High-selenium wheat: biofortification for better health

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Abstract

Selenium (Se) is an essential micronutrient for humans and animals, but is deficient in at least a billion people worldwide, and in some regions appears to be declining in the food chain. Wheat (*Triticum aestivum* L.) is a major dietary source of Se in many countries, including Australia.

The largest survey to date of Se status of Australians found a mean plasma Se concentration of 103 μg/l in 288 Adelaide residents, just above the nutritional adequacy level. In the total sample analysed (six surveys from 1977-2002; n = 834), plasma Se was higher in males and increased with age. This study showed that many South Australians consume inadequate Se to maximise selenoenzyme expression and cancer protection, and indicated that levels had declined around 20% from the 1970s.

No significant genotypic variability for grain Se concentration was observed in modern wheat cultivars, but the diploid wheat *Aegilops tauschii* L. and rye (*Secale cereale* L.) were higher. Grain Se concentrations ranged 5-720 μg/kg and it was apparent that this variation was determined mostly by available soil Se level.

Field trials, along with glasshouse and growth chamber studies, were used to investigate agronomic biofortification of wheat. Se applied as sodium selenate at rates of 4-120 g/ha Se increased grain Se concentration progressively up to 133-fold when sprayed on soil at seeding and up to 20-fold when applied as a foliar spray after flowering. A threshold of toxicity of around 325 mg/kg Se in leaves of young wheat plants was observed, a level that would not normally be reached with Se fertilisation. On the other hand sulphur (S) applied at the low rate of 30 kg/ha at seeding reduced grain Se concentration by 16%.

This study showed that, although Se concentration was highest in the embryo of wheat grain, Se and S were more evenly distributed throughout the grain, and hence a lower proportion was removed in the milling residue, than for other mineral nutrients. Post-milling processing of wheat, including baking and toasting, is unlikely to result in reduction of Se content.

Agronomic biofortification could be used by food companies as a cost-effective method to produce high-Se wheat products that contain most Se in the desirable selenomethionine

form. Further studies are needed to assess the functionality of high-Se wheat, for example short-term clinical trials that measure changes in genome stability, lipid peroxidation and immunocompetence. Increasing the Se content of wheat is a food systems strategy that could increase the Se intake of whole populations.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

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1. Literature Review

1.1 Introduction

A review of literature on selenium (Se) in soils, plants, animals and humans is presented. The importance of Se in human health is discussed, followed by Se intake by humans, with a focus on Australia. Strategies to increase Se intake are discussed briefly. The review then examines wheat as a major source of bioavailable Se, and discusses Se fertilisation and plant breeding, two strategies to increase Se density in wheat grain. The issue of phytotoxicity to young wheat plants is also addressed.

1.1.1. Selenium: the essential metalloid

Berzelius, the Swedish chemist who discovered Se in 1817, recognised it to be an unusual element with properties intermediate between the metals and the non-metals. He named it after the Greek word for the moon, to distinguish it from tellurium, a similar element named after the earth.

Se has an atomic weight of 78.96 and an atomic number of 34. It lies between sulphur and tellurium in Group 6A and between arsenic and bromine in Period 4 of the Periodic Table. This position accounts for many of its biological relationships with sulphur, arsenic and phosphorus. Like sulphur, Se can exist in five valence states: selenide (-2), elemental Se (0), thioselenate (+2), selenite (+4; eg Na_2SeO_3) and selenate (+6; eg Na_2SeO_4). Se forms many inorganic and organic compounds that are similar to those of sulphur (Greenwood & Earnshaw, 1984).

Se may substitute for sulphur in methionine to form selenomethionine (Lockitch, 1989), which is the main form of Se in food (Levander, 1983). Inorganic forms (selenite and selenate) are generally only included in the diet through supplements. The volatile Se forms are dimethyl selenide, dimethyl diselenide and hydrogen selenide (Lauchli, 1993).

Se is a relatively rare element, 70th in abundance of the 88 elements that comprise the earth's crust (Nebergall et al, 1968). However, despite this rarity, Se plays important roles in animal and human nutrition. As a co-factor in selenocysteine, the 21st amino acid, Se is

a component of selenoproteins, which have important enzyme functions (Sunde, 1997). As Reilly observes: "Without it, neither humans nor any other animal could develop properly or survive for long" (Reilly, 1996, 1).

Until it was recognised as an essential nutrient for humans (Schwarz & Foltz, 1957), Se was known mainly for its toxicity, and was considered a carcinogen, which resulted in objections by the US Food & Drug Administration to its use as an additive in livestock feed (Oldfield, 1981). It is thus ironic that Se now arouses most interest as an anticarcinogen. Nevertheless, Se was recognised by several researchers for its possible therapeutic use in cancer treatment from the early 20th century (Schrauzer, 1979).

1.1.2. Selenium in soils: ubiquitous and variable

The ultimate source of all Se is the rocks and soils of our terrestrial environment, in which it is ubiquitous but enevenly distributed. Soil concentrations range from less than 0.1 to more than 100 mg/kg; however, most soils contain between 1.0 and 1.5 mg/kg (Berrow & Ure, 1989). Soil Se level is determined mainly by the nature of the parent rock: highly siliceous rocks such as granite produce low-Se soils, whereas shales and coals may contain high levels of Se (Thornton et al, 1983). Soils which are high in organic matter, such as peats, may also be high in Se (Fleming, 1962).

In general, total soil Se of 0.1 to 0.6 mg/kg is considered deficient. Soils in New Zealand, Denmark, Finland (pre-1984, before Se was added to fertilisers), central Siberia, and a belt from north-east to south-central China are notably Se-deficient and hence have sub-optimal levels in their food systems (Combs, 2001; Gupta & Winter, 1975; Lag & Stiennes, 1978). Large areas of Africa, including much of Zaire, are also likely to be Se-deficient, but further mapping is required. On the other hand, parts of the Great Plains of the USA and Canada, Enshi County in China, and parts of Ireland, Colombia and Venezuela are seleniferous (Combs, 2001).

In acidic, poorly aerated soils, Se is poorly available to plants and occurs as insoluble selenides and elemental selenium. In lateritic soils, which have a high iron content, it binds strongly to iron to form poorly soluble ferric hydroxide-selenite complexes (Cary & Allaway, 1969). Elemental Se, though stable in soils, can be slowly oxidised, particularly at high pH (Geering et al, 1968). Se is available in aerated acid or neutral soils where

selenites form, and in aerated alkaline soils in the selenate form (Cary & Allaway, 1969). In certain soils, organic Se may be oxidised to available forms (Shrift, 1964).

Selenates are highly soluble and easily taken up by plants. Selenate ions do not form stable adsorption complexes or co-precipitate with sesquioxides (Ullrey, 1981). In the low rainfall seleniferous areas of South Dakota, selenate is highly available and in certain species of accumulator plants, for example *Astragalus,* can build up to levels which are toxic to animals (Oldfield, 1993). In wetter regions, selenate can be leached from the soil, resulting in selenium-deficient areas, for example New Zealand and Tasmania (Reilly, 1996). The availability of soil Se to crops can be affected by irrigation, aeration, liming and Se fertilisation (Gissel-Nielsen, 1998).

Australia has both high- and low-Se soils and large areas that have not been mapped for the element. Seleniferous soils occur in the Tambo Formation of central Queensland (McCray & Hurwood, 1963), where a limestone shale supports the growth of Se accumulators, and at several locations on Cape York Peninsula (Judson & Reuter, 1999). Se deficiency in Australia usually occurs on acidic soils which receive more than 500 mm rain per year, such as the Central and Southern Tablelands and Slopes (Plant, 1988) and the Northern Tablelands (Langlands et al, 1981) of New South Wales, the south-eastern coast of Queensland, associated with tertiary volcanic soils (Noble & Barry, 1982), the archaean granite soils of south-west Western Australia (Godwin, 1975), coastal and central regions of Victoria (Hosking et al, 1990), and much of Tasmania's arable land (Judson & Reuter, 1999).

In South Australia, Se deficiency occurs in sheep grazing pastures on lateritic soils, mainly in the Mount Lofty Ranges and on Fleurieu Peninsula and Kangaroo Island. Available Se is low because the soils are low in pH and high in iron oxides, rainfall is high (500-800 mm p.a.) and waterlogging is common in winter (Reuter, 1975). Moreover, annual applications of sulphate-containing low-Se superphosphate can reduce available Se in acid soils (Jones & Belling, 1967). Acidic "sand-over-clay" soils in the South-East of South Australia also tend to be low in Se (Reuter, 1975).

1.1.3. Selenium in plants: important source for animals and humans

1.1.3.1. Se levels in plants and their effects

The Se contents of plants vary according to available soil Se and species. For example, wheat grown in Shaanxi Province, China may have 0.003 mg/kg Se in the grain, compared to 2.0 mg/kg for wheat from the North or South Dakota wheatlands (Combs, 2001). Wheat from highly seleniferous areas of South Dakota may contain up to 30 mg/kg Se (University of California, 1988), while *Astragalus* on the same soils may accumulate up to 15,000 mg/kg (Beath, 1937).

Among non-accumulators, certain crucifers, especially brassicas (including broccoli), as well as soybeans contain more Se than other crops grown under the same conditions (Gupta & Gupta, 2000). Although lower plants such as algae require Se for growth (Lindstrom, 1983), it is not considered to be an essential nutrient for higher plants (Terry et al, 2000), although previous studies to ascertain essentiality failed to account for volatile Se compounds (Broyer et al, 1966). Low levels of soil Se do not appear to inhibit plant growth or crop yields. However, plants contain Se-dependent glutathione peroxidases, and Se deprivation has been shown to reduce the growth of wheat (Peng et al, 2000) and rice (Zhou, 1990), and increase sensitivities of ryegrass and lettuce to ultraviolet B radiation (Xue & Hartikainen, 2000). The question of the essentiality of Se for higher plants shall be examined further below.

Se phytotoxicity is variable: in rice, 2.0 mg/kg DW Se can cause a 10% yield reduction, whereas in white clover 330 mg/kg is required for the same yield reduction (Mikkelson et al, 1989). Incorporation of seleno-amino acids into proteins can lead to dysfunctional enzymes. In particular, the altered tertiary structure of a selenocysteine-substituted protein inhibits catalytic activity (Brown & Shrift, 1982), and is thus a major cause of Se toxicity.

1.1.3.2. Factors that effect Se uptake by plants

High soil sulphate level decreases uptake of selenate (Hopper & Parker, 1999). This antagonism is probably due to competition between sulphate and selenate for absorption by plant roots rather than the reduction by sulphate of selenium solubility in the soil (Cary & Gissel-Nielsen, 1973), as sulphate and selenate use the same transporter (Breton & Surdin-Kerjan, 1977).

Rhizosphere processes play an important role in the availability of Se for plant uptake. In particular, ascorbic and gallic acids and manganese oxides can increase oxidation of selenite to selenate (Blaylock & James, 1994). In soils with low availability of inorganic Se, organic Se may provide an important source of available Se, and selenomethionine is actively absorbed by wheat plants (Abrams et al, 1990). On the other hand, trimethylselenonium, an important urinary Se metabolite, is not absorbed by wheat (Olson et al, 1976).

Refer to Section 2.7.2b below for further discussion of the effect of plant nutrients on Se uptake.

1.1.3.3. Short-distance transport

In higher plants, selenate influx occurs actively via the sulphate transporter, a high-affinity permease. Sulphate transporters belong to two classes, with either a high or a low affinity for sulphate. The high-affinity transporter, the more important one for sulphate uptake, is expressed primarily in the roots, whereas the low-affinity transporter is expressed in both shoots and roots (Smith et al, 1995, 1997). A gene (HVST 1) encoding the sulphate transporter has been isolated in barley (Smith et al, 1997). The transporter genes exhibit a high degree of sequence conservation with other sulphate transporters cloned from animals and micro-organisms. The expression of transporter genes is regulated by the sulphur status of the plant, glutathione and the cysteine precursor, O-acetylserine. High levels of sulphate and glutathione decrease transcription, whereas high levels of O-acetylserine increase transcription. Therefore, increasing O-acetylserine levels can increase selenate uptake (Terry et al, 2000).

Influx of selenite is usually much slower than that of selenate and it appears to be a passive process that does not involve membrane transporters, although it is partly affected by metabolic inhibitors (Shrift & Ulrich, 1976). On the other hand it is clear that plants can take up organic Se forms actively. It was found that selenomethionine uptake by wheat seedlings followed Michaelis Menten kinetics and was coupled to metabolism (Abrams et al, 1990).

Plants can also absorb volatile Se from the atmosphere via the leaves, although this is minor compared with other routes. The major volatile Se form is dimethyl selenide (Zieve & Peterson, 1984).

1.1.3.4. Long-distance transport and storage

As with absorption, transport and storage of Se by plants varies with Se form. Selenate is transported via the xylem to chloroplasts in leaves where it is reduced and the Se converted to organic forms, some of which are volatile. Selenate is transported more easily from root to shoot than is selenite or organic Se (Terry et al, 2000). The reason for selenite's poor translocation may be because it is rapidly converted to organic Se forms, which are stored in the roots (Zayed et al, 1998). Using broccoli, Indian mustard, sugarbeet and rice, Zayed et al (1998) found that shoots of selenate-supplied plants accumulated the greatest amount of Se, followed by shoots supplied with selenomethionine, then selenite. In roots, the highest Se concentrations were reached with selenomethionine, followed by selenite, then selenate.

In maize, Se is transported in the xylem as amino acids when Se is supplied to the plant as selenite, but remains unchanged during transport when supplied as selenate (Gissel-Nielsen, 1979). However, in barley and ryegrass, Se form and method of application do not affect the proportions of the selenium fractions of selenite, selenate, seleno-amino acids or protein-bound Se in the barley grain or the ryegrass leaves (Gissel-Nielsen, 1987). Sulphur retranslocates in the phloem via S-methylmethionine, produced from methyltransferase, and it is likely that Se is transported likewise (Gissel-Nielsen, 1979); however, more research is required to elucidate the mechanism of Se transport in the phloem.

Selenate assimilation generally follows that of sulphate. Plants store Se in protein as seleno-amino acids, and thus plants and their components which are higher in protein are usually higher in Se (Peterson & Butler, 1962). In high-sulphur crops, eg broccoli, most Se is present as Se-methylselenocysteine (Whanger, 2002). The DNA codon sequencing UGA appears to have the dual function of termination codon and codon for selenocysteine in all groups of organisms, and this is why selenocysteine has been included as the 21st amino acid (Lauchli, 1993). Se accumulators convert selenocysteine into selenomethylcysteine, rather than incorporate it into proteins (Brown & Shrift, 1982; Whanger, 2002).

1.1.4. Selenium in animals: more likely too little than too much

Twentieth century agricultural scientists discovered that Se was responsible for losses to farmers in areas where the element occurred in excess in the soil, and later recognised that Se deficiency in agricultural soils was a more widespread and serious problem than toxicity (selenosis) (Reilly, 1996).

Selenosis has not been a major problem for livestock producers except in the USA, where it was known to farmers as *alkali disease*, a reflection of the soil types on which it occurred. It caused growth reduction, and shedding of hair and hooves. The underlying mechanisms of this toxicity are still poorly understood but may involve interference with enzyme activity, particularly by blocking the function of SH groups involved in oxidative metabolism within cells (Martin, 1978). Today, although Se's toxicity cannot be forgotten, animal and human studies focus on its nutritional and disease prevention roles.

Since Se is an essential nutrient, animals respond positively to it where their diet would otherwise be deficient, that is where it contains less than 0.1 mg/kg Se in dry matter (Oldfield, 1993). Conditions which are related to Se deficiency, some of which occur on a wide scale in certain countries, include white muscle disease, exudative diathesis, pancreatic degeneration, liver necrosis, mulberry heart disease, and ill-thrift. These conditions may respond also to vitamin E, but not as strongly as to Se, and it should be noted that Se deficiency is usually not the only cause of these diseases (Reilly, 1996).

White muscle disease is a myopathy of skeletal and heart muscle which can affect lambs, calves, horses, goats, poultry, rabbits, deer and rats (Oldfield, 1990). It is the most economically important Se-deficiency condition of livestock. It was estimated in 1960 that around 30% of New Zealand's sheep flock (i.e. around 15 million sheep) were at risk of developing Se-deficiency conditions, including white muscle disease (Wolf et al, 1963). Pathogenesis of the disease involves an increase in lipid peroxidation in muscle (Kennedy et al, 1993).

Exudative diathesis in chicks and piglets is often caused by rations of low-Se grain. It causes oedematous muscles, reduced growth rate and eventual death, and often occurs concurrently with white muscle disease and pancreatic degeneration (Andrews et al, 1968; Bains et al, 1975; Salisbury et al, 1962).

In mulberry heart disease, two- to four-month old piglets born of Se- and vitamin E-deprived sows develop extensive cardiac haemorrhage and connective tissue lesions, which may result in sudden death (Shamberger, 1981).

Se deficiency can also cause ill-thrift, which, prior to extensive Se supplementation, affected large numbers of sheep and cattle grazing improved pastures in New Zealand and Australia (McDonald, 1975). Ill-thrift may manifest as reduced fertility (Underwood, 1977), retained placenta in cows (Mayland, 1994) or impaired immune response: in the absence of protection by adequate levels of glutathione peroxidase, the free radicals which are produced by neutrophils to kill ingested foreign organisms can damage the neutrophils themselves (Arthur et al, 1981).

Various methods are used to supplement Se in livestock, including application as a fertiliser to pastures, free choice supplementation, and direct administration to animals. Se supplementation is practised widely in Finland, Sweden, New Zealand, Australia, the United Kingdom, Canada and the United States (Oldfield, 1990; Reilly, 1996).

1.2. Selenium: essential for human health

1.2.1. Introduction

Since Se was recognised as an essential nutrient by Klaus Schwarz (Schwarz & Foltz, 1957), a voluminous literature has accumulated which describes and examines the profound effect of this element on human health. It is beyond the scope of this literature review to examine in depth the many roles of Se in the human body. Moreover, several comprehensive reviews have been published, including those of Reilly (1996), Rayman (2000, 2002) and Combs (2001). Combs examines Se in humans within a food systems context and makes the distinction between Se's normal metabolic roles and its anticarcinogenic activity at supra-nutritional levels. This review shall summarise the known roles of Se in the body, briefly examine variability in global intakes and blood levels, look at toxicity and then focus on selenium intake in Australia.

1.2.2. Selenoprotein activities

Twenty-five mammalian selenoproteins have been discovered to date; however, the functions of most of these are yet to be determined (Kryukov et al, 2003). Selenoproteins have important enzyme functions in humans (Sunde, 1997) and contain selenocysteine. At least four steps are required for the synthesis of selenocysteine, starting with the tRNA for serine (Kryukov et al, 2003). Glutathione peroxidase (of which at least five forms exist) has an antioxidant role in reducing damaging hydrogen peroxide and lipid and phospholipid hydroperoxides which are present in all cells. This function reduces damage to lipids, lipo-proteins and DNA, and hence reduces risk of cardiovascular disease and cancer (Diplock, 1994; Neve, 1996). Glutathione peroxidase also protects vitamin E, a lipid-soluble antioxidant. Other known selenoenzymes are the iodothyronine deiodinases, thioredoxin reductases and selenophosphate synthetase (Allan et al, 1999).

The thioredoxin reductases are involved in reduction of nucleotides in DNA synthesis, regeneration of antioxidant systems, and maintenance of intracellular redox state (Allan et al, 1999). The iodothyronine deiodinases catalyse the production of active thyroid hormone, T3, from thyroxine, T4 (Beckett et al, 1987). Selenophosphate synthetase is necessary for the synthesis of selenophosphate, the selenocysteine precursor (Allan et al, 1999).

Other selenoproteins have been identified, but their exact metabolic roles remain unclear. These include selenoprotein P (found in plasma; appears to protect endothelial cells from peroxynitrite) (Arteel et al, 1999), selenoprotein W (needed for skeletal and cardiac muscle function) (Vendeland et al, 1995), sperm mitochondrial capsule selenoprotein (required for stability and motility of sperm) (Karimpour et al, 1992; Ursini, 1999), and prostate epithelial selenoprotein (redox function which may protect glandular cells against cancer) (Behne et al, 1997).

1.2.3. Selenium deficiency diseases

In parts of China and eastern Siberia two overt Se-deficiency diseases occur: Keshan disease, a cardiomyopathy which bears similarities to white muscle disease of sheep and cattle, and Kaschin-Beck disease, a deforming arthritis. Keshan disease occurs mainly in children and women of child-bearing age. It involves reduction of cardiac function,

cardiac enlargement and arrhythmia (Reilly, 1996). The disease's etiology is likely to be complex, involving Se and vitamin E deficiencies, and presence of the Coxsackie B virus (Levander & Beck, 1999; Yang et al, 1994). In the 1940s the case-fatality for Keshan disease in China was over 80%. The incidence of the disease has been reduced markedly by the use of selenite tablets and selenite-fortified salt (Yang et al, 1984). Other factors involved in this improvement include improved sanitation and medical infrastructure, and a more varied diet (Reilly, 1996).

Kaschin-Beck disease (KBD) is an osteoarthropy which manifests as enlarged joints, shortened fingers and toes, and in severe cases dwarfism. Se and vitamin E deficiency (Reilly, 1996) and iodine deficiency (Neve, 1999) are likely to be predisposing factors rather than a primary cause. Proposed causative factors include fulvic acids in drinking water (Peng et al, 1999) and mycotoxins in food (Xiong et al, 1998).

1.2.4. Immune function

Se has a role in many aspects of the immune response to infections, and its contribution to the integrity of the immune system is a major feature of its nutritional role (Reilly, 1996). Nutritional Se deficiency has been shown to impair the *in vitro* ability of neutrophils and macrophages to kill ingested cells of the yeast *Candida albicans* (Boyne & Arthur, 1986). Depressed oxidative metabolism in polymorphonuclear leukocytes (blood phagocytes which can leave the blood and migrate to extravascular sites of infection and inflammation) caused by Se deficiency has been shown to be associated with defective killing ability of these cells (Dimitrov et al, 1984). Furthermore, the mRNAs of several T-cell-associated genes have the ability to encode selenoproteins, which indicates that Se may have more roles in the immune system than previously thought (Taylor & Nadimpalli, 1999).

1.2.5. Thyroid function

The thyroid gland has the highest Se concentration of any human organ (Kohrle, 1999), and Se is involved in thyroid metabolism, notably through the catalysis of the T4 to T3 conversion by iodothyronine 5-deiodinase. The iodine deficiency diseases goitre and myxedematous cretinism are more prevalent in central Africa in those regions which are deficient in both iodine and Se (Vanderpas et al, 1993), and in such areas supplementation

with both nutrients is indicated. Moreover, a thyroid response has been found in a sample of elderly Westerners, where supplementation with Se resulted in enhanced conversion of T4 to T3 (Olivieri et al, 1995).

1.2.6. Cancer

There is "perhaps no more extensive body of evidence for the cancer preventive potential of a normal dietary component than there is for selenium" (Combs & Gray, 1998, 186). From the late 1960s, epidemiological studies have suggested an inverse association between human Se intake and cancer mortality (Combs & Gray, 1998). Cancer mortality in different counties in the US was found to be inversely related to the distribution of Se in soils and forage crops (Clark et al, 1991), and in Schrauzer's 27-country study dietary Se intake was inversely associated with total age-adjusted cancer mortality (Schrauzer et al, 1977). Although some of the Se-cancer epidemiological studies have design flaws, are subject to confounding, and have low numbers of certain cancers with consequent lack of statistical power, the evidence remains compelling (Lyons, 2000).

An extensive literature documents the numerous *in vitro* and animal studies which have been conducted during the past 35 years. Most demonstrate that application or intakes of Se at supra-nutritional levels can inhibit tumorigenesis (Combs & Gray, 1998; El-Bayoumy, 1991; Ip, 1998). For example, a study of pulmonary metastases of melanoma cells in mice found that 2 ppm of selenite in the diet reduced the median number of lung tumours from 53 in controls to one (Yan et al, 1997).

Prospective cohort and case-control studies that have involved up to 34,000 people have generally shown an association between low Se status and a significantly higher risk of cancer incidence and mortality (Brooks et al, 2001; Knekt et al, 1998; van den Brandt et al, 1993; Willett et al, 1983; Yoshizawa et al, 1998; Yu et al, 1999). For example, Yoshizawa et al (1998) found that men in the lowest quintile of Se level were three times more likely to develop advanced prostate cancer than those in the highest quintile.

Intervention studies using Se as a single chemopreventive agent include the Qidong trials in China, where selenite significantly reduced primary liver cancer (Yu et al, 1997). In the Nutritional Prevention of Cancer (NPC) trial in the USA, 200 μg Se/day (as yeast) reduced total cancer mortality by 41%, total cancer incidence by 25% and prostate cancer

incidence by 52% in a cohort of 1,300 people. The effect on total cancer was limited to male smokers (current or previous) with baseline Se levels below 113 μg/l, although non-smoking males below this level are likely to have benefited from Se supplementation in terms of prostate and colon cancer protection (Duffield-Lillico et al, 2002).

Current, larger Se-cancer chemoprevention trials include the PRECISE trial, with 50,000 participants, and the PINS trials investigating prostate cancer, and which include vitamin E in certain treatment groups (Lee & Fair, 1999; Nelson et al, 1999; Rayman, 2000).

The NPC trial was conducted in a region of the US where Se intakes are estimated to be around 90 μg/day, well above the level required for optimal selenoenzyme activity (Thomson et al, 1993). This suggests additional mechanisms in Se's cancer preventive role. While some cancer protection, particularly that involving antioxidant activity, involves selenoenzymes, the anti-cancer effect of Se is likely to involve the production of specific anti-tumorigenic metabolites, notably methylselenol, which neutralise carcinogens, enhance the immune system, alter gene expression, inhibit tumour cell metabolism and neo-angiogenesis (blood vessel development around tumours), and promote apoptosis (programmed cell death) (Combs & Gray, 1998; Combs, 2000, 2001; Finley & Davis, 2001; Harrison et al, 1997; Ip et al, 1991; Jiang et al, 1999; Lu, 2000; Rayman, 2000; Seo et al, 2002).

According to this two-stage model of cancer prevention, which involves Se intakes that correct nutritional deficiency as well as much higher, supranutritional intakes, individuals with nutritionally adequate Se intakes may benefit from Se supplementation (Combs & Gray, 1998). Se's anti-cancer activities remain under intensive study worldwide.

1.2.7. Viral diseases

Se deficiency is associated with increased virulence of a range of viral infections (Taylor, 1997). The association of Keshan disease with Coxsackie B virus was noted above. It is evident that in a Se-deficient host, normally harmless viruses can become virulent. For example, when Se-deficient mice are inoculated with benign Coxsackie B3 virus, the virus mutates into a virulent form that causes myocarditis similar to that seen in Keshan disease (Beck et al, 1995, 1998), and mortality increases significantly if the virus is co-administered with mercury, a Se antagonist (South et al, 2001).

Se appears to be a particularly important nutrient for people with HIV. It strongly inhibits HIV replication *in vitro*, inhibits viral cytotoxic effects and the reactivation of HIV-1 by hydrogen peroxide, and inhibits necrosis factor kappa alpha and beta, which are important cellular activators of HIV-1 (Look et al, 1997; Sappey et al, 1994). Moreover, Se deficiency is a significant predictor of HIV-related mortality (Baum & Shor-Posner, 1998; Campa et al, 1999) and viral load (Baeten et al, 2001). It was found that Se-deficient HIV patients are nearly 20 times more likely to die from HIV-related causes than those with adequate levels (Baum et al, 1997). The decline in blood Se levels occurs even in the early stages of the disease and is thus unlikely to be due to malnutrition or malabsorption (Look et al, 1997).

Se also appears to be protective in people infected with hepatitis B or C against progression to cirrhosis and liver cancer (Yu et al, 1997; Yu et al, 1999). Selenoproteins encoded by HIV, hepatitis C virus and the Ebola virus (which causes acute haemorrhage) have been discovered that consume the host's Se supply, thus reducing immune response. Adequate Se status protects against viral progression by maintaining host immunocompetence and intracellular redox control: the cell is likely to remain alive and the virus replicates at low levels. However, under Se deficiency, increased oxidative stress activates the virus, which increases replication to escape the dying cell (Taylor & Nadimpalli, 1999; Zhao et al, 2000).

Given the high global incidences of HIV, hepatitis B and C, and other RNA viruses, including measles and influenza (new strains of which regularly evolve in China, with its Se-deficient belt), the public health implications of the above findings are enormous.

1.2.8. Cardiovascular disease

Damage to the vascular epithelium by reactive oxygen species causes various cardiovascular diseases, including atherosclerosis. This damage can be prevented by Se (Hara et al, 2001). Several epidemiological studies also suggest a protective effect of selenium against cardiovascular disease (Bjorksten, 1979; Salonen et al, 1982; Suadicani et al, 1992), although the effect may only be apparent in populations of low Se status.

Glutathione peroxidase is required for the metabolism of hydroperoxides produced in eicosanoid synthesis by the lipoxygenase and cyclo-oxygenase pathways (Spallholz et al,

1990). It also reduces total cholesterol, triglycerides, free fatty acids and low density lipoproteins in the serum (Iizuka et al, 2001). Further evidence for a protective role of Se against cardiomyopathies is provided by the finding that Se deficiency aggravates the effects of lipid peroxides (Neve, 1996; Schoene et al, 1986; Warso & Lands, 1985), and is a major risk factor for Keshan disease and white muscle disease, discussed above. Moreover, cardiomyopathy associated with selenium deficiency in parenteral nutrition is well recognised as a potential problem in hospital patients (Reilly, 1996).

1.2.9. Other oxidative stress/inflammatory conditions

There is a growing body of evidence to suggest that Se (especially in the sodium selenite form) can alleviate conditions associated with high levels of oxidative stress or inflammation. These include asthma (Greene, 1995; Hasselmark et al, 1993; Misso et al, 1996; Shaheen et al, 1999), diabetes (Douillet et al, 1998; Kowluru et al, 2001; Kruse-James & Rukgouer, 2000; Naziroglu & Cay, 2001; Stapleton, 2000), arthritis (Knekt et al, 2000; Peretz et al, 2001; Rosenstein & Caldwell, 1999), muscular dystrophy (Kurihara et al, 2000; Passwater, 1980), cystic fibrosis (Kauf et al, 1994; Wallach & Garmaise, 1979), pancreatitis (Burney et al, 1989; De las Heras Castano et al, 2000; Kuklinski et al, 1995; McCloy, 1998; Uehara et al, 1988; Van Gossum et al, 1996), osteoarthritis (Kurz et al, 2002), systemic inflammatory response syndrome (Angstwurm et al, 1999) and kwashiorkor (Ashour et al, 1999; Golden et al, 1985; Hopkins & Majaj, 1967; Levine & Olson, 1970; Schwarz, 1961).

1.2.10. Fertility

Low Se status has long been known to reduce fertility in livestock (Underwood, 1977), and this also appears to be the case for humans. Low Se levels have been associated with male infertility (Se is required for testosterone biosynthesis and the normal development of spermatozoa; the two known sperm selenoproteins were noted above) (Behne et al, 1996; Vezina et al, 1996) and spontaneous abortions (Barrington et al, 1996). Se may protect against oxidative DNA damage in sperm cells. Xu et al (2003) found significant correlations between Se in seminal plasma and sperm density, number, motility and viability in a sample of men. In a Scottish study, supplementation of subfertile men with 100 μg of Se per day for three months significantly increased sperm motility (Scott & MacPherson, 1998), and supplementation of a similar group with 225 μg Se/day and 400

mg vitamin E/day decreased oxidative stress and increased sperm motility (Keskes-Ammar et al, 2003). Conversely, Hawkes & Turek (2001) found that a diet containing 300 μg Se/day caused a reduction in sperm motility in healthy men.

1.2.11. Detoxification of heavy metals

Se, the most reactive of the trace elements, forms selenides with all metals, and detoxifies mercury, cadmium, lead, silver, thallium and arsenic. This effect can be enhanced by vitamin E (Frost, 1981). In the case of cadmium and mercury, the detoxification is achieved through the diversion in their binding from low to high molecular weight proteins (Whanger, 1992). At low dietary Se intakes the presence of any of these metals may produce Se deficiency, with consequences as discussed above. This may have public health implications if the levels of Se-antagonistic elements in foods and the environment result in exposures well in excess of dietary Se intakes (Schrauzer, 1979).

1.3. Human selenium intake: variable but mostly too low

1.3.1. Selenium intake in humans

In his review of Se in global food systems, Combs (2001) summarises Se levels for major classes of foods from several countries. His data are summarised in Table 1.

Table 1. **Range of Se concentrations found in different food classes**

Food class	Range (μg/kg)[1]	Usual range (μg/kg)
Cereal products	4 – 6,900[2]	50 - 600
Vegetables	1 – 45,700[2]	2 - 80
Fruits	1 - 60	5 - 60
Red meats	10 - 910	50 - 300
Poultry	10 - 700	50 - 180
Fish	30 – 1,480	100 - 600
Milk products	2 - 430	10 - 120
Eggs	20 – 1,500	50 - 200

[1]The range comprises the combined data for the US, England, Germany, New Zealand, Finland, China and Venezuela.
[2]Selenosis areas in China.
(After Combs, 2001)

Estimates of Se intake for adults in various countries are summarised in Table 2.

Table 2. **Estimated human Se intakes in selected countries**

Country	Se intake (μg/adult/day)	References
Australia	63 - 96 (range 23 - 204)	Fardy et al, 1989; Reilly, 1996
Belgium	45[1]	Robberecht et al, 1994
Canada	98 - 224	Gissel-Nielsen, 1998
China - KD area	7 – 11[1,2]	Combs & Combs, 1986
- Moderate Se area	40[1,2] - 120	Combs & Combs, 1986; Xian et al, 1997
- Selenosis area	750 - 5,000	Yang et al, 1997
Croatia	27[1,2]	Klapec et al, 1998
England	12[1,2] – 43[2]	Barclay et al, 1995; Joint Food Safety & Standards Group, 1997
Finland - Pre 1984	25[1,2]	Aro et al, 1995
- Post 1984	67 - 110	Aro et al, 1995; Anttolainen et al, 1996
Japan	104 - 127	Suzuki et al, 1988; Yoshita et al, 1998
New Zealand	19[1,2] - 80	Duffield & Thomson, 1999; Robinson & Thomson, 1987
USA	60 - 220	Combs & Combs, 1986
Venezuela	200 - 350	Combs & Combs, 1986

[1] This level does not meet the WHO normative requirement (WHO, 1996)
[2] This level does not meet the recommended dietary allowance (Panel on Dietary Antioxidants and Related Compounds, 2000)
(after Combs, 2001 - with addition of Australia)

It is evident that many people do not consume enough Se to support maximum expression of the selenoenzymes, let alone the level required for optimum prevention of cancer, heart disease, etc. Combs (2001) estimates the number of Se-deficient people in the world to be in the range of 500-1,000 million. As well, he considers that the vast majority of the world's population have suboptimal Se intakes, and hence are at increased risk of cancer, heart disease, viral diseases, and indeed any conditions which involve increased levels of oxidative stress.

1.3.2. Human blood concentrations of selenium: the global view

Combs (2001) presents a comprehensive list of Se concentrations in plasma, serum or whole blood of healthy adults from 69 countries, of which a sample (serum/plasma data

from 14 countries) appears below in Table 3. Blood Se levels are determined mainly by dietary intake, although sex, age, smoking and exposure to heavy metals can have an effect (Robberecht & Deelstra, 1994). Selenium concentrations of plasma are around 81% of those of whole blood (Zachara et al, 1988) and around 94% of those of serum (Harrison et al, 1996). Note that there are few data available from some of the most populous areas of the world, including most of Africa, South America and central and south Asia.

Table 3. **Se concentration in adult human plasma/serum for selected countries**

Country	Mean Se level (μg/L)	References
Austria	67	Tiran et al, 1992
Australia	93	Mean of findings from 10 studies; for references, see below
Burundi	15	Benemariya et al, 1993
Canada	132	Lemoyne et al, 1993
China - KD areas	21	Whanger et al, 1994
- Selenosis area	494	Whanger et al, 1994
- Other areas	80	Whanger et al, 1994
Finland - Pre 1984	70	Aro et al, 1989
- Post 1984	92	Mutanen et al, 1989
Hungary	54	Cser et al, 1992
Japan	130	Suzuki et al, 1989
New Zealand	59	Whanger et al, 1988
Norway	119	Meltzer & Huang, 1995
Slovak Republic	58	Krajkovicova-Kudlackova et al, 1995
USA	119	Primm et al, 1979
Venezuela	216	Bratter et al, 1984
Zaire	27	Thilly et al, 1993

(after Combs, 2001 - with the addition of Australia)

The mean value (calculated as the mean of the means for a representative sample of 45 countries and using post-1990 data only is 78 μg/l, with a range of means from 15 (Burundi) to 125 (Canada). This can be compared to the figure of 70 μg/L, the WHO's reference level, which is the minimum reported level for which further selenium supplementation fails to increase plasma or serum glutathione peroxidase activities (Neve, 1995). Rayman (1997) has suggested that this level should be increased to 100 μg/L as a criterion of nutritional adequacy. The absolute range of values, including selenosis areas, is 15-494 μg/L (China-Enshui).

