

The argument for increasing selenium intake

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The essential trace mineral, Se, is of fundamental importance to human health. As a constituent of selenoproteins it plays both structural and enzymic roles, in the latter context being best known as an antioxidant and catalyst for the production of active thyroid hormone. While Se-deficiency diseases have been recognised for some time, evidence is mounting that less-overt deficiency can also cause adverse health effects and furthermore, that supra-nutritional levels of Se may give additional protection from disease. In the context of these effects, low or diminishing Se status in some parts of the world, notably in some European countries such as the UK, is giving cause for concern. While deficiency has an adverse effect on immunocompetence, Se supplementation appears to enhance the immune response. Se appears to be a key nutrient in counteracting certain viral infections; thus, in a Se-deficient host the benign coxsackie virus becomes virulent, causing heart damage, the influenza virus causes more serious lung pathology and HIV infection progresses more rapidly to AIDS. Long recognised as essential for successful animal reproduction, Se is required for human sperm maturation and sperm motility and may reduce the risk of miscarriage. Deficiency has been linked to adverse mood states. Findings have been equivocal in linking Se to cardiovascular disease risk, although other conditions involving oxidative stress and inflammation have shown some association with Se status. There is growing evidence that higher Se intakes are associated with reduced cancer risk. While persuasive evidence already exists to suggest that additional Se would be beneficial in some health conditions, results from intervention trials underway or planned have the potential to reinforce or refute the argument for increasing Se intake.

Selenium: Selenium intake: Supra-nutritional intakes: Selenium status: Human health

It is hard to overestimate the importance of Se to biological systems. Its crucial role is underlined by the fact that it is the only trace element to be specified in the genetic code. It is specified as selenocysteine, now recognised as the 21st amino acid, as it has its own codon and selenocysteine-specific biosynthetic and insertion machinery (Gladyshev, 2001a). In the presence of a downstream stem-loop structure, the UGA codon in mRNA, instead of behaving as a stop codon, specifies the insertion of a selenocysteine moiety in protein synthesis to give a selenoprotein. Se, as selenocysteine in proteins, scores over its analogue, S, in existing as an anion (selenolate) at biological pH (acid dissociation constants: selenocysteine 5.2, cysteine 8.5; Stryer 1995; Stadtman, 1996). This property enables it to carry out biological redox reactions. Its additional ability as a metalloid to both give and accept electrons, makes it an ideal catalytic centre.

About fourteen mammalian selenoproteins have been characterised to date (Gladyshev, 2001a). A number of these selenoproteins have been identified as having enzymic (catalytic) redox activity, although structural and transport functions have also been recognised (Ursini *et al.* 1999; Hill & Burk, 2001). Some known selenoproteins and their functions are listed in Table 1, which demonstrates the fundamental nature and role of the selenoproteins.

In this context, it is not surprising that Se adequacy is crucial to human and animal health (Rayman, 2000). While Se-deficiency diseases have been recognised for some time, evidence is now mounting that less-overt deficiency may also cause adverse health effects and, furthermore, that supra-nutritional levels of Se may give additional protection from some diseases. Low or diminishing Se status in some parts of the world, notably in some European countries of which the UK is one, is thus giving cause for concern

Abbreviations: GPx, glutathione peroxidase; HCC, hepatocellular carcinoma; NPC, Nutritional Prevention of Cancer; PRECISE, Prevention of Cancer by Intervention with Se.

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Table 1. Known selenoproteins that carry out the nutritional functions of selenium

Selenoprotein	Function	References
Glutathione peroxidases (GPx; four forms: GPx1, GPx2, GPx3, GPx4)	Antioxidant enzymes: remove H ₂ O ₂ , lipid and phospholipid hydroperoxides (thereby maintaining membrane integrity, modulating eicosanoid synthesis, modifying inflammation and the likelihood of propagation of further oxidative damage to biomolecules such as lipids, lipoproteins and DNA)	Spallholz <i>et al.</i> (1990), Diplock (1994), Sunde (1997), Allan <i>et al.</i> (1999)
(Sperm) mitochondrial capsule selenoprotein	Form of GPx (GPx4): shields developing sperm cells from oxidative damage and later polymerises into a structural protein required for stability/motility of mature sperm	Ursini <i>et al.</i> (1999)
Sperm nuclei GPx	Present in sperm nuclei. Stabilises condensed chromatin by cross-linking protamine thiols and thus is necessary for sperm maturation and male fertility	Pfeifer <i>et al.</i> (2001)
Iodothyronine deiodinases (three forms)	Production and regulation of level of active thyroid hormone, triiodothyronine, from thyroxine	Sunde (1997)
Thioredoxin reductases (three forms)	Reduction of nucleotides in DNA synthesis; regeneration of antioxidant systems; maintenance of the intracellular redox state, critical for cell viability and proliferation; regulation of gene expression by redox control of binding of transcription factors to DNA	Allan <i>et al.</i> (1999)
Selenophosphate synthetase (SPS2)	Required for the biosynthesis of selenophosphate, the precursor of selenocysteine, and therefore for selenoprotein synthesis	Allan <i>et al.</i> (1999)
Selenoprotein P	Found in plasma and associated with endothelial cells. Antioxidant and transport functions. Appears to protect endothelial cells against damage from peroxynitrite	Sunde (1997), Allan <i>et al.</i> (1999), Arteel <i>et al.</i> (1999)
Selenoprotein W	Believed to be involved in skeletal and cardiac muscle metabolism	Sunde (1997), Allan <i>et al.</i> (1999)
15 kDa selenoprotein	Differentially expressed in normal and malignant tissues. Gene located in a region often altered in cancers. High levels in prostate. May protect prostate cells against development of carcinoma	Behne <i>et al.</i> (1997)
DNA-bound spermatid selenoprotein (34 kDa)	GPx-like activity. Found in stomach and in nuclei of spermatozoa. May protect developing sperm	Behne <i>et al.</i> (1997)
18 kDa selenoprotein	Important selenoprotein, found in kidney and large number of other tissues. Preserved in Se deficiency	Behne <i>et al.</i> (2000)

(Rayman, 2000). Combs (2001) estimates that the total number of Se-deficient individuals (as defined by sub-maximal selenoenzyme activity) is likely to be in the range of 500–1000 million.

Arguments for increasing Se intake are based on three premises: Se deficiency, even if marginal, may leave an individual less than optimally protected against a number of adverse health conditions; Se intakes above those required to replete the glutathione peroxide (GPx) selenoenzymes appear to confer additional health benefits; Se intake and status is low or marginal in many countries. Evidence in support of these premises follows.

