

REVIEW

Selenium and endocrine systems

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Abstract

The trace element selenium (Se) is capable of exerting multiple actions on endocrine systems by modifying the expression of at least 30 selenoproteins, many of which have clearly defined functions. Well-characterized selenoenzymes are the families of glutathione peroxidases (GPXs), thioredoxin reductases (TRs) and iodothyronine deiodinases (Ds). These selenoenzymes are capable of modifying cell function by acting as antioxidants and modifying redox status and thyroid hormone metabolism. Se is also involved in cell growth, apoptosis and modifying the action of cell signalling systems and transcription factors. During thyroid hormone synthesis GPX1, GPX3 and TR1 are up-regulated, providing the thyrocytes with considerable protection from peroxidative damage. Thyroidal D1 in rats and both D1 and D2 in humans are also up-regulated to increase the production of bioactive 3,5,3'-tri-iodothyronine (T3). In the basal state, GPX3 is secreted into the follicular lumen where it may down-

regulate thyroid hormone synthesis by decreasing hydrogen peroxide concentrations. The deiodinases are present in most tissues and provide a mechanism whereby individual tissues may control their exposure to T3. Se is also able to modify the immune response in patients with autoimmune thyroiditis. Low sperm production and poor sperm quality are consistent features of Se-deficient animals. The pivotal link between Se, sperm quality and male fertility is GPX4 since the enzyme is essential to allow the production of the correct architecture of the midpiece of spermatozoa. Se also has insulin-mimetic properties, an effect that is probably brought about by stimulating the tyrosine kinases involved in the insulin signalling cascade. Furthermore, in the diabetic rat, Se not only restores glycaemic control but it also prevents or alleviates the adverse effects that diabetes has on cardiac, renal and platelet function.

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Introduction

The biological roles ascribed to selenium (Se) include the prevention of cancer (Combs & Lu 2001) cardiovascular disease (Rayman 2002, Beckett *et al.* 2004) and viral mutation (Beck 2001). In addition the trace element is essential for optimal endocrine and immune function and moderating the inflammatory response (McKenzie *et al.* 2002b, Arthur *et al.* 2003).

These biological actions are mediated in most cases through the expression of at least 30 selenoproteins coded by 25 selenoprotein genes in humans (Kryukov *et al.* 2003). The importance of Se to endocrine systems is highlighted by the fact that many endocrine tissues have evolved mechanisms to maintain relatively high concentrations of Se even when there is severe dietary deficiency. This review will focus on the various mechanisms by which Se may modify thyroid function, fertility and glucose homeostasis.

The current recommended dietary intake of Se in humans is between 55 and 75 µg per day (Rayman 2000). These amounts are based on the Se intake that maximally induces the activity of glutathione peroxidase (GPX) in plasma or erythrocytes. The anticancer properties of Se operate at intakes of the order of 200 µg/day, suggesting that a re-appraisal of dietary Se intake may be useful. Many areas of the globe including the UK have Se intakes that are significantly below the current recommended intake, leading to sub-maximal expression of GPX and other selenoproteins in blood and tissues (Brown *et al.* 2000, Rayman 2002).

The selenoproteins

The selenoproteins incorporate Se co-translationally as a selenocysteine residue that is fully ionized at physiological pH and acts as a very efficient redox catalyst. Of the up to 30 selenoproteins that have been characterized or

Table 1 Mammalian selenoproteins and their functions

	Proposed function
Selenoprotein	
Glutathione peroxidases (GPXs)	
GPX1	Antioxidant in cell cytosol; Selenium store?
GPX2	Antioxidant in GI tract
GPX3	Antioxidant in extracellular space and plasma
GPX4	Membrane antioxidant; structural protein in sperm; apoptosis?
GPX5	Unknown
GPX6	GPX1 homologue?
Thioredoxin reductase (TRs)	
	Multiple roles including dithiol-disulphide oxidoreductase
	Detoxifies peroxides, reduces thioredoxin (control of cell growth); maintains redox state of transcription factors
TR1	Mainly cytosolic, ubiquitous
TR2	Expressed by testes
TR3	Mitochondrial, ubiquitous
Iodothyronine deiodinases	
Type D1 and D2	Converts thyroxine (T4) to bioactive 3,5,3'-tri-iodothyronine (T3)
Type D1 and D3	Converts thyroxine (T4) to bioinactive 3', 3', 5' reverse T3
Selenoprotein P	Selenium-transport protein. Antioxidant on endothelium
Selenoprotein W	Antioxidant in cardiac and skeletal muscle?
Selenophosphate synthetase (SPS2)	Synthesis of selenophosphate for selenoprotein synthesis.
15 kDa Selenoprotein (Sep 15)	Protects against cancer?
H, I, K, M, N, O, R, S, T, V	Role largely unknown