Evidence suggests that there is a global trend toward a reduction of Se in the food chain, caused by fossil fuel burning (with consequent sulphur release), acid rain, soil acidification, the use of high-sulphur fertilisers (Frost, 1987) and more intensive crop production (Gissel-Nielsen & Gupta, 2001). Rayman (1997) and Giovannucci (1998) observe that blood Se levels have decreased significantly in the United Kingdom from 1984 to 1994, and current average selenium intake in the UK may be as low as 35 μg/day (Barclay et al, 1995). These authors attribute this fall in part to the use of low-Se UK and European wheat in place of US wheat. This highlights the sensitivity of Se intake and body levels to changes in the food supply. Both authors call for action to increase Se intake.

1.3.3. Selenium levels in the Australian population

The figure of 93 μg/L quoted for Australia is calculated as the mean of the means determined from ten studies of blood Se levels. The studies (with mean plasma or serum Se levels determined, in μg/L) are: Brock et al, 1991 (88); Cumming et al, 1992 (81); Daniels et al, 2000 (77, 88); Dhindsa et al, 1998 (92); Judson et al, 1982 (113); Judson et al, 1978 (121); Lux & Naidoo, 1995 (101); McGlashan et al, 1996 (80); McOrist & Fardy, 1989 (98, 86); and Pearn & McCay, 1979 (88). If post-1990 data only are used, the mean is 89 μg/ L.

In Australia, relatively low blood Se levels have been found in Adelaide infants, along with evidence for declining levels in adults. Daniels et al (2000) found a plasma Se level of around 31 μg/L (SD 13) in a sample of newborn infants, a level comparable with that of New Zealand. Infant levels are typically half those of adults. These levels place the infants at increased risk of a range of conditions which involve oxidative stress and inflammation.

Se levels in South Australians may be falling. Whole blood Se level for a 1977 sample of South Australians (Institute of Medical & Veterinary Science employees) was reported to be 152 μg/l (equivalent to around 122 for plasma) (Judson et al, 1978) and a sample from Kangaroo Island in 1979 was 142 μg/L (equivalent to around 114 for plasma) (Judson et al, 1982), while in later samples of Adelaide adults, mean plasma selenium levels were 98 (Fardy et al, 1989) and 88 (Daniels et al, 2000).

Daniels et al (2000) observe that, given the geographical variability in Se intake and blood levels and the sensitivity of these to changes in the food supply, it is necessary to use local data to monitor Se status. They refer to the paucity of Australian Se status data: there have been few studies and most have involved small sample sizes. Indeed her own study, South Australia's most recent, uses 1990 data and a sample size for adults of just 19. Clearly, more data are required to clarify the Se status of Australians, and this leads to the first research question of this thesis:

RESEARCH QUESTION 1: What is the current Se status of a sample of relatively South Australian residents?

1.3.4 Selenium toxicity: garlic breath and cracked nails

Much has been written about the toxicity of Se, but a careful examination of the literature reveals that it may have been exaggerated. A total of three deaths, including those of two infants are attributed to excess dietary Se (Reilly, 1996), and in none of these is it clear that Se was the primary cause of death. Perspective is gained by consideration of the magnitude of current levels of iatrogenic (medically-induced) death: for example, adverse effects of medications are estimated to be the fifth highest cause of death (involving around 70,000 deaths per year in hospitals alone) in the US (Phillips et al, 1998; Starfield, 2000).

In the 1980s, due to a manufacturing mistake, several people each ingested up to 2.4 grams of Se. Symptoms of poisoning included garlic breath, nausea, vomiting, hair loss, fatigue, irritability and nail changes. The changes were all reversible and all the people recovered (WHO, 1987). In the 1970s in New Zealand, a 15-year old girl attempted suicide by ingesting 400 ml of sheep drench containing 1 gram of sodium selenate. Symptoms included nausea and a strong garlic breath. Her serum selenium level was 3,100 μg/L, 20 times the usual level for the Rotorua area. She was discharged after 17 days with no symptoms (Civil & McDonald, 1978).

Chronic selenosis occurs in Enshi County, China where coal-contaminated soil contains up to 8 ppm Se, and residents have consumed up to 7 mg per day. Common symptoms include nail thickening and cracking and hair loss, and some people exhibit skin lesions (Liu & Li, 1987; Yang & Zhou, 1994). The concern that the incorporation of

selenomethionine into body proteins could increase Se to toxic levels is not warranted because a steady state is established, which prevents the uncontrolled accumulation of Se (Schrauzer, 2000).

Se intakes shall be discussed in greater depth below, but it appears very unlikely that acute toxicity would occur with doses less than 1,000 μg per day. People in parts of China, the US, Venezuela and Greenland have ingested Se at this level for their entire lives without ill effects (Taylor, 1997), and men at high risk of prostate cancer have been supplemented with 3,200 μg of Se (as yeast) per day for more than three months with no problems (Nelson et al, 1999).

1.3.5. Optimal selenium intake

In the NPC trial in the US, the strongest protective effect of Se against cancer occurred in the lowest (relative risk of 0.52, 95% CI: 0.33-0.82) and middle (RR 0.64, 95% CI 0.40-0.97) tertiles, which included those people with plasma Se levels below 120 μg/L (Rayman & Clark, 2000). None of the subjects had plasma Se levels below 60 μg/L and very few were less than 80, thus the cohort must be considered Se-adequate by current nutritional standards (Combs, 2000).

Although there is a risk in generalising results of individual epidemiological and intervention studies, this result would suggest, using the levels presented by Combs (2001) above, that the vast majority of the world's population (including that of Australia, with a probable mean plasma/serum level around 89 μg/L, and the entire population of Europe (Rayman, 2000)) would be in the responsive range.

Optimal level of Se intake shall now be discussed. The NPC participants lived in a region where dietary Se intake is around 90 μg/day (Clark et al, 1996), thus with the addition of the 200 μg supplement, individuals in the treatment group would have received around 270-290 μg/day. Although the US RDA for Se is currently 55 μg/day, around 110 μg/day is required to reach a plasma Se concentration of 120 μg/L (Combs, 2001), and the safe and adequate range of 50-200 μg/day for adults (National Research Council, 1980) is often quoted. To provide a sufficiently wide safety margin, the US reference dose for Se from all nutritional sources for a 70 kg human has been set at 350 μg/day. This level

defines as safe the total intake by an American adult on a normal diet who is taking an additional 200 μg/day as a supplement (Schrauzer, 2000).

Combs (2001) considers that a Se intake of around 300 μg/day may be required to significantly reduce cancer risk. This compares with an estimated Australian adult intake of around 75 (see Table 2 above). Of course, as Rayman (2000) notes, Se requirement varies between individuals in the same population. Even Moyad (2002), who expresses doubts about the interpretations of certain Se studies, and considers some estimates of its cancer-protective effect to be optimistic, suggests that an intake of 200 μg Se/day and around 50 mg vitamin E/day may be beneficial, particularly for current or previous smokers. The results of the NPC trial (Duffield-Lillico et al, 2002) suggest that males may have a higher Se requirement than females. Further studies may find optimum adult Se intakes in the range 125-280 μg/day, with means of around 130 (for females) and 250 μg/day (for males). Pregnant females may have a higher Se requirement than non-pregnant females (Dylewski et al, 2002).

With respect to a safe upper limit, a review by the US Environment Protection Agency, using the Enshi County study of Yang et al (1989) as a reference, concluded that no adverse effects were observed in individuals with whole blood Se concentrations as high as 1,000 μg/L, with a no adverse effect adult intake level of 853 μg Se/day (Poirier, 1994). Under normal conditions, a Se intake of less than 1,000 μg/day (or 15 μg/kg bodyweight) does not cause toxicity (Neve, 1991; Taylor, 1997), although in certain sensitive individuals the maximum safe intake may be as low as 600 μg/day (Whanger et al, 1996), and thus Rayman (2000) cautions that it would be sensible to restrict the upper adult limit to 450 μg/day.

It is unlikely that the food system of any country, with the possible exception of Venezuela, delivers an optimal level of Se to its population, and indeed the food systems of most populations do not even provide enough Se to maximise selenoenzyme expression (Combs, 2001). The impact of this deficiency and suboptimality in global health terms is difficult to quantify, but is likely to be enormous given the high prevalence of various cancers, cardiovascular diseases, viral diseases (including AIDS, hepatitis, measles and influenza), and exposure to environmental pollutants throughout much of the world. It is thus a matter of urgency that many countries begin to address this major public health issue and develop effective, sustainable ways to increase Se intakes (Combs, 2001).

1.4. Strategies to increase human selenium intake

Given that the populations of most countries would be likely to benefit from an increased Se intake, how could this be best achieved? Strategies to increase Se intake include increased consumption of higher-Se foods through education, individual supplementation, food fortification, supplementation of livestock, use of selenium fertilisers, and plant breeding for enhanced selenium accumulation. Each of these shall be discussed briefly.

1.4.1. Increased consumption of higher-selenium foods through education

Globally, wheat is probably the most important dietary source of Se. Even in Europe, with its low levels of available soil Se, bread and cereals, being commonly consumed, are important Se sources. In the UK, for example, it is estimated that they supply around 22% of Se, second to meat, poultry and fish (36%) (Ministry of Agriculture, Fisheries & Food, 1997). The importance of wheat as a Se source shall be discussed further below.

Brazil nuts provide the most concentrated natural food source of Se. In the UK they were found to contain 2-53 mg/kg of Se (Thorn et al, 1976), while an Australian study found even higher levels: 0.5-150 mg/kg (Tinggi & Reilly, 2000).

1.4.2. Individual supplementation

In Western countries today many people regularly consume Se supplements. In the US a variety of non-prescription pharmaceutical preparations designed to increase Se status is available. In Australia the range of Se supplements is narrower, although a Se-yeast powder and several selenomethionine tablets are available, up to the allowed maximum of 50 μg of Se per tablet.

Se-yeast (from California, the same form as used in the NPC trial) is probably the best form to take: it is effective, safer and more bioavailable than inorganic forms, and includes several organic Se forms, including selenomethionine and selenocysteine (Bird et al, 1997). Another form of individual supplementation is selective consumption of the fortified or "functional foods" listed below.

A well-known deficiency of individual supplementation as a population strategy to improve nutrition is that those people most in need tend to be the least likely to take supplements.

1.4.3. Food fortification

This approach has been used successfully with folate-enriched breakfast cereals, iron-enriched milk, iodised salt, carotene- and vitamin E-enriched margarine, as well as selenised salt in Se-deficient regions of China. Seleniferous areas can be considered resources for the production of Se-enriched plants: for example, in China an elixer is made from high-Se tea in Enshi County (Combs, 2001), and high-Se wheat from South Dakota, USA attracts a premium price (Fedgazette, 2000). These examples can be included in the *functional foods* category, along with high-Se broccoli (Finley, 1999), high-Se garlic (Ip & Lisk, 1994) and the high-Se wheats noted above. Also, soybeans accumulate relatively high levels of selenium when it is added as a fertiliser: for example, when 10 g/ha Se was added to the soil as selenate, soybeans accumulated 1.93 mg/kg Se (control 0.03) compared to 0.26 mg/kg (control 0.02) in barley grain (Gupta & Macleod, 1994). Thus Se-rich soymilk could become a popular functional food. Combs (2001) even lists a high-Se beer marketed in China. These products are likely to become more popular if the findings from the current large Se-cancer prevention trials are positive.

1.4.4. Selenium supplementation of livestock

Supplementation strategies to increase dietary Se intake by livestock (and thus increased levels in meat and milk) include Se fertilisation of pastures (noted above), dietary supplements (e.g. salt licks or added to the rations of dairy cows during milking), and direct administration (drenches, slow-release reticulum/rumen "bullets", injection). Se supplementation of dairy cows, apart from raising Se levels in milk, reduces the incidence of mastitis (Ali-Vehmas et al, 1997).

In New Zealand, sodium selenate is supplied as a prill to pastures at up to 10 g/ha, and this is also the allowable limit in Australia (Wilson, 1964). In Western Australia, slow-release barium selenate applied at 10 g/ha prevented subclinical selenium deficiency in sheep for four years, whereas a single application of sodium selenate at the same rate was effective for only 15 months (Whelan & Barrow, 1994).

Supplementation of livestock with Se is unlikely to be an efficient strategy to increase Se level in the human population. In New Zealand, little increase in the Se content of human foods was observed after the introduction of Se supplementation for farm animals in the 1960s (Thomson & Robinson, 1980).

1.4.5. Selenium fertilisation of crops

The use of Se as a soil amendment in fertiliser is practised mainly in Finland (by law from 1984) and New Zealand (at an individual level, and generally on pastures to increase the Se level in grazing animals - see below). In Finland, Se was initially added to NPK fertilisers at a rate of 16 mg/kg for cereal crops and 6 mg/kg for pastures. In 1990 it was reduced to 6 mg/kg for cereals and pastures; however, this change stabilised the average human blood Se level at a lower concentration than intended and in 1998 supplementation was increased to 10 mg/kg for crops and pastures (Gissel-Nielsen & Gupta, 2004).

The Finnish experiment has demonstrated the safety, effectiveness, ease and cost-efficiency of this approach to raise Se levels in a human population. Dietary Se intakes trebled and plasma Se concentrations nearly doubled within three years of the program's commencement (Aro et al, 1995). However, it is difficult to isolate the effects of a single factor, such as dietary change, from other factors that can be involved in the aetiology of such conditions as cancer and cardiovascular disease. There have been significant decreases in the rates of cardiovascular disease and certain cancers in Finland since 1985, but with no controls for comparison, this cannot be ascribed to Se alone (Mussalo-Rauhamaa et al, 1993; Varo et al, 1994). Cardiovascular disease mortality has declined in Finland by 55% for men and 68% for women between 1972 and 1992. As well as the trebling of the Se intake in the latter eight years of this period, fruit and vegetable consumption increased 2.5-fold and there was a decline in smoking by men (Pietinen et al, 1996).

Sodium selenate is the Se form generally used for crop and pasture fertilisation: it is weakly adsorbed on soil colloids and can bring about a rapid increase in plant Se level (Gupta & Watkinson, 1985). The enhancement of wheat Se level by fertiliser shall be discussed in more detail below.

1.4.6. Plant breeding for enhanced selenium accumulation

Breeding for improved Se uptake and/or retention by plants may be an effective, sustainable strategy. Preliminary studies have found a 15-fold variation in Se-accumulating ability among brassica vegetables (Combs, 2001), and a Se-accumulating soybean cultivar has been identified (Wei, 1996). Substantial variability exists within cereal crop varieties for zinc, iron and other nutrients (Graham et al, 1999). Micronutrient efficiency in this context refers to a genotype or phenotype that is better adapted to, or yields more in, a micronutrient-deficient soil than can an average cultivar of the species (Graham, 1984). Virtually all micronutrient efficiency traits studied to date are due to greater ability to extract the micronutrient from the soil rather than the plant's ability to live on less of the micronutrient (Graham & Welch, 1996). These findings suggest that it should be possible to breed cultivars with enhanced Se uptake and/or retention, or to use genetic engineering to enhance Se levels (and even specific Se metabolites) in food crops.

In summary, each of these strategies could contribute to enhanced delivery of Se to human populations through their food systems. As described by Welch & Graham (1999), a food systems paradigm encompasses an agriculture that aims not only at productivity and sustainability, but also at improved nutrition. In Australia, a program which combines selection for enhanced grain Se level in wheat; strategic ferrtilisation of wheat, barley, soybeans and broccoli; education to encourage greater consumption of these higher-Se foods; increased supplementation of livestock grazing acid soils in high rainfall areas, and education and targeted supplementation of high-risk individuals, would be likely to significantly improve population health, with a consequent significant reduction in health costs.

1.5. Wheat: an important selenium source for humans

Surveys indicate that wheat is the most efficient Se accumulator of the common cereal crops (wheat, rice, maize, barley, oats) and it is one of the most important Se sources for humans. Bread, for example, is the second most important source of Se in the US (Schubert et al, 1987), and has been found to supply one-third of the daily Se intake of Australian children (Barrett et al, 1989). With the addition of selenium supplied through breakfast cereals, cake and biscuits, and in view of its high bioavailability, wheat-Se probably supplies around half the Se utilised by Australians. In a Russian survey, serum

Se level was found to be highly correlated (r = 0.79) with selenium level in wheat flour (Golubkina & Alfthan, 1999).

1.5.1. Selenium concentrations in wheat grain

1.5.1.1. The global view

There is wide variation in wheat grain Se level between and within countries. Published values range from 0.001 mg/kg in south-west Western Australia (White et al, 1981) to 30 mg/kg in highly seleniferous areas of South Dakota (University of California, 1988), but most of the world's wheat falls within the 0.020-0.600 mg/kg range (Alfthan & Neve, 1996). Canada and the US have relatively high levels, usually in the 0.2-0.6 mg/kg range (Reilly, 1996). New Zealand and Eastern Europe generally have low levels, for example the 0.028 mg/kg average found by Mihailovic et al (1996) for Serbia.

Some countries, including China (with a range of 0.01-0.23 mg/kg (Alfthan & Neve, 1996)), Canada and the US, have highly variable wheat Se levels, even within states. Se concentrations in wheat grain from 12 locations in Manitoba, Canada in 1986-88 ranged from 0.06-3.06 mg/kg, and levels varied between years within a location (Boila et al, 1993). A US survey, also in 1986-88, analysed major brands of white bread in nine different geographical regions. The overall range was 0.06-0.74 mg/kg, while a single brand of bread collected from different bakeries in Boston alone had a range of 0.24-0.92 mg/kg (CV 41%), with a mean of 0.60 mg/kg (Holden et al, 1991).

1.5.1.2. Selenium in Australian wheat

Just four surveys of Se concentrations in Australian wheat have been identified, from Queensland, Western Australia, South Australia, and wheat imported into New Zealand from South Australia and New South Wales. The Queensland study (Noble & Barry, 1982) found a mean value for wheat grain Se of 0.150 mg/kg (range 0.020-0.800) for wheat grown between 1974 and 1978. Similar levels were found for sorghum and soybean.

Se levels varied widely between the south-east Darling Downs site (mean 0.040 mg/kg, range 0.020-0.080) on Tertiary volcanic soils and that at Biloela (mean 0.360, range

0.230-0.770), where Tertiary sediments predominate. Site differences accounted for a 40-fold difference between wheat Se values, and a 110-fold difference for sorghum. There was no significant difference between the Se concentrations of wheat and barley from the south-east Darling Downs, but sorghum at Biloela accumulated twice as much Se as wheat.

Four wheat cultivars were grown at six Darling Downs sites: two-way analysis of variance indicated no significant difference in Se concentrations between cultivars at each site. There is no mention of within-cultivar variation within sites, so it is unlikely that replicate samples were analysed. These findings suggest that environmental influences predominate over genetic variation in determining Se level in wheat.

The study also found, for 25 sites, a statistically significant negative correlation ($r = -0.41$) between rainfall and wheat Se level. This supports the findings of Miltmore et al (1975) for oats and grass in British Columbia, Canada, and those of White et al (1981) for wheat in Western Australia, and may be due to a dilution effect. A European study, which included high nitrogen applications and hence high grain protein levels, found the opposite (Johnsson, 1991).

Very low Se concentrations were found in wheat surveyed in south-west Western Australia in 1975: mean Se concentration in grain was 0.023 mg/kg (standard error 0.006), with a range of 0.001-0.117 mg/kg (White et al, 1981). The figure of 0.001 mg/kg is the lowest level reported globally for Se in wheat. The lowest levels were found on soils derived from Archaean granite, which are also known for a high prevalence of white muscle disease in lambs (Godwin, 1975).

Watkinson (1981) compared Se concentrations in New Zealand-grown wheat with wheat imported from Australia, grown in 1978 and 1979. The Australian wheats were higher in Se (mean 0.123 mg/kg, SD +/-0.026, range 0.043-0.224) than the NZ wheats (0.028, +/-0.010, 0.011-0.086), with South Australian wheats (0.154,+/-0.030, 0.100-0.224) higher than New South Wales wheats (0.091,+/-0.022, 0.043-0.130).

The South Australian study (Babidge, 1990) used pooled wheat and barley samples from 107 and 100 silos, respectively, across the state in the 1981 season for wheat and the 1981 and 1982 seasons for barley. The results were not corrected for moisture content and were

found to be not normally distributed, so median values were reported rather than means. There were significant differences ($p<.001$) between regions: for example, the Upper Eyre Peninsula region had a wheat Se median value of 0.229 mg/kg (range 0.120-0.316), compared to the South East with 0.118 (0.047-0.240). Between these were the Murraylands (0.197), Lower Eyre Peninsula (0.142) and Central region (0.142). Wheat and barley showed the same regional trends, but wheat levels were around 30% higher. Se levels in barley in 1982 were not significantly different from those of 1981 apart from in the South East region.

The findings of these four studies (means of 0.150 (Qld), 0.023 WA), 0.150 (SA), 0.091 (NSW), and SA median of 0.170), together with the findings of the human blood surveys discussed above, suggest that Australian wheat grain Se concentrations are moderate on a global scale, probably on average just below the mean; they are well above most New Zealand and Eastern European levels, but generally lower than those of Canada and the US. However, the South Australian study provides the most recent data, and it is now 23 years old. Clearly, more surveys are required to provide data on current Se concentrations in Australian wheat.

RESEARCH QUESTION 2: What are the grain Se concentrations in a sample of wheats grown recently on a range of soil types in South Australia?

1.5.2. High bioavailability of wheat-selenium

In human nutrition terms, bioavailability can be defined as the amount of a nutrient in a meal that is absorbable and utilisable by the person eating the meal (Van Campen & Glahn, 1999). In general, organic selenium (e.g. selenomethionine, selenocysteine) is absorbed more efficiently than inorganic forms, with uptake from the gastrointestinal tract of around 90% for selenomethionine compared with 60% for selenite (Stewart et al, 1987).

However, the assessment of bioavailability of food micronutrients in general (Graham et al, 2001; House, 1999) and different forms of dietary Se is not straightforward (Reilly, 1996). Several measures can be used to determine bioavailability and they are subject to a range of variables. If tissue retention is used as a measure, naturally occurring food Se is

by far the most available form of the element, although there are significant differences between different types of food.

It is apparent that Se is more bioavailable from plant forms than from animal foodstuffs (Combs, 1988; Young et al, 1982), and wheat Se is one of the most bioavailable forms (Hakkarainen, 1993; Jaakkola et al, 1983; Laws et al, 1986). In a study on chicks, using glutathione peroxidase activity and prevention of exudative diathesis as measures of bioavailability, and using selenite as the reference at 100%, the bioavailabilities of Se in different foods were: wheat 83-100%, barley 78-85%, oats 41-45%, fishmeal 64-80%, and meatmeal 22-30%. With changes in selenium levels in whole blood as the measure, results were wheat 123%, barley 104%, oats 99%, fishmeal 107% and meatmeal 69%. Measurement of Se buildup in cardiac muscle gave: wheat 108%, barley 87%, oats 60%, fishmeal 100% and meatmeal 42% (Hakkarainen, 1993). In a feed experiment with chickens on an initially Se-deficient diet, birds supplied with Se in the form of baked bread had double the percent survival (72% v 38%) of birds with a fish Se source (Laws et al, 1986).

Selenomethionine, the form in which Se mainly occurs in cereals (Olson et al, 1970), beans, mushrooms and yeast (Reilly, 1996) is initially incorporated into tissue proteins after absorption. After further metabolism to selenide and selenocysteine, this tissue-stored selenomethionine is used for the synthesis of glutathione peroxidase (Levander & Burk, 1990). Selenite and selenocysteine, on the other hand, are used directly for the synthesis of glutathione peroxidase (Deagen et al, 1987).

The importance of imported North American wheat as a former Se source for the British population was noted above, and is reinforced by a Scottish study which found that the Se content of wheat harvested in 1989 which was used for breadmaking in Scotland ranged from 0.028 mg/kg for home-grown wheat to 0.518 for Canadian wheat, and was significantly correlated ($p<0.001$) with protein content (Barclay & MacPherson, 1992). The importance of this Se source is further illustrated by Norway's population which, despite a modest total Se intake, has the highest serum Se level in Europe at 120 μg/L. The probable explanation is that their major selenium source is North American wheat. Meltzer et al (1992) demonstrated the high bioavailability of wheat-Se by feeding trial participants Se-rich bread providing 100, 200 or 300 μg of Se daily for six weeks. Serum selenium increased in a dose-response manner by 20, 37 and 53 μg/L, respectively, in the three groups ($p<0.001$).

Wheat enriched with Se by foliar application was found to be highly effective in raising plasma Se (53% increase after 6 weeks) in a Serbian study. Glutathione peroxidase activity in blood increased and oxidative stress parameters decreased (Djujic et al, 2000a). A follow-up study found that Se-enriched wheat increased levels of copper, iron and zinc in erythrocytes, compared to individuals consuming low-Se wheat (Djujic et al, 2000b).

In trials with high-Se broccoli, using a rat model, Finley & Davis (2001) found that Se bioavailability was not related to its efficacy in colon cancer prevention; however, wheat-Se (which is largely in the form of selenomethionine) was effective as a colon cancer preventive, whereas pure selenomethionine was not. The authors concluded that it is necessary to study Se in food forms, rather than to generalise from studies using pure chemical forms. Further studies are required to determine the relative anti-cancer effects of wheat phytate and wheat-Se.

1.5.3. Effects of post-harvest processing and cooking on selenium

1.5.3.1. Milling

It is well known that wheat milled to white flour loses nutrients (Burk & Solomons, 1985), and there is evidence that processing, at both industrial and domestic levels, influences Se levels in food (Bratakos et al, 1988; Reilly, 1996). Several studies report a reduction in "content" of Se in white flour compared with whole grain or wholewheat flour, but actually report reductions in Se *concentration,* ranging from 4-47%, with a mean around 27% (Ahmad et al, 1994; Burk & Solomons, 1985; Korkman, 1980; Robberecht et al, 1990). This compares with a decrease in concentration from whole wheat to white flour for Zn, Fe and K in the range 41-80% (Ahmad et al, 1994; Lorenz et al, 1980; Toepfer et al, 1972). A study which investigated Se *content* as well as concentration found that wheat flour (which comprised 73% of the grain) contained 63% of the grain's total Se (Ferretti & Levander, 1974).

These results suggest that Se, like S, is more evenly distributed throughout the wheat grain, with a higher proportion stored in the endosperm than is the case for other nutritional elements. This is not unexpected, as both Se and S are mostly protein-bound. However, there is a need for further studies that examine how much Se is stored in the

different components of the grain and what are the actual losses of Se and other nutrients in milling to white flour. The cited studies merely compare Se concentrations in the different grain fractions after milling.

RESEARCH QUESTION 3: In what proportions is selenium stored in the different fractions of the wheat grain?

1.5.3.2. Cooking

Studies of the effects of different cooking methods on Se content of foods have produced varying results. Some have found that usual cooking procedures did not result in Se losses for most foods (Dudek et al, 1989; Ferretti & Levander, 1974; Thomson & Robinson, 1990), whereas a Greek study of the effects of frying, grilling, boiling or canning found that all foods lost some Se. Whole grain cereals (wheat, rye and corn) lost, on average, 25% of their Se after two hours' boiling in water, while spaghetti lost 5% Se after 45 minutes' boiling. This compares with mushrooms, which lost 85% of Se after frying in oil at 207^0C for 20 minutes (Bratakos et al, 1988).

Baking studies have also yielded equivocal findings: some found Se losses of around 15% (Higgs et al, 1972; Olson & Palmer, 1984), while others reported no losses (Arthur, 1972; Morris & Levander, 1970). A study of the effects of various commercial thermal processes found no effect on Se concentration in whole grain wheat from steam flaking, autoclaving or popping. Se concentration in white flour was unaffected by extrusion cooking, but drum-drying decreased it by 23% (Hakansson et al, 1987).

Even if there is some loss of Se due to cooking or baking, this may be compensated by an increase in bioavailability. In a feeding trial of chickens on an initially Se-deficient diet, those chickens which were supplied with Se from baked bread had a 26% higher survival rate than those with a raw bread source (Laws et al, 1986).

As this thesis examines Se in wheat as an important source for humans, it would be worthwhile to know what losses of the element (if any) are incurred along the processing chain, especially for bread, one of the most important dietary Se providers for Australians.

RESEARCH QUESTION 4: What effects do post-harvest processing and cooking have on grain selenium content?

1.6. Strategies to increase selenium in wheat

1.6.1. Introduction

The evidence discussed above indicates that, in many countries, an increase in Se concentration in wheat is the most effective and efficient way to increase the Se intake of the human population, with consequent likely improvement in public health, and also to reverse the trend of declining Se levels in food systems.

It is clear that Se fertilisation of wheat is an effective means to increase grain Se concentration (Combs, 2001; Gupta & Gupta, 2000), and this shall be discussed below. Australia exports around 68% of its annual wheat production to over 40 countries (AWB, 2002), so a significant increase in the mean Se level of its wheat would be expected to improve population health in those countries where mostly Australian wheat is consumed, such as Iraq, Iran, Egypt, Indonesia, Japan and South Korea. The most sustainable, cost-effective approach, particularly in developing countries, may be to breed wheat cultivars which are better at accumulating grain Se, and this shall also be discussed.

Education is also important to encourage people to consume appropriate amounts and proportions of different classes of healthy foods, including whole grain cereal products.. However, in developing countries those people who are most at risk of nutrient deficiencies (frequently women and children) often rely on one staple food, e.g. wheat, rice, cassava or maize, for most of their energy and nutrient requirements, and they lack the money to improve their diet. Hence the high prevalence of iron, zinc, vitamin A, iodine and Se deficiencies, and the high incidence and prevalence of infectious diseases in these countries (Graham & Welch, 1996; Graham et al, 2001).

1.6.2. Selenium fertilisation

1.6.2.1. Se form; method, timing and rate of application

To overcome the low Se levels in food crops in certain areas, different methods of Se fertilisation have been investigated for more than 30 years. Of particular interest are the experiments of Ylaranta and Gissel-Nielsen & Gupta in Finland, Denmark and Canada. It appears to be a relatively inexpensive way to increase Se intake by humans, and also effective, especially in view of the finding of Mutanen et al (1987) that the bioavailability (calculated as the mean of four criteria) of wheat-Se was higher for Se-fertilised (both soil-applied and foliar) wheat than for American wheat which was naturally high in selenium.

The Finland Se program was discussed above. The selenium level in all domestic cereal grains in Finland pre-1984 was 0.01 mg/kg or less; now spring wheats typically contain around 0.25 mg/kg, and for the less-fertilised winter wheat, 0.05 mg/kg (Eurola et al, 1990). Current application rates vary from 5-10 g selenate/ha, and the practice appears to be safe. Concern that continuing use of Se-amended fertilisers might eventually lead to accumulation of toxic levels in the environment appears to be unfounded (Oldfield, 1999; Vuori et al, 1994).). The bioavailability of residual Se is lowered by the reducing action of micro-organisms in the soil and rumen. Furthermore, in New Zealand, where Se fertilisation has been practised for over 30 years, no such build-up has occurred, and positive responses continue to be obtained from Se addition (Oldfield, 1999).

Most studies have shown selenate, whether applied to the soil or as a foliar fertiliser, to be much more effective than selenite (Gissel-Nielsen 1981a, 2001; Gupta et al, 1993; Shand et al, 1992; Singh, 1991; Ylaranta 1983a,b, 1984a). For example, a grain Se content of 100-200 micrograms (μg)/kg in barley was obtained by applying 10-20 g/ha of selenate, but over 100 g/ha of selenite was required to reach this level (Ylaranta, 1983b). On a fine sandy loam of pH 6.0, 10g selenate/ha applied to the soil raised barley grain from 33 μg/kg (control) to 234 μg/kg, while 10 g/ha selenite caused no increase (Gupta et al, 1993). Foliar selenate was found to be more effective than basal selenite by Gupta & McLeod (1994). In many soils, selenite is readily adsorbed on clay colloids and becomes unavailable to plants.

The relative effectiveness of soil or foliar application of Se depends on Se form, soil characteristics, method of basal application, and time of foliar application. Ylaranta (1983b) found basal and foliar selenate to be equally effective at the low (10 g/ha) rate, foliar better at 50 g/ha, and both equal at the very high rate of 500 g/ha. In further trials, foliar selenate applied at the 3-4 leaf stage was found to be more effective than basal application on clay soil, pH 6.3, of similar effectiveness on high-humus fine sandy soil, pH 4.6, and slightly less effective than basal fertiliser on a fine sandy soil, pH 5.0. Ten g/ha selenate, using a wetting agent, raised wheat grain Se level from 16 to 168 μg/kg on the clay soil, while 9g basally applied raised it to just 77 μg/kg. Overall, foliar application was the more effective method, except where growth was poor due to low rainfall (Ylaranta, 1984a). This suggests that, for most of Australia's wheatgrowing areas, basal application may be preferable, especially where foliar nitrogen (to which selenium could be added) to boost growth or protein is not used.

It is clear that foliar Se is more effective if applied at more advanced stages of growth. Ylaranta (1984b) found that selenate was twice as effective and selenite 12 times as effective if applied at Feekes stage 9-10 rather than stage 2.5-3. At the earlier stage, selenite was only 20% as effective as selenate, whereas at the later stage (end of stem extension) their effects were equal. This finding was similar to that of Gissel-Nielsen (1981a). Selenate spray which falls on the ground can be leached to the roots and taken up, whereas selenite is generally adsorbed. The effectiveness of foliar-applied Se can be enhanced by the use of surfactants (wetting agents) by 12-16% for selenate (Ylaranta, 1984a) and more for selenite: 13-40% (Ylaranta, 1984a) or up to 100% (Gissel-Nielsen, 1981a,b).

Factors apart from soil type which can influence the effectiveness of basal Se fertiliser include the form of NPK fertiliser used and the method of placement. Selenate was found to be more effective when added to a chloride-based NPK fertiliser, to avoid the ionic competition from sulphate ions in a sulphate-based NPK fertiliser (Singh, 1991). In Finland, the current levels of Se in spring wheat are partly due to the practice of placing the fertiliser between every second row of seeds at 8 cm depth so nutrients are readily accessible to the roots of the young wheat plants (Ylaranta, 1984b).

Seed coated with either selenite or selenate has also been used as a means of supplying Se to crops. Selenite has not been effective, but selenate-coated seed can provide similar

results to basal fertiliser at the same rate (Ylaranta 1983b, 1984a). However, accurate seed treatment with concentrated, toxic solutions of Se salts can be difficult, and seed treatment is not recommended (Ylaranta, 1984a).

The residual effect of Se treatments was found to be low to negligible in the following year, even when it had been applied at the high rate of 500 g/ha (Gissel-Nielsen, 2001; Singh, 1991; Ylaranta 1983b, 1984a). Hence it is clear that Se needs to be applied annually.

Gissel-Nielsen (1987) conducted a glasshouse trial to test whether different Se treatments (selenite or selenate as foliar or basal fertilisers at 5 g/ha equivalent) affected the speciation of Se in barley and ryegrass. It was found that even with a ten-fold variation in the total Se content of the plants there was little difference in the distribution of the fractions of selenate, selenite, selenoaminoacids and protein-bound Se in grain and leaves, under different treatments. These results, which were similar to the findings for *Zea mays* (Gissel-Nielsen, 1979), suggest that the distribution of Se among the different Se fractions of the plants cannot be changed significantly by application method or Se form.

What rates of selenate should be used to increase wheat grain Se concentration? Ylaranta (1984a) found that at least 12 g is required (as basal or foliar) to achieve a grain concentration of 300 μg/kg of Se. Using a higher rate (mean 25 g/ha, range 20-33), the same researcher raised Se levels in spring wheat from a base level of 13 μg/kg (7-25) to 700 (650-750) μg/kg. Similarly, selenate added as basal fertiliser at 10 g/ha raised the Se level in barley and oat grain from 33 to 500 μg/kg. It was even more effective in soybeans, increasing levels from 30 to 1,930 μg/kg (basal) and 3,005 μg/kg (foliar) (Gupta & MacLeod, 1994).

Gissel-Nielsen, in his 2001 review, concludes that the following are likely to be effective annual treatments for biofortifying annual crops for human or animal consumption: foliar application of 5 g/ha as selenite or selenate, soil fertilisation using 10 g/ha as selenate or 120 g/ha as selenite, or 10 g/ha as seed treatment (Gissel-Nielsen, 2001).

The question arises as to what is an appropriate target level for selenium in wheat grain. From the foregoing studies it is evident that 10 g/ha of selenate (which is currently the maximum allowable application rate for Australia) can raise wheat crops from 30-100 to

300-500 μg/kg of grain Se. Is this as high as required? It could be considered a minimum target level in view of the estimated 300 μg Se level of a loaf of wholemeal bread made from this wheat. The consumption of this bread over a week would significantly increase an individual's Se intake.

