deficiency by increasing the hypothypoid stress on the thyroid gland.

Selenium and iodine deficiency: thyroid selenoenzymes and hormones

The hypothypoid stress of combined Se and I deficiency also occurs in female rats and their offspring at different postnatal ages (Mitchell *et al.* 1996, 1998). In 19-d-old pups from I-deficient and (Se- and I)-deficient rats plasma TSH concentrations were increased 25-fold and 50-fold respectively (Mitchell *et al.* 1996). Despite this, there were no differences between these treatment groups in the weight of the thyroid gland or in plasma T₄ and T₃ concentrations in adult or young rats. However, rat pups with combined Se and I deficiency had significantly lower thyroidal ID-I, *c*GSHPx and *ph*GSHPx activities and mRNA levels than pups that were only I-deficient. Compared with control groups thyroidal ID-I activity was increased and *c*GSHPx activity was unchanged in Se- and I-deficient pups at 19 d old. In contrast thyroidal ID-I activity was unchanged and *c*GSHPx activity was decreased by more than 50 % in Se- and I-deficient pups 11 d old (Mitchell *et al.* 1998). The compensatory mechanisms that operate to retain selenoprotein function are therefore less efficient in the thyroids of the 11-d-old rat suggesting that a greater proportion of circulating T₃ may be produced from *de novo* synthesis in neonatal, as compared with mature, rats. In addition, ID-II has recently been identified in human thyrocytes (Salvatore *et al.* 1996), but there is as yet no evidence for this enzyme in animal thyroid glands. As ID-II may be upregulated in I deficiency, the potential for its occurrence in animal thyroid glands and its involvement in responses to I deficiency need to be examined.

Selenium and iodine deficiency and the brain

The effects of Se and I deficiencies on brain development are variable and depend not only on the relative severity of the deficiencies but also on the stage of development at which they are induced. Despite the compensatory mechanisms to spare circulating T₃ concentrations in Se and I deficiencies, the developing fetus requires a supply of maternal T₄ for early brain development (Deescobar *et al.* 1993). More than 80 % of T₃ residing on the nuclear receptors in the brain appears to be derived from intracellular deiodination of T₄ by ID-II. Nuclear T₃ receptors have been detected in fetal rat brain by 13 d gestation (Perez-Castillo *et al.* 1985; Larsen & Berry, 1995). The activities and expression of ID-I, *c*GSHPx and *ph*GSHPx in the cerebral cortex, cerebellum, mid-brain and brain stem of female rats and their offspring at 19 d were unaffected by Se and I deficiency. In contrast, ID-II activities were increased to the same or a greater extent than in I deficiency alone (Mitchell *et al.* 1997). Thus, despite the fact that the concurrent Se deficiency decreased plasma *e*GSHPx activity to less than 15 % of control levels, sufficient Se was still available to the preweanling offspring to increase brain ID-II activity more than 6-fold. ID-II activity was also increased 6-fold in the cerebral cortex of third

generation Se-deficient rats, which were I-deficient for the final 2 months of the experiment (Meinhold *et al.* 1993). A similar induction of rat brain ID-II activity in experimentally-induced hypothyroidism allowed tissue T₃ concentrations to remain unchanged (Serrano-Lozano *et al.* 1993). Consistent with this, the activities of brain cell-specific marker enzymes and expression of myelin basic protein were unchanged in Se- and I-deficient 19-d-old rats (Mitchell *et al.* 1997), confirming the efficiency of compensatory mechanism to maintain thyroid hormone metabolism. However, Campos-Barros *et al.* (1997) demonstrated a 50% decrease in tissue T₃ concentrations in all brain regions, except the cerebellum, in rats which were Se-deficient for three generations and I-deficient for 2 months, despite 3-fold increases in ID-II activities. Such a change in T₃ concentrations would predispose to impaired brain development.