Health effects of selenium deficiency: overt or marginal

Selenium-deficiency diseases

Diseases associated with overt Se deficiency have been recognised for some time in parts of the world notable for their low soil Se. Keshan disease is a cardiomyopathy, frequently fatal, named after the area in the extreme north-

east of China where it was endemic. Prophylactic administration of Se has greatly reduced the incidence of the condition (Reilly, 1996a). A number of cases of cardiomyopathy have also been reported in subjects on intravenous nutrition receiving inadequate Se in their infusion solutions (Reilly, 1996b). Kashin-Beck disease is an osteoarthropathy found in rural areas of China, Tibet and Siberia (Coppinger & Diamond, 2001) associated with severe Se deficiency. Both conditions are believed to require cofactors which, in the case of Kashin-Beck disease, may be low I status or the presence of fulvic acids or mycotoxins in foods (Moreno-Reynes *et al.* 1998; Coppinger & Diamond, 2001). In severely Se-deficient parts of China, Tibet and Eastern Siberia affected by these diseases there is a clear argument for increasing Se intake. However, in other countries widely understood to have low soil Se (e.g. New Zealand) there is no evidence of these conditions. This is because Se intakes are in fact substantially higher (Duffield *et al.* 1999), there is wider availability of non-local and imported foods, and at least some of the cofactors are absent.

Health effects related to the role of selenium as antioxidant

Cardiovascular disease. Findings have been equivocal in linking Se to cardiovascular disease risk. On theoretical grounds, the protective effect of Se against cardiovascular disease is supported by the ability of GPx to combat the oxidative modification of lipids and to reduce platelet aggregation (Nève, 1996). GPx4 has been shown to reduce hydroperoxides of phospholipids and cholesteryl esters associated with lipoproteins (Sattler *et al.* 1994), and may therefore reduce the accumulation of oxidised LDL in the artery wall. GPx is required for the metabolism of hydroperoxides produced in eicosanoid synthesis by the lipoxygenase and cyclo-oxygenase pathways (Spallholz *et al.* 1990). In Se deficiency a build up of these hydroperoxides inhibits the enzyme prostacyclin synthetase, which is responsible for the production of vasodilatory prostacyclin by the endothelium, but stimulates the production of thromboxane, which is associated with vasoconstriction and platelet aggregation (Nève, 1996). The balance is therefore tipped towards the pro-aggregatory state. In men with coronary artery disease platelet aggregability has been shown to be inversely related to Se status (Salonen *et al.* 1988; Nève, 1996).

Prospective epidemiological studies have had mixed findings. While Salonen *et al.* (1982) found a two- to threefold increase in cardiovascular morbidity and mortality for subjects with serum Se levels <45 µg/l compared with subjects above that level at baseline, Virtamo's group (Virtamo *et al.* 1985) found no significant associations with Se levels above and below that cut-off point, except for stroke mortality. A study by Suadicani *et al.* (1992) showed that middle-aged and elderly Danish men with serum Se <79 µg/l had a significantly increased risk of IHD (adjusted relative risk 1.55 (95% CI 1, 2.39)). However, about six other studies have not shown a clear association between cardiovascular risk and low Se, although these studies differ from the previously mentioned studies in having included few or no subjects with low Se status (Salvini *et al.* 1995; Nève, 1996). That a low Se status may be relevant was suggested by the findings of Kardinaal *et al.* (1997) in the ten-centre EURAMIC study, in which a significant inverse association between toenail Se levels and risk of myocardial infarction was shown only for Germany, the centre with the lowest Se status (odds ratio 0.62 (95% CI 0.42, 0.91)). Thus, the effect may only be apparent in populations of low Se status, lower than the levels present in the USA and a large part of Europe. The disparity between studies may also be explained to some extent by the status of other antioxidants such as vitamin E, which may compensate for a deficiency in Se in protection against atherosclerosis (Kardinaal *et al.* 1997).

A further factor to be taken into consideration when assessing these studies is that atherosclerosis is an inflammatory state and will provoke the acute-phase response to an extent related to its severity. Being an acute-phase reactant (Nicol *et al.* 1998), some fall in plasma Se concentration might be expected in subjects suffering from atherosclerosis, even before the occurrence of an event.

Other oxidative stress conditions. Other conditions involving oxidative stress and inflammation have shown

some association with Se status. Se behaves both as an antioxidant and an anti-inflammatory agent. This is because Se in its antioxidant role, notably as GPx, can: (1) reduce H₂O₂, lipid and phospholipid hydroperoxides, thereby dampening the propagation of free radicals and reactive oxygen species; (2) reduce hydroperoxide intermediates in the cyclo-oxygenase and lipoxygenase pathways, preventing the production of inflammatory prostaglandins and leukotrienes; (3) modulate the respiratory burst, by removal of H₂O₂ and superoxide (Spallholz *et al.* 1990).

Any condition associated with increased levels of oxidative stress or inflammation might be expected to be influenced by Se status. There is some evidence that this is the case in rheumatoid arthritis, pancreatitis, asthma and systemic inflammatory response syndrome.

In a case-control study nested within a Finnish cohort of 18 709 men and women who had no arthritis at baseline, the adjusted relative risk between the highest and lowest tertiles of serum Se was 0.16 (for trend, $P=0.02$) for rheumatoid factor-negative arthritis. There was no association for rheumatoid factor-positive rheumatoid arthritis (Knekt *et al.* 2000). In a double-blind randomised controlled trial in a small group of patients with rheumatoid arthritis, supplementation with 200 µg Se as Se-enriched yeast for 3 months, gave a significant reduction in pain ($P<0.01$) and joint involvement ($P<0.05$; Peretz *et al.* 1992). However, a later similar study by the same group found only an improvement in arm movements and health perception (Peretz *et al.* 2001). There is evidence for a protective effect of Se in pancreatitis, a condition associated with a high level of oxidative stress. At Manchester Royal Infirmary, Manchester, UK, administration of Se (600 µg/d) along with other antioxidants to patients with chronic and recurrent pancreatitis, significantly reduced pain and number of days spent in hospital ($P<0.001$ and $P<0.05$ respectively; McCloy, 1998). Treatment has been revolutionised by obviating the need for surgery for pancreatic pain (McCloy, 1998). Se has also shown benefit in acute pancreatitis. In a small controlled trial carried out in Rostock in Germany intravenous administration of Se to patients suffering from acute necrotising pancreatitis reduced mortality from 89% in controls to 0% in the treatment group (Kuklinsky & Schweder, 1996).