There are no published studies of Se fertilisation of wheat in Australia. Conditions here are very different to those in Europe, where most studies have been conducted. For example, Professor Gissel-Nielsen recommends that foliar Se be applied, for maximum effect, "as late as possible, while the leaves are still green" (G. Gissel-Nielsen, pers. comm. 16/7/2001); however, in Australia there is likely to be considerable leaf loss from the mid-milky stage onwards, so that may represent the latest stage for effective foliar Se application.

RESEARCH QUESTION 6: What is the most effective fertilisation method to increase wheat grain selenium density on South Australian soils of varying pH?

1.6.2.2. Effect of plant nutrients on Se uptake

As noted above in Section 2.1.3 the literature reveals that soil sulphate level is an important determinant of Se uptake and transport in plants, with nitrogen and phosphorus playing less important roles. Each shall now be discussed briefly.

Sulphur

Studies of crop and pasture plants show that increasing soil sulphur level decreases Se uptake and transport (Hopper & Parker, 1999; Murphy & Quirke, 1997; Pezzarossa et al, 1999; Pratley & McFarlane, 1974; Schubert, 1961; Ylaranta, 1990). The effect can be strong: a ten-fold increase in sulphate decreased Se concentration and content by more than 90% in perennial ryegrass and strawberry clover (Hopper & Parker, 1999), and where pasture yields responded to sulphur topdressing, the selenium concentration in the legumes present was reduced by up to 50% (Pratley & McFarlane, 1974). This effect is most obvious late in the season (Murphy & Quirke, 1997) and can be partly explained by a dilution effect caused by a growth response of the plant to the applied sulphur. The use of gypsum (calcium sulphate) in Oregon increased the incidence and severity of white muscle disease (Schubert et al, 1961).

The reduction in plant Se level due to increased sulphur supply is largely due to competitive inhibition, as sulphate and selenate use the same transporter (Lauchli, 1993; Pezzarossa et al, 1999; Terry et al, 2000). This effect is not limited to selenate, however. Zayed et al (1998), using broccoli, Indian mustard, sugarbeet and rice, found that increasing sulphate in culture solution from 0.25 mM to 10 mM inhibited selenite and selenomethionine uptake by 33% and 15-25%, respectively. Moreover, selenate uptake was reduced by 90%. This suggests that other mechanisms are involved in the inhibition of Se uptake by sulphur.

Nitrogen

In a survey of UK wheat and bread, Se level was found to be positively associated with protein level (Barclay & MacPherson, 1992), and soft wheats were found to contain less Se (0.02-0.13 mg/kg) than hard wheats (0.05-1.09 mg/kg), probably because of the lower protein level of soft wheats (Lorenz, 1978). Research on nitrogen and Se interaction is limited, but Gissel-Nielsen (1979) found that a high nitrogen level strongly increased the Se concentration in *Zea mays* roots exposed to selenite, but decreased translocation, and increased the proportion of selenoamino acids in xylem sap. A pasture trial found that a high nitrogen application (320 kg/ha/year) increased total Se uptake at the first harvest by a factor of four, but had little effect on plant Se concentration (Shand et al, 1992).

Phosphorus

There have been conflicting results reported on the effect of phosphorus application on Se uptake by plants (Elrashidi et al, 1989; He et al, 1994). The use of superphosphate tends to increase plant Se levels as it is a source of Se (Robbins & Carter, 1970), and phosphorus itself can increase the uptake of both natural and added Se by lucerne (Carter et al, 1972). The mechanism appears to involve decreased selenite sorption: soil colloids like iron oxides exhibit greater affinity to phosphorus than to selenite. The phosphorus is preferentially sorbed and the selenite is forced to sorb on sites with less binding-energy (He et al, 1994). Thus phosphorus can increase soil Se loss through leaching (Ylaranta, 1991).

Ylaranta (1990) found that despite addition of a high level of phosphorus to three soil types in pots, the effect on the Se uptake by ryegrass was small in all soils. He concluded that it was unlikely that phosphorus could play an important role in the uptake of Se by

crops, at least in Finland. This is likely to be the case elsewhere as well, as a similar result occurred with Italian ryegrass in France (More & Coppenet, 1980).

The evidence for the influence of sulphur on the uptake of Se by plants is conclusive, but the effect of other fertiliser components such as nitrogen and phosphorus is poorly understood because of the complexity of the processes involved and the lack of extensive research. In view of the finding that grain Se concentration may be associated with protein content, it is proposed that the effect of varying levels of added sulphur and nitrogen on grain Se concentration be investigated. It would be useful to know what levels of sulphur and nitrogen to apply in order to maximise grain Se concentration.

RESEARCH QUESTION 7: Do different combinations of sulphur and nitrogen applied at commercial rates affect wheat grain selenium density?

1.6.2.3. Se toxicity to wheat: effects on germination and early growth

At the Se levels used for fertilisation of wheat in the field (5-100 g/ha) it is unlikely that there would be any toxic effects, including growth inhibition. There have been few studies conducted, however, and some results are conflicting.

Toxic plant tissue levels of Se are generally above 5 mg/kg (Reilly, 1996), but among agricultural crops the phytotoxicity of selenium is variable. Mikkelson et al (1988) found that Se concentrations that caused a 10% yield reduction varied between species from 2 mg/kg DW for rice to 330 mg/kg DW for white clover. Bollard (1983) noted that tobacco and soybeans are Se-sensitive, and can be affected by concentrations as low as 1.0 mg/kg Se in culture media.

Of all plant species tested by Carlson et al (1989) in a three-day trial, wheat was the least sensitive to Se, with no effect on germination up to 32 mg Se/L of culture solution, as selenite or selenate. Other researchers have found that high (46-253 mg Se/L as selenite) concentrations were required to reduce seed germination in some species (Levine, 1925; Lintschinger et al, 2000; Spencer & Siegel, 1978). In a preliminary five-day study, Lintschinger et al (2000) found that a solution containing 50 mg/L of selenate did not affect germination capacity in wheat, and the early growth of wheat and lucerne was

slightly reduced. Growth inhibition was also found by Carlson et al: Se reduced radicle length, starting at 2 mg/L. Selenite had a larger inhibitory effect than selenate above 4 mg/L, a finding supported by Smith & Watkinson (1984), with growth of selenate-treated plants remaining at 85% of controls even up to 25 mg Se/L.

Se toxicity in wheat is evident by leaves turning yellow and the midrib becoming white and chlorotic; there may also be pinkish spots on the roots (Fiskesjo, 1979). Se toxicity can be reduced by increased levels of sulphate or phosphate in the soil or culture solution and increased sulphur in the tissues (Bollard, 1983).

There appears to be several mechanisms for Se toxicity in plants. Incorporation of selenocysteine into protein instead of cysteine could alter disulphide bridge formation, which affects protein activity (Brown & Shrift, 1982). The chlorosis induced by excess Se may be due to inhibition of porphobilinogen synthetase, an enzyme required for chlorophyll biosynthesis (Padmaja et al, 1989). Both selenate and selenite inhibit the reduction of nitrate in leaves (Aslam et al, 1990), and selenate may inhibit glutathione biosynthesis (De Kok & Kuiper, 1986). More recently, it has been found that, while Se acts as an antioxidant and inhibits lipid peroxidation at low concentrations, it has the opposite effect at higher concentrations and can increase lipid peroxidation products (Hartikainen et al, 2000).

Although it is unlikely that toxic effects would occur at the levels of selenate applied to raise grain Se concentration to, say, 1 mg/kg, we need to be sure that yield is not reduced. It is apparent from conflicting reports that further studies are required to clarify the threshold of toxicity of Se for early growth in wheat.

RESEARCH QUESTION 8: At what higher levels of applied selenium do reduction in wheat germination and growth occur?

1.6.3. Wheat breeding to increase grain selenium density

1.6.3.1. The extent of human micronutrient malnourishment

Around half of the world's population is malnourished. More than two billion people consume diets that are less diverse than 30 years ago, leading to deficiencies in

micronutrients, especially iron, zinc, iodine and selenium, and also vitamin A. In some regions almost everyone suffers from some form of "hidden hunger". In South-East Asia, for example, it is estimated that iron deficiency affects 98.2% (around 1.4 billion) of the people (Graham et al, 2001).

Cereals are generally low in micronutrients compared to other food crops, thus cereal-dominated food systems are low in micronutrients. The people most at risk are resource-poor women, infants and children. These authors conclude that a new agricultural paradigm is needed to address global micronutrient malnutrition: "...an agriculture which aims not only for productivity and sustainability, but also for balanced nutrition, or what we have called the *productive, sustainable, nutritious food systems paradigm*" (Graham et al, 2001, 91).

1.6.3.2. Breeding for higher nutrient density in staple crops

Programs which include fortification, education and supplementation have been successful in countering micronutrient deficiencies in certain cases and will continue to play a role. However, they tend to be expensive, require ongoing inputs and often fail to reach all individuals at risk. Furthermore, such programs themselves are at risk from economical, political and logistical impacts (Gibson, 1994; Graham & Welch, 1996).

On the other hand, a strategy of breeding staple crops with enhanced ability to fortify themselves with micronutrients offers a sustainable, cost-effective alternative, which is more likely to reach those most in need and has the added advantage of requiring no change in current consumer behaviour to be effective. It represents a strategy of "tailoring the plant to fit the soil" rather than the opposite, which is afforded by the soil fertilisation approach (Bouis, 1996; Graham et al, 2001).

Exploiting the genetic variation in crop plants for micronutrient density is likely to be an effective method to improve the nutrition of entire populations. A four- to five-fold variation was found between the lowest and highest grain iron and zinc concentrations among wheat accessions studied at CIMMYT, and the highest concentrations were double those of popular modern varieties (Graham et al, 2001). Moreover, wild, small-seeded relatives of modern bread wheats have been found with 50% more iron and zinc than the highest CIMMYT germplasm studied (Ortiz-Monasterio, 1998). If, for example, a wheat

variety were identified which was both high yielding and produced twice the grain Se density of most other varieties, it could result in a significant increase in a population's Se intake.

Substantial genetic variation has also been found among rice varieties for iron and zinc density, maize for beta-carotene, and cassava for protein, iron, zinc, calcium, beta-carotene and vitamin C (Graham et al, 2001). An overall average genetic variation in micronutrient density in seeds of food staple crops is probably around a factor of three (Bouis, 1996).

The micronutrient density of wheat and other food crops is important not only for human nutrition but also for animal nutrition and seedling growth, especially in deficient soils. Well-nourished seedlings are more likely to survive, grow faster and resist disease better, leading to higher yield (Genc et al, 2000; Longnecker et al, 1991; Rengel & Graham, 1995; Yilmaz et al, 1997).

Graham et al (2001) and Bouis (1996) emphasise the importance of combining nutrient density traits with high yield. There is unlikely to be a premium paid for a higher quality product (unless for protein), so new, high-nutrient varieties must also be attractive to farmers in terms of yield. In all the crops examined so far, it is possible to combine high micronutrient density with high yield. This is not the case for sulphur-amino acid and yield, and as Se density may be associated with grain sulphur-amino acid concentration, it may be difficult to identify and breed high-yielding, high-Se varieties. The above authors also note that another key issue in plant breeding for micronutrient density is bioavailability: will the added nutrients be sufficient to have a significant effect on human nutrient status? As discussed above, Se in wheat is highly bioavailable.

A benefit-cost analysis of a breeding approach to increase wheat grain zinc density in Turkey, using very conservative assumptions and current dollar values, estimated that costs of $13 million would produce benefits of $274 million (economic only, with no account taken of improved health and quality of life), a favourable benefit-cost ratio of 21 (Graham et al, 2001). In contrast, fortification requires yearly funding, and if the investment is not sustained, the benefits cease.

1.6.3.3. Screening and selection; the importance of genotype-environment interaction

In the screening phase of a breeding program, genotype-environment interaction needs to be relatively low for a breeding approach to be viable. Field trials where different genotypes are grown at the same site in the same season should enable comparison of genotypes. Ideally, trials should be conducted for two years at the same site, as different grain nutrient levels of genotypes grown on different soils can even be reflected in the first-year grain harvested. Furthermore, the variability in soil availability for micronutrients is generally much greater than that for macronutrients (Graham, 1991), hence field trials need to be of limited area to reduce spatial variability; paired-plots, where each replicate comprises an adjacent treatment and control plot, can be a useful technique.

A major gene together with several minor additive genes are likely to control the uptake of nutrients into crop plants, and another group of genes, also featuring a major gene, appear to control grain loading (Epstein, 1972; Graham, 1984; Ripperger & Schreiber, 1982). It has been found in Excalibur wheat, for example, that high zinc concentration in grain and zinc efficiency are not strongly associated. Although Excalibur had the highest and second-highest zinc contents in grain (in grams/ha) for the high zinc and zero zinc treatments, respectively, its grain zinc concentration (in mg/kg) was among the lowest evaluated, diluted by high starch. Ideally, genes for such efficiency should be combined with those for enhanced grain loading (Graham et al, 1997).

The same principle would also apply to Se. In selecting for grain Se density, both high uptake from the soil and high capacity to translocate Se from the vegetative tissues to the grain are required. However, as discussed above, Se is not known to be essential for plants, hence agronomic efficiency may not be involved. Nevertheless, physiological/biochemical efficiency is likely to be important, involving efficiency of Se metabolism within the plant. This may be due to a higher concentration of the endogenous chelator of Se in the phloem that increases Se transport to the inflorescence, or to more transporters at the plasma membrane at unloading or uptake into the grain itself (Dr. J. Stangoulis, Adelaide University, pers. comm. 10/1/2002).

Studies of rye addition and translocation lines (which include extra chromosome pairs and chromosome arm substitutions, respectively) have shown that zinc, copper and manganese

efficiency are not linked (Graham et al, 1981), which indicates that independent, specific genes are involved. Zinc efficiency traits appear to be additive, and the genes involved can be "pyramided" in bread wheat to breed improved cultivars, a process which can be greatly accelerated by the use of doubled haploid populations (Grewal et al, 1997).

Gene technology, including the use of molecular markers (cDNAs, RFLPs, AFLPs and RAPDs) for the mRNAs expressed by efficiency and grain loading genes, provides an alternative to conventional plant breeding as a means to enhance micronutrient density in cereals (Graham et al, 1997). The introduction, for example, of a gene which facilitates expression of the permease sulphate transporter would be likely to increase uptake and transport of selenate, although this would not be effective on acid soils where Se exists in other, less available forms.

Surveys of Se level in grains have suggested that environment may be more important than genotype in determining grain Se density. The Queensland study of Noble & Barry (1982) discussed above found differences in Se concentration in wheat of up to 100-fold at different sites, but little difference between wheat varieties. The maximum variation for both species and varieties at any site was five-fold. A Japanese study found that Se levels in rice grown in different parts of the country varied from 11 to 182 μg/kg. A significant difference was found between levels in rice from different districts, but not between different rice varieties grown on the same soil (Yoshida & Yasumoto, 1987).

Genotype differences are apparent, however, in a study of Se in hulled (spelt and emmer accessions) and modern bread and durum wheats, grown together. The hulled wheats had higher concentrations of Se, lithium, magnesium, phosphorus and zinc. Five spelt accessions had twice the Se concentrations of emmer, and from two to eight times those of normal wheat (Piergiovanni et al, 1997). This is in contrast to Grela (1996), who observed no Se differences between spelt and wheat. Hence it is not clear at this stage whether sufficient genetic variability for grain Se density exists between wheat cultivars to enable selection for this trait.

RESEARCH QUESTION 9: Does sufficient genotypic variation exist in wheat for grain selenium density to enable selection for this trait?

1.7. Conclusion

Research results continue to illustrate the importance of selenium in human health, in particular its cancer preventive capacity. It is evident, due mainly to its poor availability in many soils, that as many as one billion people may be Se-deficient. The vast majority of the world's population would receive well below the level needed to maximise cancer prevention, estimated at around 275 micrograms/day for an adult. The average Australian adult would ingest around 75 μg Se/day.

Se levels in Australian wheat are generally moderate, but due to widespread wheat consumption and the high bioavailability of selenium in wheat, this source probably accounts for around half the Se utilised by Australians. An increase in the Se content of wheat grain is likely to be the most cost-effective method to increase Se levels in the human population. A substantial increase in population Se intake could result in decreased rates of several cancers, cardiovascular disease, viral disease sequelae, and a range of other conditions that involve oxidative stress and inflammation, with consequent reductions in health costs.

Australia exports around 68% of its annual wheat production, so a significant increase in its mean wheat Se level would be expected to improve population health in those countries where mostly Australian wheat is consumed, such as Iraq, Iran, Egypt, Indonesia, South Korea and Japan.

The most promising strategies to increase Se in wheat appear to be Se fertilisation and breeding wheat varieties with superior ability to accumulate Se in the grain. Studies in Europe and North America have shown that the addition of as little as 10 g Se/ha can increase grain Se level by up to 0.40 mg/kg. However, studies are needed to determine the most efficient methods for Australian conditions, to clarify the Se phytotoxicity threshold for wheat, and to determine the extent of genetic variability for grain Se accumulation.

References for the Literature Review and General Discussion of the thesis begin on p. 57.

2. List of Research Questions for this Thesis

1. What is the current selenium status of a sample of healthy South Australian residents?

2. What are the grain selenium concentrations in a sample of wheats grown recently on a range of soil types in South Australia?

3. In what proportions is selenium stored in the different fractions of the wheat grain?

4. What effects do post-harvest processing and cooking have on grain Se content?

5. What is the most effective fertilisation method to increase selenium density in wheat grain on South Australian soils of different pH?

6. Do different combinations of sulphur and nitrogen applied at commercial rates affect wheat grain selenium density?

7. At what higher levels of applied selenium do reduction in germination and growth occur?

8. Does sufficient genetic variation exist in wheat for grain selenium density to enable selection for this trait?

3. Articles presented for this Thesis

1. Lyons GH, Stangoulis JCR, Graham RD. High-selenium wheat: biofortification for better health.
   Published in *Nutrition Research Reviews* 2003; **16**: 45-60.

2. Lyons GH, Stangoulis JCR, Graham RD. Nutriprevention of disease with high-selenium wheat.
   Published in the *Journal of the Australian College of Nutritional and Environmental Medicine 2003;* **22**(3): 3-9.

3. Lyons GH, Judson GJ, Stangoulis JCR, Palmer LT, Jones JA, Graham RD. Trends in selenium status of South Australians.
   Published in *The Medical Journal of Australia* 2004; **180**(8): 383-386.

4. Lyons GH, Judson GJ, Stangoulis JCR, Palmer LT, Jones JA, Graham RD. Trend in selenium status, and current blood levels of mineral nutrients, of healthy South Australian residents.
   Not submitted for publication.

5. Lyons GH, Lewis J, Lorimer MF, Holloway RE, Brace DM, Stangoulis JCR, Graham RD. High-selenium wheat: agronomic biofortification strategies to improve human nutrition.
   Published in *Food, Agriculture and Environment* 2004; **2**(1): 171-178.

6. Lyons GH, Stangoulis JCR, Graham RD. Exploiting micronutrient interaction to optimize biofortification programs: the case for inclusion of selenium and iodine in the *HarvestPlus* program.
   Published in *Nutrition Reviews* 2004; 62(6): 247-252..

7. Lyons GH, Ortiz-Monasterio I, Stangoulis JCR, Graham RD. Selenium concentration in wheat grain: is there sufficient genotypic variation to use in breeding?
   Accepted for publication in *Plant and Soil* 2004.

8. Lyons GH, Genc Y, Stangoulis JCR, Palmer LT, Graham RD. Distribution of selenium and other minerals in wheat grain, and the effect of processing on wheat selenium content.
   Accepted for publication in *Biological Trace Element Research* 2004.

9. Lyons GH, Stangoulis JCR, Graham RD. Tolerance of wheat (*Triticum aestivum* L.) to high soil and solution selenium levels.
   Accepted for publication in *Plant and Soil* 2004.

Nutrition Research Reviews 2003; **16**: 45-60.

High-selenium wheat: biofortification for better health

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Abstract

The metalloid selenium (Se) is ubiquitous in soils, but exists mainly in insoluble forms in high-iron, low pH and certain leached soils, and hence is often of limited availability to plants. Consequently, it is often supplied by plants to animals and humans at levels too low for optimum health. Se deficiency and sub-optimality are manifested in populations as increased rates of thyroid dysfunction, cancer, severe viral diseases, cardiovascular disease, and various inflammatory conditions. Se deficiency probably affects at least a billion people. Optimal cancer protection appears to require a supra-nutritional Se intake, and involves several mechanisms, which include promotion of apoptosis, and inhibition of neo-angiogenesis. Evidence suggests that in some regions Se is declining in the food chain, and new strategies to increase its intake are required. These could include education to increase consumption of higher-Se foods; individual supplementation; food fortification; supplementation of livestock; Se fertilisation of crops, and plant breeding for enhanced Se accumulation. Se levels in Australian residents and wheat appear to be above a global estimated mean, but few studies have been conducted. Wheat is estimated to supply nearly half the Se utilised by most Australians. Increasing the Se content of wheat represents a food systems approach that would increase population intake, with consequent likely improvement in public health, and health cost savings. The strategies that show most promise to achieve this are biofortification by Se fertilisation and breeding wheat varieties that are more efficient at increasing grain Se density. Research is needed in Australia to determine the most cost-effective fertilisation methods, and to determine the extent of genetic variability for grain Se accumulation. Before recommending large-scale fortification of the food supply with Se, it will be necessary to await the results of current intervention studies with Se on cancer, HIV/AIDS and asthma.

Selenium: Biofortification: Wheat: Disease prevention

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Introduction

Since Se was recognised as an essential nutrient (Schwarz & Foltz, 1957), a voluminous literature has accumulated that describes the profound effect of this element on human health. The findings of recent human intervention trials (Clark *et al.* 1996; Yu *et al.* 1997) have stimulated interest in a cancer-preventive role for Se. In addition to its cancer preventive capacity, Se has an anti-viral effect (Baum *et al.* 1997; Beck *et al.* 1995; Yu *et al.* 1997, 1999). Given the high global incidences of HIV, hepatitis B and C, and other RNA viruses, including measles and influenza, the public health implications of selenium

deficiency (estimated by Combs (2001) to affect more than a billion individuals) and suboptimality are enormous.

Several comprehensive reviews have examined Se and human health, including those of Reilly (1996), Rayman (2000, 2002) and Combs (2001). Combs discusses Se in humans within a food systems context and makes the distinction between Se's normal metabolic roles and its anti-carcinogenic activity at supra-nutritional levels.

It is important to note that the biological actions of Se are not properties of the element *per se*, but rather are properties of its various chemical forms. Inorganic Se forms (selenate, selenite) undergo reductive metabolism, yielding hydrogen selenide, which is incorporated into selenoproteins. Successive methylation of hydrogen selenide detoxifies excess Se. Selenomethionine can be incorporated non-specifically into proteins in place of methionine, and selenocysteine is catabolised to hydrogen selenide by a beta-lyase (Combs, 2001).

The present review summarises briefly the roles of Se in soils, plants and animals. The importance of Se in human health is discussed, followed by Se intake by humans, with a focus on Australia. Strategies to increase Se intake are presented. The review then examines wheat as an important source of bioavailable Se, and discusses Se fertilisation and plant breeding, two strategies to increase Se density in wheat grain.

Background

Soil Se is uneven in distribution and availability: concentrations range from less than 0.1 to more than 100 mg/kg; however, most soils contain between 1.0 and 1.5 mg/kg (Berrow & Ure, 1989). In general, total soil Se of 0.1 to 0.6 mg/kg is considered deficient. Soils in New Zealand, Denmark, Finland (pre-1984, before Se was added to fertilisers), central Siberia, and a belt from north-east to south-central China are notably Se-deficient and hence have suboptimal levels in their food systems (Gupta & Winter, 1975; Lag & Stiennes, 1978; Combs, 2001). Large areas of Africa, including much of Zaire, are also likely to be Se-deficient, but further mapping is required. On the other hand, parts of the Great Plains of the USA and Canada, Enshi County in China, and parts of Ireland, Colombia and Venezuela are seleniferous (Combs, 2001).

In acidic, poorly aerated soils, Se is relatively unavailable to plants and occurs mainly as insoluble selenides and elemental selenium. In lateritic soils, which have a high iron content, it binds strongly to iron to form poorly soluble ferric hydroxide-selenite complexes (Cary & Allaway, 1969). In wetter regions, selenate can be leached from the soil, resulting in selenium-deficient areas, for example New Zealand and Tasmania (Reilly, 1996). The availability of soil Se to crops can be affected by irrigation, aeration, liming and Se fertilisation (Gissel-Nielsen, 1998).

Australia has both high- and low-Se soils and large areas that have not been mapped for the element. Seleniferous soils occur in central Queensland and parts of Cape York Peninsula. Se deficiency in Australia usually occurs on acidic soils with more than 500 mm rain per year, such as the Central and Southern Tablelands and Slopes and the Northern Tablelands of New South Wales, the south-eastern coast of Queensland, south-west Western Australia; coastal and central regions of Victoria, much of Tasmania, and South Australia's Mount Lofty Ranges and Kangaroo Island (see Fig. 1) (Reuter, 1975; Reilly, 1996).

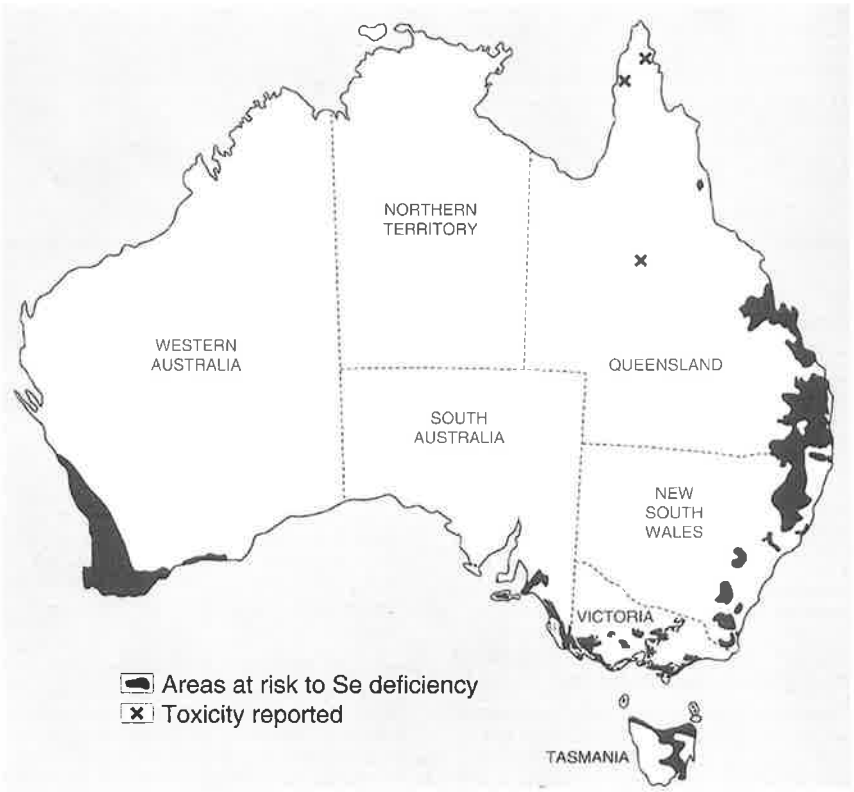

Fig. 1. Selenium in Australian soils (from Judson & Reuter, 1999)

The Se contents of plants vary according to available soil Se and plant species. For example, wheat grown in Shaanxi Province, China may have 0.003 mg/kg Se in the grain, compared to 2.0 mg/kg for wheat from the North or South Dakota wheatlands (Combs, 2001). Wheat from highly seleniferous areas of South Dakota may contain more than 50 mg/kg Se (G Combs, personal communication), while *Astragalus* on the same soils may accumulate up to 15,000 mg/kg dry weight (Beath *et al.* 1937).

Although lower plants such as algae require Se for growth (Lindstrom, 1983), it is not considered to be an essential nutrient for higher plants (Terry *et al.* 2000), although previous studies to ascertain essentiality failed to account for volatile selenium compounds (Broyer *et al.* 1966).

In higher plants, selenate is absorbed by roots via the sulphate transporter, a high-affinity permease. High soil sulphate level decreases selenate influx (Cary & Gissel-Nielsen, 1973). Se is transported via the xylem to chloroplasts in leaves where it is reduced and the selenium converted to organic forms, some of which are volatile. Selenate is transported more easily from root to shoot than is selenite or organic Se (Terry *et al.* 2000).

Because Se is an essential nutrient, animals respond positively to it where their diet contains less than 0.1 mg/kg Se in dry matter (Oldfield, 1993). Conditions that are related to Se deficiency, some of which occur on a wide scale in certain countries, include white muscle disease, exudative diathesis, pancreatic degeneration, liver necrosis, mulberry heart disease, and ill-thrift. Se deficiency is usually not the only cause of these diseases (Reilly, 1996).

Selenium: essential for human health

Selenium deficiency diseases

In parts of China and eastern Siberia two overt Se-deficiency diseases occur: Keshan disease and Kaschin-Beck disease. Keshan disease occurs mainly in children and women of child-bearing age. and involves impairment of cardiac function, cardiac enlargement and arrhythmia (Reilly, 1996). The disease's aetiology is likely to be complex, involving Se and vitamin E deficiencies, and presence of the Coxsackie B virus (Yang *et al.* 1994; Levander & Beck, 1999; Liu *et al.* 2002).

Kaschin-Beck disease is an osteoarthropathy which manifests as enlarged joints, shortened fingers and toes, and in severe cases dwarfism. Se and vitamin E deficiency (Reilly, 1996) and iodine deficiency (Neve, 1999) are likely to be predisposing factors whereas fulvic acids in drinking water (Peng *et al.* 1999) or mycotoxins in food (Xiong *et al.* 1998) are probable causes.

Antioxidant, anti-inflammatory, thyroid and immunity roles

Selenocysteine, the 21st amino acid, is present in selenoproteins, which have important enzyme functions in humans. Glutathione peroxidase (of which at least five forms exist) has an antioxidant role in reducing damaging hydrogen peroxide and lipid/phospholipid hydroperoxides produced in eicosanoid synthesis by the lipoxygenase and cyclo-oxygenase pathways (Spallholz *et al.* 1990). This function reduces damage to lipids, lipo-proteins and DNA, and hence reduces risk of cardiovascular disease and cancer (Diplock, 1994; Neve, 1996). Moreover, selenite inhibits tumour necrosis factor-alpha-induced expression of adhesion molecules that promote inflammation (Zhang *et al.* 2002).

There is a growing body of evidence to suggest that Se (especially in the sodium selenite form) can alleviate conditions associated with high levels of oxidative stress or inflammation. These include asthma (Jahnova *et al.* 2002; Shaheen *et al.* 1999), diabetes (Kowluru *et al.* 2001), arthritis (Peretz *et al.* 2001), muscular dystrophy (Kurihara *et al.* 2000), cystic fibrosis (Kauf *et al.* 1994), acute pancreatitis (De las Heras Castano *et al.* 2000), osteoarthritis (Kurz *et al.* 2002), systemic inflammatory response syndrome (Angstwurm *et al.* 1999) and kwashiorkor (Ashour *et al.* 1999). In addition, Schrauzer (1998) discusses the application of selenite therapy to viral haemorrhagic fever, acute septicaemia and lymphoedema. Another group of selenoenzymes, the thioredoxin reductases are involved in reduction of nucleotides in DNA synthesis, regeneration of antioxidant systems, and maintenance of intracellular redox state (Allan *et al.* 1999).

The thyroid gland has the highest Se concentration of any human organ (Kohrle, 1999), and Se is involved in thyroid metabolism through the iodothyronine deiodinases, which catalyse the production of active thyroid hormone, T3, from thyroxine, T4 (Beckett *et al.* 1987). The iodine deficiency diseases goitre and myxedematous cretinism are more prevalent in central Africa in those regions which are deficient in both iodine and Se (Vanderpas *et al.* 1993), and in such areas supplementation with both nutrients is indicated.

Se has a role in many aspects of the immune response to infections. Se deficiency reduces immunocompetence, involving impairment of neutrophil, macrophage and polymorphonuclear leukocyte activity (Boyne & Arthur, 1986; Dimitrov *et al.* 1984; Spallholz *et al.* 1990). Se supplementation of even supposedly Se-replete individuals is

immunostimulatory, and involves enhancement of natural-killer-cell and lymphocyte activity as well as enhancement of proliferation of activated T-cells (Kiremidjian-Schumacher *et al.* 1994).

Cancer

Selenium and its relationship to cancer has been thoroughly reviewed recently (Whanger, 2004). There is "perhaps no more extensive body of evidence for the cancer preventive potential of a normal dietary component than there is for selenium" (Combs & Gray, 1998, 186). From the late 1960s, epidemiological studies have suggested an inverse association between human Se intake and cancer mortality (Combs & Gray, 1998). An extensive literature documents the numerous *in vitro* and animal studies that have been conducted during the past 35 years. Most demonstrate that application or intakes of Se at supranutritional levels can inhibit tumorigenesis (El-Bayoumy, 1991; Combs & Gray, 1998; Ip, 1998). Prospective cohort and case-control studies that have involved as many as 34,000 people have generally shown an association between low Se status and a significantly higher risk of cancer incidence and mortality (Yoshizawa *et al.* 1998; Yu *et al.* 1999; Brooks *et al.* 2001). Indeed, of eight human trials which have studied the effects of Se on cancer incidence or biomarkers, all but one have shown a Se benefit (Whanger, 2004).

Intervention studies using Se as a single chemopreventive agent include the Qidong trials in China, where selenite significantly reduced primary liver cancer (Yu *et al.* 1997). In the Nutritional Prevention of Cancer (NPC) trial in the US, 200 μg Se/d (as yeast) reduced total cancer mortality by 41%, total cancer incidence by 25% and prostate cancer incidence by 52% in a cohort of 1,300 people. The effect on total cancer was limited to male smokers (current or previous) with baseline Se levels below 113 μg/l, although non-smoking males below this level are likely to have benefited from Se supplementation in terms of prostate and colon cancer protection (Duffield-Lillico *et al.* 2002).

The NPC trial was conducted in a region of the US where Se intakes are estimated to be around 90 μg/d, well above the level required for optimal selenoenzyme activity. This suggests additional mechanisms in Se's cancer-preventive role. While some cancer protection, particularly that through antioxidant activity, involves selenoenzymes, the anti-cancer effects of Se are likely to involve the production of specific anti-tumorigenic metabolites, such as methylselenol. Studies have suggested that Se provided in certain forms can neutralise carcinogens, enhance the immune system, alter gene (including p53) expression, inhibit tumour cell metabolism and neo-angiogenesis (blood vessel development around tumours), and promote apoptosis (programmed cell death) (Ip *et al.* 1991; Harrison *et al.* 1997; Combs & Gray, 1998; Jiang *et al.* 1999; Combs, 2000, 2001; Lu, 2000; Rayman, 2000; Finley & Davis, 2001; Seo *et al.* 2002).

According to this two-stage model of cancer prevention, which involves Se intakes that correct nutritional deficiency as well as much higher, supranutritional intakes, individuals with nutritionally adequate Se intakes may benefit from Se supplementation (Combs & Gray, 1998). Se's anti-cancer activities remain under intensive study worldwide.

Viral and mycobacterial diseases

Se deficiency is associated with increased virulence of a range of viral infections (Taylor, 1997). It is evident that in a Se-deficient host, normally harmless viruses can become virulent. For example, when Se-deficient mice are inoculated with benign Coxsackie B3

virus, the virus mutates into a virulent form that causes myocarditis similar to that seen in Keshan disease (Beck *et al.* 1995, 1998; Beck 2001). Furthermore, Se-deficient mice develop severe pneumonitis when infected with a mild strain of influenza virus (Beck, 2001).

Se appears to be of particular importance for people with HIV. Se deficiency is a significant predictor of HIV-related mortality (Baum & Shor-Posner, 1998; Campa *et al.* 1999) and viral load (Baeten *et al.* 2001). A US study found Se-deficient HIV patients to be twenty times more likely to die from HIV-related causes than those with adequate levels (Baum *et al.* 1997). The decline in blood Se levels occurs even in the early stages and is thus unlikely to be due to malnutrition or malabsorption (Look *et al.* 1997). Moreover, a study of HIV-1-seropositive drug users found low Se level to be a significant risk factor for developing mycobacterial disease, notably tuberculosis (Shor-Posner *et al.* 2002).

Se also appears to be protective in individuals infected with hepatitis B or C against progression to cirrhosis and liver cancer (Yu *et al.* 1997; Yu *et al.* 1999). Selenoproteins encoded by HIV, hepatitis C virus and the Ebola virus (which causes acute haemorrhage) have been discovered that consume the host's Se supply, thus reducing immune response (Taylor & Nadimpalli, 1999; Zhao *et al.* 2000).