Selenoprotein activities in the central nervous system of 1-, 4- and 11-d-old rat pups from Se- and I-deficient mothers are less efficiently retained than in 19-d-old pups (Mitchell *et al.* 1998). Cerebrum cGSHPx activities are decreased by 50% in 1-, 4- and 11-d-old rats with Se deficiency and concurrent Se and I deficiency. ID-II activity could not be detected in cerebrum 1 d after birth, but was increased 1.5-fold and 3-fold in Se- and I-deficient rats at 4 and 11 d old when compared with controls. This may be associated with tissue T₄ supply, as in I deficiency the element is retained by the mother during pregnancy and supplied to the pup via milk, resulting in a 25-fold increase in pup thyroidal T₄ during the first week after birth (Obregon *et al.* 1991). Se deficiency caused a decrease in the cerebral expression of brain-derived neurotrophic factor in 4- and 11-d-old animals. The decreases were exacerbated by I deficiency in 4-d-old animals and ameliorated by I deficiency in 11-d-old animals (Mitchell *et al.* 1998). Although localized changes in thyroid hormone concentrations could have influenced brain-derived neurotropic factor expression, other selenoproteins such as TR, which can indirectly modulate gene expression, could underlie the altered gene expression caused by Se deficiency (Gladyshev *et al.* 1996; McLeod *et al.* 1997). Myelination in the rat brain occurs at about 10 d after birth and is delayed in hypothyroidism as assessed by the expression of myelin basic protein (Kristensson *et al.* 1986). Myelin basic protein expression in I-deficient rats was the same as controls at 11 d old but was decreased by 50% in combined Se and I deficiency (Mitchell *et al.* 1998). Thus, concurrent Se deficiency may cause a further delay in brain development; however, it is unlikely that this is directly due to the similar differences in tissue T₄ concentrations which occur in I deficiency and combined Se and I deficiency (Campos-Barros *et al.* 1997).

Selenium and iodine deficiency: the potential for thyroidal damage

The lack of, or in some cases a very small, difference in plasma T₃ concentrations between I-deficient, and Se- and I-deficient rats suggests that the thyroid gland is able to retain sufficient Se to produce T₃ either by *de novo* synthesis or by deiodination of T₄ by ID-I. This may be at the expense of increased peroxidative damage to the gland; however, thyroid atrophy characteristic of myxoedematous cretinism is not observed in rats (Beckett *et al.* 1993; Meinhold *et al.* 1993; Mitchell *et al.* 1996, 1998). The role for Se deficiency in thyroidal damage has also been investigated in rats by feeding Se-deficient and normal rats with goitrogens to destroy their thyroid gland and then re-feeding them with iodide (Contempre *et al.* 1993). Although the duration of deficiency and the stage of development at which it was induced were not exactly comparable with Se and I deficiency in human subjects, the results showed that thyroid cells from Se-deficient rats were more necrotic on iodide re-feeding than were those from Se-adequate, goitrogen-fed rats. In addition, the thyroid gland morphology was

restored to normal within 15 d of iodide administration in Se-sufficient, but not in Se-deficient, rats (Contempre *et al.* 1995). The thyroid degeneration in Se- and I-deficiency was thought to be due to impaired defence against oxidative damage since methimazole, which inhibits I oxidation by the thyroid peroxidase system, protected against the iodide-induced necrosis. Furthermore, selenium deficiency increased the necrosis induced by free radicals in other organs including liver and kidney (Burk *et al.* 1995). The fibrosis and permanent damage to thyroid tissue characteristic of myxoedematous cretinism could also reflect Se-induced disturbance in the inflammatory response which follows necrosis (Contempre *et al.* 1996). An influx of macrophages which in turn secreted an excess of the cytokine, transforming growth factor type β led to changes in the proliferation indexes of epithelial cells and fibroblasts and ultimately led to fibrosis. Anti-transforming growth factor β antibodies decreased the macrophage invasion and prevented necrosis within 15 d of iodide administration (Contempre *et al.* 1996). The authors hypothesized that the mechanism underlying this overproduction of transforming growth factor β by the macrophages may also play a role in the aetiology of other Se-related disorders such as Kashin-Beck disease. However, Se deficiency alone is not sufficient to initiate this disease (Morenoreyes *et al.* 1998).