GI, gastrointestinal.

identified bioinformatically (Table 1), six are GPXs, three are iodothyronine deiodinases (Ds) and three are thioredoxin reductases (TRs; Kryukov *et al.* 2003). Selenoprotein P is quantitatively the major selenoprotein in plasma and has both antioxidant and transport roles (Burk *et al.* 2003, Hill *et al.* 2003, Mostert *et al.* 2003). Thus Se can influence at least three broad areas of cell biochemistry, namely antioxidant function, redox status and thyroid hormone metabolism.

TRs

The TRs, with thioredoxin as a substrate and NADPH as a cofactor, form a powerful dithiol–disulphide oxidoreductase system that regulates the cellular redox state of cells and may also protect against oxidative stress (Holmgren 2001, Kryukov *et al.* 2003). The system is also involved in many diverse cellular functions including cell signalling, regulation of cell growth and inhibition of apoptosis (Saitoh *et al.* 1998, Rundlof & Arner 2004). A range of diseases in humans are suspected to be related to the activity of TR and the enzyme has become a major

target for the development of therapeutic drugs (Becker *et al.* 2000, Gromer *et al.* 2004).

GPXs

At least six mammalian GPX isoenzymes have been described (Table 1). Cytosolic enzyme (GPX1) is expressed by all cells types in mammals. Extracellular GPX (GPX3) is a secreted glycoprotein that is the second most abundant selenoprotein in plasma while phospholipid hydroperoxide GPX (GPX4) can specifically reduce phospholipid hydroperoxides (Imai & Nakagawa 2003) and may be involved in moderating apoptotic cell death (Nomura *et al.* 2001) and sperm maturation.

Thyroid hormone deiodinases

Three iodothyronine deiodinases (D1, D2 and D3) have been identified. All are integral membrane proteins of 29–33 kDa, sharing 50% sequence identity. Each has a selenocysteine residue at the active centre that confers the high catalytic activity of the enzymes. The deiodinases