Other health effects

Low Se status has long been known to reduce fertility in livestock (Underwood, 1977), and this also appears to be the case for humans. Low Se levels have been associated with male infertility (Behne *et al.* 1997) and spontaneous abortions (Barrington *et al.* 1996). In a Scottish study, supplementation of subfertile men with 100 μg Se/d for three months significantly increased sperm motility (Scott & MacPherson, 1998). Conversely, Hawkes & Turek (2001) found that a diet containing 300 μg Se/d caused a reduction in sperm motility in healthy men.

Se appears to be influential in the brain, and Rayman (2000) documents several studies that indicate low Se levels are associated with cognitive impairment, depression, anxiety and hostility. These conditions can be alleviated in individuals with low baseline Se levels by Se supplementation. Recent studies suggest that selenoprotein P (Whanger, 2001), selenoprotein W (Jeong *et al.* 2002) and the newly discovered selenoprotein M (Korotkov *et al.* 2002) have important roles in the brain.

Se forms selenides with all metals, and detoxifies Hg, Cd, Pb, Ag, Tl and As. This effect can be enhanced by vitamin E (Frost, 1981). In the case of Cd and Hg, detoxification is achieved through the diversion in their binding from low- to high-molecular-weight proteins (Whanger, 1992).

Human selenium intake

Selenium intake: low and getting lower

Selenium intake in humans is determined mainly by the level of available Se in the soil on which their food is grown, and by dietary composition. Se levels in major food classes usually occur within the following ranges: 0.10 – 0.60 mg/kg (fish), 0.05 – 0.60 (cereals),

0.05 – 0.30 (red meats), and 0.002 – 0.08 (fruit and vegetables) (Combs, 2001). Bioavailability, which varies with food source of Se, will be discussed below.

The absolute range of global daily Se intake by adults is around 7 (in Chinese Keshan disease areas) – 5,000 μg/d (in Chinese selenosis areas). Estimates provided by Combs (2001) of Se intake for several countries include England (12-43), Belgium (45), Canada (98-224), USA (60-220), Croatia (27), New Zealand (19-80), Japan (104-127) and Venezuela (200-350). In Australia few comprehensive studies have been conducted, but estimates of 63 and 96 μg/d have been provided, with a range of 23-204 (Fardy *et al.* 1989; Reilly, 1996). In view of the estimated mean plasma level of Se in Australian adults (see below), a mean intake of around 75 μg/day appears likely for Australians.

The US Recommended Daily Allowance, which is based on the Se levels considered to be necessary to maximise glutathione peroxidase activity, is 55 μg/d for both men and women, while in Australia it is 85 and 70 μg/d for men and women, respectively. The Third National Health and Nutrition Examination Survey in the USA (N = 17,630) indicated that 99% of the subjects were Se replete (i.e. above 80 μg/l plasma Se) and thus supplementation is not recommended (Burk, 2002). However, referring to the study of Neve (2000), Rayman (2000) points out that if platelet, rather than plasma, saturation of glutathione peroxidase activity is used as the measure of Se repletion, a higher intake is required, in the range of 80-100 μg/d.

It is evident that many people do not consume enough Se to support maximum expression of selenoenzymes, let alone the level required for optimum prevention of cancer. Combs (2001) estimates the number of Se-deficient people in the world to be in the range of 500-1,000 million. In addition, he considers that the vast majority of the world's population have suboptimal Se intakes, and hence are at increased risk of cancer, heart disease, viral diseases, and indeed any conditions that involve increased levels of oxidative stress.

Furthermore, evidence suggests that there is a trend toward a reduction of Se in the global food chain, caused by fossil fuel burning (with consequent S release), acid rain, soil acidification, the use of high-S fertilisers (Frost, 1987) and more intensive crop production (Gissel-Nielsen, 1998). Rayman (1997, 2000, 2002) and Giovannucci (1998) observe that blood Se levels have decreased significantly in the United Kingdom from 1984 to 1994, and current average Se intake in the UK may be as low as 34-39 μg/day (Barclay *et al.* 1995; MAFF, 1997, 1999). These authors attribute this fall in part to the use of low-Se UK and European wheat in place of North American wheat. This highlights the sensitivity of Se intake and body levels to changes in the food supply. Both authors call for action to increase Se intake.

Human blood concentrations of selenium: the global view

Blood Se levels are determined mainly by dietary intake, although gender, age, smoking and exposure to heavy metals can have a minor effect (Robberecht & Deelstra, 1994). Combs (2001) presented a comprehensive list of Se concentrations in plasma, serum or whole blood of healthy adults from sixty-nine countries. A sample of plasma or serum Se concentrations (μg/l) is as follows: Austria (67), Burundi (15), Canada (132), China-Keshan disease area (21), China-selenosis area (494), Finland pre-1984 (70) and post-1984 (92), Hungary (54), Japan (130), New Zealand (59), Norway (119), USA (119) and Zaire (27) (Combs, 2001). Note that there are few data available from some of the most populous areas of the world, including most of Africa, South America and central and south Asia. The mean value (calculated as the mean of the post-1990 means for a

representative sample of forty-five countries) is 78 μg/l, with a range of means of 15 (Burundi)-216 μg/l (Venezuela). However, this is likely to be optimistic as small studies of Se levels in much of Africa and central, south and South-East Asia indicate levels well below this.

This can be compared to the figure of 70 μg/l, the WHO's reference level, which is the minimum level for maximisation of plasma or serum glutathione peroxidase activities (Neve, 1995). Rayman (1997) has quoted studies that show that a level of 100 μg/l is required for optimal expression of plasma glutathione peroxidase. The vast majority of the world's population would not reach this level of plasma or serum Se.

Selenium levels in the Australian population

An estimate of 94 μg/l of plasma or serum Se for Australia can be derived from the means determined from seventeen studies of blood Se levels. The studies (with mean plasma or serum Se levels in μg/l) are: Judson *et al.* 1978, 1982 (124, 114); Pearn & McCay, 1979 (88); McOrist & Fardy, 1989 (98, 86); Brock *et al.* 1991 (88); Cumming *et al.* 1992 (81); Lux & Naidoo, 1995 (101); McGlashan *et al.* 1996 (80); Dhindsa *et al.* 1998 (92); Daniels *et al.* 2000 (77, 88); GJ Judson (unpublished results from 1987 and 1988) (91, 92, 101, 95); GH Lyons *et al.* (unpublished results from 2002) (103). This places Australia well above the estimated world mean reported Se level of 78 μg/l.

In Australia, relatively low blood Se levels have been reported in Adelaide infants. Daniels *et al.* (2000) found a plasma Se level of around 31 μg/l (SD 13) in a sample of newborn infants, a level comparable with that of New Zealand. Infant Se levels are typically half those of adults. These levels place the infants at increased risk of a range of conditions that involve oxidative stress and inflammation.

An apparent global decline in Se in the food chain was noted above. Se levels in South Australians may have fallen from 1977-1987. For instance, the mean whole blood Se level for a 1977 sample of Adelaide health workers was 155 μg/l (Judson *et al.* 1978), whilst Kangaroo Island residents in 1979 recorded a mean of 143 μg/l (Judson *et al.* 1982). However, in later samples of healthy Adelaide adults from 1987-2002, mean whole blood Se levels were 117, 126, 128, 118 (GJ Judson *et al.*, unpublished results from 1987 and 1988) and 125 (GH Lyons *et al.*, unpublished results from 2002), with Mount Gambier residents recording 121 in 1987 (GJ Judson *et al.*, unpublished results from 1987), for a grand mean for the period 1987-2002 of 122 μg/l. The apparent decline in the early 1980s may be due to changes in dietary composition and/or a decrease in Se concentration in South Australian-grown wheat.

Optimum selenium intake

In the NPC trial in the US, the protective effect of Se against cancer occurred in the lowest (relative risk of 0.52, 95% CI: 0.33-0.82) and middle (relative risk 0.64, 95% CI 0.40-0.97) tertiles, which included those individuals with plasma Se levels below 121 μg/l (Rayman & Clark, 2000), and the latest analysis shows that the Se benefit was largely restricted to male smokers with baseline plasma Se level below 113 μg/l The strongest protective effect was against prostate cancer, with a hazard ratio of 0.48 (95% CI: 0.28-0.80) (Duffield-Lillico *et al.* 2002). None of the subjects had plasma Se levels below 60 μg/l and very few were less than 80 μg/l, thus the cohort must be considered Se-adequate by current nutritional standards (Combs, 2000).

Although there is a risk in generalising results of individual epidemiological and intervention studies, this result would suggest, using the levels presented by Combs (2001) above, that the vast majority of the world's population (including that of Australia, with a probable mean plasma or serum level around 89 μg/l, and many populations in Europe (Rayman, 2000)) would be in the responsive range.

The NPC participants lived in a region where dietary Se intake is around 90 μg/d (Clark *et al.* 1996), thus with the addition of the 200 μg supplement, individuals in the treatment group would have received around 270-310 μg/d. Combs (2001) suggested that a Se intake of 200-300 μg/d may be required to significantly reduce cancer risk. This compares with an estimated Australian adult intake of 75 μg/d. Of course, as Rayman (2000) notes, Se requirement varies between individuals in the same population. Even Moyad (2002), who expressed doubts about the interpretations of certain Se studies, and considers some estimates of its cancer-protective effect to be optimistic, suggested that an intake of 200 μg Se/d and around 50 mg vitamin E/d may be beneficial, particularly for current or previous smokers. The results of the NPC trial (Duffield-Lillico *et al.* 2002) suggested that males may have a higher Se requirement than females. Further studies may find optimum adult Se intakes in the range 125-280 μg/d, with means of around 130 (for females) and 250 (for males). Pregnant females may have a higher Se requirement than non-pregnant females (Dylewski *et al.* 2002).

Chronic selenosis occurs in Enshi County, China where coal-contaminated soil contains up to 8 mg Se/kg, and residents have consumed up to 7 mg/d. Common symptoms include nail thickening and cracking and hair loss, and some people exhibit skin lesions (Liu & Li, 1987; Yang & Zhou, 1994). The concern that the incorporation of selenomethionine into body proteins could increase Se to toxic levels appears unwarranted because a steady state is established, which prevents the uncontrolled accumulation of Se (Schrauzer, 2000).

Combs (2001) considered it probable that the WHO and European Union estimates of the upper safe limit of Se intake of 400 and 300 μg/adult/d, respectively, are too conservative. Under normal conditions, a Se intake of less than 1,000 μg/d (or 15 μg/kg bodyweight) does not cause toxicity (Neve, 1991; Poirier, 1994; Whanger *et al.* 1996; Taylor, 1997). Those living in parts of China, the USA, Venezuela and Greenland have ingested Se at this level for their entire lives without ill effects (Taylor, 1997).

However, it would be prudent at this stage to limit medium- to long-term Se intake to around the US reference dose, which has been set at 350 μg/d for a 70 kg human (Schrauzer, 2000) for several reasons. First, Vinceti *et al.* (2001) have documented possible adverse effects of levels of supplemented Se around 300 μg/d on thyroid status. Second, a reduction in sperm motility in a group of eleven men supplemented with 300 μg Se/d for 3 months has been shown by (Hawkes & Turek, 2001). Third, there has been a surprising finding from the NPC trial of a non-significant increase in risk (based on small case numbers) of five cancer types (melanoma, lymphoma and leukaemia, breast, bladder, and head and neck cancers). Nevertheless, biofortification of cereals with Se at the rates discussed below would be very unlikely to place consumers at risk of any adverse health effects from Se.

The food systems of very few countries appear to deliver an optimum level of Se to their populations, and indeed the food systems of most countries do not even provide enough Se to maximise selenoenzyme expression. The impact of this deficiency and suboptimality in global health terms is difficult to quantify, but is likely to be enormous given the high prevalence of various cancers, cardiovascular diseases, viral diseases (including AIDS,

hepatitis, measles and influenza), and exposure to environmental pollutants throughout much of the world. It is thus a matter of urgency that many countries begin to address this major public health issue and develop effective, sustainable ways to increase Se intakes (Combs, 2001).

Strategies to increase human selenium intake

Given that the populations of most countries would be likely to benefit from an increased Se intake, how could this be best achieved? Strategies to increase Se intake include increased consumption of higher-Se foods through education, individual supplementation, food fortification, supplementation of livestock, use of selenium fertilisers, and plant breeding for enhanced selenium accumulation. Each of these shall be discussed briefly.

Increased consumption of higher-selenium foods through education

Globally, wheat is probably the most important dietary source of Se. Even in Europe, with its low levels of available soil Se, bread and cereals, being commonly consumed, are important Se sources. In the UK, for example, it is estimated that bread and cereals supply around 22% of Se, second to meat, poultry and fish (36%) (Ministry of Agriculture, Fisheries & Food, 1997).

Brazil nuts provide the most concentrated natural food source of Se. A study conducted in the UK found concentrations of 2-53 mg Se/kg (Thorn *et al.* 1976), while an Australian study found even higher levels: 0.5-150 mg/kg (Tinggi & Reilly, 2000).

Individual supplementation

In Western countries many individuals currently consume Se supplements, which are available in both inorganic and organic forms. Sodium selenite, available in tablet or fluid form, is preferable to selenate (Chen *et al.* 2000; Finley & Davis, 2001). High-Se yeast includes several organic Se forms, including selenomethionine and selenocysteine (Bird *et al.* 1997). Another form of individual supplementation is selective consumption of the fortified or "functional foods" listed below.

Studies suggest that dietary sources of Se, vitamin E and beta-carotene are preferable to supplements (Moyad, 2002). Moreover, a well-known drawback of individual supplementation as a population strategy to improve nutrition is that those who are most in need tend to be the least likely to take supplements.

Food fortification

This approach has been used successfully with folate-enriched breakfast cereals, Fe-enriched milk, iodised salt, carotene- and vitamin E-enriched margarine, as well as selenised salt in Se-deficient regions of China. Seleniferous areas can be considered resources for the production of Se-enriched plants; for example, in China an elixer is made from high-Se tea in Enshi County (Combs, 2001), and high-Se wheat from South Dakota, USA attracts a premium price (Fedgazette, 2000). These examples can be included in the *functional foods* category, along with high-Se broccoli (Finley, 1999), high-Se garlic (Ip & Lisk, 1994) and the high-Se yeast noted above.

Selenium supplementation of livestock

Supplementation strategies to increase dietary Se intake by livestock (and thus increase levels in meat and milk) include Se fertilisation of pastures, dietary supplements (e.g. the now widespread practice of adding selenomethionine to the rations of livestock, including dairy cows during milking), and direct administration (drenches, slow-release reticulum/rumen "bullets", injection).

In New Zealand, sodium selenate is supplied as a prill to pastures (Reilly, 1996). In a Western Australian study, slow-release barium selenate applied at 10 g/ha prevented subclinical selenium deficiency in sheep for 4 years, whereas a single application of sodium selenate at the same rate was effective for only 15 months (Whelan & Barrow, 1994).

Supplementation of livestock with Se is unlikely to be an efficient strategy to increase Se level in the human population, however. In New Zealand, little increase in the Se content of human foods was observed after the introduction of Se supplementation for farm animals in the 1960s (Thomson & Robinson, 1980).

Selenium fertilisation of crops

The use of Se as a soil amendment in fertiliser is practised mainly in Finland (by law from 1984), where it is currently added to NPK fertiliser at a rate of 10 mg/kg (Eurola & Hietaniemi, 2000), and New Zealand (at an individual level, and generally on pastures).

The Finnish experiment has demonstrated the safety, effectiveness, ease and cost-efficiency of this approach to raise Se levels in a human population. Dietary Se intakes trebled and plasma Se concentrations nearly doubled within 3 years of the program's commencement (Aro *et al.* 1995). However, it is difficult to isolate the effects of a single factor, such as dietary change, from other factors that can be involved in the aetiology of such conditions as cancer and cardiovascular disease. There have been significant decreases in the rates of cardiovascular disease and certain cancers in Finland since 1985, but with no controls for comparison, this cannot be ascribed to Se alone (Varo *et al.*, 1994).

Sodium selenate is the Se form generally used for crop and pasture fertilisation; it is weakly adsorbed on soil colloids and can bring about a rapid increase in plant Se level (Gupta & Watkinson, 1985). The enhancement of wheat Se level by fertiliser will be discussed further below.

Plant breeding for enhanced selenium accumulation

Breeding for improved Se uptake and/or retention by plants may be an effective, sustainable strategy. Preliminary studies have found a 15-fold variation in Se-accumulating ability among brassica vegetables (Combs, 2001), and a Se-accumulating soybean cultivar has been identified (Wei, 1996). Substantial variability exists within cereal crop varieties for zinc, iron and other nutrients (Graham *et al.* 1999). These findings suggest that it should be possible to breed cultivars with enhanced Se uptake and/or retention, or to use genetic engineering to enhance Se levels (and even specific Se metabolites) in food crops.

In summary, each of these strategies could contribute to enhanced delivery of Se to human populations through their food systems. As described by Welch & Graham (1999), a food systems paradigm encompasses an agriculture that aims not only at productivity and sustainability, but also at improved nutrition. In Australia, a program that combines selection for enhanced grain Se content in wheat, strategic fertilisation of wheat and barley, education to encourage greater consumption of higher-Se foods, increased supplementation of livestock grazing acid soils in high rainfall areas, and education and targeted supplementation of high-risk individuals, would be likely to significantly improve population health.

Wheat: an important selenium source for humans

Surveys indicate that wheat is the most efficient Se accumulator of the common cereal crops (wheat, rice, maize, barley, oats) and is one of the most important Se sources for humans. In a Russian survey, serum Se level was found to be highly correlated ($r = 0.79$) with Se level in wheat flour (Golubkina & Alfthan, 1999). Bread is the second most important source of Se in the US (Schubert *et al.* 1987), and has been found to supply one-third of the daily Se intake of Australian children (Barrett *et al.* 1989). With the addition of Se supplied through breakfast cereals, cake and biscuits, and in view of its high bioavailability, wheat-Se probably supplies around half the Se utilised by Australians.

Selenium concentrations in wheat grain

The global view. There is wide variation in wheat grain Se level between and within countries. Published values range from 0.001 mg/kg in south-west Western Australia (White et al, 1981) to 30 mg/kg in highly seleniferous areas of South Dakota (University of California, 1988), but most of the world's wheat falls within the 0.020-0.600 mg/kg range (Alfthan & Neve, 1996). Canada and the US have relatively high levels, usually in the 0.2-0.6 mg/kg range (Reilly, 1996). New Zealand and Eastern Europe generally have low levels, for example the 0.028 mg/kg average found by Mihailovic *et al.* (1996) for Serbia.

Some countries, including China (with a range of 0.01-0.23 mg/kg (Alfthan & Neve, 1996)), Canada and the US, have highly variable wheat Se levels, even within states. Se concentrations in wheat grain from 12 locations in Manitoba, Canada in 1986-88 ranged from 0.06-3.06 mg/kg, and levels varied between years within a location (Boila *et al.* 1993). A US survey, also in 1986-88, analysed major brands of white bread in nine different geographical regions. The overall range was 0.06-0.74 mg/kg, while a single brand of bread collected from different bakeries in Boston alone had a range of 0.24-0.92 mg/kg (CV 41%), with a mean of 0.60 mg/kg (Holden *et al.* 1991).

Selenium in Australian wheat. Four published surveys of Se concentrations in Australian wheat have been identified. The Queensland study (Noble & Barry, 1982) found a mean value for wheat grain Se of 0.150 mg/kg (range 0.020-0.800) for wheat grown between 1974 and 1978. Similar levels were found for sorghum and soybean. Site differences accounted for a 40-fold difference between wheat Se values, and a 110-fold difference for sorghum.

Very low Se concentrations were found in wheat surveyed in south-west Western Australia in 1975: mean Se concentration in grain was 0.023 mg/kg (SE 0.006), with a range of 0.001-0.117 mg/kg (White *et al.* 1981). The figure of 0.001 mg/kg is the lowest

level reported globally for Se in wheat. The lowest levels were found on soils derived from Archaean granite, which are also associated with a high prevalence of white muscle disease in lambs (Godwin, 1975).

Watkinson (1981) compared Se concentrations in New Zealand-grown wheat with wheat imported from Australia, grown in 1978 and 1979. The Australian wheats were higher in Se (mean 0.123 mg/kg (SD 0.026) mg/kg, range 0.043-0.224 mg/kg) than the NZ wheats (0.028 (SD 0.010) mg/kg, range 0.011-0.086 mg/kg).

The South Australian study (Babidge, 1990) used pooled wheat and barley samples from 107 and 100 silos, respectively, across the state in the 1981 season for wheat and the 1981 and 1982 seasons for barley. There were significant differences ($p<0.001$) between regions: for example, the Upper Eyre Peninsula region had a wheat-Se median value of 0.229 (range 0.120-0.316) mg/kg, compared to the South East with 0.118 (range 0.047-0.240) mg/kg. Wheat and barley showed the same regional trends, but wheat levels were around 30% higher.

A recent targeted survey of wheat grown in South Australia in the 2000 and 2001 seasons yielded a range of 0.005 – 0.700 mg Se/kg, with values typically 0.080 – 0.180 mg/kg, a grand mean of 0.155 (SE 15) mg/kg, and a median of 0.100 mg/kg (GH Lyons et al, unpublished results from 2002).

The findings of these studies (means of 0.150 (Queensland), 0.023 (Western Australia), 0.150 (South Australia), 0.091 (New South Wales), and South Australia median of 0.170), together with the findings of the human blood surveys discussed above, suggest that Australian wheat grain Se concentrations are above the global average, being well above New Zealand, UK and Eastern European levels, but generally lower than those of Canada and the USA.

High bioavailability of wheat-selenium

In human nutrition terms, bioavailability can be defined as the amount of a nutrient in a meal that is absorbable and utilisable by the person eating the meal (Van Campen & Glahn, 1999). Se is well absorbed (generally 73-93%) from most sources, with selenomethionine and selenate usually absorbed more efficiently than selenite (Raghib *et al*, 1986; Stewart *et al.* 1987; Moser-Veillon *et al*, 1992; Van Dael *et al*, 2002).

However, the assessment of bioavailability of food micronutrients in general (Graham *et al.* 2001; House, 1999) and different forms of dietary Se is not straightforward (Reilly, 1996). Several measures can be used to determine bioavailability and they are subject to a range of variables. If tissue retention is used as a measure, naturally occurring food Se is by far the most available form of the element, although there are significant differences between different types of food.

Selenium form is important: selenomethionine, the form in which Se mainly occurs in cereals (Olson *et al.* 1970), beans, mushrooms and yeast (Reilly, 1996), enters the general protein pool and is well retained. However, it must be released from the protein pool and be catabolised to hydrogen selenide before it can support selenoenzyme expression. Selenocysteine, on the other hand, cannot be incorporated directly into proteins, but is catabolised directly to hydrogen selenide. It is thus better utilised for the selenoenzymes but not retained as well as selenomethionine (Deagen *et al.* 1987; Levander & Burk, 1990; Combs, 2001).

Se is generally more bioavailable from plant forms than from animal foodstuffs (Combs, 1988; Bugel *et al.* 2002), and wheat Se is one of the most bioavailable forms (Jaakkola *et al.* 1983; Laws *et al.* 1986; Hakkarainen, 1993). In a study on chicks, using glutathione peroxidase activity and prevention of exudative diathesis as measures of bioavailability, and using selenite as the reference at 100%, the bioavailabilities of Se in different foods were: wheat 83-100%, barley 78-85%, oats 41-45%, fishmeal 64-80%, and meatmeal 22-30% (Hakkarainen, 1993). The importance of imported North American wheat as a former Se source for the British population was noted above, and is reinforced by a Scottish study which found that the Se content of wheat harvested in 1989 which was used for breadmaking in Scotland ranged from 0.028 mg/kg for home-grown wheat to 0.518 mg/kg for Canadian wheat, and was significantly correlated ($p<0.001$) with protein content (Barclay & Macpherson, 1992). Norway's population, despite a modest total Se intake, has the highest serum Se level in Europe at 119 μg/l. The probable explanation is that their major selenium source is North American wheat. In a Norwegian study, Meltzer *et al.* (1992) demonstrated the high bioavailability of wheat-Se by feeding trial participants Se-rich bread providing 100, 200 or 300 μg Se daily for 6 weeks. Serum selenium increased in a dose-response manner by 20, 37 and 53 μg/l, respectively, in the three groups ($p<0.001$).

Wheat enriched with Se by foliar application was found to be highly effective in raising plasma Se (53% increase after 6 weeks) in a Serbian study. Glutathione peroxidase activity in blood increased and oxidative stress parameters decreased (Djujic *et al.* 2000a). A follow-up study found that Se-enriched wheat increased levels of copper, iron and zinc in erythrocytes, compared to individuals consuming low-Se wheat (Djujic *et al.* 2000b). This is the first time such interactions have been reported, and further studies are warranted in view of the billions of people who are iron and/or zinc deficient.

Strategies to increase selenium in wheat

Background

The foregoing evidence suggests that for many countries an increase in Se concentration in wheat would be the most effective and efficient way to increase the Se intake of the human population, with consequent likely improvement in public health, and also to reverse the trend of declining Se levels in food systems.

It is clear that Se fertilisation of wheat is an effective means to increase grain Se concentration (Gupta & Gupta, 2000; Combs, 2001), and this will be examined further below. Education is also important to encourage people to consume appropriate amounts and proportions of different classes of healthy foods, including whole grain cereal products.

However, in developing countries those individuals who are most at risk of nutrient deficiencies (frequently women and children) often rely on one staple food, for example wheat, rice, cassava or maize, for most of their energy and nutrient requirements, and lack the money to improve their diet. Hence the high prevalence of Fe, Zn, vitamin A, I and Se deficiencies, and the high incidence and prevalence of infectious diseases in these countries (Graham & Welch, 1996; Graham *et al.* 2001). For certain soil types in developing countries, the most sustainable, cost-effective approach may be to breed wheat cultivars that are better at accumulating grain-Se.

Selenium fertilisation

To overcome the low Se levels in food crops in certain areas, different methods of Se fertilisation have been investigated for more than 30 years. Of particular interest are the experiments of Ylaranta (Finland), Gissel-Nielsen (Denmark) and Gupta (Canada). It is a very inexpensive method to increase Se intake by humans: the material cost of applying 10 g/ha of selenate is less than US$1.00/ha. Its effectiveness is illustrated by the increase in blood Se levels in Finland post-1984. Moreover, Mutanen *et al.* (1987) found that the bioavailability (calculated as the mean of four criteria) of wheat-Se was higher for Se-fertilised (both soil-applied and foliar) wheat than for American wheat which was naturally high in Se.

The Finland Se program was discussed above. The Se level in all domestic cereal grains in Finland pre-1984 was 0.01 mg/kg or less; now spring wheats typically contain around 0.25 mg/kg, and for the less-fertilised winter wheat, around 0.05 mg/kg (Eurola *et al.* 1990). Current application rates vary from 5-10 g selenate/ha, and the practice appears to be safe.

Concern that continuing use of selenium-amended fertilisers might eventually lead to accumulation of toxic levels in the environment appears to be unfounded (Vuori *et al.* 1994; Oldfield, 1999). The residual effect of Se treatments was found to be low to negligible in the following year, even when it had been applied at the high rate of 500 g/ha (Gissel-Nielsen, 1981; Ylaranta, 1983a,b, 1984; Singh, 1991; Shand *et al.* 1992; Gupta *et al.* 1993; Gissel-Nielsen & Gupta, 2001). Furthermore, the bioavailability of residual selenium is lowered by the reducing action of micro-organisms in the soil and rumen. A Californian study (Norman *et al.* 1992) found that long-term Se supplementation of cattle at maximum permitted levels did not result in Se contamination in streams that received runoff from feeding areas. However, a Californian study of a deer forest range treated with seleniferous fertiliser found significant Se accumulation in aquatic biota in streams in the same area (Maier *et al.* 1998), a not unexpected finding. In New Zealand, where Se fertilisation has been practised for over 30 years, no Se build-up has occurred, and positive responses continue to be obtained from Se application (Oldfield, 1999). It would appear that risk of environmental Se accumulation due to responsible agricultural use is low.

Most studies have shown selenate, whether applied to the soil or as a foliar fertiliser, to be much more effective than selenite (Gissel-Nielsen 1981; Ylaranta 1983a,b, 1984; Singh, 1991; Shand *et al.* 1992; Gupta *et al.* 1993; Gissel-Nielsen & Gupta, 2001). For example, a grain Se content of 100-200 μg/kg in barley was obtained by applying 10-20 g selenate /ha, but over 100 g selenite/ha was required to reach this level (Ylaranta, 1983b). On a fine sandy loam of pH 6.0, 10g selenate/ha applied to the soil raised barley grain from 33 μg/kg (control) to 234 μg/kg, while 10 g selenite/ha caused no increase (Gupta *et al.* 1993). In many soils, selenite is readily adsorbed on clay colloids and becomes unavailable to plants.

The relative effectiveness of soil or foliar application of Se depends on Se form, soil characteristics, method of basal application, and time of foliar application. Ylaranta (1983b) found basal and foliar selenate to be equally effective at the low (10 g/ha) rate, foliar better at 50 g/ha, and both equal at the high rate of 500 g/ha. In further trials, foliar selenate applied at the 3-4 leaf stage was found to be more effective than basal application on clay soil of pH 6.3, of similar effectiveness on high-humus, fine sandy soil of pH 4.6, and slightly less effective than basal fertiliser on a fine sandy soil of pH 5.0. Ten g/ha of foliar selenate, using a wetting agent, raised wheat grain Se level from 16 to 168 μg/kg on

the clay soil, while 9g basally applied raised it to just 77 μg/kg. Overall, foliar application was the more effective method, except where growth was poor due to low rainfall (Ylaranta, 1984). This suggests that, for most of Australia's wheatgrowing areas, basal application may be preferable, especially where foliar nitrogen (to which selenium could be added) to boost growth or protein is not used.

At the Se levels used for fertilisation of wheat in the field (5-100 g/ha) it is unlikely that there would be any phytotoxic effects, including growth inhibition.

The question arises as to what is a desirable target level for Se in wheat grain. From the studies above it is evident that 10 g selenate/ha can raise wheat crops from a base level of 30-100 to 200-500 μg grain-Se/kg. Is this as high as required? It could be considered a minimum target level in view of the estimated 300 μg Se level of a loaf of wholemeal bread made from this wheat. The consumption of this bread over a week would significantly increase an individual's Se intake.

There are no published studies of Se fertilisation of wheat in Australia. Conditions here are very different to those in Europe and Canada, where most studies have been conducted. Higher grain Se concentrations than those found above may be obtained in Australian-grown wheat, where soils are mostly alkaline and yields usually lower. Our group is currently conducting field trials to assess different application methods and rates of selenate on wheat in South Australia.

Wheat breeding to increase grain selenium density

The extent of human micronutrient malnourishment. Around half of the world's population is malnourished. More than two billion people consume diets that are less diverse than 30 years ago, leading to deficiencies in micronutrients, especially iron, zinc, iodine and Se, and also vitamin A. In some regions almost everyone suffers from some form of "hidden hunger". In South-East Asia, for example, it is estimated that iron deficiency affects 98.2% (around 1.4 billion) of the people (World Health Organisation, 1999).

Cereals are generally low in micronutrients compared to other food crops, thus cereal-dominated food systems are low in micronutrients. The people most at risk are resource-poor women, infants and children. Graham *et al.* (2001) concluded that a new agricultural paradigm is needed to address global micronutrient malnutrition: "...an agriculture which aims not only for productivity and sustainability, but also for balanced nutrition, or what we have called the *productive, sustainable, nutritious food systems paradigm*" (Graham *et al.* 2001).

Breeding for higher nutrient density in staple crops. Programs which include fortification, education and supplementation have been successful in countering micronutrient deficiencies in certain cases and will continue to play a role. However, they tend to be expensive, require ongoing inputs and often fail to reach all individuals at risk. Furthermore, such programs themselves are at risk from economical, political and logistical impacts (Gibson, 1994; Graham & Welch, 1996).

On the other hand, a strategy of breeding staple crops with enhanced ability to fortify themselves with micronutrients offers a sustainable, cost-effective alternative, which is more likely to reach those most in need and has the added advantage of requiring no change in current consumer behaviour to be effective. It represents a strategy of "tailoring

the plant to fit the soil" rather than the opposite, which is afforded by the soil fertilisation approach (Graham *et al.* 2001).

Exploiting the genetic variation in crop plants for micronutrient density is likely to be an effective method to improve the nutrition of entire populations. A four- to five-fold variation was found between the lowest and highest grain iron and zinc concentrations among wheat accessions studied at Centro Internacional de Mejoramiento de Maiz y Trigo (International Maize and Wheat Improvement Centre) (CIMMYT), and the highest concentrations were double those of popular modern varieties (Graham *et al.* 2001). Moreover, wild, small-seeded relatives of modern bread wheats have been found with 50% more iron and zinc than the highest CIMMYT germplasm studied (Ortiz-Monasterio, 1998). If, for example, a wheat variety were identified which was both high yielding and produced twice the grain Se density of most other varieties (i.e. 100 instead of 50 μg Se/kg on a relatively low-Se soil), it could, assuming wheat supplies 50% of available Se ingested in Australia, result in an increase in Se intake from the current estimate of 75 μg/adult/day to 113 μg/adult/d, with significant health benefits.

Graham *et al.* (2001) emphasised the importance of combining nutrient density traits with high yield. There is unlikely to be a premium paid for a higher quality product by resource-poor consumers, so new, high-nutrient varieties must also be attractive to farmers in terms of yield. In all the crops examined so far, it is possible to combine high micronutrient density with high yield. These authors also stress the importance of bioavailability: will the added nutrients be sufficient to have a significant effect on human nutrient status? As discussed above, wheat-Se is highly bioavailable.

A benefit-cost analysis of a breeding approach to increase wheat grain Zn density in Turkey, using very conservative assumptions and current dollar values, estimated that costs of US $13 million would produce benefits of US $274 million (economic only, with no account taken of improved health and quality of life), a favourable benefit-cost ratio of 21 (Graham *et al.* 2001). In contrast, conventional fortification requires yearly funding, and if the investment is not sustained, the benefits cease.

Screening and selection; the importance of genotype-environment interaction. In the screening phase of a breeding program, genotype-environment interaction needs to be relatively low for a breeding approach to be viable. Field trials where different genotypes are grown at the same site in the same season should enable comparison of genotypes. Ideally, trials should be conducted for two years at the same site, as different grain nutrient levels of genotypes grown on different soils can even be reflected in the first-year grain harvested. Furthermore, the variability in soil availability for micronutrients is generally much greater than that for macronutrients (Graham, 1991), hence field trials need to be of limited area to reduce spatial variability; paired-plots, where each replicate comprises adjacent treatment and control plots, can be a useful technique.

A major gene together with several minor additive genes are likely to control the uptake of nutrients into crop plants, and another group of genes, also featuring a major gene, appear to control grain loading (Epstein, 1972; Ripperger & Schreiber, 1982; Graham, 1984). In selecting for grain selenium density, both high uptake from the soil and high capacity to move selenium from the vegetative tissues to the grain are required. However, as selenium is not known to be essential for higher plants, agronomic efficiency may not be involved. Nevertheless, an increase in loading efficiency into the grain is desirable. This could be achieved by the endogenous chelator of Se in the phloem which increases Se

transport to the inflorescence, or by more transporters at the plasma membrane at unloading or uptake into the grain.

Gene technology, including the use of molecular markers (cDNA, restriction fragment length polymorphisms, amplified fragment length polymorphisms and randomly amplified polymorphic DNA) for the mRNA expressed by efficiency and grain-loading genes, provides an alternative to conventional plant breeding as a means to enhance micronutrient density in cereals (Graham *et al.* 1997). The introduction, for example, of a gene that facilitates expression of the permease sulphate transporter would be likely to increase uptake and transport of selenate.

Surveys of Se level in grains have suggested that environment may be more important than genotype in determining grain Se density. The Queensland study of Noble & Barry (1982), discussed above, found differences in Se concentration in wheat of up to 100-fold at different sites, but little difference between wheat varieties. The maximum variation for both species and varieties at any site was 5-fold. A Japanese study found that Se levels in rice grown in different parts of the country varied from 11 to 182 μg/kg. A significant difference was found between levels in rice from different districts, but not between different rice varieties grown on the same soil (Yoshida & Yasumoto, 1987). Our group has found up to a six-fold variation in grain-Se concentration in one wheat cultivar at a trial site in South Australia.

Genotypic differences were apparent, however, in a study of Se in hulled (spelt and emmer accessions) and modern bread and durum wheats, grown together. The hulled wheats had higher concentrations of Se, Li, Mg, P and Zn. Five spelt accessions had twice the Se concentrations of emmer, and from 2 to 8 times those of normal wheat (Piergiovanni et al, 1997). This is in contrast to the findings of Grela (1996), who observed no Se differences between spelt and wheat. It is not clear at this stage whether sufficient genetic variability for grain Se density exists between wheat cultivars to enable selection for this trait, and further research is proceeding.