Selenium and iodine deficiency and brown adipose tissue

Rodent and human brown adipose tissue (BAT) contains ID-II activity and the T_3 generated by this enzyme is thought to play an important role in thermogenesis via regulation of the tissue-specific uncoupling protein (Bianco *et al.* 1988). In Se-deficient rats, BAT ID-II activity was decreased by 60% and the normal increase in ID-II activity induced by cold stress was attenuated; BAT ID-II activity after 18 h at 4 ° was 10-fold lower in Se-deficient than in Se-sufficient animals (Arthur *et al.* 1991). Thus, Se deficiency may impair the thermogenic response to cold and pose a substantial threat to neonates. BAT ID-II activity is increased in I deficiency, but the increase is reversed by a concurrent Se deficiency and the levels of uncoupling protein are decreased (Geloan *et al.* 1990; Mitchell *et al.* 1997). A decrease in BAT T_3 concentrations is likely as a consequence of low plasma T_4 levels and inhibited ID-II activity in combined Se and I deficiency. This would be consistent with T_3 regulation of the levels of uncoupling protein mRNA in BAT (Bianco *et al.* 1988). Since the levels of uncoupling protein in BAT play an important role in its thermogenic capacity, combined Se and I deficiency is likely to impair the ability of neonatal animals to generate heat and could have implications for their survival during cold stress. Ruminant BAT contains ID-I rather than ID-II, the activity being present only during the first 3 weeks of life. If the generally greater sensitivity of ID-I, compared with ID-II, to Se deficiency applies to ruminant BAT, there may be an important Se–I interaction in the response of neonatal ruminants to cold stress. However, this hypothesis requires further investigation to assess its relevance to survival of farm animals in cold conditions.

Selenium and iodine deficiency: species differences in responses?

In contrast to rats and human subjects, many other animals such as cattle, sheep and goats do not express significant thyroidal ID-I activity under normal conditions and thus probably have to rely entirely on *de novo* synthesis of T_3 in Se deficiency (Beech *et al.* 1993). However, I deficiency increased thyroidal ID-I activity 10–12-fold in cows and their calves, although the final activity was still lower than normal levels in rats and human subjects. Thyroidal cGSHPx activities were also increased, probably in response to the higher levels of H_2O_2

produced to stimulate thyroid hormone synthesis (Zagrodzki *et al.* 1998). Thus I-deficient cows and their calves can induce similar compensatory mechanisms as rats in order to maintain thyroid hormone synthesis and metabolism, allowing them to appear 'clinically normal'. However, these animals had adequate intake of Se and it is uncertain whether Se deficiency would impair this compensatory response (McCoy *et al.* 1997; Zagrodzki *et al.* 1998).

I supplementation of Se-deficient, pregnant ewes decreased plasma T₃ concentrations in their lambs (Donald *et al.* 1993). Goat kids from Se- and I-deficient mothers also had decreased plasma T₃ concentrations on I supplementation (Wichtel *et al.* 1996). Se and I deficiencies have been associated with perinatal calf mortality; however, supplementing cows before and during gestation restored any thyroid hormone imbalance, but did not reduce calf mortality (Mee & Rogers, 1996). In addition, when lambs were partially immersed in water of progressively declining temperatures, their thermoregulatory ability was unaffected by Se status and there were no Se and I interactions (Donald *et al.* 1994). However, the deficiencies reported in these experiments were subclinical and the studies rarely compared the thyroid status of the animals before supplementation, which makes any possible Se and I interactions difficult to interpret. Further work is required to determine the effects of concurrent Se and I deficiencies on selenium protein function and thyroid hormone status in farm animals.

Selenium and iodine deficiency in human subjects

Studies with experimental Se and I deficiencies in animals have provided a basis for the understanding of the deficiencies in human populations. In man attention has focused on how Se deficiency may alter the effects of I deficiency and how the deficiencies relate to the different forms of endemic cretinism.

Endemic cretinism

Endemic cretinism is found in areas of severe endemic goitre, the incidence being as high as 15% of the population. Affected individuals have subnormal intellect and physical development. Two forms of the disorder occur and in both the abnormalities are irreversible once present (Delange & Ermans, 1996): myxoedematous cretinism and neurological cretinism. In myxoedematous cretinism there is severe hypothyroidism and stunted growth. The prevalence of goitre in myxoedematous cretinism is much lower than that in non-cretins and in many subjects no thyroid tissue may be palpable, with only small amounts of functional tissue being found on thyroid scanning. Typically plasma T₄ and T₃ are very low whilst TSH is extremely high. The fact that bone age in many cretins corresponds to a fetus of 7–8 months suggests that thyroid destruction must have occurred *in utero* or soon after birth. In neurological cretinism mental deficiency may be accompanied by neurological problems including hearing and speech defects. Growth and thyroid function are usually normal. The neurological form is more common than myxoedematous cretinism.