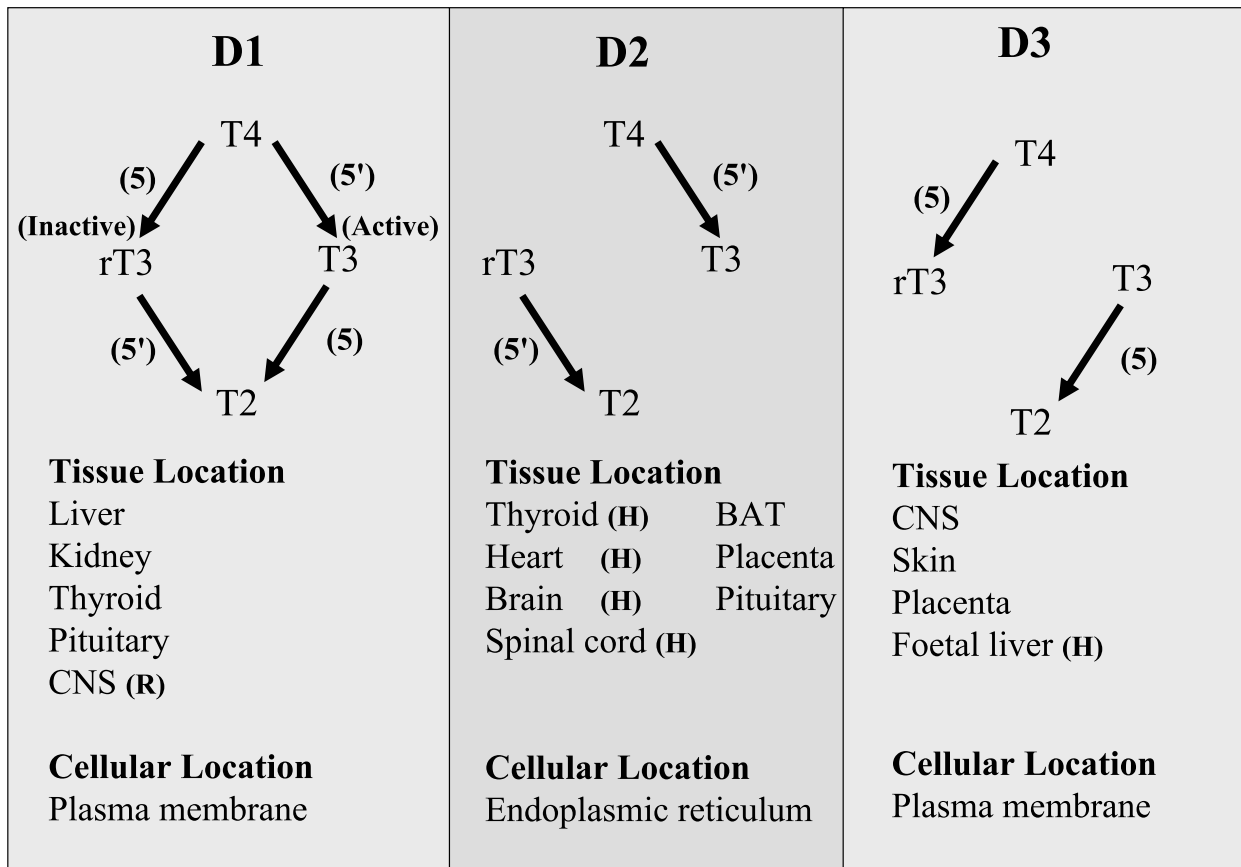


Figure 1 Characteristics of the iodothyronine deiodinases. T3 sulphate is also a substrate for 5-deiodination by D1. CNS, central nervous system; BAT, brown adipose tissue; T2 3,3'-di-iodothyronine; (R), rat not human; (H), human not rat.

have differing substrate specificities and tissue distribution (Bianco *et al.* 2002). The enzymes can catalyse the removal of iodine from the 5 or 5' positions of iodothyronine substrates and in doing so have an important regulatory role in the activation and inactivation of the thyroid hormones in all tissues (Fig. 1).

Recently details of the protein structure of the deiodinases has become available. The extra-membrane portion of the deiodinases belongs to the thioredoxin-fold superfamily, a superfamily that also includes the GPXs. Furthermore, a large deiodinase region embedded in the thioredoxin fold shares strong similarities with the active site of iduronidase, a member of the clan GH-A-fold glycoside hydrolases. The substrates for the deiodinases (iodothyronines such as thyroxine (T4), reverse tri-iodothyronine (rT3) and 3,5,3'-tri-iodothyronine (T3)) and substrates for the iduronidase (sulphated α -L-iduronic acid) are structurally similar, having O-linked hexagonal rings substituted with bulky groups lying *ortho* to the linker. It would thus appear that the deiodinases have iduronidase-like sequences embedded in the

selenocysteine-containing thioredoxin fold that are critical for iodothyronine binding. The predicted protein structure of the deiodinases together with site-directed mutagenesis experiments have allowed the elucidation of some of the critical amino acids that are responsible for the differences in substrate specificity and enzyme kinetics observed between D1, D2 and D3 (Callebaut *et al.* 2003).

The deiodinases show marked tissue- and time-specific expression during the foetal period and may be important regulators of this maturation process by modifying the supply of T3 to T3-responsive genes (Hume *et al.* 2001, Kester *et al.* 2004). However, the ontogeny of the deiodinases and their tissue distribution is quite different in rats than humans, thus data obtained from rat models cannot always be appropriately applied to humans.

Regulation of selenoprotein expression

The predominant control of selenoprotein expression is Se supply with a strict hierarchy of selenoprotein expression

when Se supply is limited. Endocrine tissues are well adapted to maintaining selenoprotein expression in Se deficiency and within any single tissue the expression of the deiodinases, GPX4 and TRs is maintained at the expense of GPX1, which is quickly lost (Behne *et al.* 1988, Bermanno *et al.* 1995, 1996, Crosley *et al.* 2003). Oxidative stress induces TR1 and GPX (Sun *et al.* 1999) and isothiocyanates such as sulforaphane induce TR1 (Zhang *et al.* 2003). Activation of second-messenger pathways also modifies the expression of specific selenoproteins in a tissue-specific manner (Beech *et al.* 1995, Howie *et al.* 1995, 1998, Anema *et al.* 1999).