With regard to asthma, a protective relationship was found between dietary Se intake and asthma in adults in a large population-based case-control study in London (odds ratio per quintile increase 0.84; $P=0.002$; Shaheen *et al.* 2001). A suggestion of reduced risk was found in a nested case-control study of 7217 Finnish men and women (relative risk 0.64 (95% CI 0.31, 1.31)) between highest and lowest quartiles of serum Se (Knekt 2002). In a small nested case-control study, current wheeze among New Zealand children was found to be more common in those with low levels of Se in serum samples collected 8 years previously (odds ratio 3.1; Shaw *et al.* 1994). Another small study in intrinsic asthmatics showed significant clinical improvement ($P=0.042$) on daily supplementation with Se at 100 µg/d as Na₂SeO₃ (Hasselmark *et al.* 1993).

Although only relevant to extremely ill patients in the intensive treatment unit, some remarkable results were achieved in a trial of Se supplementation in systemic inflam-

matory response syndrome (Angstwurm *et al.* 1999). In a controlled randomised prospective open-label pilot study on forty-two patients with Acute Physiology and Chronic Health Evaluation II scores (a point score based on initial values of twelve routine physiological measurements, age and previous health status, to provide a general measure of severity of disease on admission to hospital; Knaus *et al.* 1985) of fifteen points on the day of admission, those given substantial doses of Na₂SeO₃ (maximum dose 535 µg/d) had significantly lower Acute Physiology and Chronic Health Evaluation III scores (which relate to follow-up during treatment and predict hospital mortality risk for critically ill hospitalised adults; Knaus *et al.* 1991) on days 7 and 14, ($P=0.018$ and $P=0.045$ respectively), lower mortality (33.5% v. 52%) and there was a significant reduction in the number of patients requiring haemodialysis as a result of acute renal failure ($P=0.035$).

Effects on the immune system

While deficiency has an adverse effect on immunocompetence, Se supplementation appears to enhance the immune response. In Se deficiency both cell-mediated immunity and B-cell function can be impaired (Spallholz *et al.* 1990). Thus, the delayed-type hypersensitivity skin reaction is significantly suppressed ($P<0.01$) in Se-deficient rats (Kukreja & Khan, 1998), while vaccination of Se-deficient animals is not followed by effective immunisation, showing the inability to mount an antibody response (Sanders, 1984). Furthermore, neutrophils and macrophages from Se-deficient animals show failure to kill ingested *Candida* yeasts (Turner & Finch, 1991).

In contrast, supplementation with Se, even in 'Se-replete' individuals, has been shown to have marked immunostimulant effects, including an enhancement of proliferation of activated T-cells, otherwise known as clonal expansion (Kiremidjian-Schumacher *et al.* 1994). Lymphocytes from subjects supplemented with Se (as Na₂SeO₃) at 200 µg/d, showed an enhanced response to antigen stimulation and an increased ability to develop into cytotoxic lymphocytes and destroy tumour cells. Natural killer cell activity was also increased. The supplementation regimen resulted in a 118% increase in cytotoxic lymphocyte-mediated tumour cytotoxicity and an 82% increase in natural killer cell activity as compared with baseline values (Kiremidjian-Schumacher *et al.* 1994).

The mechanism appears to be closely related to the ability of Se to up-regulate the expression of receptors for the growth regulatory cytokine interleukin 2 on the surface of activated lymphocytes and natural killer cells, thereby facilitating their interaction with interleukin 2. This interaction is crucial for clonal expansion and differentiation into cytotoxic T-cells. Results of this study indicate that even at so-called replete levels of plasma Se produced by normal dietary intake in the USA, i.e. 120–134 µg/l, supplementation with 200 µg/d Se has considerable immunoenhancing effects (Kiremidjian-Schumacher *et al.* 1994). These effects have been demonstrated by the same research group in patients undergoing therapy for head and neck cancer (Kiremidjian-Schumacher *et al.* 2000).

Se appears to be able to reverse the age-related decline in immune response in the elderly. In a group of twenty-two institutionalised elderly subjects supplemented with 100 µg Se-enriched yeast/d or placebo for 6 months, response to mitogen challenge in those receiving the Se was restored to the level of that in healthy young individuals (Peretz *et al.* 1991).

Additionally, cells of the immune system may have an important functional need for Se. Activated T-cells show up-regulated selenophosphate synthetase 2 activity (Guimaraes *et al.* 1996), directed towards the synthesis of selenocysteine, the essential building block of selenoproteins, demonstrating the importance of selenoproteins to activated T-cell function and the control of the immune response. The mRNA of several T-cell-associated genes (e.g. interleukin 2 receptor α -subunit, CD4) have the theoretical capacity to encode functional selenoproteins, suggesting that the roles of Se in the immune system may be more diverse than previously suspected (Taylor & Nadimpalli, 1999).

Viral infections

Se appears to be a key nutrient in counteracting certain viral infections. Elegant work by Beck *et al.* (1995) has shown that in a Se-deficient host harmless viruses can become virulent. When Se-deficient mice were inoculated with a benign strain of the coxsackie virus, B3/0, mutations occurred in the genome to give a cardiovirulent form of the virus which caused myocarditis with similarities to human pathology. Furthermore, when the virus recovered from these mice was inoculated into Se-adequate mice, it still induced appreciable heart damage, demonstrating the irreversibility of the mutation. In the case of the coxsackie virus six separate point mutations were identified with the development of virulence, causing myocarditis in the host. A similar study on mice unable to make GPxl (GPxl-knockout mice) demonstrated that this enzyme is essential for the avoidance of oxidative damage to the genome of the RNA virus, which results in the myocarditic mutations (Beck *et al.* 1998).

Coxsackie virus has been isolated from the blood and tissues of patients with Keshan disease and is thought likely to be a cofactor in the development of the cardiomyopathy that constitutes this condition (Beck *et al.* 1995, 1998). It seems probable, therefore, that human Se deficiency similarly affects the viral genome, resulting in the development of the heart pathology.