Conclusion

Research results continue to illustrate the importance of selenium in human health, in particular its anti-inflammatory, anti-cancer and anti-viral activities. It is evident, due mainly to its poor availability in many soils, that as many as one billion people may be Se-deficient. The vast majority of the world's population would receive well below the level needed to maximise cancer prevention, which is likely to be within the range of 125-280 μg/adult/d, depending on gender, pregnancy and exposure to oxidative stress. The average Australian adult would ingest around 75 μg Se/d.

Se levels in Australian wheat are generally moderate, but due to widespread wheat consumption and the high bioavailability of selenium in wheat, this source probably accounts for nearly half the Se utilised by Australians. An increase in the Se content of wheat grain is likely to be the most cost-effective method to increase Se levels in the human population. A substantial increase in population Se intake may result in decreased rates of several important cancers, cardiovascular disease, viral disease sequelae, and a range of other conditions that involve oxidative stress and inflammation, with resultant reductions in health costs.

The most promising strategies to increase Se in wheat appear to be biofortification by Se fertilisation and breeding wheat varieties with superior ability to accumulate Se in the grain. Studies in Europe and North America have shown that the addition of as little as 10 g Se/ha can increase grain Se level by up to 0.40 mg/kg. However, studies are needed to determine the most efficient methods for Australian conditions, and to determine the extent of genetic variability for grain Se accumulation.

Furthermore, before recommending large-scale fortification of the food supply with Se, it will be necessary to await the results of current intervention studies with Se on cancer (including the SELECT and PRECISE trials), asthma and HIV/AIDS.

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Nutriprevention of disease with high-selenium wheat

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Abstract

The metalloid selenium (Se) is ubiquitous in soils, but exists mainly in insoluble forms. Consequently, it is often supplied by plants to animals and humans at levels too low for optimum health. Se deficiency and sub-optimality are manifested in populations as increased rates of thyroid dysfunction, cancer, severe viral diseases, cardiovascular disease, and various inflammatory conditions. Se deficiency probably affects at least a billion people. Optimal cancer protection appears to require a supra-nutritional Se intake, and involves several mechanisms, which include promotion of apoptosis, and inhibition of neo-angiogenesis. In some regions Se is declining in the food chain, and new strategies to increase its intake are required. Se levels in healthy Australians are generally above the estimated global average but below an estimated optimal intake. Increasing the Se content of wheat, which supplies around half the dietary Se of most Australians, represents a food systems approach that would increase population intake, with likely improvement in public health. The strategy that shows most promise to achieve this is biofortification of wheat with selenate. Before recommending large-scale fortification of the food supply with Se, it will be necessary to await the results of current intervention studies.

Keywords: selenium, biofortification, wheat, disease prevention

Introduction

Ever since Se was recognised as an essential nutrient, a voluminous literature has accumulated which describes the profound effect of this element on human health. The findings of recent human intervention trials [1,2] have stimulated interest in a cancer-preventive role for Se. In addition to its cancer preventive capacity, Se has an anti-viral effect.[3,4] Given the high global incidences of HIV, hepatitis B and C, and other RNA viruses, including measles and influenza, the public health implications of selenium deficiency - estimated to affect more than a billion people[5] - and suboptimality are enormous.

Several comprehensive reviews have examined Se and human health[5-8], including that of Combs (2001), who discusses Se in humans within a food systems context and makes the distinction between selenium's normal metabolic roles and its anticarcinogenic activity at supra-nutritional levels.[5]

It is important to note that the biological actions of Se are not properties of the element *per se*, but rather are properties of its various chemical forms. Inorganic Se forms (selenate, selenite) undergo reductive metabolism, yielding hydrogen selenide, which is incorporated

into selenoproteins. Successive methylation of hydrogen selenide detoxifies excess Se. Selenomethionine can be incorporated non-specifically into proteins in place of methionine, and selenocysteine is catabolised to hydrogen selenide by a beta-lyase.[5]

This review summarises briefly the roles of Se in soils, plants and animals. The importance of Se in human health is discussed, followed by Se intake by humans, with a focus on Australia. The review then examines wheat as an important source of bioavailable Se, and discusses biofortification, a promising strategy to increase organic Se concentration in wheat grain.

Background

Soil Se is uneven in distribution and availability: concentrations range from less than 0.1 to more than 100 mg/kg; however, most soils contain between 1.0 and 1.5 mg/kg.[9] In general, total soil Se of 0.1 to 0.6 mg/kg is considered deficient. Soils in New Zealand, Denmark, Finland (pre-1984, before Se was added to fertilisers), central Siberia, and a belt from north-east to south-central China are notably Se-deficient and hence have sub-optimal levels in their food systems.[5,10,11] Large areas of Africa, including much of Zaire, are also likely to be Se-deficient, but further mapping is required. On the other hand, parts of the Great Plains of the USA and Canada, Enshi County in China, and parts of Ireland, Colombia and Venezuela are seleniferous.[5]

In acidic, poorly aerated soils, Se is relatively unavailable to plants and occurs mainly as insoluble selenides and elemental selenium. In lateritic soils, which have a high iron content, it binds strongly to iron to form poorly soluble ferric hydroxide-selenite complexes.[12] In wetter regions, selenate can be leached from the soil, resulting in selenium-deficient areas, for example New Zealand and Tasmania.[6] The availability of soil Se to crops can be affected by irrigation, aeration, liming and Se fertilisation.[13]

Australia has both high- and low-Se soils and large areas that have not been mapped for the element. Seleniferous soils occur in central Queensland and parts of Cape York Peninsula. Se deficiency in Australia usually occurs on acidic soils with more than 500 mm rain per year, such as the Central and Southern Tablelands and Slopes and the Northern Tablelands of New South Wales, the south-eastern coast of Queensland, south-west Western Australia; coastal and central regions of Victoria, much of Tasmania, and South Australia's Mount Lofty Ranges and Kangaroo Island (see Fig. 1).[6,14]

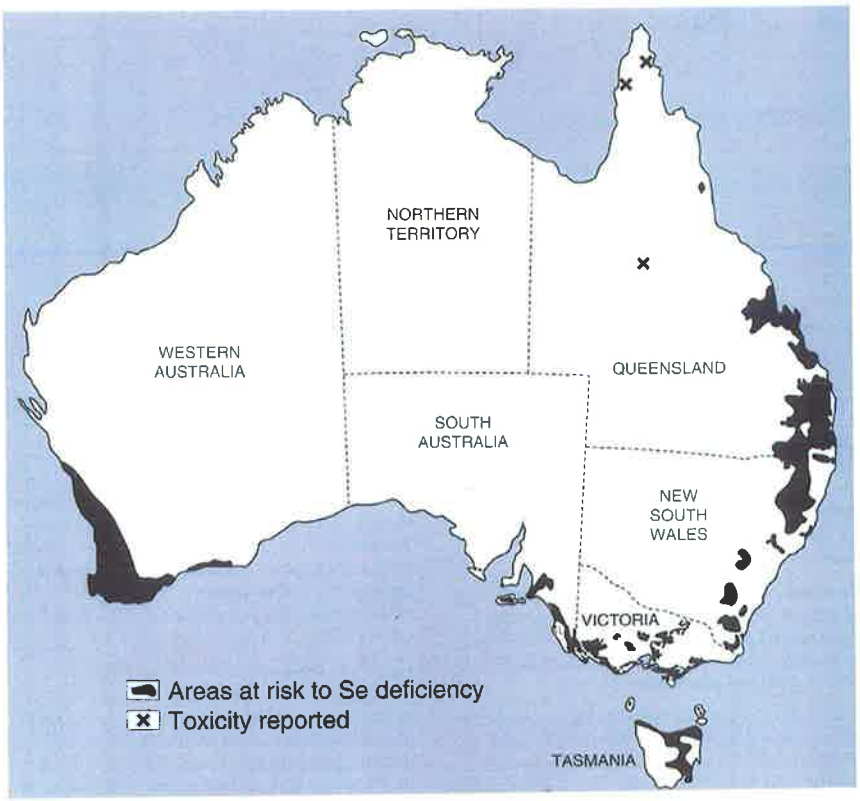

Figure 1. Selenium in Australian soils[15]

The Se contents of plants vary according to available soil Se and plant species. For example, wheat grown in Shaanxi Province, China may have 0.003 mg/kg Se in the grain, compared to 2.0 mg/kg for wheat from the North or South Dakota wheatlands.[5] Wheat from highly seleniferous areas of South Dakota may contain more than 50 mg/kg Se (Prof. G. Combs, pers. comm. 7th June 2002), while *Astragalus* on the same soils may accumulate up to 15,000 mg/kg dry weight.[16]

Because Se is an essential nutrient, animals respond positively to it where their diet contains less than 0.1 mg/kg Se in dry matter.[17] Conditions which are related to Se deficiency, some of which occur on a wide scale in certain countries, include white muscle disease, exudative diathesis, pancreatic degeneration, liver necrosis, mulberry heart disease, and ill-thrift. Se deficiency is usually not the only cause of these diseases.[6]

Selenium: essential for human health

Selenium deficiency diseases

In parts of China and eastern Siberia two overt Se-deficiency diseases occur: Keshan disease and Kaschin-Beck disease. Keshan disease occurs mainly in children and women of child-bearing age and involves impairment of cardiac function, cardiac enlargement and arrhythmia.[6] The disease's aetiology is likely to be complex, involving Se and vitamin E deficiencies, and presence of the Coxsackie B virus.[18-20]

Kaschin-Beck disease is an osteoarthropy which manifests as enlarged joints, shortened fingers and toes, and in severe cases dwarfism. Se and vitamin E deficiency [6] and iodine deficiency [21] are likely to be predisposing factors whereas fulvic acids in drinking water [22] or mycotoxins in food [23] are probable causes.

Antioxidant, anti-inflammatory, thyroid and immunity roles

Selenocysteine, the 21st amino acid, is present in selenoproteins, which have important enzyme functions in humans. Glutathione peroxidase (of which at least five forms exist) has an antioxidant role in reducing damaging hydrogen peroxide and lipid/phospholipid hydroperoxides produced in eicosanoid synthesis by the lipoxygenase and cyclo-oxygenase pathways.[24] This function reduces damage to lipids, lipo-proteins and DNA, and hence reduces risk of cardiovascular disease and cancer.[25,26] Moreover, selenite inhibits tumour necrosis factor-alpha-induced expression of adhesion molecules that promote inflammation.[27]

There is a growing body of evidence to suggest that Se (especially in the sodium selenite form) can alleviate conditions associated with high levels of oxidative stress or inflammation. These include asthma,[28,29] diabetes,[30] arthritis,[31] muscular dystrophy,[32] cystic fibrosis,[33] acute pancreatitis,[34] osteoarthritis,[35] systemic inflammatory response syndrome [36] and kwashiorkor.[37] In addition, Schrauzer (1998) discusses the application of selenite therapy to viral haemorrhagic fever, acute septicaemia and lymphoedema.[38] Another group of selenoenzymes, the thioredoxin reductases are involved in reduction of nucleotides in DNA synthesis, regeneration of antioxidant systems, and maintenance of intracellular redox state.[39]

The thyroid gland has the highest Se concentration of any human organ,[40] and Se is involved in thyroid metabolism through the iodothyronine deiodinases, which catalyse the production of active thyroid hormone, T3, from thyroxine, T4.[41] The iodine deficiency diseases goitre and myxoedematous cretinism are more prevalent in central Africa in those regions which are deficient in both iodine and Se,[42] and in such areas supplementation with both nutrients is indicated.

Se has a role in many aspects of the immune response to infections. Se deficiency reduces immunocompetence, involving impairment of neutrophil, macrophage and polymorphonuclear leukocyte activity.[24,43,44] Se supplementation of even supposedly Se-replete individuals is immunostimulatory, and involves enhancement of natural killer cell and lymphocyte activity as well as enhancement of proliferation of activated T-cells.[45]

Cancer

There is "perhaps no more extensive body of evidence for the cancer preventive potential of a normal dietary component than there is for selenium." From the late 1960s, epidemiological studies have suggested an inverse association between human Se intake and cancer mortality.[46] An extensive literature documents the numerous *in vitro* and animal studies that have been conducted during the past 35 years. Most demonstrate that application or intakes of Se at supranutritional levels can inhibit tumorigenesis.[46-48] Prospective cohort and case-control studies that have involved as many as 34,000 people have generally shown an association between low Se status and a significantly higher risk of cancer incidence and mortality.[49-51]

Intervention studies using Se as a single chemopreventive agent include the Qidong trials in China, where selenite significantly reduced primary liver cancer,[2] and the Nutritional Prevention of Cancer (NPC) trial in the US, where 200 micrograms (μg) of Se per day (as yeast) reduced total cancer mortality by 41%, total cancer incidence by 25% and prostate cancer incidence by 52% in a cohort of 1,300 people. The effect on total cancer was limited to male smokers (current or previous) with baseline Se levels below 113 μg/l,

although non-smoking males below this level are likely to have benefited from Se supplementation in terms of prostate and colon cancer protection.[52]

The NPC trial was conducted in a region of the US where Se intakes are estimated to be around 90 μg/day, well above the level required for optimal selenoenzyme activity. This suggests additional mechanisms in Se's cancer preventive role. While some cancer protection, particularly that through antioxidant activity, involves selenoenzymes, the anti-cancer effects of Se are likely to involve the production of specific anti-tumorigenic metabolites, such as methylselenol. Studies have suggested that Se provided in certain forms can neutralise carcinogens, enhance the immune system, alter gene (including p53) expression, inhibit tumour cell metabolism and neo-angiogenesis (blood vessel development around tumours), and promote apoptosis (programmed cell death). [5,7,46,53-59]

According to this two-stage model of cancer prevention, which involves Se intakes that correct nutritional deficiency as well as much higher, supranutritional intakes, individuals with nutritionally adequate Se intakes may benefit from Se supplementation.[46] Se's anti-cancer activities remain under intensive study worldwide.

Viral and mycobacterial diseases

Se deficiency is associated with increased virulence of a range of viral infections.[60] It is evident that in a Se-deficient host, normally harmless viruses can become virulent. For example, when Se-deficient mice are inoculated with benign Coxsackie B3 virus, the virus mutates into a virulent form that causes myocarditis similar to that seen in Keshan disease.[4,61,62] Furthermore, Se-deficient mice develop severe pneumonitis when infected with a mild strain of influenza virus.[62]

Se appears to be of particular importance for people with HIV. Se deficiency is a significant predictor of HIV-related mortality [63,64] and viral load.[65] A US study found Se-deficient HIV patients to be 20 times more likely to die from HIV-related causes than those with adequate levels.[3] The decline in blood Se levels occurs even in the early stages and is thus unlikely to be due to malnutrition or malabsorption.[66] Moreover, a study of HIV-1-seropositive drug users found low Se level to be a significant risk factor for developing mycobacterial disease, notably tuberculosis.[67]

Se also appears to be protective in people infected with hepatitis B or C against progression to cirrhosis and liver cancer.[2,51] Selenoproteins encoded by HIV, hepatitis C virus and the Ebola virus (which causes acute haemorrhage) have been discovered that consume the host's Se supply, thus reducing immune response.[68,69]

Other health effects

Low Se status has long been known to reduce fertility in livestock,[70] and this also appears to be the case for humans. Low Se levels have been associated with male infertility [71] and spontaneous abortions.[72] In a Scottish study, supplementation of subfertile men with 100 μg of Se per day for three months significantly increased sperm motility.[73]

Se appears to be influential in the brain, and several studies that indicate low Se levels are associated with cognitive impairment, depression, anxiety and hostility.[7] These conditions can be alleviated in individuals with low baseline Se levels by Se supplementation. Recent studies suggest that selenoprotein P,[74] selenoprotein W [75] and selenoprotein M [76] have important roles in the brain.

Se forms selenides with all metals, and detoxifies mercury, cadmium, lead, silver, thallium and arsenic. This effect can be enhanced by vitamin E.[77] In the case of cadmium and mercury, detoxification is achieved through the diversion in their binding from low to high molecular weight proteins.[78]

Human selenium intake

Selenium intake: low and getting lower

The absolute range of global daily Se intake by adults is around 7 (in Chinese Keshan disease areas) – 5,000 μg/day (in Chinese selenosis areas). Estimates provided of Se intake (in μg/day) for several countries include England (12-43), Belgium (45), Canada (98-224), USA (60-220), Croatia (27), New Zealand (19-80), Japan (104-127) and Venezuela (200-350).[5] In Australia few comprehensive studies have been conducted, but estimates of 63 and 96 μg/day have been provided, with a range of 23-204.[6,79] In view of the estimated mean plasma level of Se in Australian adults of 89 μg/l, a mean intake of around 75 μg/day appears likely for Australians.

The US Recommended Daily Allowance, which is based on the Se levels considered to be necessary to maximise glutathione peroxidase activity, is 55 μg/day for both men and women, while in Australia it is 85 and 70 μg/day for men and women, respectively. It is evident that many people do not consume enough Se to support maximum expression of selenoenzymes (80-90 μg/day), let alone the level required for optimum prevention of cancer. It is likely that the vast majority of the world's population have suboptimal Se intakes,[5] and hence are at increased risk of cancer, heart disease, viral diseases, and indeed any conditions which involve increased levels of oxidative stress.

Furthermore, evidence suggests that there is a trend toward a reduction of Se in the global food chain, caused by fossil fuel burning (involving sulphur release), acid rain, soil acidification, the use of high-sulphur fertilisers [80] and more intensive crop production.[13] Blood Se levels have decreased significantly in the United Kingdom from 1984 to 1994,[7,8,81,82] and current average Se intake in the UK may be as low as 34-39 μg/day.[83,84] This fall is attributed largely to the use of low-Se UK and European wheat in place of North American wheat. This highlights the sensitivity of Se intake and body levels to changes in the food supply. Prominent researchers Margaret Rayman [8,81] and Edward Giovannucci [82] have called for action to increase Se intake.

Human blood concentrations of selenium: the global view

Blood Se levels are determined mainly by dietary intake, although sex, age, smoking and exposure to heavy metals can have a minor effect.[85] Combs (2001) presents a comprehensive list of Se concentrations in plasma, serum or whole blood of healthy adults from 69 countries, of which a sample of plasma/serum Se concentrations (in μg/l) follows. Notc that there are few data available from some of the most populous areas of the world, including most of Africa, South America and central and south Asia: Austria (67), Burundi (15), Canada (132), China-Keshan disease area (21), China-selenosis area (494), Finland pre-1984 (70) and post-1984 (92), Hungary (54), Japan (130), New Zealand (59), Norway (119), USA (119) and Zaire (27).[5] The mean value (calculated as the mean of the means for each of the 69 countries) is 83 μg/l, with a range of means of 15 (Burundi)-216 (Venezuela). However, this is likely to be optimistic as small studies of Se levels in much of Africa and central, south and South-East Asia indicate levels well below this.

Selenium levels in the Australian population

An estimate of 93 μg/l of plasma/serum Se for Australia can be derived from the means determined from 18 studies of blood Se levels. If post-1990 data only are used, the mean is 89 μg/l. Using either mean, Australia is above the estimated world mean reported Se level of 78 μg/l. A large sample of Adelaide blood donors with mean age of 49 years had a mean plasma Se concentration of 103 μg/l in 2002 (Lyons et al, unpublished).

However, low blood Se levels have been reported in Adelaide infants: a plasma Se level of around 31 μg/l (SD 13) in a sample of newborn infants, similar to New Zealand levels.[86] Infant Se levels are typically half those of adults. The reported level places the infants at increased risk of a range of conditions that involve oxidative stress and inflammation.

Optimum selenium intake

In the NPC trial in the US, the protective effect of Se against cancer occurred in the lowest (relative risk [RR] of 0.52, 95% CI: 0.33-0.82) and middle (RR 0.64, 95% CI 0.40-0.97) tertiles, which included those people with plasma Se levels below 121 μg/l,[87] and the latest analysis shows that the Se benefit was largely restricted to male smokers with baseline plasma Se level below 113 μg/l. The strongest protective effect was against prostate cancer, with a hazard ratio of 0.48 (95% CI: 0.28-0.80).[52] None of the subjects had plasma Se levels below 60 μg/l and very few were less than 80, thus the cohort must be considered Se-adequate by current nutritional standards.[53]

Although there is a risk in generalising results of individual epidemiological and intervention studies, this result would suggest that the vast majority of the world's population (including that of Australia, with a probable mean plasma/serum level around 89 μg/l, and many populations in Europe[7]) would be in the responsive range.

The NPC participants lived in a region where dietary Se intake is around 90 μg/day,[1] thus with the addition of the 200 μg supplement, individuals in the treatment group would have received around 270-310 μg/day. A Se intake of 200-300 μg/day may be required to significantly reduce cancer risk.[5] This compares with an estimated Australian adult intake of 75. Of course, Se requirement varies between individuals in the same population.[7] Even Moyad (2002), who expresses doubts about the interpretations of certain Se studies, and considers some estimates of its cancer protective effect to be optimistic, suggests that an intake of 200 μg/day Se and around 50 mg/day of vitamin E may be beneficial, particularly for current or previous smokers.[88] The results of the NPC trial suggest that males may have a higher Se requirement than females.[52] Further studies may find optimum adult Se intakes in the range 125-280 μg/day, with means of around 130 (F) and 250 (M). Pregnant females may have a higher Se requirement than non-pregnant females.[89]

Chronic selenosis occurs in Enshi County, China where coal-contaminated soil contains up to 8 mg/kg Se, and residents have consumed up to 7 mg per day. Common symptoms include nail thickening and cracking and hair loss, and some people exhibit skin lesions.[90,91] The concern that the incorporation of selenomethionine into body proteins could increase Se to toxic levels appears unwarranted because a steady state is established, which prevents the uncontrolled accumulation of Se.[92]

Combs (2001) considers it likely that the WHO and EU estimates of the upper safe limit of Se intake of 400 and 300 μg/adult/day, respectively, are too conservative.[5] Under normal

conditions, a Se intake of less than 1,000 μg/day (or 15 μg/kg bodyweight) does not cause toxicity.[60,93-95] People in parts of China, the US, Venezuela and Greenland have ingested Se at this level for their entire lives without ill effects.[60]

It is unlikely that the food system of any country, with the possible exception of Venezuela, delivers an optimum level of Se to its population, and indeed the food systems of most populations do not even provide enough Se to maximise selenoenzyme expression. The impact of this deficiency and suboptimality in global health terms is difficult to quantify, but is likely to be enormous given the high prevalence of various cancers, cardiovascular diseases, viral diseases (including AIDS, hepatitis, measles and influenza), and exposure to environmental pollutants throughout much of the world. It is thus a matter of urgency that many countries begin to address this major public health issue and develop effective, sustainable ways to increase Se intakes.

Strategies to increase human selenium intake

Given that the populations of most countries would be likely to benefit from an increased Se intake, how could this be best achieved? Strategies to increase Se intake include increased consumption of higher-Se foods (such as Brazil nuts, bread, cereals meat, fish) through education; individual supplementation (with selenomethionine, selenite, Se-yeast); food fortification (eg sodium selenite added to salt in low-Se regions in China); supplementation of livestock (eg sodium selenate added to New Zealand pastures); Se fertilisation, or more correctly *agronomic biofortification*, of food crops (see below for further discussion), and plant breeding for enhanced selenium accumulation.

Wheat: an important selenium source for humans

Surveys suggest that wheat is the most efficient Se accumulator of the common cereal crops (wheat, rice, maize, barley, oats) and is one of the most important Se sources for humans. In a Russian survey, serum Se level was found to be highly correlated ($r = 0.79$) with Se level in wheat flour,[96] and bread has been found to supply one-third of the daily Se intake of Australian children.[97] With the addition of Se supplied through breakfast cereals, cake and biscuits, and in view of its high bioavailability, wheat-Se probably supplies around half the Se utilised by Australians.

Selenium concentrations in wheat grain

The global view

There is wide variation in wheat grain Se level between and within countries, reflecting variability in available soil Se concentrations. Published values range from 1 μg/kg in south-west Western Australia [98] to 30,000 μg/kg in highly seleniferous areas of South Dakota,[99] but most of the world's wheat falls within the 20-600 μg/kg range.[100] Canada and the US have relatively high levels, usually in the 200-600 μg/kg range.[6] New Zealand and Eastern Europe generally have low levels, for example the 28 μg/kg average found for Serbia.[101]

Selenium in Australian wheat

Four published surveys of Se concentrations in Australian wheat have been identified. These studies sampled wheat grown from 1974 to 1982 and, excluding a study in a very

low Se area in south-west Western Australia,[98] provide a combined mean of 146 μg/kg (range 20-800).[102] A targeted survey of wheat grown in South Australia in the 2000 and 2001 seasons yielded a mean of 155 μg/kg (range 5–700), but a lower median of 100 μg/kg. Furthermore, levels were highly variable, with a ten-fold difference in grain Se concentration recorded in replicates of a single cultivar grown at one trial site (Lyons et al, unpublished). These surveys suggest that, like blood Se levels, Australian wheat grain Se concentrations are generally above the global average. They are well above New Zealand, United Kingdom and Eastern European levels, but lower than those of Canada and the USA.

High bioavailability of wheat-selenium

In human nutrition terms, bioavailability can be defined as the amount of a nutrient in a meal that is absorbable and utilisable by the person eating the meal.[103] Se is well absorbed (generally 73-93%) from most sources, with selenomethionine and selenate usually absorbed more efficiently than selenite.[104,105]

If tissue retention is used as a measure, naturally occurring food Se is by far the most available form of the element, although there are significant differences between different types of food. Se form is important: selenomethionine, the form in which Se mainly occurs in cereals,[106] beans, mushrooms and yeast,[6] enters the general protein pool and is well retained. However, it must be released from the protein pool and be catabolised to hydrogen selenide before it can support selenoenzyme expression. Selenocysteine, on the other hand, cannot be incorporated directly into proteins, but is catabolised directly to hydrogen selenide. It is thus better utilised for selenoenzymes but not retained as well as selenomethionine.[5,107,108]

Se is generally more bioavailable from plant forms than from animal foodstuffs,[109,110] and wheat Se is one of the most bioavailable forms.[111,112] Norway's population, despite a modest total Se intake, has the highest serum Se level in Europe at 119 μg/l. The probable explanation is that their major selenium source is North American wheat.[113]

The foregoing evidence suggests that for many countries an increase in Se concentration in wheat would be the most effective and efficient way to increase the Se intake of the human population, with consequent likely improvement in public health, and also to reverse the trend of declining Se levels in food systems. Moreover, it is clear that agronomic Se biofortification of wheat is an effective means to increase grain Se concentration.[5,114]

Agronomic selenium biofortification

To overcome the low Se levels in food crops in certain areas, different methods of Se biofortification have been investigated for more than 30 years. Of particular interest are the experiments of Ylaranta, Gissel-Nielsen and Gupta in Finland, Denmark and Canada, respectively. It is a very inexpensive method to increase Se intake by humans: the material cost of applying 10 g/ha of selenate is around A$2/ha. Its effectiveness is illustrated by the increase in blood Se levels in Finland post-1984. The Se level in all domestic cereal grains in Finland pre-1984 was 10 μg/kg or less; now spring wheats typically contain around 250 μg/kg, and for the less-fertilised winter wheat, around 50 μg/kg.[115] Current application rates vary from 5-10 g selenate/ha, and the practice appears to be safe.

The concern that continuing use of selenium-amended fertilisers might eventually lead to accumulation of toxic levels in the environment appears to be unfounded.[116,117] The residual effect of Se treatments was found to be low to negligible in the following year, even when it had been applied at the high rate of 500 g/ha.[13,118-124] Furthermore, the bioavailability of residual selenium is lowered by the reducing action of micro-organisms in the soil and rumen. In New Zealand, where Se fertilisation has been practised for over 30 years, no Se build-up has occurred, and positive responses continue to be obtained from Se application.[116] It would appear that risk of environmental Se accumulation due to responsible agricultural use is low.

Most studies have shown selenate, whether applied to the soil or to the leaves, to be much more effective than selenite.[13,118-124] For example, a grain Se content of 100-200 μg/kg in barley was obtained by applying 10-20 g/ha of selenate, but over 100 g/ha of selenite was required to reach this level.[123] In many soils, selenite is readily adsorbed on clay colloids and becomes unavailable to plants.

The relative effectiveness of soil or foliar application of Se depends on Se form, soil characteristics, method of basal application, and time of foliar application. Ylaranta found basal and foliar selenate to be equally effective at the low (10 g/ha) rate, foliar better at 50 g/ha, and both equal at the high rate of 500 g/ha.[123] In further trials, foliar selenate application was generally found to be the more effective method, except where growth was poor due to low rainfall.[124] This suggests that, for most of Australia's wheatgrowing areas, basal application may be preferable, especially where foliar nitrogen (to which selenium could be added) to boost growth or protein is not used. Our own trials in South Australia have confirmed this (Lyons et al, unpublished). Furthermore, at the Se levels used for biofortification (5-100 g/ha) it is unlikely that there would be any phytotoxic effects, including growth inhibition.

The question arises as to what is a desirable target level for Se in wheat grain. A concentration of around 1 mg/kg (1,000 μg/kg) could be considered desirable in a "high-Se" breakfast cereal, flour, pasta or bread, as it is well within food regulatory guidelines and, based on average consumption, would significantly increase a consumer's daily Se intake. On the other hand, a *nutripreventive* anti-cancer "functional food" could be based on wheat containing 10 mg/kg Se. In a US trial that investigated the effect of different Se forms on the proliferation of colon cancer precursors in carcinogen-treated rats, high-Se wheat (around 10 mg/kg) sourced from South Dakota was the most effective anti-cancer treatment. This wheat, which provided 2 mg Se/kg of diet, reduced the number of aberrant crypts by 48%, compared to 36% for high-Se broccoli while there was no effect for sodium selenite.[54] Further animal trials to investigate the effect of high-Se wheat on different cancers are warranted.

Conclusion

Research results continue to illustrate the importance of Se in human health, in particular its anti-inflammatory, anti-cancer and anti-viral activities. It is evident, due mainly to its poor availability in many soils, that at least a billion people may be Se-deficient. Furthermore,the vast majority of the world's population would receive well below the level needed to maximise cancer prevention, which is likely to be within the range of 125-280 μg/adult/day, depending on gender, pregnancy and exposure to oxidative stress. The average Australian adult would ingest around 75 μg Se/day.

Se levels in Australian wheat are generally higher than the world average. Due to widespread wheat consumption and the high bioavailability of selenium in wheat, this source probably accounts for nearly half the Se utilised by Australians. An increase in the Se content of wheat grain is likely to be the most cost-effective method to increase Se levels in the human population. A substantial increase in population Se intake may result in decreased rates of several important cancers, cardiovascular disease, viral disease sequelae, and a range of other conditions that involve oxidative stress and inflammation, with resultant reductions in health costs.

The most promising strategy to increase Se in wheat appears to be agronomic biofortification. Studies in Europe and North America have shown that the addition of as little as 10 g Se/ha can increase grain Se level by up to 400 μg/kg.

Before recommending large-scale fortification of the food supply with Se, it will be necessary to await the results of current intervention studies with Se on cancer (including the SELECT and PRECISE trials), asthma and HIV/AIDS.

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Trend in selenium status, and current blood levels of mineral nutrients, of healthy South Australian residents

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The article published in *The Medical Journal of Australia* was derived from this article, which is longer and includes data on other nutrients as well as Se, and which has not been submitted for publication.

Abstract

The essential trace element selenium (Se) has a number of critical roles in humans, including catalysing the production of active thyroid hormone, countering oxidative stress, lowering cancer risk and protecting against serious viral disease sequelae. While Se deficiency diseases have been recognised for several decades, there is mounting evidence that less overt deficiency can cause adverse health effects, and that supranutritional intakes may provide additional protection from disease. In healthy sub-groups of the South Australian population sampled from 1977 to 2002 (total sample size 834), whole blood and plasma Se concentrations were well above an estimated global mean, notwithstanding an apparent 20% decline from the late 1970s to the late 1980s. Age and gender effects, although not large, were statistically significant: plasma Se increased with age, and males had higher plasma Se levels. Levels of copper, zinc, phosphorus, sulphur, sodium, potassium, calcium, magnesium and iron in whole blood and plasma in the 2002 sample of Adelaide blood donors were satisfactory and indicative of a healthy sample. Se was weakly associated with calcium and phosphorus in plasma, and with sulphur in whole blood. The mean plasma Se concentration of 103 μg/l found in the Adelaide, 2002 survey is just above the estimated requirement for maximisation of selenoenzyme expression. Only 8% of men in the 2002 survey were above 119 μg/l, a plausible target for maximum cancer protection. Many Australians – and especially pregnant or lactating women, infants, male smokers, men at increased risk of prostate cancer, and the frail elderly – could benefit from increased Se intake. Se status can be improved by increased consumption of Brazil nuts, wheat products, fish and meat; and by biofortification (for example, by applying Se to wheat crops), careful supplementation, and reducing smoking.

Introduction

The trace element selenium (Se) is of profound importance for human health. Its deficiency and sub-optimality (as defined by sub-maximal selenoenzyme activity) are widespread and likely to be manifested in populations as increased risk of thyroid and immune dysfunction, serious sequelae of RNA viral infections, cancer and various inflammatory conditions (Rayman, 2002). Moreover, mounting evidence suggests that supranutritional Se intakes provide additional protection against disease, in particular, cancer (Combs, 2001; Duffield-Lillico et al, 2002; Rayman, 2002).

Rayman (2000, 2002) and Giovannucci (1998) observe that blood Se levels have decreased in the United Kingdom during the decade 1984-1994, and current Se intake in the UK may be as low as 37 μg/day (Barclay et al, 1995; MAFF, 1999). These authors attribute this fall largely to the consumption of low-Se UK and European wheat in place of high-Se North American wheat. This highlights the sensitivity of Se intake and body levels to changes in the food supply. Also of concern in this context is a trend toward a reduction of Se in the global food chain, possibly caused by fossil fuel burning (with releasc of sulphur, a Se antagonist), acid rain, soil acidification (Se in low pH soils is less available to plants), use of high-sulphur fertilisers (Frost, 1987) and more intensive crop production (Gissel-Nielsen, 1998).

Whole blood, plasma or serum Se concentrations provide useful indicators of human Se intake and status. Se concentration in plasma is around 94% of that in serum and around 80% of that in whole blood (Harrison et al, 1996; Zachara et al, 1988). Plasma Se is more responsive than whole blood Se to current Se intake, so whole blood Se level has generally been considered the more accurate measure of longer-term Se status. An Australian study, however, found whole blood Se showed greater intra-individual variance (estimated by measurements 12 weeks apart) than plasma Se (Lux & Naidoo, 1995).

Combs presents a comprehensive list of blood Se concentrations of healthy adults from 69 countries, of which a sample of plasma/serum concentrations (in μg/l) follows: Austria (67), Canada (125), China - low-Se Keshan disease area (19), China – high-Se selenosis area (494), Hungary (58), Japan (111), New Zealand (57), USA (124) and Zaire (27) (Combs, 2001). Note that only data from studies published after 1990 were used to provide the above figures. The mean value (calculated as the mean of the means for a representative sample of 45 countries is 78 μg/l, with a range of means from 15 (Burundi) to 125 (Canada). Median values were also calculated, and found to be very similar to the calculated mean values. However, this overall mean value may be optimistic as small studies of Se levels in much of Africa and central, south and South-East Asia (areas poorly mapped for Se) indicate levels well below this.

The WHO reference level for Se is 70 μg/l, which is the minimum level for maximisation of the selenoenzyme glutathione peroxidase activity in plasma or serum (Neve, 1995). However, this value may be conservative: Rayman (1997) has quoted studies that show that a level of 100 μg/l is required for optimal expression of plasma glutathione peroxidase. The vast majority of the world's population would not reach this level.

For Australia, an estimate of 92 μg/l (range 77-121) for adult plasma/serum Se can be derived from the means of eleven published studies These are Agar & Hingston (1983), Brock et al (1991), Cumming et al (1992), Daniels et al (2000), Dhindsa et al (1998), Judson et al, 1978, 1982), Lux & Naidoo (1995), McGlashan et al (1996), McOrist & Fardy (1989) and Pearn & McCay (1979). This places Australia above most countries in population Se status.

However, relatively low plasma Se levels have been reported in Adelaide infants. Daniels et al (2000) found a plasma Se level of around 31 μg/l (SD 13) in a sample of newborn infants, a level comparable with New Zealand, a low-Se country. Infant Se levels are typically half those of adults. These levels place the infants at increased risk of conditions that involve oxidative stress and inflammation. Daniels et al observe that, given the geographical variability in Se intake and blood levels and the sensitivity of these to changes in the food supply, it is necessary to use local data to monitor Se status. They also note the paucity of Australian Se data: most of the few published studies have

involved small sample sizes. Clearly, more data are required to clarify the Se status of Australians. Moreover, there are few recent published reports on human blood levels of other mineral nutrients, including zinc, copper, magnesium and calcium.

The present study was undertaken to compare plasma Se levels in a sizeable sample of healthy South Australians in 2002 with current global levels; compare 2002 South Australian plasma and whole blood Se levels with previous South Australian Se surveys, back to 1977, to assess trends; assess the relationships of blood Se concentration to gender, age and other mineral nutrients, and discuss estimates of optimal Se status and compare these with current South Australian Se levels.