Se and thyroid function

The thyroid contains more Se per gram of tissue than any other organ (Dickson & Tomlinson 1967) and Se, like iodine, is essential for normal thyroid function and thyroid hormone homeostasis. Labelling cultured human thyrocytes with [⁷⁵Se]selenite reveals numerous selenoproteins with TRs and GPXs predominating (Fig. 2).

Thyroid hormone synthesis

Synthesis of thyroid hormone requires iodination of tyrosyl residues on thyroglobulin which is stored in the lumen of the thyroid follicle. This iodination is catalysed by thyroid peroxidase (TPO) and requires the generation of high H₂O₂ concentrations which are potentially harmful to the thyrocyte. The generation of H₂O₂ appears to be the rate-limiting step in thyroid hormone synthesis and is regulated through the action of thyroid-stimulating hormone (TSH) via a complex network of interacting, second-messenger systems (Corvilain *et al.* 1991, 1994, Raspe *et al.* 1991, Kimura *et al.* 1995). The iodination of thyroglobulin and generation of H₂O₂ takes place on the luminal surface of the apical membrane of the thyrocyte (Fig. 3). This organization allows the H₂O₂ formed on the surface of the thyrocyte to be made readily available for iodination reactions, while any harmful H₂O₂ that diffuses into the thyrocyte can be degraded by the intracellular GPX, TR and catalase systems (Ekholm & Bjorkman 1997).

GPX3 as a potential regulator of thyroidal hormone production

The thyrocyte is capable of synthesizing and secreting GPX3 in a controlled manner. In basal conditions, cultured human thyrocytes secrete GPX3 and this secretion is prevented by the co-addition of the calcium ionophore A23187 and phorbol ester (PMA), known stimulators of H₂O₂ production (Howie *et al.* 1995). This raises the intriguing possibility that GPX3 may provide an additional mechanism for controlling thyroid hormone synthesis through regulating the concentration of H₂O₂ in the

follicular lumen. Thus when increased thyroid hormone production is signalled through the TSH receptor, increased synthesis of H₂O₂ at the apical membrane is accompanied by impaired secretion of GPX3 and thus diminished degradation of the peroxide. These concurrent changes would have the effect of amplifying the concentration of H₂O₂ available for iodination of thyroglobulin. When thyroid hormone synthesis is not strongly signalled, thyroid hormone production would be prevented both by diminished H₂O₂ synthesis and by active secretion of GPX3 across the apical membrane that would promote degradation of H₂O₂ produced in the basal state.

Se as an antioxidant in the thyroid

The thyrocyte is continually exposed to potentially toxic concentrations of H₂O₂ and lipid hydroperoxides. The cytotoxic effects of H₂O₂ on thyroid cells include caspase-3-dependent apoptosis that occurs at H₂O₂ concentrations that are insufficient to induce necrosis. In Se deficiency the apoptotic response to H₂O₂ is increased (Demelash *et al.* 2004). When Se intake is adequate the intracellular GPX and TR systems protect the thyrocyte from these peroxides. Furthermore, in iodine deficiency or Grave's disease, where hyperstimulation of the TSH receptor signals increased H₂O₂ production, activation of the calcium-phosphoinositol cascade stimulates GPX1 production and particularly TR1 (Fig. 2; Howie *et al.* 1998) thus providing an up-regulation of antioxidant protection (Fig. 3).

Se as a regulator of T3 production

The deiodinase D1 is the major isoform in liver, kidney, thyroid and pituitary. It can catalyse 5 or 5'-monodeiodination and thus can convert T4 to the inactive metabolite rT3 or the active isomer T3. The important physiological roles of D1 include providing an important source of plasma T3 and degrading rT3 and T3 sulphate.