In more recent work the same group showed that Se-deficient mice infected with a mild strain of influenza virus, influenza A/Bangkok/1/79, developed much more severe and prolonged lung inflammation than infected Se-adequate mice (Beck *et al.* 2001). Passage through the Se-deficient animals caused mutations in the virus, which included twenty-nine nucleotide changes in the gene for the M1 matrix protein, an internal viral protein previously thought to be relatively stable. Beck *et al.* (2001) believe that the changes involve an oxidative-stress mechanism, as in the case of the coxsackie virus described earlier.

It seems clear that Se deficiency can have a profound impact on the genome of RNA viruses, contributing to the

emergence of new viral strains that may be capable of promoting epidemics (Beck *et al.* 2001). Other RNA viruses that may be similarly susceptible to mutations resulting in increased virulence are the common cold, HIV, Ebola (Beck *et al.* 2001), polio and hepatitis. These findings, therefore, have considerable public health implications.

Se seems to be a crucial nutrient for HIV-infected subjects. It is a potent inhibitor of HIV replication *in vitro* (Sappey *et al.* 1994). The progress of HIV can be thought of as being synonymous with the progressive loss of CD4+ T-helper cells. More than twenty papers report a progressive decline in plasma Se paralleling the ongoing loss of CD4+ T-cells in HIV-1 (Look *et al.* 1997). This process occurs even in the early stages of disease where malnutrition or malabsorption cannot be a factor (Look *et al.* 1997). In fact, plasma Se is a strong predictor of the outcome in HIV infection. Work carried out by Baum *et al.* (1997) at the University of Miami, Miami, FL, USA, showed that Se-deficient HIV-infected patients are 19.9 times more likely ($P < 0.0001$) to die from HIV-related causes than those with adequate Se levels. Se deficiency is defined by Baum *et al.* (1997) as a plasma level $\leq 85 \mu\text{g/l}$, a level not attained in many northern European countries (see p. 210), e.g. a mean level of $60 \mu\text{g/l}$ was found in a Scottish study (Shortt *et al.* 1997). Baum *et al.* (1997) showed that low plasma Se was significantly associated with mortality (relative risk 10.8, $P < 0.002$), and conferred a much more significant risk than a low helper T-cell count or deficiency of any other nutrient investigated. In HIV-infected children, low levels of plasma Se were significantly and independently related to mortality (relative risk 5.96, $P = 0.02$) and faster disease progression (Campa *et al.* 1999). Clinical trials are now in progress in HIV-infected subjects to investigate the effect of Se supplementation on survival, viral load, CD4 count, immune function and disease progression.

Se also appears to be protective in subjects infected with hepatitis-virus (B or C) against the progression of the condition to liver cancer (see pp. 208, 209; Yu *et al.* 1997; Yu *et al.* 1999).

It is notable that viruses may be capable of hijacking the Se supply of the host by incorporating Se into viral selenoproteins, thereby reducing the ability of the host to mount an effective immune response. Experimental evidence based on the work of Moss's group (Shisler *et al.* 1998) suggests that this process is possible in the case of the pox virus, molluscum contagiosum, which makes a homologue of GPx, and both theoretical and experimental evidence from Taylor's group (Zhang *et al.* 1999) suggests that the ability to make viral selenoproteins (such as GPx) is common to many human viral pathogens, such as HIV-1 and 2, coxsackie virus B3, hepatitis B and C, and the measles virus. Good Se status may protect against HIV progression by maintaining host immune competence and appropriate redox control.

Reproduction

Se has long been recognised in animal husbandry as being essential for successful reproduction (Underwood, 1977; Sanders, 1984). Idiopathic miscarriage has been shown to be associated with Se deficiency in veterinary practice (Stuart

& Oehme, 1982), while in sheep administration of Se supplements has been shown to prevent early pregnancy loss (Hidioglou, 1979). Investigating whether this role could also be relevant to man, Barrington *et al.* (1996, 1997) found significantly lower serum Se in women who suffered either first-trimester ($P = 0.0054$) or recurrent miscarriages ($P = 0.014$). These authors suggest that early pregnancy loss may be linked to reduced antioxidant protection of biological membranes and DNA by relatively low levels of the Se-dependent GPx. A subsequent study found lower Se levels in non-pregnant women suffering recurrent miscarriage than in controls, but the difference did not reach significance (Nicoll *et al.* 1999). However, the choice of control group can be criticised in this study, as it did not exclude women who had suffered a miscarriage.

Se is essential for male fertility, being required for testosterone biosynthesis and the formation and normal maturation and development of spermatozoa (Behne *et al.* 1996; Pfeifer *et al.* 2001). Se concentration of seminal plasma correlated positively with sperm count (R^2 0.15, $P > 0.001$) and total sperm concentration (R^2 0.21, $P > 0.001$) in a group of sub-fertile Norwegian men (Olderid *et al.* 1998). Animals fed Se-deficient diets show structural abnormalities in the sperm midpiece which are linked to poor motility and a tendency for the tail to break off (Wu *et al.* 1973), thus decreasing the chance of fertilisation. An explanation for these findings has recently been afforded by the work of Ursini *et al.* (1999). They found that a form of GPx (GPx4), believed to shield developing sperm cells from oxidative damage, polymerises in mature sperm into a structural protein in the mitochondrial capsule of the midpiece region. As GPx4 accounts for about 50% of the capsule material, it seems likely that it is this polymerisation that confers the structural integrity required for sperm stability and motility.

Work carried out at Glasgow Royal Infirmary, Glasgow, UK, supports this interpretation. In studies by Scott & MacPherson (1998) supplementation of subfertile men with $100 \mu\text{g Se/d}$ for 3 months significantly increased sperm motility ($P = 0.023$). Of the men receiving the active supplement 11% achieved paternity as compared with none in the placebo group. However, administration of double the quantity of Se to subfertile Polish men over a similar period showed no beneficial effect on sperm motility (Iwanier & Zachara, 1995).

Thyroid function

Although it is recognised that deiodinase activity is relatively protected in conditions of marginal Se availability (Sunde, 1997), there are some indications that, nonetheless, European levels of Se intake may compromise thyroid hormone metabolism. For example, values of the plasma triiodothyronine:thyroxine ratio in young Scottish subjects were as low as those normally found in elderly populations (JR Arthur, personal communication). Furthermore, Se supplementation in a small group of elderly subjects decreased plasma thyroxine levels, consistent with increased deiodinase activity and improved conversion to the active hormone, triiodothyronine (Olivieri *et al.* 1995). Similarly, a supplementation study carried out in Otago, New Zealand

showed that an additional 10–40 µg Se/d significantly decreased ($P < 0.05$) thyroxine concentrations over a period of 20 weeks, implying that the baseline intake of about 30 µg/d was limiting conversion of thyroxine to triiodothyronine (Duffield *et al.* 1999). When Se and I deficiency are combined, hypothyroidism is exacerbated and may manifest itself as myxoedematous cretinism, such as is seen in the Democratic Republic of Congo (Zaire) where deficiencies of both these minerals exist (Vanderpas *et al.* 1990).