The pathogenesis of cretinism is unclear but I deficiency certainly plays an important role since the diseases may be prevented by I supplementation. However, additional factors, which may vary with geographical location, may influence the prevalence and type of disease. These additional factors may include goitrogens, and deficiency in certain trace elements (see later).

The effect of combined iodine and selenium deficiency in human subjects

The first clue that Se intake may modulate the effects of I deficiency in human subjects came with the study of the cretinism endemia in the Island of Idjwi in Zaire (Goyens *et al.* 1987; Corvilain *et al.* 1993). As in other parts of Zaire, there was a high incidence of myxoedematous rather than of neurological cretinism. The distribution of endemia cretinism was related to clear geological areas with severe myxoedematous cretinism occurring in the north and a mild endemic occurring in the south of the island. It was proposed that the thyroid atrophy found in myxoedematous cretinism resulted from thyroid destruction brought about by loss of protection from toxic levels of H_2O_2 . Trace elements are important for the synthesis of a number of enzymes that are involved in detoxification of oxygen-derived free radicals. Se is needed for activation of glutathione peroxidases and other selenoenzymes described earlier and Cu and Zn are required for synthesis of superoxide dismutase. Dietary Cu and Zn supplies appeared normal on Idjwi island with no differences between the north and south (Cornil *et al.* 1974), but Se deficiency was prevalent. Moreover, Se deficiency, I deficiency and thiocyanate overload were all more marked in the north than the south (Goyens *et al.* 1987; Corvilain *et al.* 1993).

These observations gave rise to the following hypothesis. When I supply is limited, thyroid hormone synthesis is impaired and TSH concentrations rise, which in turn leads to increased generation of H_2O_2 . Furthermore, TSH concentration greatly increases after birth and this in turn leads to a marked increase in the generation of H_2O_2 in the neonatal thyroid. Under normal circumstances, the H_2O_2 so formed is utilized by thyroid peroxidase to incorporate iodide into thyroglobulin. Any excess H_2O_2 that diffuses into the thyrocyte from the follicular lumen is detoxified by enzymes such as *cGSHPx* and catalase (*EC* 1.11.1.6). Utilization of H_2O_2 would also be diminished in I deficiency, due to lack of iodide as substrate for thyroid peroxidase, and this could also increase H_2O_2 concentration in the gland. It was suggested that if the thyrocyte lost its ability to protect itself from the peroxidative stress generated in I deficiency, then thyroid atrophy would result. Since the expression of *cGSHPx* in the thyrocyte in the presence of TSH is largely regulated by Se supply, Se deficiency concurrent with I deficiency could explain the high prevalence of myxoedematous cretinism in the north of the island, the Se deficiency leading to loss of synthesis of glutathione peroxidase. The proposed role of Se deficiency in the pathogenesis of myxoedematous cretinism is shown in Fig. 2.

Further support for this hypothesis came from studies in the endemic goitre belt of northern Zaire (Vanderpas *et al.* 1990, 1992, 1993; Contempre *et al.* 1991, 1992; Thilly *et al.* 1992). In this area the prevalence rate of cretinism was 2–6%, with 80% of these of the myxoedematous form. Serum Se levels in children living in the goitre belt (Kawara villages) were much lower than those in adults from villages on the border of the endemia area (Vanderpas *et al.* 1990) and indeed in some children were as low as values found in areas of China regarded as severely Se-deficient. Furthermore, erythrocyte *cGSHPx* activity was 5-fold lower in the Kawara children than in adults living in a non-I-deficient area of Zaire. In the Kawara villages serum Se and erythrocyte *cGSHPx* levels were similar in cretins and normal children (Vanderpas *et al.* 1990).