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

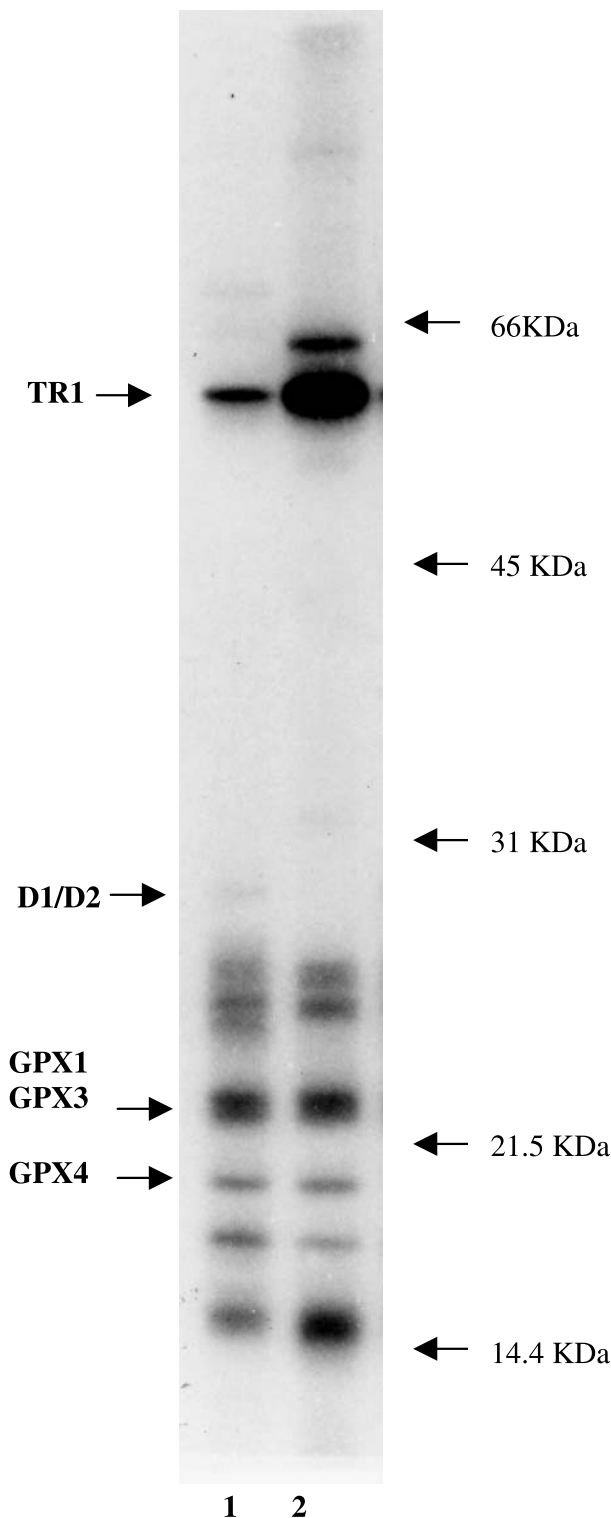


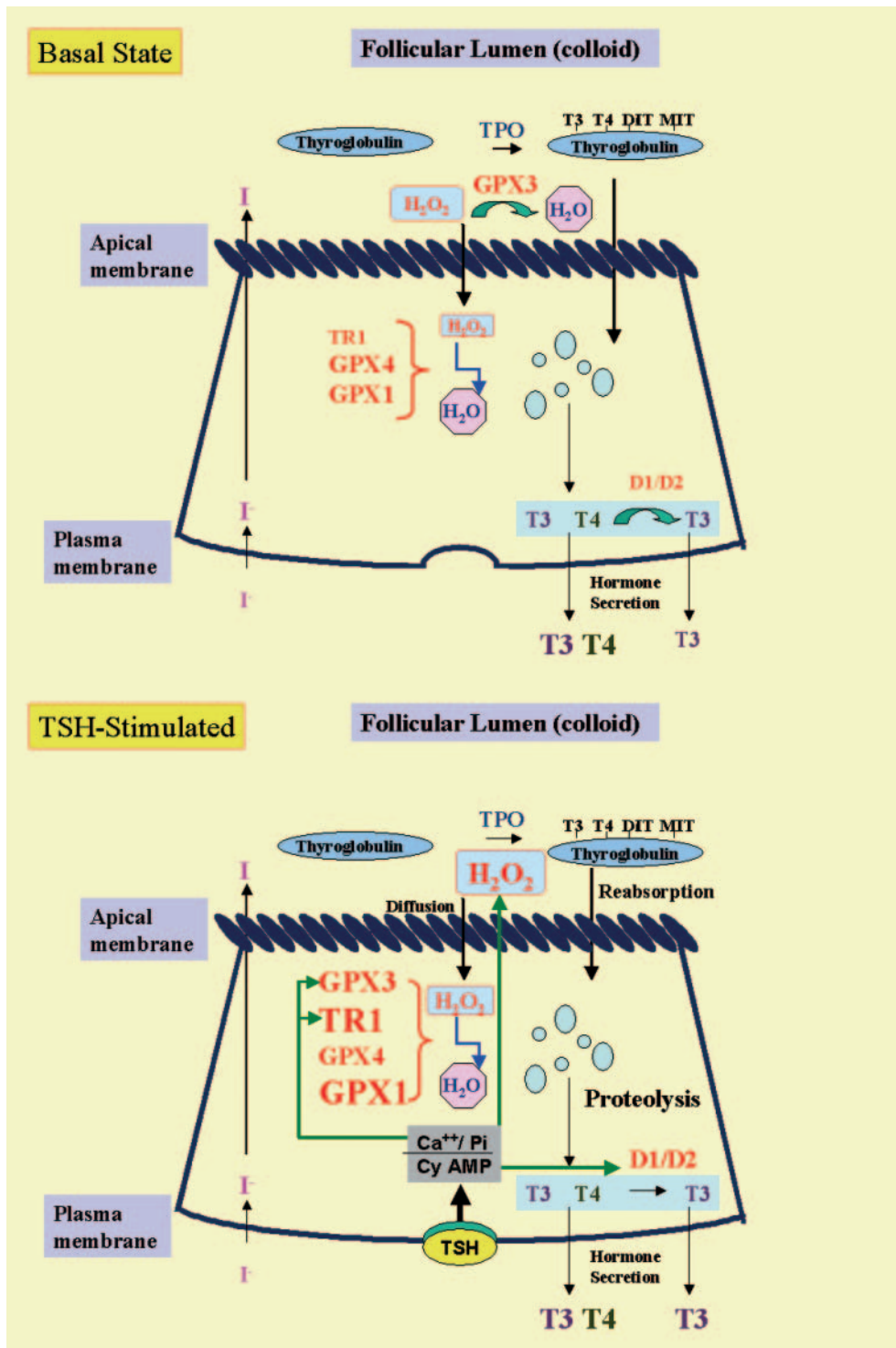
Figure 2 Selenoproteins in human thyrocytes. Autoradiograph of an SDS/PAGE gel taken from sonicates of human thyrocytes grown in the presence of [^{75}Se]selenite. Lane 1, thyrocytes grown in basal medium; lane 2, thyrocytes treated with 10^{-6} M phorbol ester (PMA) and the calcium ionophore A23187.

adipose tissue. D3 is found in the plasma membrane of brain, placenta and foetal liver and performs only 5-monodeiodination (Fig. 1; Baqui *et al.* 2003). The biochemistry, cellular and molecular biology and physiological roles of the deiodinases have been reviewed extensively (St Germain 2001, Bianco *et al.* 2002).

In Se-sufficient rats, hepatic D1 provides an important source of circulating T3 yet in Se-deficient animals, when hepatic D1 expression falls to approximately 10% of that in Se-adequate animals, plasma T3 concentrations are largely maintained. The maintenance of plasma T3 in these Se-deficient animals arises from an adaptive response driven by a rise in TSH that in turn signals increased *de novo* synthesis of T3 on thyroglobulin and also increased expression of thyrocyte D1 that promotes high rates of T4-to-T3 conversion (Beckett *et al.* 1987, Arthur *et al.* 1990). In humans, thyrocyte D2 may also contribute to maintaining plasma T3 in Se deficiency. The paradoxical increase in thyrocyte D1 found in Se-deficient rats is made possible because the gland retains adequate amounts of the trace element in dietary Se deficiency (Bermano *et al.*, 1995). Not all animal species express thyrocyte D1 and theoretically those lacking the enzyme may be less able to maintain plasma T3 concentrations in Se deficiency (Beech *et al.* 1993). Since D2 expression and T3 production are vital for regulating thermogenesis in brown adipose tissue, Se-deficient animals may show impaired production of D2 and uncoupling protein, with poor survival when subjected to a cold stress (Arthur *et al.* 1991).