Materials & Methods

Samples

2002 Survey
A total of 288 samples of whole blood were obtained (using 10ml Vacutainer glass tubes with sodium heparin as coagulant) from blood donated at the Australian Red Cross Blood Service (ARCBS), Adelaide from 26-28 June, 2002. Donor approval was obtained, and age and sex of each donor provided. This group could be considered to be a relatively healthy sample of the Adelaide population, having been screened free of infectious diseases, and likely to be free of cancer.

Earlier Surveys
As noted above, human blood Se data collected by Judson et al (SARDI) from 1977-1988 (n=555) were included in the study. These include unpublished data from ARCBS donors and published studies of employees of the Institute of Medical and Veterinary Science (Judson et al, 1978) and Kangaroo Island residents (Judson et al, 1982). All of these groups are of similar health status, mean age and age range to the 2002 sample. When the data of all the surveys are combined, the total sample size of the study was 834 (444 M, 390 F), with mean age of 42 years and range 17-71 years. Collection of blood samples from ARCBS was similar to that for the 2002 survey.

Laboratory procedures

2002 Survey
Samples were stored at 4°C until collection was completed. A sub-sample of 30 was split for comparison of whole blood and plasma Se concentrations and for analysis of other mineral nutrients in whole blood. The remaining samples were centrifuged for ten minutes at 3,000 rpm, and plasma pipetted into 5ml Sarstedt polypropylene tubes. The remaining samples were stored at -20°C prior to digestion and analysis. The blood and plasma samples (2ml) were digested with a nitric/perchloric acid mixture and diluted to 25ml with MilliQ water to give a final acid concentration of 5% perchloric acid. For analysis of Se by hydride inductively coupled plasma optical emission spectrometry (ICPOES), all Se must be in the Se (+4) state. To effect this conversion the digest solutions (4ml) were diluted with 1ml concentrated perchloric acid and 5 ml concentrated hydrochloric acid, heated at 90°C for 30 minutes, then the Se (+4) in the solutions was reacted with sodium borohydride to convert it to hydrogen selenide (H_2Se) which was analysed as a part of the hydride ICPOES technique. The Method Reporting Limits (MDL) for the blood and plasma samples using the hydride technique were calculated as 10 times the standard deviation for the reagent blanks multiplied by the overall sample dilution ratio, thus the MDL = 6 μg/l. In addition to Se analysis, 27 whole blood samples

and a subset of 90 plasma samples from the digests prepared above were further analysed by normal liquid nebulisation ICPOES for nine mineral nutrients: iron, copper, zinc, calcium, magnesium, sodium, potassium, phosphorus and sulphur.

Earlier Surveys
The Se analyses of the pre-2002 samples were conducted using the fluorimetric method (Watkinson (1966) for the 1977 and 1979 surveys, and Koh & Benson (1983) for the 1987 and 1988 surveys), whereas the 2002 samples were analysed by ICPOES. To test the validity of comparison of samples analysed by different methods, 30 of the plasma samples were split and half sent to South Australian Research and Development Institute (SARDI) Livestock Systems for fluorimetric Se analysis. The methods provided similar results: fluorimetric: mean of 100 μg/l (SE 1.9), hydride ICPOES: 102 (SE 2.0), $r = 0.96$ ($p<0.01$). Thus, on the basis of analytical method, the earlier Se data can be compared with the 2002 sample. Quality controls were whole blood and plasma samples of known Se content.

Statistical analyses

Statistical analyses were conducted in two stages:

- Using all of the Se data from 1977-2002 and a linear mixed model, using fixed (age as a covariate, and gender as a factor) and random (group) effects, the effects of age, gender and group (and hence time) were examined. The Best Linear Unbiased Predictor (BLUP) for the group effect was used to interpret the group effect. The statistical model assumes normality with constant variance. The fixed effects were tested using a Wald test, and the random effect with a Log-Likelihood Ratio test (both tests distributed asymptotically as chi-square). All analyses were performed in S-PLUS 2000.

- Using the sub-samples of whole blood (n=27) and plasma (n=90), the data were examined for possible associations between Se and each of the 9 other mineral nutrients. The method of analysis was a multiple linear regression using backward elimination. The maximal model included all nine explanatory variables, which were then eliminated one by one using an F-test, until the contribution of every explanatory variable in the model was significant at 5%. The significance of the coefficient of each term in the final model was tested using a t-test.

Ethics approval

Ethics approval for the project was obtained from the University of Adelaide and ARCBS, Adelaide.

Results

1. Adelaide plasma Se survey, 2002

A histogram depicting the distribution of plasma Se concentrations of a sample of Adelaide blood donors (collected 26-28 June, 2002) is shown in Figure 1 below. This depicts a Normal distribution skewed somewhat toward the higher levels. Most Se values lie within the relatively narrow range of 86-110 μg/l

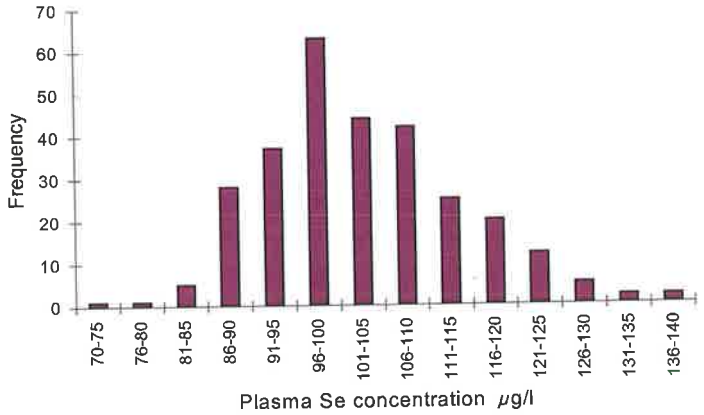

Fig. 1. Distribution of plasma selenium concentrations: Adelaide survey, 2002
N=288 (149 males, 139 females). Age: mean 49 years (SD 11) (males 52, females 46), range 17-71. Plasma Se: mean 103 µg/l (SD 11, SE 0.6).

2. South Australian human Se surveys, 1977-2002

Data from the six surveys that comprise this study are summarised in Table 1 and Figure 2 below.

Table 1. Summarised data from surveys of whole blood and plasma selenium concentrations (µg/l) in South Australian residents, 1977-2002

Sample parameter	Survey Number					
	1	2	3	4	5	6
Collection date	Dec 1977	Feb 1979	Oct 1987	Oct 1987	July 1988	June 2002
Description	Adel[1]	K Is[2]	Adel	Mt Ga[3]	Adel	Adel
N	117	30	96	103	200	288
Males	70	20	47	59	100	149
Females	47	10	49	44	100	139
Age mean	32	37	42	41	39	49
Age range	17-64	18-59	22-62	20-46	19-64	17-71
Whole blood Se	152	143	123	121	122	125[5]
SD	27	17	20	20	18	20
Range	115-212	119-186	84-211	82-205	85-212	100-160
Plasma Se	122[4]	114[4]	98	96	91	103
SD	21[4]	14[4]	15	14	12	11
Range	92-170[4]	95-149[4]	74-183	62-156	51-122	66-140

Notes over page.

1977-2002: mean age: 42 years; mean whole blood Se: 128 μg/l; mean plasma Se: 102 μg/l.
[1]Institute of Medical & Veterinary Science employees (Judson et al, 1978). The remaining Adelaide samples (designated "Adel") were Red Cross blood donors.
[2]Residents of Kangaroo Island, 70km S of Adelaide (Judson et al, 1982).
[3]Red Cross blood donors at Mount Gambier, 400 km S of Adelaide.
[4]Estimated values (based on 80% of whole blood Se concentration).
[5]N=28.
Whole-blood and plasma Se concentration means were similar for males and females within samples. For example: Survey 1 whole blood (M 155 μg/l, F 155); Survey 6 plasma (M 103, F 103).

Mean blood Se levels from the South Australian Se surveys 1977-2002 are depicted in Figure 2.

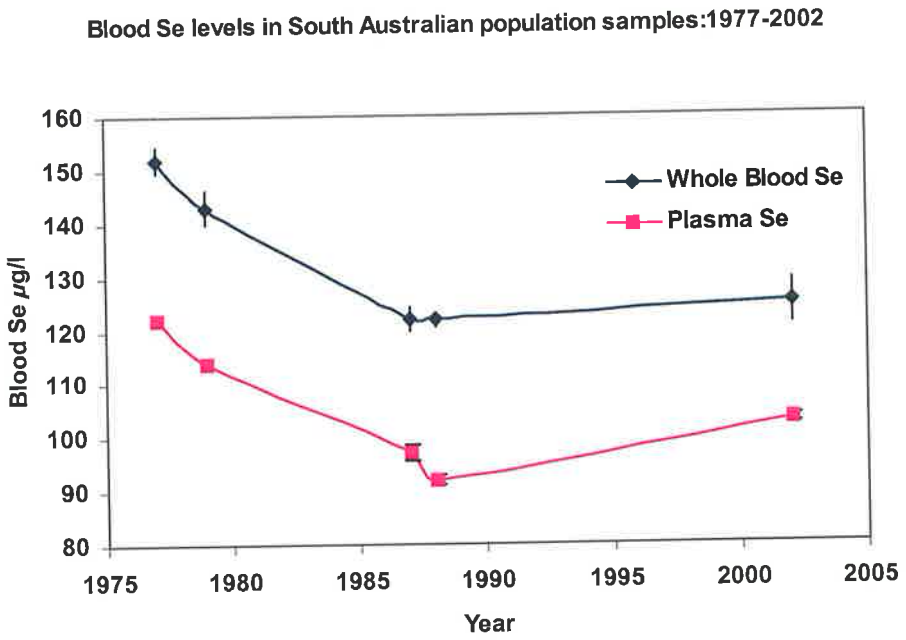

Fig. 2. Trend in whole blood and plasma selenium levels: South Australia 1977-2002
From Table 1. Means with SE bars. Note that data from Surveys 3&4 (Adelaide & Mount Gambier, 1987) have been combined.

Whole blood
The Survey effect was significant at the 5% level, indicating that the variation between Surveys was significantly different from zero. Variance component = 184, which is 31% of the total variation in the data. In terms of the BLUPs, Survey 1 has the highest above-average effect, and Survey 2 is also above average. All other Surveys are below average, with 4 being the lowest. This is evident by calculating the raw means. Mean whole blood Se for individuals in Surveys 1&2 (1977/1979) is 153 μg/l, while for 3-6 (1987/1988/2002) it is 122, a decrease of 20% (see Figure 2 above). The age: gender fixed effect (P=0.12), the gender effect (P=0.67) and the age effect (P=0.08) were not significant at the 5% level.

Plasma
The final model found the Survey effect to be significant (variance component = 20.80, which is 14.27% of the total variation in the data), which indicates that the variation

between the Surveys is significantly different from zero. The Survey effect has found that Surveys 3&6 have higher plasma Se levels than average, and Survey 5 has lower than average plasma Se, with Survey 4 close to the average. Note that Surveys 1&2 were excluded from this plasma Se analysis as they included whole blood data only.

The age:gender fixed effect was not significant at the 5% level (P=0.08). The age effect was significant at the 5% level (P=0.008) and has an estimated slope of 0.095. This indicates that plasma Se levels rise as age increases. The gender effect was also significant at the 5% level (P=0.01). The gender effect is that females are lower than males: when age is equal to zero, the average plasma Se level is equal to 90.8 for females and 92.9 on average for males. The slopes for each gender increase at the same rate of 0.095 (as two distinct parallel lines).

When Survey 6 (Adelaide, 2002) data are age-stratified (see Table 2 below), a trend toward lower plasma Se is suggested in the youngest and oldest individuals. However, greater numbers of people under 26 and over 69 years are required for this to achieve significance.

Table 2. Plasma selenium in Adelaide 2002 sample stratified for age

Age range (years)	Mean age (years)	N	Mean plasma Se (µg/l)
17-25	21.4	14	95
26-69	49.9	270	104
70-71	70.3	4	93

3. Blood mineral nutrient analysis

Results of the ICPOES analyses of sub-samples of whole blood and plasma from the Adelaide 2002 survey are presented in Table 3 below.

Table 3. Mineral nutrient levels in whole blood and plasma - Adelaide 2002

Nutrient	Concentration (mg/l)			
	Whole blood		Plasma	
	Mean	Range	Mean	Range
Fe	480	360-590	2.8	1.3-7.3
Cu	0.95	0.72-1.7	1.2	0.49-2.8
Zn	6.1	4.9-7.4	1.2	0.92-1.6
Ca	59	49-68	99	91-108
Mg	34	28-40	21	17-26
Na	2029	1770-2200	2987	2700-3300
K[1]	1707	1470-1990	866	600-1350
P	351	310-390	132	101-173
S	1567	1410-1730	1171	1050-1360
Se	0.130	0.098-0.160	0.104	0.085-0.129

[1]The potassium values are indicative only as a nitric/perchloric acid digest can cause losses due to the precipitation of potassium perchlorate.

Sulphur was the only variable to significantly explain whole blood Se (P<0.001). A one-unit change in S was associated with a 0.106 μg/l change in whole blood Se. The R-square value for this regression equals 0.35, which indicates that only 35% of the variation in Se concentrations is associated with variation in blood sulphur concentrations. The small sample size for this analysis should also be taken into account.

The only variables that significantly explain plasma Se are calcium (P=0.036) and phosphorus (P=0.007). A one-unit change in Ca was associated with a 0.743 μg/l change in plasma Se (when all other terms are constant), and a one-unit change in P was associated with a 0.193 μg/l change in plasma Se (when all other terms are constant). The R-square value equals 0.143, which indicates a very poor fit, likely due to large variability in the dataset.

Discussion

Global context

Data from surveys of healthy population samples were taken from the following references to show how the new South Australian data relate to selected representative global reference data (Bates et al, 2002a; Benemariya et al, 1993; Burk et al, 1992; Imai et al, 1990; Marinov et al, 1998; Nomura et al, 2000; Robinson et al, 1997; Terrier et al, 1995). This comparison is shown in Figure 3 below.

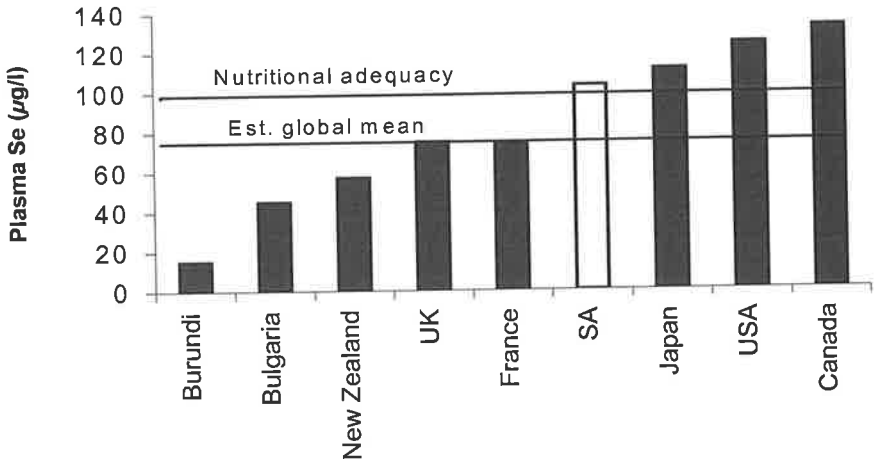

Fig. 3. Comparative plasma Se concentrations from around the world, placing the new South Australian data in a global context of eight selected countries with both higher and lower plasma Se concentrations

The mean plasma Se concentration of 103 μg/l for the sample of Adelaide blood donors in 2002 is well above the WHO reference level of 70, the estimated global mean of 78, and the mean (92) and median (88) of Australian published studies. However, it is just above the estimate of 100 suggested by Rayman (1997) as necessary for optimal expression of glutathione peroxidase, and 39% (111/288) of the sample were below this level.

Combs (2001) estimates the number of Se-deficient people in the world to be in the range of 500-1,000 million. As well, he considers that the vast majority of the world's population have suboptimal Se intakes, and hence are at increased risk of cancer, heart disease, viral diseases, and indeed any conditions which involve increased levels of

oxidative stress. Low Se intakes in much of Africa may be a reason why HIV has spread much faster there than in North America (Foster, 2003).

Determinants of Se status

Se status, which can be defined as the total content of the element in the body, can be estimated by Se concentration in plasma, serum, whole blood, erythrocytes, various organs, toenails or hair, or by the activity of the selenoenzyme glutathione peroxidase, usually measured in erythrocytes (Reilly, 1996).

The samples used in this South Australian study of Se status all comprised relatively healthy individuals. They were likely to have been above average in socioeconomic status, with a low incidence of tobacco smoking, and few pregnant women and no children were represented. Chronically ill people, the frail elderly, children and certain other groups could be expected to have lower Se status. For example, studies have shown that third-trimester pregnant women may be 25-30% lower in Se than controls (Golubkina & Alfthan, 1999; Reyes et al, 2000; Tan et al, 2001). It also appears that plasma Se and plasma and erythrocyte glutathione peroxidase activity vary during the menstrual cycle, with peaks coinciding with that of oestrogen during the periovulatory phase (Ha & Smith, 2003).

Se status can be directly related to socioeconomic status (Bates et al, 2002), and tends to be lowered by smoking (Bates et al, 2002; Kafai & Ganji, 2003; Kocyigit et al, 2001; Luty-Frackiewicz et al, 2002; Robberecht & Deelstra, 1994). A German study found serum Se of smokers (>20 cigarettes/day) to be 35% lower than non-smoking controls (Luty-Frackiewicz et al, 2002).

Gender effect
This study found males to have a marginally (although statistically significant) higher plasma Se concentration than females (93 μg/l M v 91 F when age effect reduced to zero), similar to studies in the USA (Kafai & Ganji, 2003) and the Azores (Viegas-Crespo et al, 2000). There was no difference for whole blood Se. This finding agrees with most studies, which have found little effect of gender on blood Se concentration in adults (unless late-term pregnant or lactating women are included) (Bates et al, 2002a; Benes et al, 2000; Morisi et al, 1989). The difference in the current survey is likely to be due to differences in dietary composition and total intake.

Age effect
Plasma Se was found to increase with age, while the increase for whole blood Se was non-significant. Most studies have found little difference in human Se levels in the age range 20-65 years. For example, a study involving 1,458 samples of whole blood from 24 locations in China found no age trend in Se concentration from 27-72 years (McOrist & Fardy, 1989).

Our 2002 Adelaide survey found the 17-25 year olds (n=14) to have plasma Se concentrations around 9 μg/l lower than the 26-69 year olds, but this was non-significant due to low numbers. Children under 15 years generally have lower plasma Se levels than adults within the same population (Barony et al, 2002; Bates et al, 2002b; Morisi et al, 1989), and infants are at particular risk for Se deficiency (Daniels et al, 2000; Muntau et al, 2002), which has potentially serious medical implications. A German study of healthy children found infants aged 1-4 months to be the highest-risk group for Se status: median serum Se declined from 50 μg/l in the newborns (<1 month) to 34 in the 1-4 month

infants, before rising to 48 in the 4-12 month infants (Muntau et al, 2002). In their study of Se status in infants born in Adelaide, Daniels et al (2000) found plasma Se concentrations in the first 5 days of life for term and pre-term infants to be 33 (SD 11) and 29 μg/l (SD 14) respectively, with females higher than males. From these initially low levels they may have declined further by 4 months of age.

An Italian study found low serum Se values for people over 60 years, especially males (Morisi et al, 1989), and a British study of people over 65 years found that plasma Se decreased with age (Bates et al, 2002a). Our 2002 survey found no decrease at the older end of the range, until 70 years was reached. Only 4 individuals were aged 70 or 71 and they averaged 93 μg/l, around 10 μg/l less than the 26-69 year group. Campbell et al (1989) suggest that ageing *per se* may not affect Se, but rather illness and lower food intake.

Possible decline in Se status

The South Australian population samples studied suggest that a decline in Se status of around 20% may have occurred from the late 1970s to the late 1980s. However, no decline is evident thereafter (see Fig. 2 above). The inter-group comparison appears to be valid: samples are similar in age, gender balance, health and socioeconomic status. It is possible that seasonality may have influenced Se status: samples 1 & 2 were obtained in summer, 3-5 in spring and 6-8 in winter. The summer-collected samples (1977 & 1979) were higher in Se than the rest. A British survey found a seasonal effect as follows: spring/summer (73 μg/l mean plasma Se)>winter (69)>autumn (66) (Bates et al, 2002a), while a Spanish study found spring/autumn>summer>winter (Garcia et al, 1999). An earlier Australian study found no seasonal differences for Se (McGlashan et al, 1996). If samples 1 & 2 had higher Se levels due to summer collection, the effect is likely to be small.

A possible decline in Se status from 1970s levels may be due to changes in dietary composition and/or a decline in the mean Se concentration of South Australian wheat, given the importance of this source. Almost all flour used in SA is made from SA-grown wheat. Watkinson (1981) showed that an increase in blood Se levels in New Zealand residents was due to the importation of Australian wheat. A targeted survey of wheat grown in SA in the 2000 and 2001 seasons conducted by our group has found grain Se concentrations ranging from 5-720 μg/kg, with most in the range 50-200 and a grand mean around 130 (Lyons et al, unpublished). Although this was not a systematic survey of the state's entire wheatbelt, these levels appear lower than those presented for the 1981 season, which found large differences between regions and an overall median Se concentration of 170 μg/kg (range 47-316) (Babidge, 1990). If there was a real decline in soil Se levels in the SA wheatbelt in the 1980s, it may have been due to more intensive cropping (Gissel-Nielsen, 1998), lower pH, increased use of gypsum (which contains sulphur) to treat sodic soils, or a combination of these factors.

Optimal Se status

Optimal Se intake and blood levels are currently under debate, but it is apparent that supranutritional intakes may be required to provide maximum cancer protection (Combs, 2001; Rayman, 2002). In the Nutritional Prevention of Cancer (NPC) trial in the US (where the treatment group received 200 μg Se/day), the protective effect of Se against cancer occurred in the lowest (relative risk [RR] of 0.52, 95% CI: 0.33-0.82) and middle (RR 0.64, 95% CI: 0.40-0.97) tertiles, which included those people with plasma Se levels

below 121 μg/l (Rayman & Clark, 2000), and the latest analysis shows that the Se benefit was largely restricted to male smokers with baseline Se levels below 113 μg/l. The strongest protective effect was against prostate cancer, with a hazard ratio of 0.48 (95% CI: 0.28-0.80) (Duffield-Lillico et al, 2002).

Other studies have shown a protective effect of Se against prostate cancer (Brooks et al, 2001; Hardell et al, 1995; Vogt et al, 2003; Yoshizawa et al, 1998). Brooks et al (2001), for example, in a nested case-control study in the US, found men with baseline plasma Se in the range 82-107 μg/l to have nearly a 7-fold increased risk of prostate cancer compared with men in the second quartile (range 108-118 μg/l).

Although there is a risk in generalising results of individual epidemiological and intervention studies, the results of the NPC trial and the other studies noted above would suggest, using the Se status figures presented by Combs (2001) above, that most of the world's male population (including that of Australia, with an estimated mean plasma Se concentration of 93 μg/l) would be in the responsive range for protection by Se against prostate cancer.

Evidence from the NPC trial and other studies suggests that a plasma Se concentration of around 120 μg/l provides maximum protection against a range of cancers. The NPC participants had a natural dietary Se intake of around 90 μg/day (Clark et al, 1996); thus with the addition of a 200 μg supplement the treatment group would have received around 290 μg/day. Combs (2001) suggests that a Se intake of 200-300 μg/day may be required to significantly decrease cancer risk, which compares with a current estimated Australian adult intake of around 75 μg/day. It is important, however, to remember that Se status can vary widely between individuals in the same population.

Moyad (2002) is critical of the interpretation of certain Se studies, but nevertheless suggests that an intake of 200 μg Se/day and 50 mg vitamin E/day may reduce prostate cancer risk, especially in current or previous smokers. The latest NPC trial findings indicate that males may have a higher Se requirement than females, who apparently gained no cancer protection from Se in this trial (Duffield-Lillico et al, 2002). A gender effect has been found by one of our authors in studies on cattle, with steers more susceptible than heifers to Se deficiency. Although speculative, it is possible that further studies – which include several large, current intervention trials - may find optimal adult human Se intakes in the range 125-280 μg/day, with means of around 130 (F) and 230 (M).

Se status can be raised by increased consumption of Brazil nuts (the most concentrated food source of Se), wheat products, fish and meat (Reilly, 1996). It should be noted, however, that Se concentration in Brazil nuts is highly variable (0.03-512 mg/kg, a 17,000-fold variation), depending on where they are grown in South America (Chang et al, 1995). Most Australians derive between a third and half of their Se from wheat products (Barrett et al, 1989). Careful supplementation (using, for example, high-Se yeast) is an effective way to increase Se status, as is biofortification. For example, applying Se to a growing wheat crop will increase the concentration of selenomethionine, a desirable Se form, in the grain (Djujic et al, 2000). Groups which could benefit especially from Se supplementation include male smokers, pregnant or lactating women (particularly if they are smokers with low socioeconomic status) (Bates et al, 2002; Kafai & Ganji, 2003; Morisi et al, 1989), infants under 8 months (Daniels et al, 2000; Muntau et al, 2002), cancer sufferers and those at high risk of cancer, sufferers of high-oxidative-stress conditions generally , sufferers of serious viral infections (including HIV, hepatitis B and

hepatitis C) (Clark et al, 1996; Combs, 2001; Rayman, 2002), and the frail elderly (Campbell et al, 1989).

Se is a micronutrient, required only in small amounts, with a relatively narrow therapeutic index. Documented cases of serious illness or death due to excess Se are few, however, and it could be argued that its toxicity has been overrated. Combs (2001) considers the WHO and EU estimates of the safe upper limit of Se intake of 400 and 300 μg/adult/day to be conservativc. Under normal conditions, a Se intake of less than 1,000 μg/day (or 15 μg/kg bodyweight) does not cause toxicity (Neve, 1991; Poirier, 1994; Taylor, 1997; Whanger et al, 1996), and people living in parts of China, the US, Venezuela and Greenland have consumed Se at this level throughout their lives without ill effects (Taylor, 1997). However, it would be prudent to limit medium- to long-term Se intake to around the US reference dose of 350 μg/day for a 70kg human (Schrauzer, 2000).

Other nutrient elements in blood

Although plasma Se values are useful indicators of Se status, most plasma and serum nutrient measurements are either closely controlled and thus vary little (e.g. calcium) or reflect recent intake rather than long-term nutritional status (e.g. water soluble vitamins), and are not necessarily valid measures of status (Friis et al, 2002; Rutishauser, 1997). Erythrocyte (and thus whole blood) values tend to reflect longer-term status.

The minerals measured in the Adelaide 2002 survey are all in the expected range for a healthy sample. The Adelaide copper and zinc levels were similar to those found in adolescents in Sweden (Barany et al, 2002) and blood donors in Czechoslovakia (Benes et al, 2000), and 15% and 23% higher in copper and zinc, respectively than a sample of healthy Sydney volunteers, aged 23-45 years (Lux & Naidoo, 1995). A correlation between two trace elements in blood indicates that interactions may be occurring. The Swedish survey found copper and zinc to be correlated in whole blood and serum, but we found no association. Bates et al (2002a), in a British survey of people aged over 65, found plasma Se to be strongly associated with zinc, but again we found no association.

In our survey, calcium and phosphorus in plasma were found to be weakly associated with Se. The calcium/Se finding is supported by Bates et al (2002a). Calcium is the most abundant cation in the human body. Over 99% is present in bone. Plasma calcium is present in ionised (60%) and non-ionised forms. Ionised calcium and calcium complexed with lactate, bicarbonate, citrate, phosphate or sulphate are readily diffusable, whereas protein-bound calcium (35%) is not. Plasma calcium is maintained at a relatively constant level of around 100 mg/l (Root & Harrison, 1976). Plasma phosphorus is mostly in the form of phosphoproteins, lipids and sugars (Jones, 1997).

In whole blood, sulphur was the only nutrient element analysed which was found to be associated with Se, albeit not strongly. The sample size was relatively small, and with a larger sample the association may not be evident. The sulphur would be present mainly in the amino acids cysteine and methionine and as disulphide bonds in polypeptide chains. In healthy individuals plasma Se up to a concentration of around 80 μg/l is mostly in the form of selenoprotein P, with glutathione peroxidase making up the balance. Above this level, nearly all plasma Se is in the form of selenomethionine (Burk et al, 2002).

Conclusion

In healthy sub-groups of the South Australian population sampled from 1977 to 2002 (n=834, mean age 42 years, range 17-71 years), whole blood and plasma Se concentrations were above an estimated global mean. However, there was an apparent decline from the late 1970s to the late 1980s. Age and gender effects, although not large, were statistically significant: plasma Se increased with age, and males had higher plasma Se levels. Levels of other mineral nutrients in whole blood and plasma in the 2002 sample of Adelaide blood donors were satisfactory. Se was weakly associated with calcium and phosphorus in plasma, and with sulphur in whole blood.

The mean plasma Se concentration of 103 μg/l found in our most recent survey of healthy individuals (Adelaide, 2002) was just above the estimated requirement for maximising selenoenzyme expression. There would be sub-groups of the South Australian population that would be less healthy than the groups surveyed in this study, which could be expected to have substantially lower levels of blood Se. In the case of prostate cancer, these data, when considered together with the findings of several studies discussed above, suggest that a majority of Australian men would be in the responsive range for increased Se intake to lower the risk of prostate cancer.

Many Australians could benefit from increased Se intake, in terms of reduced oxidative stress, lowered cancer risk and protection against serious viral disease sequelae. Se status can be improved by increased consumption of Brazil nuts, wheat products, fish and meat; biofortification (for example, by applying Se to growing wheat crops); careful supplementation, and cessation of smoking.

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Exploiting micronutrient interaction to optimize biofortification programs

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Biofortification of staple food crops with micronutrients by either breeding for higher uptake efficiency or by fertilization can be an effective strategy to address widespread dietary deficiency in human populations. Selenium and iodine deficiencies affect a large proportion of the population in countries targeted for biofortification of staple crops with Zn, Fe and vitamin A, and inclusion of Se and I would be likely to enhance the success of these programs. Interactions between Se and I in the thyroid gland are well established. Moreover, Se appears to have a normalizing effect on certain nutrients in the body. For example, it increases the concentration of Zn and Fe at key sites such as erythrocytes when these elements are deficient, and reduces potentially harmful high Fe concentration in the liver during infection. An important mechanism in Se/Zn interaction is selenoenzyme regulation of Zn delivery from metallothionein to Zn enzymes. More research is needed to determine whether sufficient genetic variability exists within staple crops to enable selection for Se and I uptake efficiency. In addition, bioavailability trials with animals and humans are needed, using varying dietary concentrations of Se, I, Zn, Fe and vitamin A to elucidate important interactions, in order to optimize delivery in biofortification programs.

Keywords: biofortification, crops, iodine, iron, selenium, zinc, vitamin A

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Introduction

Micronutrient deficiency is widespread. More than 2 billion people consume diets that are less diverse than 30 years ago, leading to deficiencies in micronutrients, especially iron (Fe), zinc (Zn), iodine (I) and selenium (Se), and also vitamin A. These deficiencies increase the risk of severe disease in around 40% of the world's population.[1] Se, Fe, Zn and vitamins A, B and C have immunomodulating functions and thus influence the susceptibility of a host to infectious diseases and their courses and outcomes.[2,3]

Diets dominated by cereals tend to lack micronutrients. Dietary diversity is desirable but may not be achievable in many countries until population declines. In the meantime, a major effort is required to improve the micronutrient content of cereals and other staple foods to maximize impact on the lowest economic strata in societies. Programs that include fortification, education and supplementation have been successful in countering micronutrient deficiencies in certain cases and will continue to play a role. However, they tend to be expensive, require ongoing inputs and often fail to reach all individuals at risk.

Furthermore, such programs themselves are at risk from economic, political and logistical impacts.[4,5]

A strategy that exploits genetic variability to breed staple crops with enhanced ability to fortify themselves with micronutrients (*genetic* biofortification) offers a sustainable, cost-effective alternative, which is more likely to reach those most in need; such a strategy has the added advantages of requiring no change in current consumer behaviour to be effective and being able to be transported to many countries. Substantial variations in Zn, Fe, manganese (Mn), and copper (Cu) density in wheat varieties grown together have been demonstrated. Exploiting the genetic variability in crop plants for micronutrient density may be an effective method to improve the nutrition of entire human populations.[1]

Fertilization (by addition to soil, seed or leaves) to improve micronutrient concentration in crops (*agronomic* biofortification) can be an effective alternative[6] for micronutrients like Se and I, that are relatively mobile in plants and for which substantial genetic variation in uptake efficiency in staple crops has not yet been demonstrated.[7-10] Wheat grain Se concentration, for example, can be increased inexpensively more than 100-fold on a Se-deficient soil by soil amendment.[10,11]

Crops biofortified with Fe, Zn and pro-vitamin A carotenoids are proposed for Southeast Asia and Africa in the *HarvestPlus* program.[12] We argue that inclusion of Se and I in this program is likely to be beneficial for several reasons. Firstly, deficiencies of both Se and I affect at least 20% of the world's population,[13,14] with a higher prevalence in those countries targeted by the biofortification program. Because of beneficial interactions (to be discussed below), the benefits of Fe, Zn and vitamin A fortification are unlikely to be maximized in many countries without the inclusion of Se and I. In addition, fortification or supplementation with one or two nutrients in the presence of deficiencies of others may even intensify existing morbidity.[15]

Moreover, low dietary micronutrient intakes are not confined to developing nations. The food systems of developed countries also often fail to provide optimum nutrition. For example, New Zealand's low Se supply impacts on high-risk groups like the aged,[16] and a recent Australian study found that 76% of schoolchildren surveyed in Melbourne had below-normal I status, with 27% suffering moderate to severe deficiency.[17]

In this paper we discuss briefly the importance of Se and I to human health; examine Se and I interactions, with a focus on thyroid hormone metabolism; document evidence of interactions between Se and I and between Zn, Fe and vitamin A, particularly in terms of enhancing nutritional bioavailability; and suggest research priorities to address knowledge gaps in this area.