Mood

To date, three published studies have linked Se deficiency to adverse mood states. There are a number of indications that Se is important to the brain: during Se depletion the brain receives a priority supply (Hawkes & Hornbostel, 1996); the turnover rate of some neurotransmitters is altered in Se deficiency (Castano *et al.* 1997); supplementation with Se reduced intractable epileptic seizures in children (Weber *et al.* 1991; Ramaekers *et al.* 1994) low plasma Se levels in the elderly were found to be significantly associated with senility and accelerated cognitive decline (odds ratio 1.58 (95 % CI 1.08, 2.31); Hawkes & Hornbostel, 1996; Berr *et al.* 2000); brain Se concentration in Alzheimer's patients was only 60 % of that in controls (Hawkes & Hornbostel, 1996). Furthermore, the brain is deficient in catalase (Halliwell & Gutteridge, 1999), thus peroxidation products such as H₂O₂ and primary peroxides must be removed by the antioxidant selenoenzymes.

A number of studies have shown a beneficial effect of Se status on mood, at least when Se status is 'marginal'. In three separate studies low Se status was associated with significantly greater incidence of depression and other negative mood states such as anxiety, confusion and hostility (Benton & Cook, 1991; Hawkes & Hornbostel, 1996; Finley & Penland, 1998). Mood was measured by use of a questionnaire, the 'Profile Of Mood States – Bipolar form'. The higher the score, the better the mood.

In a study carried out in the USA, Se depletion led to depressed mood and more hostile behaviour in those with low initial erythrocyte Se (Hawkes & Hornbostel, 1996). The lower the initial Se status, the more the mood scores decreased as a result of the low-Se diet. In a second US study, where subjects were fed either a low- or a high-Se diet for 15 weeks, those on the low-Se diet had significantly decreased clearheaded–confused and elated–depressed subscores ($P < 0.05$; Finley & Penland, 1998). The dietary Se intake on this low-Se diet was 32.6 µg/d, similar to current UK intakes of 34–39 µg/d (Ministry of Agriculture, Fisheries and Food, 1997, 1999).

In contrast to these findings, high dietary Se or supplementation with Se appears to improve mood. In the US study referred to earlier, those subjects on the high Se (226.5 µg/d) diet significantly improved in the clearheaded–confused, confident–unsure and composed–anxious subscores, and total mood disturbance was significantly less ($P < 0.05$; Finley & Penland, 1998). A similar finding was obtained in a double-blind crossover study carried out in the UK, where a 100 µg/d Se supplement significantly decreased anxiety, depression and tiredness ($P < 0.0001$;

Benton & Cook, 1991), the effect being most marked in those consuming lesser amounts of dietary Se.

The reasons for the effect of Se on mood are unclear, but a number of possibilities come to mind. As thyroid hormones play an important part in the regulation of mood and are involved to some extent in the pathophysiology of mood disorders (Joffe & Levitt, 1993), the effect could be mediated through the Se-dependent thyroid hormones. Alternatively, Se may help preserve the concentrations of certain neurotransmitters, or influence the level of fatty acids in the brain, either of which could influence mood.

The author is running a 500 subject trial on the effect of Se on mood and thyroid function, results of which should be available in 2002.

Cancer

There is growing evidence that higher Se intakes are associated with reduced cancer risk. Two-thirds of animal studies have shown a reduced incidence of tumours from cancer-causing chemicals or viruses with Se supplementation (Combs & Lü, 2001). Most epidemiological studies carried out since the 1970s have provided evidence of an inverse relationship between Se intake and cancer mortality (Combs & Gray, 1998).

In prospective studies published in the 1980s and early 1990s involving from 8000–11 000 subjects, low Se status was associated with a significantly increased risk of cancer incidence and/or mortality. Risk has been from twofold to sixfold higher in the lowest tertile or quintile (according to the study) of serum Se concentration, although in one case the effect was only apparent among males (Kok *et al.* 1987; Combs & Gray, 1998).

Studies carried out with the large Finnish Mobile Health Examination Survey cohort have shown a reduced risk of certain cancers, notably cancers of the stomach, pancreas and lung in men (Knekt *et al.* 1990, 1991). A later nested case–control study within a cohort of 9000 Finnish individuals showed the adjusted relative risk of lung cancer between the highest and lowest tertiles of serum Se to be 0.41 (Knekt *et al.* 1998). A much smaller study on lung cancer in Chinese tin miners did not show a significant association between Se and lung cancer incidence in prospectively-collected serum samples. However, median serum Se concentrations in both cases and controls in the latter study were very low (about 46 µg/l) raising the question of whether likely anti-cancer mechanisms of Se (see p. 210) could operate at these concentrations, especially in a cohort so highly exposed to cigarette smoke, As and Ra (Ratnasinghe *et al.* 2000). As is known to have the capacity to reduce the anti-cancer effect of some forms of Se (Combs & Lü, 2001).

In the case of hepatocellular carcinoma (HCC), a significant inverse association ($P = 0.01$) was shown between Se levels in stored plasma and later development of the disease in a cohort of 7342 Taiwanese men with chronic hepatitis-virus (B or C) infection, a noted risk factor for the development of this condition (Yu *et al.* 1999). However, evidence suggests that Se status may not be an important aetiological factor in breast cancer. In a Finnish study investigating the association between toenail Se and breast

cancer in 289 cases and 433 community controls, although prediagnostic Se concentrations were lower in the case group, the difference did not reach significance (Mannisto *et al.* 2000).