Se and iodine deficiency

In humans, attention has focused on how Se status may modify the effects of iodine deficiency and the pathogenesis of endemic myxoedematous cretinism (reviewed by Corvilain *et al.* 1993, Arthur *et al.* 1999, Rundlof & Arner 2004), a condition associated with severe hypothyroidism, thyroid involution and stunted growth. Some epidemiological studies have suggested that the increased generation of H_2O_2 caused by the high TSH associated with iodine deficiency, together with a loss of thyrocyte selenoperoxidase activity due to concurrent Se deficiency, produces the marked thyroid atrophy found in myxoedematous cretinism. In contrast, if Se supply is adequate thyroid destruction may be prevented due to the maintenance of thyrocyte GPX and TR. The importance of Se in protecting the thyroid from oxidative damage is supported by rodent experiments (Contempre *et al.* 1995). These animal studies suggest also that myxoedematous cretinism may also result from a Se-deficiency-induced disturbance in the inflammatory response (Contempre *et al.* 1996). More recent reports have failed to provide convincing support for this hypothesis and the possible roles of other additional factors such as dietary thiocyanates must again be considered (Moreno-Reyes *et al.* 1998).



Se and autoimmune thyroid disease

The links between Se deficiency, altered immune function and inflammation have prompted studies in humans to examine if Se supplementation can modify auto-antibody production in patients with chronic autoimmune thyroiditis. Double-blind, randomized, placebo-controlled trials using daily Se supplements of 200 µg selenite produced a significant decline in TPO antibody (TPOAb) concentration accompanied in some patients by an improved ultrasound echogenicity of the thyroid (Gartner *et al.* 2002, Gartner & Gasnier 2003). This effect of Se on TPOAb concentration has been demonstrated both in an area of Germany with marginal dietary iodine and Se intakes (Gartner *et al.* 2002) and in an area around Athens where iodine and Se intakes were close to requirement (Duntas *et al.* 2003). In these studies Se supplements had no significant effect on the concentration of thyroglobulin antibodies or the concentration of TSH or thyroid hormone concentrations. The mechanism by which Se exerts effects on TPOAb production is likely to be due to the ability of high doses of Se to modify the inflammatory and immune responses (reviewed in (McKenzie *et al.* 2002a, 2002b). Further work is required to examine what long-term clinical benefits Se supplementation may have when given to patients with autoimmune thyroiditis. It would be important to determine if Se supplementation could modify the course of Graves' disease since there is one report of Se supplements decreasing the titre of TSH receptor antibodies in such patients (Vrca *et al.* 2004).

Se and fertility

Se and fertility in males

The testes contain high concentrations of Se and work with selenoprotein P-knockout mice indicates that Se is essential for testicular function (Hill *et al.* 2003). Low sperm production and poor sperm quality including impaired motility with flagella defects localized primarily to the midpiece have been a consistent feature in Se-deficient animals (Watanabe & Endo 1991, Behne *et al.* 1996) but it is only relatively recently that an explanation

for this phenomenon has been recognized and studies extended to humans (Maiorino *et al.* 1999, Flohe *et al.* 2001, Foresta *et al.* 2002, Maiorino & Ursini 2002).

GPX4 provides the pivotal link between Se, sperm quality and male fertility since GPX4 is essential to allow the production of the correct architecture of the midpiece of spermatozoa. In testes GPX4 is present as three isoforms that are derived from the same gene and are found in the cytosol, mitochondria and nucleus. The nuclear form differs from the other forms in having an arginine-rich N-terminus (Puglisi *et al.* 2003, Tramer *et al.* 2004a, 2004b). In the developing spermatozoa GPX4 probably provides protection from harmful reactive oxygen species but during sperm maturation the selenoenzyme takes on a structural role. In the midpiece of mature sperm GPX4 is a major component present as a polymeric form with no enzymic activity. Thus during sperm development GPX4 first appears in pachytene spermatocyte stages VII–X and its expression gradually increases through the stages of round spermatids with peak levels being found in elongating spermatids. As the spermatozoa mature there is a marked redox switch that is accompanied by an almost complete loss of glutathione. As this occurs reduction of peroxides catalysed by GPX4 in the spermatozoa utilizes protein thiols as an alternative donor substrate to glutathione. This results in GPX4 forming covalent cross-links with itself and other proteins that ultimately build up as a keratin-like material. This material is largely incorporated into the helix of mitochondria in the midpiece of spermatozoa that ultimately forms up to 50% of the capsule material.