Selenium and endemic cretinism

Some recent studies have failed to provide convincing support for the hypothesis that Se deficiency is the only compounding factor responsible for the endemic cretinism seen in some I-deficient areas. Myxoedematous cretinism does not occur in certain provinces of Africa with a similar degree of Se deficiency but a greater degree of I deficiency than Zaire (Ngo *et al.* 1997). Similarly there is severe I and Se deficiency in areas of Tibet but the incidence of myx-

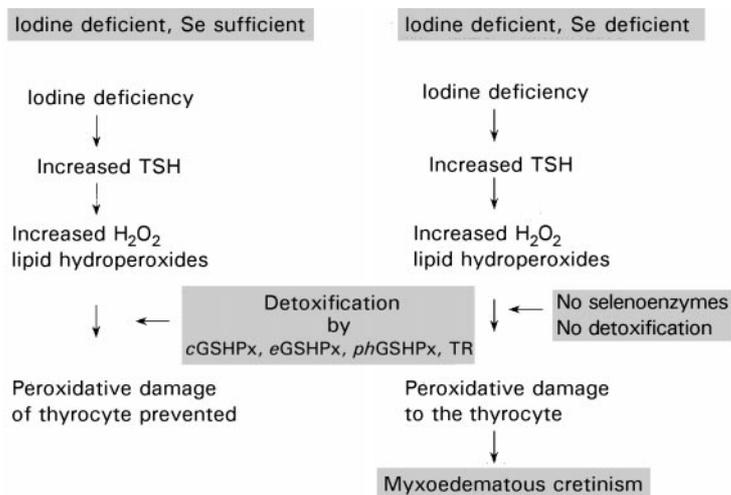


Fig. 2. A mechanism for the development of myxoedematous cretinism in combined selenium and iodine deficiency. In the presence of adequate selenium, selenoproteins protect the thyroid from excess hydrogen peroxide production occurring in response to iodine deficiency. When selenium is limiting, impaired hydrogen peroxide metabolism allows damage to the thyroid and involution associated with myxoedematous cretinism. *cGSHPx*, cytosolic glutathione peroxidase; *eGSHPx*, extracellular glutathione peroxidase; *phGSHPx*, phospholipid hydroperoxide glutathione peroxidase; *TR*, thioredoxin reductase; *TSH*, thyrotropin.

oedematous cretinism is very low with neurological cretinism predominating (Morenoyeyes *et al.* 1998; Vanderpas, 1998). Combined I and Se deficiency therefore does not appear to be sufficient to explain the elevated frequency of myxoedematous cretinism in central Africa. The possible role of other additional factors such as thiocyanates (Thilly *et al.* 1992) must again be considered (Morenoyeyes *et al.* 1998).

Selenium supplementation of iodine-deficient subjects

When normal school-children and cretins in the Kawara villages were given a supplement of 50 µg Se/d (as selenomethionine) or placebo for 2 months (Contempre *et al.* 1991, 1992; Vanderpas *et al.* 1992), serum Se and erythrocyte *cGSHPx* were normalized. In addition, plasma T_4 levels decreased. In the cretins there was a marked fall in total T_4 levels from a mean of 12.8 (SD 5.1) nmol/l to 2.8 (SD 2.5) nmol/l (Contempre *et al.* 1991, 1992; Vanderpas *et al.* 1993). The lower limit of normal values in many European countries is approximately 60 nmol/l. Following Se supplementation, mean TSH concentrations increased significantly from 262 mU/l to 363 mU/l. There was no significant change in total T_3 or surprisingly no change in plasma free T_4 , but this may have been due to methodological problems in the free T_4 assays available at the time of the study. Indeed total T_4 concentrations fell without any changes in thyroxine-binding globulin concentrations, suggesting that free T_4 concentrations should have also fallen in response to Se supplementation (Contempre *et al.* 1991, 1992; Vanderpas *et al.* 1992). In the normal school-children, Se supplementation resulted in a fall in plasma T_4 without a concomitant rise in TSH.