A prospective study published by Willett's group (Yoshizawa *et al.* 1998; also, for commentary, see Taylor & Albanes, 1998) involved 34 000 men from the Harvard-based Health Professionals' Cohort Study. Those participants in the lowest quintile of Se status, as measured by toenail Se, were three times more likely to develop advanced prostate cancer than those in the highest quintile (for trend, $P=0.03$). Only cases diagnosed >2 years after collection of the samples were counted. A similar case-control study on men from the Baltimore Longitudinal Study of Aging registry showed that after correcting for number of years before diagnosis, BMI and smoking and alcohol-use history, higher Se was associated with a lower risk of prostate cancer (Brooks *et al.* 2001). Compared with the lowest quartile of Se (range 82–107 µg/l), the odds ratios of the second (108–118), third (119–132) and fourth (133–182) quartiles were 0.15 (95% CI 0.05, 0.50), 0.21 (95% CI 0.07, 0.68) and 0.24 (95% CI 0.08, 0.77), respectively ($P=0.01$). Thus, in this study low plasma Se was associated with a four- to fivefold increased risk of prostate cancer.

There have been few intervention trials using Se as a single agent. A number of these trials have been carried out in China, where HCC is the third highest cause of cancer mortality. In the Qidong county, about 60 km north of Shanghai, the incidence of HCC is particularly high. In this region about 15% of adults carry the hepatitis B surface antigen and these individuals are 200 times more likely to develop HCC. In a study where 226 carriers of hepatitis B antigen were randomised to either 200 µg Se-enriched yeast or placebo, no case of HCC occurred in the supplemented group after 4 years, while seven subjects in the placebo group had developed HCC (Yu *et al.* 1997). In another study, table salt fortified with Se reduced HCC incidence in a Chinese township by 35% over a period of 6 years compared with control townships (Yu *et al.* 1997).

The Nutritional Prevention of Cancer (NPC) trial carried out by Clark *et al.* (1996) in the USA was the first double-blind placebo-controlled intervention trial in a Western population designed to test the hypothesis that Se supplementation could reduce the risk of cancer. In 1312 subjects with a history of non-melanoma skin cancer who were randomised to placebo or 200 µg Se (as Se-enriched yeast)/d, there was no effect on the primary end point of

non-melanoma skin cancer. However, those subjects receiving Se showed secondary end-point effects of 50% lower total cancer mortality ($P=0.002$) and 37% lower total cancer incidence ($P=0.001$), with 63% fewer cases of cancer of the prostate, 58% fewer cases of cancer of the colon and 46% fewer cases of cancer of the lung.

Analysis of treatment effect in different subgroups in the NPC trial has given some interesting results, although these must be treated with caution, as the numbers of events are low and the role of chance cannot be excluded. When subjects were divided into tertiles on the basis of initial plasma Se status, the strongest treatment effect was observed in subjects in the lowest tertile of plasma Se, i.e. those whose plasma Se concentration was <106 µg/l at entry to the trial (see Table 2; Rayman & Clark, 2000). Se supplementation reduced the risk of cancer in this tertile by 48%.

Plasma or serum Se concentrations (more or less equivalent) measured in the 1990s in a selected number of European locations are shown for comparison in Fig. 1. The upper level of the bottom tertile in the NPC trial is indicated on Fig. 1, and it is apparent that these locations fall well within that bottom tertile. Consequently, it might be predicted that a repeat of the NPC trial in these European locations would show a very marked treatment effect. (This is effectively what is planned in the Prevention of Cancer by Intervention with Se (PRECISE) trial, where Denmark, Sweden and the UK (where the author is cohort leader) will participate in an extended repeat of the NPC trial, recruiting equal numbers of men and women. PRECISE pilot studies are already well advanced.) NPC trial results also imply that an increase in Se intake to an 'appropriate' level has the potential to reduce cancer risk in many European countries.

Contrary to the effect seen in the bottom tertile, subjects in the top tertile at entry to the trial, whose plasma concentration was ≥ 121 µg/l, appeared to derive no additional reduction in risk from the supplementary Se. Combs (2001) suggests that the level of dietary intake (about 1.5 µg/kg body weight per d) required to achieve this plasma concentration (about 120 µg/l) might therefore be a target to aim for in order to minimise cancer risk.

Evidence that supra-nutritional levels of selenium may be beneficial

From the information cited earlier, it is clear that there are some cases where levels of intake above those believed to be required for repletion of the selenoenzymes (Alfthan *et al.* 1991; Thomson *et al.* 1993; Duffield *et al.* 1999), may give

Table 2. Total cancer incidence 1983–96 by plasma selenium level at baseline for subjects participating in the Nutritional Prevention of Cancer trial (from Rayman & Clark, 2000)

Baseline plasma Se (µg/l)*	Se group no. of cases†	Placebo group no. of cases	RR	95% CI	Statistical significance of difference between treatment groups: $P =$
<106	28	56	0.52	0.33, 0.82	0.005
106–121	34	49	0.64	0.40, 0.97	0.40
>121	45	41	1.00	0.65, 1.54	0.99

RR, relative risk.

*Se status at entry to trial.

† subjects received Se-enriched yeast (200 µg Se/d).