Many human subjects who have infertility due to low sperm count and poor sperm quality have marked decreases in polymerized GPX4 in their sperm. The loss in GPX4 is particularly marked in oligoasthenozoospermic specimens (Foresta *et al.*, 2002). It is unlikely that dietary Se deficiency alone could be the cause since in most patients other pathologies causing infertility could be identified. However, one study performed in Scotland (where Se intakes are below requirements at only 30–40 µg/day) showed the sperm quality and fertility of the patients improved after Se supplementation (Scott *et al.* 1998). Further research is clearly required to determine the association between male fertility in humans

Figure 3 Changes in selenoprotein expression in thyrocytes in the basal state and following stimulation of the TSH receptor. In the TSH-stimulated cells, activation of the Ca²⁺/phosphoinositol (Pi) signalling pathways stimulate hydrogen peroxide production and the expression of GPX1 and TR1. In addition the secretion of GPX3 is prevented. These changes provide a large increase in the cells' antioxidant protection systems to prevent peroxidative damage from any hydrogen peroxide that may diffuse into the thyrocyte (see also Fig. 2). The selenoenzymes also detoxify any harmful lipid hydroperoxides. The cAMP (Cy AMP) signalling pathway stimulates the expression of D1 (and D2 in humans but not rats) to promote deiodination of the pro-hormone thyroxine (T4) to the metabolically active hormone tri-iodothyronine (T3). In the basal state GPX3 is actively secreted into the follicular lumen where it will damp-down hormone synthesis by degrading hydrogen peroxide produced in the basal state. In addition, expression of GPX1, TR1 and D1 is diminished in the basal state. The expression of GPX4 is unaltered by TSH stimulation or activation of the Ca²⁺/phosphoinositol signalling pathways. MIT, mono-iodotyrosine; DIT, di-iodotyrosine.

and the range of Se intakes that are seen throughout the world.

Female fertility

Information regarding the importance of Se in female reproduction is sparse; however, experiments in rodents suggests that Se deficiency has no significant effect on female reproduction even in sixth-generation animals (Bates *et al.* 2000). In humans a significant depletion of Se in follicular fluid of women with unexplained fertility has been described (Paszkowski *et al.* 1995). A decrease in the concentration of serum Se occurs throughout normal pregnancy but women with first-trimester miscarriages have significantly lower serum Se concentrations than women in the first trimester whose pregnancies went to term (Barrington *et al.* 1996). *In vitro* studies using bovine granulosa cells obtained from different-sized follicles found that Se significantly stimulated the proliferation of cells from small follicles and augmented the stimulatory effects of gonadotrophins in the same cells. Se also enhanced oestradiol production (Basini & Tamanini 2000). The relevance of these observations to humans is not known.

Se and diabetes

Se has insulin-mimetic properties *in vitro* and *in vivo*. Insulin-stimulated glucose metabolism is impaired in adipocytes isolated from Se-deficient rats (Souness *et al.* 1983). An insulin-like effect of Se in cultured rat adipocytes include stimulating glucose transport, phosphodiesterase activity and ribosomal S6 protein phosphorylation (Ezaki 1990). When administered to streptozotocin-diabetic rats, Se restores glycaemic control and modifies the activity of a range of enzymes involved in hepatic glycolysis and gluconeogenesis. These changes are not linked to changes in insulin levels (McNeill *et al.* 1991, Ghosh *et al.* 1994, Becker *et al.* 1996, Battell *et al.* 1998, Mukherjee *et al.* 1998, Ghose *et al.* 2001). In animal models, Se also prevents or alleviates the adverse effects that diabetes has on cardiac (Battell *et al.* 1998, Ayaz *et al.* 2002, 2004), renal and platelet function (Douillet *et al.* 1996a, 1996b). We are unaware of any publications describing Se-supplementation trials in diabetic humans. Se may exert these insulin-like effects on glucose metabolism by stimulating the tyrosine kinases involved in the distal signalling of the insulin signalling cascade (Pillay & Makgoba 1992, Stapleton *et al.* 1997, Hei *et al.* 1998, McKenzie *et al.* 2002a).

Conclusions

The role of Se in the aetiology of several diseases and the impact that Se status has on several endocrine systems has

now been established. The multiple roles that selenoproteins play in cell signalling systems and in modifying the immune response, cell growth and cell survival suggest that there are more roles waiting to be discovered for Se in endocrine systems. Se may also have a role in treating malignancies that are responsive to endocrine manipulation. For example Se is effective at reducing the risk of prostatic cancer possibly by inhibiting tumour cell growth via down-regulation of androgen receptor expression (Dong *et al.* 2003, Dong *et al.* 2004).

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