A similar observation has been made in children with phenylketonuria. Their I intakes were adequate but Se intake was low due to the low-protein diet they eat. Administering Se produced a 16% fall in plasma T_4 and reverse T_3 (Calomme *et al.* 1995). The fall in T_4 in cretins was attributed to an increase in the expression of hepatic ID-I which in turn would increase the metabolism of plasma T_4 to T_3 (Vanderpas *et al.* 1993). In normal children, with adequate amounts of functional thyroid tissue, the gland would be able to meet the increased requirement for T_4 synthesis following the induction of ID-I expression caused by Se supplementation. In contrast to normal children the cretins have small fibrosed thyroid glands that exhibit impaired I uptake (Contempre *et al.* 1996). This small amount of functional thyroid tissue was thought to be insufficient to meet the increased requirement for thyroid hormone synthesis (Contempre *et al.* 1991, 1992) after Se supplementation enhanced ID-I expression (Vanderpas *et al.* 1992). However, thyroid function in most cretins improved when I supplements were given, suggesting that I availability was also a limiting step of thyroid hormone production (Contempre *et al.* 1991). It is likely therefore that in cretins the increased requirement for thyroid hormone synthesis after Se supplements often leads to rapid depletion of I reserves.

Selenium deficiency protects against iodine deficiency?

These observations have also led to the intriguing hypothesis that Se deficiency may protect from some of the effects of I deficiency particularly fetal brain development (Vanderpas *et al.* 1990, 1993; Contempre *et al.* 1992). The T_3 occupying the nuclear receptors in different organs arises from different sources. In the kidney approximately 80% of T_3 is derived from plasma T_3 and not from deiodination of T_4 taken up from the plasma. In contrast the brain appears to utilize mainly T_3 derived from uptake of plasma T_4 , which is then deiodinated by ID-II within the cell (Beckett & Arthur, 1994). Maternal T_4 is also thought to provide an important source of T_4 for fetal brain during pregnancy (Deesobar *et al.* 1993). Thus because Se deficiency often leads to higher plasma T_4 concentrations, it may actually help to protect the fetal brain during development. This implies that it is unethical to supplement a population deficient in both Se and I with Se alone since this might exacerbate the risk of abnormal brain development. Thus although Se deficiency in an I-deficient population may favour thyroid destruction and myxoedematous cretinism, it may also protect against the neurological form of the disease (Vanderpas *et al.* 1990, 1993; Contempre *et al.* 1992).

These hypotheses are interesting but are not absolutely proven. Although T_4 supply to the brain is important, the crucial determinant of brain development is the amount of T_3 located on the nuclear receptors and thus ID-II activity in the brain must also be considered. In rats and man I deficiency leads to an induction of ID-II in the brain and as discussed earlier, this is enhanced further in the rat if it is also Se-deficient (Beckett *et al.* 1993; Karmarkar *et al.* 1993). If the effects of combined Se and I deficiency on ID-II in the brain in man are similar to the rat (i.e. ID-II is induced to levels higher than in I deficiency alone) Se deficiency might indeed protect against neurological cretinism in I-deficient areas. The possible role of Se deficiency in preventing neurological cretinism is shown in Fig. 3.

Selenium as an antioxidant in the thyroid

In the rat thyroid and in cultured human thyrocytes, Se deficiency has only a modest effect on the expression of cGSHPx (Beech *et al.* 1995). This might suggest it is not the loss of this

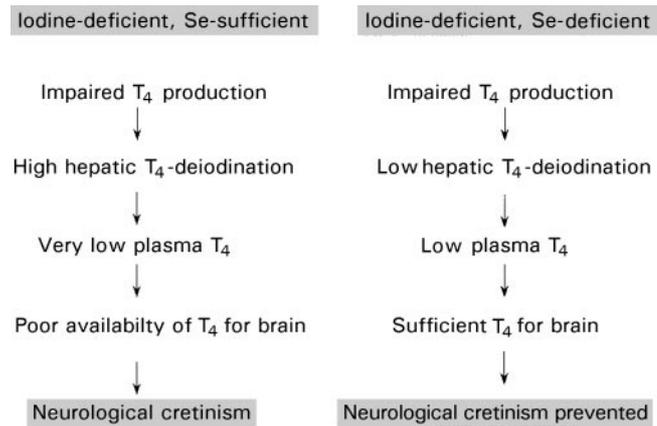


Fig. 3. A mechanism for protection against neurological cretinism in combined selenium and iodine deficiency. In selenium-adequate, iodine-deficient conditions circulating thyroxine (T₄) is metabolized by peripheral deiodination and the hormone is not available to protect the brain from neurological cretinism. In combined selenium and iodine deficiency peripheral T₄ metabolism is impaired and the hormone is available to prevent brain damage.

enzyme that gives rise to increased susceptibility to oxidative damage, but rather some other selenoprotein may be more important in this regard. Alternatively, in circumstances such as I deficiency, Se deficiency may prevent compensatory increases in selenoperoxidases that normally cope with oxidative stress.