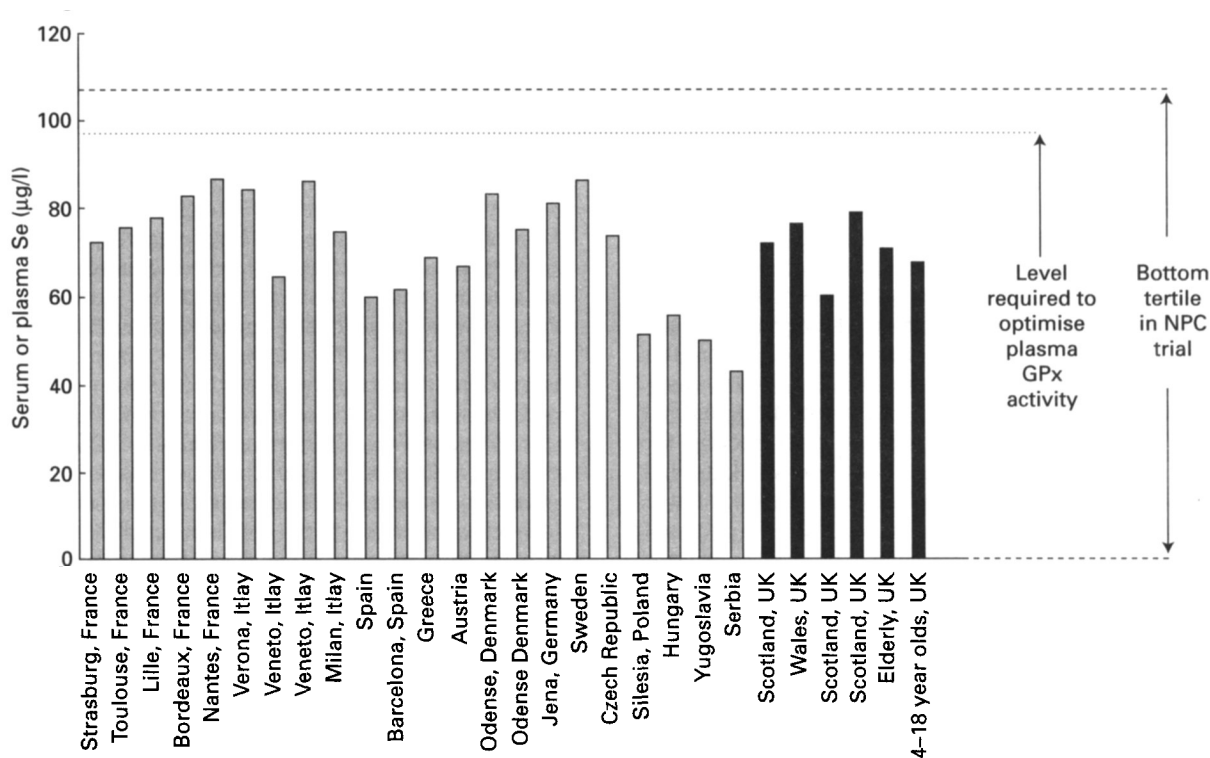


Fig. 1. Recent mean levels of serum or plasma selenium in Europe compared with (-----), bottom tertile in Nutritional Prevention of Cancer (NPC) trial of Clark *et al.* (1996); (.....), level required for optimal plasma glutathione peroxidase (GPx) activity (Alfthan *et al.* 1991; Thomson *et al.* 1993; Duffield *et al.* 1999).

additional health benefits. This situation appears to be valid for the immune system, where intakes of 200 µg/d in a Se-replete group of volunteers greatly magnified the production of cytotoxic T-cells and natural killer cells, thus enhancing cancer-protective capacity (Kiremidjian-Schumacher *et al.* 1994). Similarly, supplementation in the NPC trial was largely of subjects whose baseline Se intakes of about 90 µg/d (Clark *et al.* 1996) were such that their selenoenzyme activities, as represented by plasma GPx, would already have been maximal. In those subjects, an additional 200 µg Se/d was found to be effective in reducing cancer incidence and mortality. However, distinct selenoproteins may exhibit different expression patterns at high concentrations of Se, or when individuals are under environmental or genetic stress, so we cannot assume that selenoproteins, some of which may be unknown as yet, are not involved in cancer prevention (Gladyshev, 2001b). (Indeed, there is a suggestion that GPx2 may be important in protection from colon cancer and that the 15 kDa selenoprotein may protect against malignancy, particularly of the prostate (Wingler *et al.* 1999, 2000; Gladyshev, 2001b).)

It is clear from these studies that intakes of Se above the normal 'nutritional' range may confer further benefit. In the case of cancer, evidence suggests that these supra-nutritional intakes may further enhance the immune response and provide sufficient concentration of Se metabolites, such as methyl selenol, that can induce apoptosis of cancer cells, block cell division and inhibit tumour

neo-angiogenesis (Kaeck *et al.* 1997; Sinha & Medina, 1997; Ip, 1998; Jiang *et al.* 2000; Ghose *et al.* 2001; Fleming *et al.* 2001; for a review of anti-cancer mechanisms of Se, see Combs & Lu, 2001).

Selenium intake and status may be inadequate in many countries

Se enters the food chain through plants, but its incorporation is dependent not only on soil content and geochemistry but also on soil pH, rainfall, land contour and microbial activity (Diplock, 1993; Reilly, 1996c); some bacteria can convert insoluble forms of Se to soluble forms that can be taken up by the plant (Diplock, 1993). Availability of Se to populations in different parts of the world is thus highly variable, and is notably low in parts of China, New Zealand, parts of Eastern Europe, Russia and Africa (Combs, 2001). Tables of Se intakes in several countries from the 1980s onwards and of European intakes from the 1990s have been published by Combs (2001) and Rayman (2000) respectively.

The situation in much of Europe is giving some cause for concern, largely because of recent falls in Se intake (e.g. Denmark where intake fell from 51 to 42 µg/d over the 5-year period 1990–1995; Bro *et al.* 1990; Danish Governmental Food Agency, 1995). Mean UK daily intakes since 1974 are shown in Fig. 2. It can be seen that there has been a reduction in intake from 60–63 µg/d to the current level of 34–39 µg/d (Barclay *et al.* 1995; Ministry of Agriculture Fisheries and Food, 1997, 1999). The UK

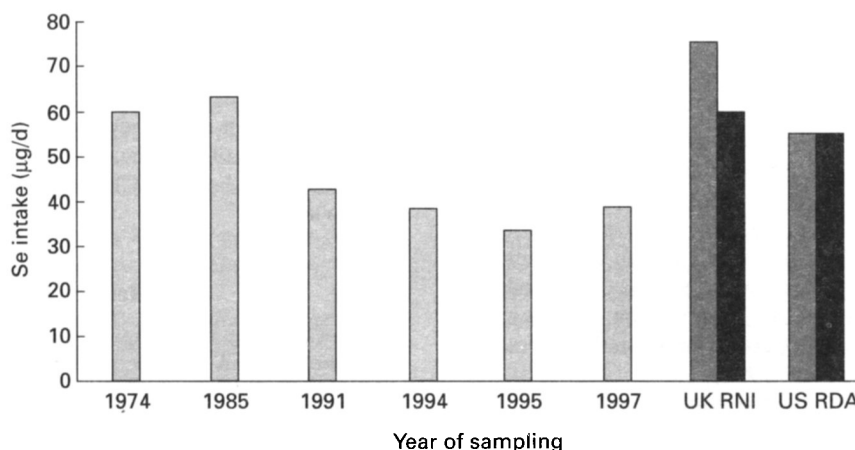


Fig. 2. Current and recent selenium intake in the UK (Ministry of Agriculture, Fisheries and Food, 1997, 1999). (▨), Male; (▩), female. RNI, reference nutrient intake; RDA, recommended daily allowance.

reference nutrient intakes for males and females (75 and 60 µg/d respectively; Department of Health, 1991) and the US recommended daily allowance (55 µg/d; Food and Nutrition Board, Institute of Medicine, 2000) are also shown. Clearly, our current intake does not meet either the reference nutrient intake or the recommended daily allowance. Many individuals will be aware that the fall in intake in recent years is believed to result from the reduced importation of North American (largely Canadian) wheat for bread-making (MacPherson *et al.* 1997). This wheat was high in Se but we imported it because it was high in protein (so-called hard wheat) and enabled the bread dough to rise satisfactorily. We now use lower-protein EU varieties and increase the protein content by adding gluten, a protein by-product of the starch industry, to help the dough to rise.