Selenium in human health

Se is an essential micronutrient for humans and animals; it is an integral component of at least three systems required for normal cell metabolism.[18] It has been suggested that around 100 selenoproteins may exist in mammalian systems.[19] As a key component of the iodothyronine deiodinase enzymes, Se has an important role in the thyroid, and hence also in hepatic enzyme expression and neutrophil function.[20] The glutathione peroxidases have an antioxidant role in reducing damaging hydrogen peroxide and lipid or phospholipid hydroperoxides produced in eicosanoid synthesis by the lipoxygenase and cyclo-oxygenase pathways.[21] Another group of selenoenzymes, the thioredoxin reductases, are

involved in the reduction of nucleotides in DNA synthesis, the regeneration of antioxidant systems, and the maintenance of cellular redox state.[22]

It is well established that dietary Se is important for a healthy immune response. The effects of Se deficiency can include reduced T-cell counts and impaired neutrophil, macrophage and polymorphonuclear leucocyte activity.[23-25] Se supplementation of even Se-replete individuals is immunostimulatory, and involves enhancement of natural-killer-cell and lymphocyte activity as well as enhancement of proliferation of activated T-cells.[25]

Mounting evidence suggests that chronic marginal Se intake increases susceptibility to viral infection and viral disease sequelae, cancer, cardiovascular diseases, thyroid dysfunction, and various inflammatory conditions.[11,26] Se's anti-viral activity is of particular interest, given the high global prevalence of severe viral infections, including HIV/AIDS, influenza, hepatitis B and hepatitis C. In a Se-deficient host the benign coxsackie virus becomes cardiomyopathic, influenza viruses cause more serious lung pathology,[27] and HIV infection progresses more rapidly to AIDS.[28] It has been suggested that Se deficiency, due to widespread low soil levels, is the main reason for the much faster spread of AIDS in Sub-Saharan Africa than in North America.[29]

Available Se concentration in soil is highly variable, and low-Se soils are common in New Zealand, Denmark, Eastern Europe, UK, central Siberia, and a belt from north-east to south-central China. Large areas of Africa and Southeast Asia are also likely to be Se-deficient, but more mapping is required. It is estimated that approximately one billion people are Se-deficient.[13]

A practical strategy to alleviate Se deficiency is agronomic biofortification of food crops with sodium selenate, which is highly mobile in plants and in many soil types. Moreover, a high proportion of applied Se is incorporated into the sulphur amino acid, methionine, in cereal grain. Selenomethionine is a desirable, highly bioavailable Se form for humans.[11]

Iodine in human health

I is an essential micronutrient involved in growth, development and metabolic regulation, through its role as a component of thyroid hormones. Severe I deficiency is associated with goitre and retardation of growth and maturation in most organ systems. Intellectual retardation, hearing impairment, lassitude and cretinism are common; indeed, I deficiency is the world's most prevalent cause of brain damage. Pregnant women and infants are at particular risk of I deficiency diseases (IDD).[30,31]

In areas of high rainfall and ancient soils I has usually been leached to low levels. Mountainous regions of Asia (including much of China) and the food production flood plains of Nepal, India, Bangladesh, Myanmar, Papua New Guinea, the Philippines and parts of Indonesia are regions where I deficiency is endemic.[32] It is estimated that over 1.5 billion people are at risk of IDD.[30]

Developed countries are also affected by I deficiency. A German study found that 20% of pregnant women surveyed in Berlin were suffering from I deficiency,[33] and recent surveys in Australia have provided evidence of I deficiency. A study in Sydney found 20% of pregnant women attending an obstetric clinic and 34% of people attending a diabetes clinic had moderate to severe I deficiency.[34] A Melbourne survey of schoolchildren (n = 577, years 5-12 at two private schools) found that 76% of children had I status below normal. Girls had a mean urinary I concentration of 64 μg/l, and boys had a mean urinary

I concentration of 82 μg/l (>100 normal; 50-99 mild deficiency; <50 moderate-severe deficiency).[17]

I supplementation, usually through iodized salt, has been effective at relieving IDD, but requires sustained inputs, and in many cases has not succeeded in the long term. In Ethiopia, for example, iodized salt is not reaching the neediest people as it is more expensive than ordinary salt, there is a lack of knowledge of I deficiency among government officials, and distribution has been disrupted by war.[35]

An iodized salt program was also unsuccessful in Xinjiang province, China, for cultural and infrastructural reasons. But when potassium iodate was added to irrigation water (10 kg in a single treatment), the I content of all irrigated crops and foodstuffs which substantially increased the I status of livestock and people for at least 2 years. Infant mortality declined by 50% and IDD were largely eliminated.[9] This program is a successful example of agronomic biofortification by *fertigation.*

Selenium-Iodine interaction

Se and I interactions in the body mostly concern thyroid hormones. Both Se and I are required for thyroid hormone synthesis, activation and metabolism, and the thyroid gland has the highest Se and I concentrations of all organs.[36] The Se-dependent iodothyronine deiodinase enzymes catalyze the production of active thyroid hormone (T3) from thyroxine (T4) and also control reversion of T3 to di-iodothyronine (T2). There are three types of iodothyronine deiodinases that function in specific tissues such as liver and brain to maintain plasma and organ thyroid hormone homeostasis.[20]

Goitre is not necessarily due to I deficiency alone, as shown by nodular goitre, which is relatively common in I-replete populations.[37] Se can inhibit goitrogenesis when I status is marginal or deficient. Children with goitre in south-eastern Poland had lower blood Se and glutathione peroxidase activity, but were similar to controls in T4 and TSH levels.[38] In Turkey, I-deficient goitrous children had lower blood Se levels and higher DNA base lesions than non-goitrous controls.[39] In a French survey, investigators observed a protective effect of Se against goitre and thyroid tissue damage in women.[40]

In addition, free radical damage and fibrosis caused by Se deficiency, together with I deficiency (which can be exacerbated by consumption of goitrogenic thiocyanates from cassava) are involved in the pathogenesis of myxoedematous cretinism.[15,41,42] In the rarer case of I excess, the antioxidative Se-dependent glutathione peroxidases protect the thyroid gland from oxidative damage due to excessive iodide exposure.[43]

Severe deficiency of both I and Se occurs in parts of central Africa[15] and in Asia, in a region extending from north-eastern China and adjoining areas of Siberia and Korea to south-western China, including Tibet. In this part of Asia, Kaschin-Beck disease, an osteoarthropy involving enlarged joints, shortened fingers and toes, and even dwarfism, is prevalent. Se, I and vitamin E deficiencies appear to be predisposing factors.[44-46]

Caution is recommended for Se supplementation in areas of concurrent I and Se deficiency. Several studies have shown that normalization of I intake is necessary before initiation of Se supplementation in order to avoid exacerbation of hypothyroidism by stimulation of thyroxine metabolism,[15,43,47]

Selenium interactions with Zinc and Iron

Evidence is accumulating for important interactions of Se with Zn and Fe. Animal studies have shown that Zn can increase Se concentration in various organs, including brain, spleen, kidney, liver, lung and heart,[48] and Se can increase Zn concentration in liver, small intestine, blood, kidney, spleen, brain and lung.[48,49] Conversely, Se deficiency was found to increase Zn concentration in heart and kidney in a rat model.[50]

Se/Zn interaction involves a link between cellular Zn and redox state. Se compounds regulate Zn delivery from metallothionein to Zn enzymes. The metallothionein/thionein couple safeguards Zn and controls the concentration of available Zn in the cell.[51] Moreover, Se, Zn and Cu are linked in cytosolic defence against reactive oxygen and nitrogen species. Cu, Zn-superoxide dismutase catalyses the conversion of superoxide to oxygen and hydrogen peroxide, which is then reduced to water and oxygen by glutathione peroxidase,[52] the gene expression of which can be upregulated by Zn.[53]

An intriguing role for Se in regulating, or normalizing, the levels of other mineral nutrients at key sites in the body has been suggested by several studies. A clinical trial in Serbia included participants who were moderately deficient at baseline in Se, Zn, Fe and Cu, while Mn status was higher than normal. Those who consumed Se-enriched wheat during the trial had increased concentrations of Zn, Fe and Cu in erythrocytes, while Mn concentration declined in both plasma and erythrocytes.[54] Furthermore, interaction between Se and Fe was apparent in a trial with Schistosoma-infected mice, in which supplemented Se lowered abnormally high liver Fe concentration.[55] In addition, in rat models Se deficiency increased Fe concentration in the kidney, heart and liver,[50] and Fe deficiency decreased Se concentrations and glutathione peroxidase activity in erythrocytes and liver.[56] Lower serum Se levels were observed in children with Fe-deficiency anemia, but Se was normalized after Fe supplementation.[57] Further studies are needed to determine to what extent Se affects the absorption, distribution and retention of Zn, Fe, Cu and Mn. Deeper understanding of beneficial interactions between these nutrients could lead to their exploitation to improve the efficiency and effectiveness of supplementation, fortification and biofortification programs.

Iodine interactions with Iron, Zinc, and Vitamin A

Evidence also exists for I interactions with Zn, Fe and vitamin A. I, Se, Zn and Fe are essential for normal thyroid hormone metabolism. Fe deficiency inhibits thyroid hormone synthesis by reducing the activity of heme-dependent thyroid peroxidase, while Fe deficiency anaemia reduces, and Fe supplementation enhances, the effectiveness of I supplementation.[43]

Several studies have suggested that Zn is necessary for normal thyroid function. A study in Turkey found that concurrent deficiencies in Zn and I were associated with endemic goitre in males, and Zn deficiency may contribute to hypothyroidism and goitre.[58] In people with hypothyroidism in China, erythrocyte Zn was correlated with T3/T4 ratio and TSH;[59] in a rat model serum T3 was lower in Zn-deficient animals, and Zn deficiency appeared to induce apoptosis in thyroid cells.[60] Zn is involved in the binding of T3 to its nuclear receptor.[61] Moreover, Zn and Se tend to be lower in thyroid cancer cells than in normal thyroid cells.[62]

As noted above, the pathogenesis of goitre is generally multifactorial. On the Croatian island of Krk, goitre prevalence of 30% was found in a sample of 1,975 schoolchildren. It

was associated with low plasma levels of vitamins A and E and low I intake.[63] Vitamin A may stimulate the sodium/iodide symporter (NIS). For example, decreased uptake of iodide by the thyroid, due to impaired expression and/or function of the NIS, is a problem with radioiodide therapy of advanced thyroid cancer; however, retinoids (vitamin A derivatives) stimulate NIS mRNA expression and iodide uptake in human thyroid cancer cells.[64]

Conclusion

Se and I deficiencies affect a large proportion of the population in countries targeted for biofortification of staple crops with Zn, Fe and vitamin A. Mounting evidence of important beneficial interactions between all of these micronutrients suggests that the inclusion of Se and I in *HarvestPlus* would be likely to enhance the program's effectiveness.

Interactions between Se and I in the thyroid gland are well established. Few studies have been conducted on interactions among Se, I, Zn, Fe and vitamin A, especially those that impact on the bioavailability of Fe and Zn in cereals, such as the selenoenzymes that regulate Zn delivery from metallothionein to Zn enzymes. In addition, Se appears to have a normalizing effect on certain other nutrients at important sites in the body.

There is an urgent need for investigation into the extent of variability within staple crops for uptake and grain loading efficiency of both Se and I. Further bioavailability studies are also needed, using varying dietary concentrations of Se, I, Zn, Fe and vitamin A in animal and human trials in order to elucidate important interactions, and to optimize delivery of these nutrients to alleviate malnutrition. It is critical that in these studies, subjects are at least mildly deficient in combinations of these nutrients.

Attention to the role of agronomy in enhancing the concentrations of Se, I and Zn can deliver more of these nutrients faster to populations and allow breeders to concentrate on Fe and carotenoids, where fertilizers are ineffective.

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Selenium concentration in wheat grain: Is there sufficient genotypic variation to use in breeding?

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Abstract

Selenium (Se) is an essential micronutrient for humans and animals, with antioxidant, anti-cancer and anti-viral effects, and wheat is an important dietary source of this element. In this study, surveys of Se concentration in grain of ancestral and wild relatives of wheat, wheat landrace accessions, populations, and commercial cultivars grown in Mexico and Australia were conducted. Cultivars were also grown under the same conditions to assess genotypic variation in Se density. Eleven data sets were reviewed with the aim of assessing the comparative worth of breeding compared with fertilising as a strategy to improve Se intake in human populations. Surveys and field trials that included diverse wheat germplasm as well as other cereals found grain Se concentrations in the range 5-720 $\mu g\ kg^{-1}$, but much of this variation was associated with spatial variation in soil selenium. This study detected no significant genotypic variation in grain Se density among modern commercial bread or durum wheat, triticale or barley varieties. However, the diploid wheat, *Aegilops tauschii* and rye were 42% and 35% higher, respectively, in grain Se concentration than other cereals in separate field trials, and, in a hydroponic trial, rye was 40% higher in foliar Se content than two wheat landraces. While genotypic differences may exist in modern wheat varieties, they are likely to be small in comparison with background soil variation, at least in Australia and Mexico. Field sites that are spatially very uniform in available soil Se would be needed to allow comparison of grain Se concentration and content in order to assess genotypic variation.

Key words: Aegilops tauschii L, genotypic variation, grain, rye (*Secale cereale* L.), selenium, bread wheat (*Triticum aestivum* L.)

Abbreviations: CIMMYT: Centro Internacional de Mejoramiento de Maiz y Trigo (International Maize and Wheat Improvement Centre)
DH: doubled-haploid

Introduction

Selenium (Se) is an integral component of at least three systems required for normal cell metabolism in humans and animals (Arthur, 1999). Soils are frequently low in available Se, and hence the food systems of many countries are deficient in Se (Combs, 2001; Lyons et al, 2003; Rayman, 2002). Wheat is an important dietary source of Se. For example, in Australia it is estimated to supply nearly half the Se intake of most people (Barrett et al, 1989; Lyons et al, 2003). However, Se concentration in wheat grain is highly variable. Published values range from 0.001 mg kg^{-1} in south-west Western Australia (White et al, 1981) to 30 mg kg^{-1} in seleniferous areas of South Dakota (University of California, 1988), while most of the world's wheat lies within the range 0.02-0.60 mg kg^{-1} (Alfthan &

Neve, 1996). Se availability in soils depends upon soil pH, redox potential, calcium carbonate level, cation exchange capacity (Banuelos & Schrale, 1989), and organic carbon, iron (Fe) and aluminium (Al) levels (Ylaranta, 1983). In alkaline soils, most Se is present as selenates, which are highly soluble and easily taken up by plants (Elrashidi et al, 1989). In acidic, poorly aerated soils, Se occurs mainly as insoluble selenides and elemental Se. In lateritic soils high in Fe, Se binds strongly to Fe to form poorly soluble ferric hydroxide-selenite complexes (Cary & Allaway, 1969).

At least half of the world's population is malnourished. More than two billion people consume diets that are less diverse than 30 years ago, leading to deficiencies in micronutrients, especially Fe, zinc (Zn), iodine (I) and Se, and also vitamin A (Graham et al, 2001). Programs that include fortification, education and supplementation have been successful in countering micronutrient deficiencies in certain cases and will continue to play a role. However, they tend to be expensive, require ongoing inputs and often fail to reach all individuals at risk. Furthermore, such programs themselves are at risk from economic, political and logistical impacts (Gibson, 1994; Graham & Welch, 1996). Fertilisation of food crops with selenate to increase human Se intake (an example of *agronomic* biofortification) has nevertheless been successful, especially in Finland (Aro et al, 1995).

A strategy of breeding staple crops with enhanced ability to load more micronutrients into the edible portion of the plant (e.g. grain) (*genetic* biofortification) offers a sustainable, cost-effective alternative to conventional fortification, which is more likely to reach those most in need, and has the added advantage of requiring no change in current consumer behaviour to be effective. Exploiting the genetic variation in crop plants for micronutrient density is likely to be an effective method to improve the nutrition of entire populations. A four- to five-fold variation was found between the lowest and highest grain Fe and Zn concentrations among wheat accessions studied at CIMMYT, and the highest concentrations were double those of popular modern varieties (Graham *et al.* 2001). Moreover, wild, small-seeded relatives of modern bread wheats have been found with 50% more Fe and Zn than the highest CIMMYT germplasm studied (Monasterio & Graham, 2000). These ancient wheats provide a valuable genetic resource for increasing Fe and Zn efficiency in modern wheat cultivars (Cakmak et al, 1999a).

It could be argued that plants may be expected to show more genotypic variability for plant-essential micronutrients than for a non-essential element like Se. However, studies have demonstrated significant genetic variability in the edible parts of soy (Yang et al, 2003; Zhang et al, 2003), tomatoes (Pezzarossa et al, 1999), radishes (Lyons et al, unpublished), and *Brassica* vegetables (Combs, 2001). Findings from wheat studies have been variable. Some have found no evidence for genetic variability among wheat cultivars for Se density in grain (Grela, 1996; Noble & Barry, 1982; Tveitnes et al, 1996), while another found higher concentrations of Se, Zn, lithium (Li), magnesium (Mg) and phosphorus (P) in hulled wheat (*Triticum spelta* L. and *Triticum dicoccum* Schrank) grown together with modern bread wheats (*Triticum aestivum* L.) (Piergiovanni et al, 1997), and a Russian study suggested that commercial wheat cultivars may vary in their ability to accumulate Se (Seregina et al, 2001). More research is needed to determine whether sufficient genetic variation in grain Se density in wheat exists to enable the selection of this trait for plant breeding purposes.

This study conducted surveys of Se concentration in grain of wheat landrace accessions, populations, and commercial varieties grown in Mexico and South Australia, and grew a

selection of varieties under the same conditions, to assess for genetic variability in Se density.

Materials & Methods

CIMMYT survey & trials

CIMMYT's field experiments were conducted in the Yaqui Valley near Ciudad Obregon, Sonora, Mexico (27^{0}N 109'W, 40 m above sea level). The soils at the research station are coarse sandy clay textured with predominantly montmorillonitic clay, and classified as Typic Calcicorthid. Surface pH (H_20) is 8.3.

The survey was planted during the cycle 1997-1998. This was a multiplication block where 665 entries were grown in the same area. The plots were sown in late November, 1997 on dry soil and irrigated on the same day. The optimum planting time for this area is mid-November to mid-December. The entries were planted in 80 cm beds, the plot size was one bed wide with two rows on top of the bed (20 cm apart) and one metre long. The multiplication block was 28 beds wide and 48 metres long to accommodate a total of 672 plots. There were no replications. Weeds were controlled with *Topik* and *Brominal*. The trial was fertilised with 100 kg N ha^{-1} as urea and 46 kg ha^{-1} of phosphate as triple superphosphate at planting. An additional 100 kg N ha^{-1} as urea was applied in early January. The experimental area received four additional irrigations during the crop cycle. Many of the cultivars were susceptible to leaf rust, therefore fungicides (*Tilt* and *Folicur*) were applied when necessary. The trial was harvested by hand but yield was not measured. One hundred wheat cultivars were selected to provide a diverse range of genotypes. They included bread wheat landraces from Mexico and Iran, various cultivars from India, Israel, Nepal and Egypt, an historical set of released varieties from Mexico, CIMMYT advance lines and pre-breeding material, and 5 durum cultivars from Israel. This set of 100 entries was sent to Cornell University, USA for analysis.

The first trial comprised the same set of 100 wheat genotypes selected from the 1997-1998 multiplication trial and was conducted in the same field as the initial trial. A randomised complete block design with two replications was used. To provide a check and insure proper identification of the entries, the cultivar Genaro 81 was planted in every sixth plot, but was not analysed for Se concentration. The bed details, fertiliser, weed and rust control were as described in the initial trial. The trial was harvested by hand but yield was not measured. This replicated set of 100 entries was sent to The University of Adelaide (Waite Analytical Services), South Australia for Se analysis.

The second trial was planted during the cycle 1999-2000. The experimental design was a lattice with three replications. The plots were two 80 cm beds (each bed with two rows planted at 20 cm) wide and 4 m long. There were 40 entries in the trial: 24 wheat hybrids, eight female lines, three male lines and five checks. The trial was planted in November 1999 and irrigated. Pathogens and weeds were controlled. Grain yield was measured in all three replications but Se concentration was measured only in two replications. The objective of the trial (apart from assessment of Se concentration) was to identify males and females that could combine into high-yielding hybrids.

The third trial evaluated grain concentrations of Se and other minerals in the ancient diploid wheat, *Aegilops tauschii* (DD genome), an ancient durum wheat, *Triticum dicoccum* (BBAA genomes) and their cross (BBAADD genomes). Grain yield was not measured in this trial. These entries were evaluated in the field during the cycle 1999-

2000. The plots were 80 cm wide by 2 m long and were not replicated. Seventeen different *Ae. tauschii* lines that had been screened from a larger population of *Ae. tauschii* for high Fe and Zn were planted along the bed from entry 1 to 17. In the adjacent bed, 17 different *T. dicoccum* lines, that had also been screened from a larger population of *T. dicoccum* for high Fe and Zn, were planted along the bed from entry 1 to 17, and in the next bed the cross of *Ae. tauschii* x *T. dicoccum* were planted from entry 1 to 17. Thus, going perpendicular to the beds on the same row, on one bed was the male, in the next bed the female and in the last bed the cross between those two parents. This trial had no replications.

University of Adelaide surveys and trials

Landrace survey
A diverse sample of 300 wheat landrace accessions was obtained from the Winter Cereals Collection, Tamworth, New South Wales. From these, 90 accessions were selected, that included a wide range of genotypes, and winter and spring bread and durum wheats. The samples had been grown at sites near Tamworth from 1971 to 1998. The sites were characterised by three main soil types: loam/sandy loam, surface pH (H_20) 6.5-7.5; black cracking clay, pH 5.8-6.8; leached sandy loam, pH 5.3-6.0. To enable assessment of environmental effects, single landraces grown at different sites in different years, along with several replicates of single landraces grown together, were included in the analysis.

South Australian wheat survey
This targeted survey of wheat grown in South Australia from 1999 to 2001 was not designed to be comprehensive, but rather to provide an indication of the range and commonly occurring concentrations of grain Se as well as the extent of within-site environmental variability. It included the analysis of ten commercial wheat varieties (Krichauff, Excalibur, Frame, Janz, Kukri, Anlace, Sunco, Tasman, Yitpi, Stylet) grown at six sites in wheat-growing areas in South Australia in 2000. The sites were Roseworthy, Palmer, Stow (Lower North), Nangari, Loxton (Murray Mallee) and Bordertown (Upper South –East). Not all varieties were grown at all of the sites. A selection of unreplicated samples from other parts of the state, grown from 1999 to 2001 and representing a wide range of soil types, was also included. These sites included Piednippie, Kingscote (Kangaroo Island), Minnipa, Callington, Lock, Keith, Paskeville, Mintaro and Turretfield. A total of 22 sites were sampled.

W7984 x Opata 85 recombinant inbred lines analysis
This population, also known as the International Triticeae Mapping Initiative (ITMI) Mapping population or the Synthetic x Opata cross has been comprehensively mapped. Its 114 recombinant inbred lines were derived by single-seed descent (F_8) from the cross of the synthetic hexaploid W7984 with the CIMMYT wheat variety Opata M 85 (Nelson et al, 1995).

The seed analysed in this study was grown from seed provided by Dr P. McGuire, University of California, Davis, by Ursula Langridge on University of California (Waite version) potting mix soil in a glasshouse at the University of Adelaide's Waite Campus. As this was a pilot survey, 31 single (unreplicated) samples of the 114 lines grown were randomly selected for analysis.

Sunco x Tasman doubled haploid population analysis
This cross was chosen because 1) we had found (in the South Australian survey, above) that the Tasman parent was higher in grain Se concentration at the three sites where it had

been grown together with Sunco; 2) the population is well-mapped for genetic markers, although not as extensively as Cranbrook x Halberd or CD 87 x Katepwa (Chalmers et al, 2001); and 3) the parents and a selection of the F_1-derived doubled haploid (DH) homozygous progeny had been grown together at the University of Adelaide's Roseworthy Campus in 2000, a site with a medium mean soil available Se level. The 180 DH lines grown at Roseworthy were produced at the Leslie Research Centre (Kammholz et al, 2001). For this survey, the parents and 28 randomly selected unreplicated progeny lines were analysed. Progeny lines 96-8-8 (lowest Se) and 96-8-446 (highest Se) were selected for assessment in the next trial.

Charlick wheat variety trial 2001

A trial that investigated the effect of varying levels of applied sulphur (S) and nitrogen (N), as well as wheat variety (using three bread wheat varieties and two durum varieties), on grain Se concentration was conducted at the University of Adelaide's Charlick experimental farm in 2001 and is described elsewhere (Lyons et al, 2004a). The varieties included three popular commercial bread wheats (Krichauff, Kukri and Yitpi) and two durum wheats (Tamaroi, a popular commercial variety, and a boron-tolerant University of Adelaide-Waite Campus breeder's line, WD 99006).

South Australian cereal trials 2001

Cereal grain grown at a subset of sites within the South Australian Research and Development (SARDI) *S-4* trials in 2001 was analysed for Se and other nutrients. The four sites, which were selected for diversity of location and soil type, were Piednippie, Far West Coast, calcareous sandy loam, surface pH (H_20) 8.7; Greenpatch, Lower Eyre Peninsula, sandy loam over ironstone, pH 5.9; Bute, Upper Yorke Peninsula, sandy clay loam, pH 7.6; Frances, Lower South-East, loamy sand, pH 6.8. The following cereals were grown at each site in a randomised block design with four replicates: bread wheat, var Krichauff; barley (*Hordeum vulgare* L., var Barque); triticale (*X Triticosecale* Wittmack, var Tahara); rye (*Secale cereale* L., var Bevy).

Glasshouse soil pot assay

A short-term trial was undertaken to assess Se uptake efficiency into the leaves of different wheat varieties and cereal species. Soil was obtained from the University of Adelaide's Roseworthy Campus, where the Sunco-Tasman DH population was grown. In order to reduce variability in soil available Se concentration in this trial, the soil was obtained from an area of just 3 m^2 to a depth of 15 cm, and was thoroughly mixed, using a cement mixer. The soil was sieved (3.9 mm aperture) and 3.5 kg added to each pot (18 cm high x 21 cm diameter). Five hundred grams of deionised water was added to each pot to achieve a 14% soil moisture content. The wheat varieties used included those identified in the landrace survey as being potentially high (Poland 4 and Portugal 153) and low (Navarre 46) Se accumulators; the Sunco and Tasman parents and the highest- (96-8-446) and lowest-Se (96-8-8) DH progeny from the Roseworthy site (above); rye (var Bevy), identified in the *S-4* trials as being higher in Se than the other cereals; and, for further comparison, a durum wheat (var Tamaroi) and the parents of 2 other wheat DH populations, Seri 82/Babax and Kukri/Janz.

Samples of the grain used in the trial were analysed for Se, as variability in seed Se concentration could be a possible confounding effect. The values are presented in Table 8 in the Results, below. Seeds were pre-germinated on 12.5 cm Whatman ashless filter paper with 10 ml Milli-Q water in plastic Petri dishes, then 4 planted per pot. There were 2 pots (duplicates) per variety, giving a total of 26 pots. The pots were placed randomly on a bench in a glasshouse, and watered (de-ionised water, by weight) and re-positioned

randomly 3 times weekly. The trial was conducted in winter, and mean day temperatures were in the range 15-24^{0}C, and mean night temperatures 5-10^{0}C, with a mean relative humidity of 55%. After 35 days the tops were harvested, with 4 plants/pot bulked for weighing and analysis. The tops were dried at 70^{0}C for 48 hours and analysed for Se and other mineral nutrients.

Phytotron hydroponic pot assay

In order to minimise the background variation in available Se, a hydroponic trial was conducted. The trial compared the highest (Poland 4) and lowest (Navarre 46) landraces identified in the earlier survey, along with rye. In view of the apparent difference in Se concentration between dark and light seed from the same rye sample (see Results: *SA cereal trials 2001* below), the two were studied separately in this trial.

Seeds were pre-germinated as above, then transferred to 2-litre black plastic pots containing aerated 0.25-strength Hoagland's solution plus 1.0 µM sodium selenate (equivalent to 80 µg l^{-1} Se). Two plants were grown per cup, with two cups per pot comprising a replicate for each variety/type, making a total of 16 plants per pot. There were four replicates (pots). The trial was conducted in a phytotron, with light intensity (at the pot surface) of 630 µmol quanta PAR m^{-2} sec^{-1} provided by high-pressure sodium *Sonti Agro* fluorescent lamps; day-length 14 hours; day temperature 20^{0}C, night temperature 15.5^{0}C; mean relative humidity 60%. The pots were moved randomly three times weekly to minimise position effects in the phytotron. The solution was replaced on days 11 and 18, and the plants harvested on day 22. Tops of the four plants of each variety/type per pot were bulked, dried at 70^{0}C for 48 hours and analysed for Se and other mineral nutrients.

Chemical analysis

Grain samples for the CIMMYT survey were analysed for Se concentration at Cornell University, USA, using atomic fluorescence spectrophotometry. Grain samples from the three CIMMYT field trials, along with the University of Adelaide surveys and field trials and leaf samples from the pot assays, were analysed for Se using hydride-inductively coupled plasma optical emission spectrometry (ICP-OES), based on the method of Tracy & Moller (1990), and for 12 mineral nutrients (Fe, Zn, Mg, P, S, manganese [Mn], boron [B], copper [Cu], molybdenum [Mo], calcium [Ca], sodium [Na] and potassium [K] using ICP-OES at the University of Adelaide's Waite Analytical Services laboratory, Adelaide, South Australia. The standard used was NIST durum wheat flour (8436) for grain samples, and NIST peach leaves (1547) for leaf samples. The Se variation in the NIST standards was low: the mean measured Se concentration for durum wheat flour was 1.22 (SD 0.03) mg kg^{-1} (certified concentration 1.23 [SD 0.09] mg kg^{-1}), and for peach leaves the measured Se concentration was 0.12 (SD 0.007) mg kg^{-1} (certified concentration 0.12 [SD 0.009] mg kg^{-1}).

Statistical analysis

Analyses of variance were conducted using *Genstat* 6th Edition (Lawes Agricultural Trust, Rothamsted Experimental Station, UK).

Results and discussion

CIMMYT survey and trials

As shown in Table 1, although the mean Se concentration was similar for the two replicates in the first trial, there was high variability between the replicates of individual varieties. There was even higher variability between the 100 lines analysed in the initial survey (which was part of a multiplication trial that covered a larger area than Trial 1, hence the greater range in Se concentration) and the same lines grown at the same site in the following year.

Table 1. CIMMYT Survey and Trial 1. Comparison of grain Se concentration (μg kg^{-1}) in 100 wheat varieties grown together (Survey, unreplicated) and the same varieties grown together at the same site in the following year (Trial, two replications)

	Mean (SE)	Range	r	R^2
Survey	56 (4)	9-244	[a]0.14	[a]0.02
Trial repl 1	45 (2)	10-110	[b]0.62	[b]0.38
Trial repl 2	46 (2)	10-130		

[a] Comparison of the survey with the mean of the two field trial replicates
[b] Comparison of the field trial replicates

None of the 100 varieties tested stood out as being consistently high or low in grain Se concentration. The highest-Se wheat in replicate 1, for example (Mexican variety Durango DG 095.1.13) (244 μg kg^{-1}), was ranked 26th highest (56 μg kg^{-1}) in replicate 2, and 22nd (74 μg kg^{-1}) in the survey. The highest Se wheat in replicate 2 (DG 095.1.24) (130 μg kg^{-1}) was ranked only 40th (42 μg kg^{-1}) in replicate 2, and 55th (43 μg kg^{-1}) in the survey. There was also a large background variation in Se concentration in the field trial (13-fold) compared to that of other minerals (mean two-fold variation for S, Zn, Fe, Mn).

The second field trial of 40 different entries, including hybrids (all different from the varieties in the first trial) was conducted on a higher-Se soil and had lower Se variation (three-fold) than the first trial, but there was even lower correlation between the replicates: see Table 2. Only one of the varieties in the top four for Se concentration in replicate 1, for example, was in the top eight in replicate 2. Again, no varieties/crosses differed significantly in grain Se concentration. Se concentration was not reduced by higher yield, i.e. there was no dilution effect. Indeed, grain yield and grain Se concentration were positively correlated in this trial (r = 0.25; regression equation: Se concentration (μg/ha) = 0.007 x yield (kg ha^{-1}) + 35.5). For example, the six highest-Se entries (which included two entries of one genotype) had a mean yield of 6200 kg ha^{-1}, while the six lowest-Se entries (all different genotypes) had a mean yield of 5700 kg ha^{-1} The mean yield (SE) determined from three replicates for the whole trial was 6,000 (78) kg ha^{-1}.

Table 2. CIMMYT Trial 2. Comparison of grain Se concentration (μg kg^{-1}) in 40 different wheat lines and hybrids grown together in a field trial. Two replications

	Mean (SE)	Range	r	R^2
Repl 1	74 (2)	40-110	[a]0.32	[a]0.10
Repl 2	80 (3)	37-120		

[a] Comparison of the replicates

The third CIMMYT field trial compared grain Se concentrations between two ancient wheats, *Triticum dicoccum*, *Aegilops tauschii* and their cross, was conducted at a site with a higher available soil Se concentration than the two previous trials, and included 17 different entries of each parent. The analysis revealed that *Ae tauschii* (mean 179 μg kg^{-1}) was higher in grain Se concentration than either *T dicoccum* (135 μg kg^{-1}) or the *T dicoccum/A tauschii* cross (117 μg kg^{-1}) ($p < 0.001$). The grand mean was 144 μg kg^{-1} (SD 47), and the range of values was 83-290 μg kg^{-1}: see Table 3. The same pattern was apparent for S, Fe, Zn and Mn, with *Ae tauschii* being higher than the other two ($p < 0.01$ for each).

Table 3. CIMMYT Trial 3. Comparison of grain Se, S, Fe, Zn and Mn concentrations in two ancient wheats and their cross grown together in a field trial. Means of 17 different entries for each parent

	Se μg kg^{-1}	Se range μg kg^{-1}	S mg kg^{-1}	Fe mg kg^{-1}	Zn mg kg^{-1}	Mn mg kg^{-1}
Ae tauschii	179	110-290	2647	71	68	83
T dicoccum	135	87-240	2300	42	42	50
T dic/Ae tau	117	83-190	2218	45	43	55

The *Aegilops* genus has been found previously to have higher grain mineral density (Balint et al, 2001), and our finding for Se supports that of Piergiovanni et al (1997), who found hulled wheats to be higher in grain Se, Mg, P and Zn than modern commercial bread and durum wheat varieties. These authors found the highest Se and Zn concentrations in spelt (*T spelta*, BBAADD genomes) accessions.

University of Adelaide survey and trials

Landrace survey

The results of the survey of grain Se concentrations in 90 diverse landrace accessions are summarised in Table 4.

Table 4. Grain Se concentration in wheat landrace accessions grown on different soil types near Tamworth, New South Wales from 1971 to 1998 by The Australian Winter Cereals Collection, Tamworth

Soil type[a]	Year	Sample size	Grain Se concentration μg kg^{-1}	
			Mean (SE)	Range
1	1971	27	256 (27)	61-510
	1980	9	224 (23)	170-310
	1993	7	233 (99)	120-430
	1996	13	302 (30)	140-490
2	1973	5	71 (35)	32-140
	1995	10	77 (18)	21-160
	1997	8	128 (36)	44-270
	1998	10	80 (19)	35-210
3	1976	8	47 (2)	41-52
	1977	5	20 (5)	15-29
	1978	6	34 (3)	23-44
	1984	5	20 (1)	18-22

[a] Soil types: 1: loam/sandy loam, surface pH (H_2O) 6.5-7.5; 2: black cracking clay, pH 5.8-6.8; 3: leached sandy loam, pH 5.3-6.0

Grand mean: 164 μg kg^{-1} (SE 15); range: 15-510 μg kg^{-1}. This is a 34-fold variation, and compares with the Zn range of 22-60 mg kg^{-1}, a three-fold variation. High variability was evident, even within soil type/year and variety. For example, four samples of China 19, grown at the same site in 1996, varied three-fold, from 160-490 μg kg^{-1}. Wheat grown on the black cracking clay with mean pH of 6.3 (soil type 2) had the most variability in grain Se concentration (13-fold). This soil would be expected to contain selenite, selenate and organic Se forms (Cary & Allaway, 1969). This variability notwithstanding, two varieties, Poland 4 (accession numbers 10872 & 12113) and Portugal 153 (5491), were identified that appeared to be consistently higher in grain Se concentration across several soil types/years, while Navarre 46 (5131 & 5133) was consistently low in grain Se concentration.

To test whether seed viability and grain Se concentration were associated, a germination trial was conducted. Thirteen wheat varieties grown in 1971, five grown in 1973, and four grown in 1980 were tested by imbibing 20 seeds of each (on filter paper in Petri dishes, using Milli-Q water, and incubating for 48 hours at 26^0C). There was no correlation between grain Se concentration and either germination % or early root/shoot growth. The 1971 wheats varied in germination from 10-100%, with most (surprisingly for their age) in the 90-100% range. The 1973 wheats ranged from 20-100% (with most 100%), while the 1980 wheats ranged from 95-100% germination. Root/shoot growth declined with increasing age: 1980>1973>1971. Of course, the possibility of substantial variation in grain Se concentration among seeds from the same sample should always be considered when assessing the validity of such trials.

South Australian wheat survey

This targeted survey included the analysis of nine commercial wheat bread varieties grown at 6 sites in wheat-growing areas in South Australia in 2000. The survey also included unreplicated samples from other parts of the state, grown from 1999 to 2001. A total of 22 sites were sampled. As with the landrace survey, a large variation in grain Se concentration - even within a single variety grown at the same site - was evident, reflecting the variability of soil available Se over distances of just a few metres. For example, at the Bordertown site (in the Upper South-East region of the state), five replicates of var. Excalibur varied six-fold in grain Se concentration, as shown in Table 5. Against this high environmental variability, no genetic variability in grain Se concentration was observed. The total sample size for the targeted survey (which included data from the SA *S-4* cereal and Charlick trials and the Sunco-Tasman population below) was 170; grand mean: 155 μg kg^{-1} (SD 101, SE 15); median: 100 μg kg^{-1}; range: 5 (Kingscote, Kangaroo Island: var. Janz) – 720 μg kg^{-1} (Minnipa, Upper Eyre Peninsula: var. Krichauff). This is a 144-fold variation, which compares with a three-fold variation in grain Zn concentration in the same sample (range: 11-34 mg kg^{-1}).

Table 5. Comparison of grain Se concentration in three bread wheat varieties grown at two field sites in South Australia in 2000 (subset of South Australian wheat Se survey)

Site	Variety	Replicate	Grain Se concentration (μg kg^{-1})		
				Mean (SE)	Range
Roseworthy	Krichauff	1	110	80 (14)	35-110
		2	100		
		3	97		
		4	35		
		5	58		
	Excalibur	1	98	92 (5)	82-98
		2	97		
		3	82		
	Janz	1	110	109 (7)	97-120
		2	97		
		3	120		
Bordertown	Krichauff	1	120	162 (40)	88-270
		2	88		
		3	170		
		4	270		
	Excalibur	1	120	392 (117)	110-690
		2	110		
		3	690		
		4	520		
		5	520		
	Janz	1	140	142 (16)	115-170
		2	170		
		3	115		

The survey was not designed to be comprehensive, but rather to provide an indication of the range and commonly occurring concentrations of grain Se as well as the extent of within-site environmental variability. The only comprehensive published survey of grain Se concentrations in South Australia used pooled wheat and barley samples from 107 and 100 silos, respectively, across the state in the 1981 season for wheat and the 1981 and 1982 seasons for barley. That study found ranges of 47-316 μg kg^{-1} and 32-276 μg kg^{-1} and medians of 170 μg kg^{-1} and 125 μg kg^{-1} for wheat and barley (1981 season only), respectively (Babidge, 1990). This is well above the median of 100 μg kg^{-1} found in the current 2000/2001 survey, though similar to its mean of 155 (SD 101) μg kg^{-1}. A survey of plasma Se levels in South Australian residents, conducted by our group, indicated that a decline of around 20% in plasma Se concentration occurred in South Australia from 1977 to 1987, but there has been no further decrease (Lyons et al, 2004b). This may have been due partly to a reduction in the Se level in wheat over this decade. The wheat grain Se concentrations found in the current South Australian survey are generally higher than those of New Zealand, China, UK and Europe, but lower than those of Canada and the USA (Adams et al, 2002; Combs, 2001; Lyons et al, 2003).