TR may be involved in protecting the thyrocyte from oxidative damage. It has a broad substrate specificity and, with NADPH as a cofactor, reacts with a wide range of substrates including thioredoxin, peroxides, selenodiglutathione, selenite and glutathione peroxidase (Holmgren & Bjornstedt, 1995). The thioredoxin–TR redox system can act as cofactor for reactions catalysed by ID-I (Sharifi & St Germain, 1992). TR can also detoxify both lipid hydroperoxides and H₂O₂ and may serve as an important alternative detoxification pathway to the Se-dependent glutathione peroxidases (Bjornstedt *et al.* 1995). It is significant that cGSHPx appears to be ineffective at degrading high concentrations of H₂O₂ in the rat thyrocyte cell line FRTL5 (Bjorkman & Ekholm, 1995). This detoxification role for TR may be of special importance in the thyrocyte since high concentrations of peroxides are generated during thyroid hormone synthesis. Indeed the expression of TR can be greatly increased in human thyrocytes through activation of the Ca–phosphoinositol signalling pathway that also stimulates H₂O₂ production (Beckett *et al.* 1998; Howie *et al.* 1998). Thus, it could be argued that the development of myxoedematous cretinism in areas of Se and I deficiency may result from loss of TR expression in the thyroid rather than loss of cGSHPx activity (Beckett *et al.* 1998; Howie *et al.* 1998).

eGSHPx is another selenoenzyme expressed by the thyroid gland and secreted into the extracellular space under the regulation of the Ca–phosphoinositol signalling pathway (Howie *et al.* 1995). It may also have a role in protecting the thyrocytes from damage. The decreased expression of eGSHPx when Se supply is low may thus contribute to the thyroid atrophy seen in combined I and Se deficiency.

Conclusions

Because of the multiplicity of roles of selenoproteins in thyroid hormone metabolism and elsewhere, Se can have both beneficial and adverse effects on man and animals with I defi-

ciency. In human subjects, a concurrent Se deficiency has a sparing effect on plasma T_4 concentrations as compared with I-deficient individuals, providing a role for Se deficiency in the protection against neurological cretinism (Contempre *et al.* 1991). This has not been demonstrated in animals and in some cases, a concurrent Se deficiency can exacerbate the effects of I deficiency on brain biochemistry (Mitchell *et al.* 1998). Whether this is due to differences between species or in the duration and severity of the deficiencies requires further investigation. Concurrent Se deficiency decreases Se supply to thyroidal ID-I (and ID-II) and glutathione peroxidases, causing a decrease in plasma and tissue T_3 concentrations, but does not induce the thyroid involution characteristic of myxoedematous cretinism (Beckett *et al.* 1993; Mitchell *et al.* 1996; Zagrodzki *et al.* 1998). However, the hypothyroid stress on the thyroid gland is significantly increased in concurrent Se and I deficiency as compared with I deficiency alone. Furthermore, the increased sensitivity of the Se-deficient thyroid gland to free radical attack in I deficiency has been confirmed in rats in which thyroid tissue was damaged by goitrogens (Contempre *et al.* 1995, 1996; Mitchell *et al.* 1996, 1998). Goitrogens may also play a significant role in the aetiology of myxoedematous cretinism, although there is no doubt that a concurrent Se deficiency will aggravate the hypothyroid stress in man and animals suffering from the damaging effects of I deficiency. Animal models of Se and I deficiency show many similarities to I deficiency diseases in human subjects, but do not mirror them exactly. Thus if Se deficiency is involved in the outcome of I deficiency in man, it is likely that other interacting factors such as goitrogens are also involved. Additionally, the ability of Se deficiency to modify the toxicity of viruses could underlie the outcome of I deficiency, particularly if the viruses attack the thyroid or the brain (Levander & Beck, 1997). Nevertheless, the protection of the thyroid gland from H_2O_2 and the regulation of tissue T_3 levels are the functions of Se that are most likely to underlie the interactions of Se and I. Further research may, however, reveal that this is an oversimplification of a very complex interaction.

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