The reduction in Se intake in the UK has been paralleled by a reduction in Se status, as measured by serum, plasma or whole-blood Se (Rayman, 1997). Recent UK measurements, each on approximately 1000 subjects, from the National Diet and Nutrition Surveys of the elderly and of adults and children, found mean plasma Se to be 71 µg/l (in 1994–5) and 68 µg/l (in 1997) respectively (Gregory *et al.* 2000; Bates *et al.* 2002). Thus, current plasma or serum Se concentrations in the UK and many other European locations, as shown in Fig. 1, do not allow maximal expression of plasma GPx (Alfthan *et al.* 1991; Thomson *et al.* 1993; Duffield *et al.* 1999), the criterion for Se sufficiency adopted by most authorities when setting their dietary reference values.

Furthermore, in the light of the apparently beneficial effects of supra-nutritional intakes of Se described earlier, it may no longer be appropriate to rely on such a marker as an indicator of adequacy of Se intake. Not only, then, are many European locations failing to meet the standards of adequacy set according to antioxidant GPx activity, but the additional potential benefit of reducing cancer risk by intakes capable of raising our plasma Se to ≥ 120 µg/l (Combs, 2001) is presently quite unattainable (except by individual supplementation).

Increasing selenium intake is feasible

There are not many naturally rich sources of Se in European foods. Brazil nuts, kidneys and crab are the richest sources (Barclay *et al.* 1995), but are hardly likely to be eaten in any great quantity. Thus, increasing Se intake from normal food sources is difficult to achieve. Other solutions must be considered, one of which has already proved successful.

In order to safeguard the health of its population and to overcome Se deficiency diseases in domestic animals, Finland, since 1984, has been adding Se to its food supply by treating crops with fertiliser containing Na₂SeO₄. This action has altered Se intake from 38 µg/d per 10 MJ in 1984, before Se-enriched fertiliser treatment, to a maximum level of 124 µg/d per 10 MJ (Eurola & Hietaniemi, 2000). Plasma Se has varied from 87 to 121 µg/l, depending on the level of fertiliser application, which has been changed twice since 1984, being last altered in 1998 from 6 to 10 mg Se/kg fertiliser for all crops (Eurola & Hietaniemi, 2000). Se intake was measured as 65 µg/d per 10 MJ in 1999, while plasma Se concentration was determined as 97 µg/l in 2000 (Alfthan, 2001), although neither of these levels may yet have plateaued.

Careful monitoring of levels in foods, grains, soils and water, and of human Se status has been carried out during these years. There have been no adverse environmental effects; residual Se concentrations in areas under cultivation, surface waters and lake sediments have not changed (Eurola & Hietaniemi, 2000). The Finnish experience shows that, at least in the geochemical environment of Finland, it is possible to increase Se intake to attain the plasma concentration where plasma GPx is optimally active, or even to reach 121 µg/l, the plasma concentration above which supplementary Se gave no further reduction in cancer risk in the NPC trial (Rayman & Clark, 2000; Combs, 2001).

Fortification of foods with Se is another option. This process has already been satisfactorily achieved for the minerals Ca and Fe with which flour is fortified by statute. Clearly, great care would have to be taken to ensure that an appropriate form of Se was selected, and that a concen-

tration with a wide margin of safety was used, owing to the toxicity of Se. However, levels of intake required for toxicity are higher than those generally assumed; thus, for a 70 kg man, a 'no observed adverse effect' level of intake is about 1000 µg/d, with low adverse effects (hair loss and nail changes) appearing at about 2000 µg/d and toxicity (selenosis) at 6300 µg/d (µg/kg body weight per d; no observed adverse effect level 15, low adverse effect level 28, selenosis level 90; Whanger *et al.* 1996). Nonetheless, supplementation of fertilisers with Se, as practised in Finland, has the advantage that plants form a biological barrier that protects the target population from the effects of accidental overdose (Aro *et al.* 1998).

In the absence of government action in most countries, it has been a tempting proposition for manufacturers to develop functional foods containing Se. High-Se vegetables, beer, tea, mushrooms and mussels are described by Combs (2001). Korea has a chain of restaurants selling pork fed with organic Se, 'Selenpork' (Cole, 2000). A Se-enriched egg was developed at the Scottish Agricultural College, Edinburgh, UK (Surai *et al.* 2000). Interestingly, both the pork and the eggs have improved keeping qualities. Marks and Spencer (London, UK) has developed a range of foods, the 'and More' range, that contain 50 µg Se per serving. A feasible way to increase Se intake would be to fortify bread with Se, which has the virtue of being a 'staple'. The UK supermarket chain ASDA (Asda Stores Ltd, Leeds, UK) already sells a type of bread in its bakeries made from Canadian flour, which is a good source of Se. At least one bakery is selling Brazil-nut bread (The Village Bakery, Melmerby, Cumbria, UK).

Clearly there are many possibilities if the need is accepted, but a public health solution must be found which extends to the less-well-off, whose health needs are greatest.

Conclusion

Avoidance of deficiency diseases is no longer seen as an adequate goal for nutrition; we are now seeking to optimise our dietary intake so that we may postpone the onset of degenerative diseases and avoid long spells of incapacity in our older years. Brown & Arthur (2001) have suggested that chronic marginal intakes of Se may culminate in chronic diseases such as cancer, cardiovascular disease and increased susceptibility to viral infection. The health effects of Se described earlier reinforce this statement and provide a cogent argument for increasing the intake of Se. This level should be at least to that accepted as optimising selenoenzyme activity in countries such as the UK where such optimal activities are not now achieved. While it is clear that an intake to this level would be beneficial in some health conditions, others such as the immune function or cancer may require an additional 'supra-nutritional' intake, which may be about 200 µg/d. This crucial point can only be clarified when current or planned intervention trials have yielded results. PRECISE, Se and Vitamin E (prostate) Cancer Prevention Trial (SELECT) and the HIV and AIDS trials have the potential to reinforce or refute the argument for increasing Se intake to supra-nutritional levels.

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