W7984 x Opata 85 recombinant inbred lines analysis

The 31 lines analysed had a mean grain Se concentration of 7 μg kg^{-1} (SE 1). While there was a range of 4-14 μg kg^{-1} (3.5-fold), it was considered that these Se levels were too low to allow meaningful assessment of genetic variability, and no further lines were analysed. Zn concentrations ranged from 67-129 mg kg^{-1}, Na from 9-57 mg kg^{-1}, and Mn from 18-54 mg kg^{-1}. Grain Zn and Fe concentrations were strongly correlated ($r = 0.8$, $p = 0.001$). Opata 85, which was bred at CIMMYT, is known to be a good average variety for grain

Zn and Fe concentration and can thus be useful as a standard check for these nutrients in trials.

Sunco-Tasman doubled-haploid population analysis
The parents and 28 DH progeny grown at Roseworthy Agricultural College, South Australia in 2000 had the following grain Se concentrations. All values are $\mu g\ kg^{-1}$: parents: Sunco: 90; Tasman: 140. DH progeny: range: 26-130 (5-fold); mean 78 (SD 26, SE 5). This was a more promising distribution than that found for the Opata synthetics, although none of the progeny had a grain Sc concentration as high as the higher parent, and 71% of the progeny were below the lower-Se parent. Progeny lines 96-8-8 (lowest Se) and 96-8-446 (highest Se) were selected for assessment in the next trial.

Neither of the wheat population studies could be considered definitive as the samples represented only a subset of the total lines that comprise the populations, and were not replicated. Moreover, the soil pot assay suggested that the relatively high variability in the Roseworthy Sunco-Tasman DH survey may be due to soil Se variation, while the Opata synthetic inbred recombinant lines were grown on a soil too low in available Se to allow expression of differences in Se uptake efficiency. In view of the superior grain Se density found in *Aegilops tauschii* in the CIMMYT trial, a future study of the Opata synthetics (which are formed by crossing *Ae tauschii* [DD] with durum wheat, *Triticum turgidum* [AABB] var. Altar 84, and then crossing the resultant synthetic hexaploid with the bread wheat, *Triticum aestivum* var. Opata85), but this time growing the lines in solution containing around 100 $\mu g\ l^{-1}$ Se through to maturity, may yield information on the location of genes involved in Se uptake and grain loading.

Charlick wheat variety trial
No effect of wheat variety on grain Se concentration was found in this trial. However, the bread wheats had a higher grain S concentration than the durum wheats ($p = 0.005$) (mean 1386 $mg\ kg^{-1}$ v 1293 $mg\ kg^{-1}$).

South Australian cereal trials 2001
The analysis of variance of data comparing grain Se concentration of four different cereal species (bread wheat, triticale, rye and barley) grown at four diverse sites (a subset of the SARDI *S-4* trials), and using 4 replicates, showed an interaction between the site and cereal-type variables ($p = 0.003$) as well as main effects of site ($p < 0.001$) and cereal ($p = 0.03$). Rye had a higher grain Se concentration (93 $\mu g\ kg^{-1}$) than triticale (71), wheat (71) and barley (64). There was no significant difference between the latter three. The grand mean was 75 $\mu g\ kg^{-1}$ (SE 7). As with the previous wheat surveys/trials, grain Se concentration was variable (22-fold; range 9-200 $\mu g\ kg^{-1}$), while grain Zn concentration varied just 2.3-fold (range 12-28 $mg\ kg^{-1}$). Rye was higher in grain Zn concentration, but no different from the other cereals in grain S or copper (Cu) concentration. The highest grain Se content was 707 $mg\ ha^{-1}$, recorded for rye grown at the Bute site.

It is known that rye is more Cu- and Zn-efficient than wheat (Graham, 1978; Cakmak et al, 1999b) and may have higher grain Cu concentration than wheat or triticale (Kozak & Tarkowski, 1979), so it is not surprising that rye had higher grain Se and Zn concentrations in the field trials, although the S and Cu concentrations were no different from the other cereals, and a survey in Finland found rye grain to be generally lower in Se concentration than wheat grown on soils of comparable Se level (Eurola et al, 1990). It was observed that each South Australian rye sample contained around 30% dark-coloured seed. In a subsequent analysis of rye grown at Callington, South Australia, the dark seed

was found to contain less than half the Se concentration of the light-coloured seed from the same sample: 170 v 390 μg kg^{-1} (mean of duplicate samples).

The influence of soil characteristics on available soil Se concentration is shown in Table 6. The table includes sites surveyed or used for trials as above.

Table 6. Typical soil types of South Australian sites classified by soil available Se status[a]

Soil Se status	Sites	Typical soil type
Very low	Kangaroo Island, Greenpatch, Palmer, Keith	Sandy loam over ironstone; pH (H_2O) 5-6
Low	Charlick, Piednippie	Sandy loam; pH 6.3-7.5
Medium	Roseworthy, Stow, Callington, Paskeville, Mintaro, Bute, Frances, Turretfield	Variable; pH 5.7-8.3
High	Minnipa, Fisher, Nangari, Loxton, Lock	Sandy loam/loamy sand; low Fe, C & S; pH 6-8.6

[a] As estimated by wheat grain Se concentration
Table compiled from SARDI Crop Harvest Report 2000/2001; University of Adelaide and SARDI site records.

Glasshouse soil pot assay
This trial was conducted to assess Se uptake into the leaves of diverse wheat varieties, including those identified in the preceding surveys and trials as being possibly high or low in Se uptake efficiency, as well as rye. Although the soil was carefully mixed to try to ensure homogeneity, there was still large variation between the duplicate samples, e.g. Sunco: mean leaf Se concentration 124 μg kg^{-1}, with a SE of 37. None of the varieties that were considered to be genetically either high or low in grain Se density were indicated as such by this trial. Indeed, the mean for the three "high Se" wheat varieties (Poland 4, Portugal 153, Tasman) plus the highest Sunco-Tasman DH progeny (96-8-446) plus the rye was 99 μg kg^{-1}, while the mean for the "low Se" variety (Navarre 46) and the lowest Sunco-Tasman DH progeny (96-8-8) was 117 μg kg^{-1}. However, there is the possibility that the genotypes tested differ in Se retranslocation from the shoots into the seeds (grain loading), which was not tested in this or the following trial. Grain Se concentration of the seed used in the trial was found to be not associated with leaf Se concentration.

Phytotron hydroponic pot assay
The results of this trial, conducted in order to reduce background Se variation, are summarised in Table 7. All plants appeared healthy throughout the trial, with no symptoms of deficiency or toxicity.

Table 7. Comparison of foliar Se and S concentration and foliar Se content in wheat and rye grown hydroponically for 22 days. Means of four replicates

Variety	Yield (g)[a]	Se conc. (mg kg^{-1})	Se content (µg $plant^{-1}$[b]) Mean (SE)	S conc. (mg kg^{-1})	S content. (mg $plant^{-1}$[b])
Poland 4	0.19	27 (1.4)	5 (0.7)	6850 (263)	1.3 (0.1)
Navarre 46	0.23	21 (1.2)	5 (0.5)	5825 (375)	1.3 (0.1)
Rye (Bevy) - light	0.27	25 (1.2)	7 (0.8)	6350 (335)	1.9 (0.2)
Rye (Bevy) - dark	0.19	19 (0.8)	4 (0.3)	5300 (155)	1.2 (0)

[a] Shoot (top) yield per plant (DW)
[b] Shoot only
Mean root yield per plant: 0.06 g (DW).
LSD (5% level): yield: 0.07g; Se concentration: 3 mg kg^{-1}; Se content: 1.5 µg $plant^{-1}$: S concentration: 750 mg kg^{-1}: S content: 0.4 mg $plant^{-1}$.

Although limited, in that it tests influx into roots and transport to foliage, but not grain loading, of only the highly soluble, highly mobile selenate Se form, this trial supports the finding from the field trial that Bevy rye (at least, when grown from normal, light-coloured seed) may have higher Se uptake efficiency than wheat. The dark-seeded rye was lower than rye grown from light-coloured seed in concentration and content of Se, S and Mn, but higher in Zn and P concentration and content. The explanation for the difference between the two seed forms of the same rye genotype remains elusive. This trial would also be unable to detect variety-specific differences in root-induced changes in the soil, which could affect Se solubility.

Selenate, the most soluble Se form in soil, is taken up by the roots mostly via the main sulphate transporter, a high-affinity permease (Cary & Gissel-Nielsen, 1973). In this trial Se and S concentrations were highly correlated ($r = 0.97$, $p < 0.01$), suggesting that most of the selenate influx and transport to the foliage was via the main sulphate transporter. The rye grown from normal, light-coloured seed was higher in both foliar Se and S content (which is probably a better short-term measure than concentration) than either of the two wheat landraces or the rye grown from dark seed ($p < 0.001$). There were no differences in foliar Se or S content between the latter three. Several sulphate transporters exist in higher plants, some of which show tissue-specific expression (Hawkesford & Prosser, 2000), and several sulphate transporter genes have been isolated in wheat (P. Buchner, Rothamsted, UK, personal communication). Breeding wheat for increased expression of S transporters and in particular the main S transporter could be expected to increase grain Se density. However, a recent survey of bread wheats in the UK found no correlation between grain Se and grain S concentrations (Adams et al, 2002). Moreover, enhanced uptake efficiency for selenate, as might be identified in hydroponic studies, may be of limited value in wheat grown on soils of very low available Se, where most Se is present as selenite, selenide and elemental Se forms (Cary & Allaway, 1969). For the same reason, the transfer of relevant genes from a Se accumulator like *Astragalus bisulcatus* may not be productive, as they appear to involve uptake and transport of soluble Se forms only (Goodson et al, 2003).

In the absence of an identified high-yielding, high-protein, disease-resistant wheat variety with superior Se uptake efficiency on soils with low available Se, agronomic biofortification using sodium selenate offers an effective alternative strategy to increase grain Se density, although there is minimal residual effect in the soil and thus the selenate must be applied annually (Gissel-Nielsen, 1998; Lyons et al, 2003, 2004a).

Conclusions

Surveys and field trials that included diverse wheat germplasm as well as other cereals found grain Se concentrations in the range 5-720 $\mu g\ kg^{-1}$. Grain Se concentration of wheat grown in Mexico was mostly in the range 30-200 $\mu g\ kg^{-1}$, while South Australian-grown wheat was commonly in the range 70-280 $\mu g\ kg^{-1}$, which is higher than that of most wheat-growing countries, but lower than North America. The diploid wheat, *Aegilops tauschii* and rye had significantly higher grain Se concentrations than other cereals in field trials (and, for rye, in solution culture), but no significant genetic variability was detected among commercial bread or durum wheat varieties, triticale or barley. Grain Se concentration appears to be determined overwhelmingly by soil available Se concentration, which is influenced by pH, redox potential, cation exchange capacity, and levels of organic carbon, S, Fe and Al. For producing wheat with higher grain Se density, agronomic biofortification may be a more practical and productive strategy than trying to breed improved Se uptake efficiency and retranslocation to the grain into existing high-yielding varieties.

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Selenium distribution in wheat grain, and the effect of post-harvest processing on wheat selenium content

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ABSTRACT

Selenium (Se) is an essential micronutrient for animals and humans, and wheat is an important source of this element. This study, using grain dissection, milling with a Quadrumat mill, and baking and toasting studies, investigated the distribution of Se and other mineral nutrients in wheat grain, and the effect of post-harvest processing on their retention. Se concentration was higher in the embryo than the endosperm (which included the aleurone layer in this study), which was higher than the seed coat. However, the difference in Se concentration between the embryo and the aleurone/endosperm was only 1.5-fold, compared with a mean 7-fold difference for Fe, Mn, Zn and Cu. Se and S were more evenly distributed than other mineral nutrients through the bran and flour fractions after milling. Genotypic variation in grain distribution of several minerals was observed in the dissection and milling studies. For example, the bread wheat cultivar Krichauff had a higher proportion of total grain Cu, Mn, Fe and Zn stored in the embryo than cultivar Kukri. Further studies comparing more cultivars are needed. Selection of varieties with a higher proportion of nutrients in the endosperm would be desirable in order to increase their concentration in white flour. In the meantime, consumers should continue to be encouraged to maximize the nutritional value of cereals by consuming whole grain products. Milling, baking and toasting resulted in no absolute losses of any of the minerals tested.

Keywords: selenium, zinc, iron, copper, manganese, wheat, distribution, processing, milling.

INTRODUCTION

Se is an essential micronutrient for animals and humans. However, it is estimated that around a billion people may be deficient (*1*) and many more sub-optimal (*2, 3*). Wheat is an important source of bioavailable Se (*4, 5*), and its content can be increased by application of Se to the growing crop or to the soil (*6, 7*). In order to maximize the amount of Se in wheat products, it is necessary to limit processing losses, yet there is little information in the literature on this topic.

It is well known that a lot of nutrients are removed in the milling residue during milling of wheat to white flour (*8*), and there is evidence that processing, at both industrial and domestic levels, influences Se levels in food (*9, 10*). Several studies report a reduction in the Se *concentration* of white flour compared with unmilled grain or whole wheat flour, ranging from 4-47%, with a mean around 27% (*11, 12, 13*). This compares with a decrease in concentration from whole wheat to white flour for Zn, Fe and K in the range

41-80% (*11, 14, 15*). A study that investigated the Se *content* of wheat flour (73% flour extraction) found that it contained 63% of the grain's total Se (*16*).

In contrast to other nutritional elements, these results suggest that Se, like S, is more evenly distributed throughout the wheat grain, with a higher proportion stored in the endosperm. This is not unexpected, as both Se and S are mostly protein-bound. However, there is a need for further studies that examine how much Se is stored in the different components of the grain, and whether there is any total loss of Se or other nutrients during post-harvest processing.

Studies into the effect of different cooking methods on Se content of foods have produced varying results. Some have found that usual cooking procedures do not result in Se losses for most foods (*16-18*), whereas a Greek study into the effects of frying, grilling, boiling or canning found that all foods lost some Se. Cereals lost 5-25% of their Se after extended boiling in water (*9*).

Baking studies have also yielded equivocal findings. Some found Se losses of around 15% (*19, 20*), while others reported no losses (*21, 22*). A study into the effect of various commercial thermal processes found no effect on Se concentration in whole grain wheat from steam flaking, autoclaving or popping. Se concentration in white flour was unaffected by extrusion cooking, but drum-drying decreased it by 23% (*23*).

This study will investigate the distribution of Se and other mineral nutrients (including Fe, Zn, Mn, Cu) in wheat grain by dissection and analysis of the seed coat layers, endosperm plus aleurone layer, and embryo of the grain, and also whether there is any total loss of Se and other minerals during the processing of wheat grain through to human consumption of bread or toast, by analysing Se and other minerals in whole grain, and then after milling, baking and toasting.

MATERIALS & METHODS

Grain dissection study

Four bread wheat (*Triticum aestivum* L) cultivars, Krichauff, Kukri, Stylet and VM506 were studied. Of these cultivars, samples of Krichauff and Kukri were taken from a previous field trial, conducted at Charlick Experimental Farm of The University of Adelaide, Strathalbyn, South Australia in 2002. The samples of Stylet and VM506 were obtained from plants grown in University of California soil mix (*24*). However, only Krichauff and Kukri were included in the analysis of Se in the grain, with both cultivars having a similar and relatively high grain Se concentration (around 400 μg/kg).

After 24-hour imbibition with high-purity water (>18 M ohm resistivity) water at 4^0C, seed tissues were carefully separated under microscope, using a scalpel. The seed was dissected into seed coat (which comprised 6 layers: the epidermis, hypodermis, cross cells, tube cells, testa, and nucellar tissue), embryo, and endosperm plus the aleurone layer. The staining of the aleurone layer with Amido black (Naphtol blue-black) (*25*) showed that the aleurone layer remained attached to the endosperm during dissection. Following excision, the tissues were placed in Eppendorf tubes. Three replicates of 23 seeds each were dissected.

Analytical method

Se in wheat grain, its milling fractions, bread and toast was analyzed by hydride inductively coupled plasma optical emission spectrometry (ICPOES) (Ciros, Spectro Analytical Instruments, Cleve, Germany). Samples were digested with nitric/perchloric acid and finished with perchloric/hydrochloric acid and sodium borohydride. The method was based on that of Tracy & Moller (*26*). In addition to the Se analysis, samples from the digests prepared above were further analyzed by normal liquid nebulisation ICPOES for the following mineral nutrients: Fe, Zn, Mn, and Cu. In the processing studies below, Ca, Mg, Na, K, P, and S were also analyzed.

Milling study

For the study of Se concentration in whole grain and its milled fractions, four wheat varieties were selected, two bread wheats and two durum wheats (*T. turgidum* L). The samples of the commercial bread wheats, Krichauff and Carnamah, had been found in a previous study to vary widely in Se concentration. The durum wheats tested were Tamaroi and a University of Adelaide breeder's line, Waite Durum 99006 (WD99006). Although normally used for pasta rather than breadmaking, it was considered that these harder, higher-protein wheats could provide a useful comparison with the bread wheats in this study. The durum wheats and one of the bread wheats (Krichauff) were grown at Strathalbyn, South Australia, while the Carnamah bread wheat was grown near Esperance, Western Australia.

A sample of 600 g of grain from each of the four varieties was prepared for milling. This involved determination of hardness and moisture content in a 15 g sub-sample by Near Infrared 300 analyzer (Perten Instruments DS 7000), and conditioning by addition of around 10 ml of high-purity water per 300 g sample (3% moisture) 24 hours before milling. Each sample was then milled in a Quadrumat Junior laboratory mill (Brabender, Duisburg, Germany) to produce two fractions: a milling residue comprising bran and germ (embryo), and white flour (endosperm and aleurone). The flour was then sieved for 6 minutes in a Simon sieve through 150 μ silk to separate pollard from the flour. The flour extraction rate was 60% for the bread wheats and 45% for the durum wheats, both of which are relatively low. The milling fractions were weighed and samples analyzed as above. The procedure was duplicated, and the data presented as mean values. A sample of whole grain of each variety drawn from the same sample that was milled was also analyzed. The whole grain samples were not ground prior to acid digestion. All samples were oven-dried for 48 hours at 70 ^{0}C prior to acid digestion.

Comparison with the whole grain analysis enabled determination of any losses of Se or other mineral nutrients that may have occurred as a result of milling. We were also able to calculate the amounts (and hence proportions) of Se and other minerals that are stored in the bran/germ, pollard and flour fractions of the wheat grain.

Baking procedure

In order to assess possible losses of Se and other mineral nutrients due to baking, we used samples of 100 g each of white flour of the two bread wheat varieties, Krichauff and Carnamah, milled as above. A commercial bread yeast was added at 0.42% by weight and high-purity water added at 67% by weight. The dough was kneaded on a floured plate until elastic, then left at room temperature (25 ^{0}C) until double its initial size. It was then placed in an oven at 210 ^{0}C and baked until golden-brown (35 minutes). The procedure

was duplicated, and the data presented are mean values. The flour and bread samples were dried in an oven for 48 hours at 70 ^{0}C prior to acid digestion and ICPOES analysis, and the results compared. A sample of the yeast was also analysed to determine its contribution to the tested nutrients.

Toasting trial

Two loaves of fresh, pre-sliced, plastic-wrapped, commercially made bread were purchased: one white and one wholemeal. A commercial kitchen toaster was used for the trial, and a temperature of around 200^{0}C was reached close to the element. Two toasting treatments were tested: 65 seconds, which resulted in lightly-browned toast ("light"), and 80 seconds, which produced well-browned – but not burned – toast ("heavy"). The toaster was warmed-up by running for one minute, then after a further minute the trial began. There was an interval of one minute between each toasting treatment. Three slices of bread per loaf were subjected to each treatment. All toasted slices, along with three untoasted slices per loaf (controls) were dried at 70 ^{0}C for 48 hours, prior to acid digestion and ICPOES analysis.

Statistical analysis

Where appropriate, variance analyses were conducted using GenStat 6th edition (Lawes Agricultural Trust, Rothamsted, UK), including a Kruskal-Wallis one-way analysis of variance for the toasting trial.

RESULTS & DISCUSSION

Distribution of Se, Fe, Zn, Mn and Cu in grain

The results of the dissection study are presented in Tables 1 and 2.

Table 1. Concentration, content and proportion of Se in grain fractions of two bread wheat cultivars. Standard errors are based on three replicates except for whole grain (two replicates)

Cultivar	Fraction	Concentration (µg/kg)	Content (ng)	Proportion (%)
Krichauff	Embryo	823 ± 89	0.6 ± 0.1	2.9
	Endosperm	543 ± 78	20 ± 3	96.2
	Seed coat	200 ± 20	0.2 ± 0	1
	Whole grain	510 ±72	18 ± 3	
Kukri	Embryo	593 ± 58	0.3 ± 0	1.5
	Endosperm	433 ± 11	19 ± 1	97.4
	Seed coat	157 ± 20	0.2 ± 0	1
	Whole grain	410 ± 49	18 ± 2	

NB: Endosperm includes the aleurone layer in this study.

Table 2. Concentration and proportion of Cu, Fe, Mn and Zn in different fractions of the grain of four bread wheat cultivars. Standard errors are based on three replicates.

		Concentration (mg/kg)			Proportion (%)[1]		
	Cultivar	Embryo	Endosperm[2]	Seed coat	Embryo	Endosperm[2]	Seed coat
Cu	Krichauff	22±0.3	6±0.1	4±0.1	6.4	91.8	1.8
	Kukri	13±0.7	5±0.0	4±0.7	2.5	95.5	1.9
	Stylet	24±1.1	4±0.2	10±0.0	15.5	79.3	5.2
	VM506	28±0.2	6±0.0	10±0.0	12.3	83.6	4.1
Fe	Krichauff	149±1.0	41±0.8	167±16.0	5.5	85.1	9.3
	Kukri	119±3.0	35±0.7	87±2.9	3.2	91.1	5.6
	Stylet	143±1.4	23±0.8	27±0.7	18.5	79.0	2.5
	VM506	151±0.9	26±0.4	21±0.6	15.2	82.8	2.1
Mn	Krichauff	250±5.8	25±0.3	74±6.7	14.1	79.6	6.3
	Kukri	209±10.7	24±0.3	64±2.3	8.0	86.0	6.0
	Stylet	313±8.8	24±0.6	112±2.3	30.4	61.9	7.7
	VM506	207±6.7	20±0.4	63±1.9	23.0	70.3	6.6
Zn	Krichauff	154±5.0	14±0.6	13±0.2	16.1	81.9	2.0
	Kukri	157±3.0	13±0.7	11±0.6	10.6	87.4	1.9
	Stylet	230±5.8	23±1.0	18±1.0	26.9	71.6	1.5
	VM506	194±3.3	25±0.3	42±0.8	19.5	78.7	1.7

[1] Proportion derived from seed nutrient content (µg/seed), not concentration.
[2] Endosperm includes the aleurone layer in this study.
Cultivars Krichauff and Kukri were grown together, and cultivars Stylet and VM506 were grown together.

The embryo contained the highest concentration of all the minerals analyzed ($p < 0.001$), except for Fe in Krichauff, where a higher Fe concentration was observed in the seed coat. It is apparent that Se is more even in concentration throughout the grain than the other nutrients tested. Se concentration in the embryo is just 1.5 fold that in the endosperm/aleurone, compared to 9.4-fold for Mn and 11.5-fold for Zn. However, Se concentration was higher in the embryo than the endosperm/aleurone, which was higher than the seed coat ($p < 0.001$). Although Krichauff had a numerically higher Se concentration in the embryo than Kukri, the difference was not significant. Mn followed the embryo > seed coat > endosperm/aleurone concentration gradient ($p < 0.001$), while for both Cu and Zn, concentrations were similar in seed coat and endosperm/aleurone.

The endosperm/aleurone fraction, due to its much greater weight compared to the other fractions (eg a mean of 73 times the embryo weight), contained the bulk of all nutrients tested. Our results differed from those of another grain dissection study, which found the seed coat to contain 55-77% of total seed nutrients (except S, which was 40%) for two wheat genotypes. The discrepancy between this and our study may be due to the authors' inclusion of the aleurone with the seed coat (*27*).

Although not all cultivars tested in this study were grown under the same conditions (Krichauff and Kukri in the field; Stylet and VM506 in the glasshouse), comparisons of genotypes grown under the same conditions point to genotypic differences in concentration, content and proportion of Cu, Fe, Mn and Zn. Krichauff had a higher concentration of most nutrients in most seed sections studied, but content was not necessarily higher, due to the larger grain size of Kukri. Stylet had higher concentrations of Zn and Mn. In the seed coat, Fe and Mn were higher in Stylet, while Zn was higher in

VM506. Although some of the differences in concentration may be due to differences in the proportion of the seed sections between genotypes (concentration or dilution effect), the differences in content or proportion indicates that breeding for increased concentrations of these nutrients may be feasible. Testing of a large number of cultivars grown under the same conditions in the future may reveal a greater variation in concentration, content and proportion of these important nutrients than that found in this study.

Distribution of 11 mineral nutrients in milled grain fractions

In the milling trial, the bread wheat varieties yielded more flour (60%) (pollard included) than the durum wheat varieties (45%). In all varieties tested, Se and S were distributed more evenly throughout the grain than were the other nutrient elements tested. In Krichauff, for example, the flour fraction contained 36% of the total grain Se content but only 5% of total Mn, 9% of iron and 13% of Zn. It is likely that these low flour extraction rates (compared with commercial rates of 70-76%) would have resulted in no aleurone tissue remaining in the flour. The aleurone comprises 5-10% of total grain weight and contains higher concentrations of minerals and vitamins than the endosperm (*28,29*). Hence, high flour extraction rates result in retention of more of the aleurone layer in the flour, with resulting higher amounts of mineral nutrients in the flour.

This variation in distribution was also reflected in nutrient concentrations. For example, in the bread wheats, Se concentration in flour was 85% (SE 5) of that in whole grain and 71% (SE 2) of that in bran, similar to the findings of our dissection study and of other studies (*11, 16*), but represented a more even Se distribution than that found by a Belgian study (*13*). Se was around 1.4 times more concentrated in bran than in flour, whereas Mn was 19 times more concentrated. For Fe, the concentration in flour compared with whole grain and bran was 24% and 12%, respectively, a finding similar to an earlier trial (*30*).

Figure 1 illustrates the variation in distribution of mineral nutrients in the bread wheat cultivars, particularly between Se/S, which are largely protein-bound, and the other minerals, with Ca in between. Less Se (51%), S (49%) and Ca (67%) were removed in the milling residue than occurred with the other minerals (77-91%).

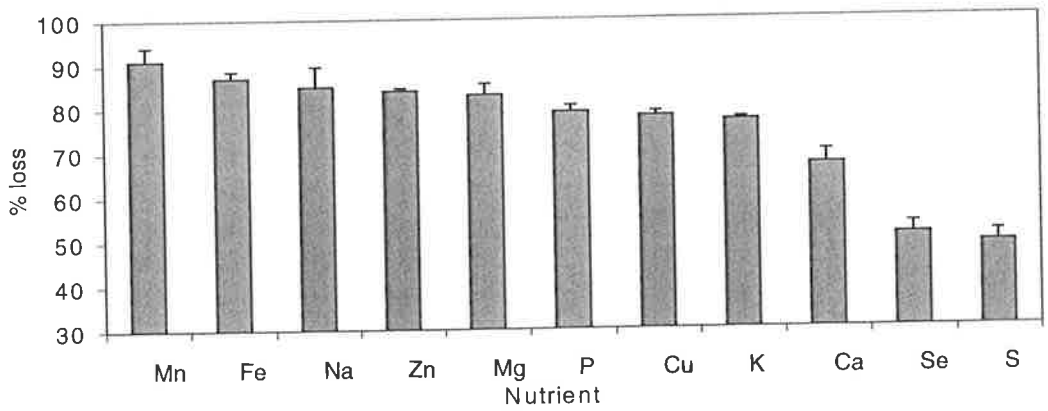

Figure 1. Removal of mineral nutrients in milling residue (% of whole grain content). Mean (SE) of two bread wheats (two replicates of each). Flour extraction: 59%. Dry weight basis.

Thus white bread can contain reasonable concentrations of Se, S and Ca, but is generally (unless fortified) low in Fe, Zn, Mn and Cu. Moreover, it has been shown that the loss of

Zn through milling to white flour is greater than the inhibitory effect of fibre components and phytate upon Zn absorption from the gut, when whole wheat products are consumed (*31,32*).

Although the four wheats tested had variable flour extraction rates in this trial, genotypic variation in the distribution of most minerals throughout the grain (with the exception of Se and S) was apparent. Using the parameter of concentration in flour/concentration in bran x 100%, Mn varied 3-fold (4-12%), Fe 3-fold (9-26%), Cu 2.5-fold (16-40%), and Zn 2-fold (13-26%). There was less variation within either bread or durum wheats, gencrally 1.5 - 2-fold. These findings support those of a study that analyzed 27 bread wheat cultivars grown under the same conditions (*30*). When the above formula is applied to their data, 2-fold variations are evident for Mn, Fe and Zn, and 1.5-fold for Cu. Their study did not include Se or S. A study of 6 bread wheat cultivars indicated genotypic differences in concentrations of Cu, Fe, Mn and Zn in different milling fractions (bran, straight-grade flour, and whole-wheat flour). For example, the range in Zn concentration was 44-81, 9-34 and 21-28 mg/kg DW in bran, straight-grade flour and whole-wheat flour, respectively (*33*). These results suggest that selection pressure could be applied for evenness of distribution of these nutrients throughout the wheat grain, i.e. for higher concentration in the endosperm. This would result in lower losses of these nutrients in milling to white flour and higher bioavailability due to lower levels of phytate and fibre components in endosperm compared with bran.

Milling losses

When the Se content of each milled fraction was determined, then totalled and compared with the Se content in the same weight of whole grain, it was found that there was a mean loss of 1% (for bread and durum wheats combined), well within the error of the analytical method. The data for the bread wheat cultivars are presented in Table 3. Furthermore, there were no losses of any of the other minerals analyzed. Concentrations and proportions of Cu, Fe, Mn and Zn are shown in Table 4. It can be concluded that milling resulted in no absolute loss of any of the minerals tested.

Table 3. Concentration, content and proportion of Se in the whole grain and milled fractions of two bread cultivars. Data represent means of two replications and standard errors.

Cultivar	Fraction	Concentration (µg/kg)	Content (µg)	Proportion (%)
Krichauff	Bran	60±4	7.1±1.0	45
	Pollard	38±7	2.0±0.5	13
	Flour	41±2	5.6±0.3	36
	Whole grain	51±4	15.7±0.5	100
Carnamah	Bran	410±15	56.1±2.0	54
	Pollard	300±10	21.2±1.5	20
	Flour	300±20	30.2±2.0	29
	Whole grain	335±25	103.5±9.0	100

Table 4. Concentration and proportion of Cu, Fe, Mn and Zn in whole grain and milled fractions of two bread wheat cultivars. Data represent means of two replications and standard errors.

		Concentration (mg/kg)				Proportion (%)		
		Bran	Pollard	Flour	Whole grain	Bran	Pollard	Flour
Cu	Krichauff	8.3±0.2	1.6±0.2	1.3±0.0	3.7±0.2	79	7	14
	Carnamah	6.4±0.3	1.6±0.3	1.6±0.1	4.8±0.3	77	9	14
Fe	Krichauff	82±7	7.2±0.8	7.0±1.0	33±3	88	3	9
	Carnamah	56±1	7.8±0.4	8.2±0.3	30±2	85	6	9
Mn	Krichauff	97±6	5.7±0.1	3.6±0.2	34±3	94	2	4
	Carnamah	52±2	6.2±0.3	5.3±0.1	23±2	88	5	7
Zn	Krichauff	57±2	8.3±0.1	7.2±0.2	24±1	83	5	12
	Carnamah	39±1	6.1±0.0	5.6±0.2	21±2	84	7	9

Baking losses

There were no significant losses of any of the tested minerals due to the baking process. The mean 4% (SE 1) loss of Se can be explained in terms of the small sample size and the Se detection limits of the analytical method (ICPOES). This agrees with a study that found no effect of most thermal processes tested on Se content of wheat or white flour (*23*). For all minerals there was a mean gain of 6% (SE 4) in concentration/content after baking, part of which can be attributed to nutrients in the yeast, which, although added to the flour at the low ratio of 1:240 (by weight) had relatively high concentrations of Fe (56 mg/kg), Zn (96), Na (390), K (21,000) and P (10,300). Bioavailability of some of the minerals may be increased by baking due to hydrolysis of phytate (*33*).

Toasting losses

There was no loss in concentration or content of Se or any other mineral tested, due to toasting, whether light or heavy. This finding supports those of Ferretti & Levander (*16*), who found negligible Se loss during the manufacture of wheat or corn breakfast cereals, and Hakansson (*23*). The Se data are presented in Table 5. The wholemeal bread was higher than the white bread in all minerals except Se, S and Ca. The white bread had clearly been fortified with Ca, to achieve its concentration of 1,900 mg/kg (compared to 570 in the wholemeal bread). The white bread's Ca:Mg ratio of 4.4 would be considered less desirable for cardiovascular health than the wholemeal's 0.5 (*35,36*)

Table 5. Se concentration in bread before and after toasting (µg/kg). Data represent the means of three replications and standard errors

Sample	Treatment				
	Untoasted	Lightly toasted	Loss (Gain) (%)	Heavily toasted	Loss (Gain) (%)
White bread	177 ±3	170 ±6	4	167 ±3	6
Wholemeal bread	170 ±0	177 ±3	(4)	170 ±0	0

No significant difference between treatments (Kruskal-Wallis test).

Even if some loss of Se occurred due to cooking/toasting/baking, this may be compensated by an increase in bioavailability. In a feeding trial of chickens on an initially Se-deficient diet, those chickens which were supplied with Se from baked bread had a 26% higher survival rate than those with a raw bread source (*37*). On the other hand, another trial, using rats, found that after 60 days' feeding Se supplied in white dough was more effective than that supplied in either baked dough (biscuit) or whole wheat meal in raising Se concentration in whole blood or liver, but Se in whole wheat was more effective than the others at restoring/raising the important antioxidant selenoenzyme, glutathione peroxidase levels in whole blood and liver. This may be because flour obtained after milling processes may have a higher selenomethionine concentration (*38*). Selenomethionine is less effective than several other Se forms (including selenocysteine) in raising glutathione peroxidase activity (*39*). Further research is needed to determine the distribution of different Se species in cereal grain.

CONCLUSION

In conclusion, this study showed that Se and S were present in proportionately higher amounts in the endosperm than were the other mineral nutrients analyzed, and hence white flour, which is the most widely used cereal grain product, contained a higher proportion of the whole grain content of Se and S than it did of the other nutrients. The embryo had higher concentrations of Se, Mn, Zn and Cu than the endosperm+aleurone or the seed coat. The combined findings of the dissection and milling studies suggested the importance of the aleurone layer as a store of nutrients. The low flour extraction rate (60%) would have minimised the aleurone content of the flour fraction, and for the bread wheat Krichauff, for example, the flour fraction contained 36% of the total grain Se content but only 5% of total Mn, 9% of Fe and 13% of Zn. Genotypic variation in the concentration and content of most minerals between the endosperm and the aleurone+bran fractions was suggested in the milling study, and in distribution of Cu, Fe, Mn and Zn in the dissection study. However, further studies comparing more cultivars grown together are needed to confirm this. No absolute losses occurred of Se or any of the other mineral nutrients, due to processing of whole wheat grain by milling, baking or toasting.

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Tolerance of wheat (*Triticum aestivum* L.) to high soil and solution selenium levels

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Abstract

The fertilisation of wheat crops with Se is a cost-effective method of enhancing the concentration of organic Se in grain, in order to increase the Se intake of animals and humans. However, it is important to avoid phytotoxicity due to over-application of Se. Studies of phytotoxicity of Se in wheat grown in Australia, where rainfall and grain yield are usually relatively low, have not been reported previously, and overseas results have been varied. This study used trials conducted in the field, glasshouse and laboratory to assess Se phytotoxicity in wheat. In field trials that used rates of up to 120 g Se ha^{-1} as selenate, and in pilot trials that used up to 500 g Se ha^{-1} soil-applied or up to 330 g Se ha^{-1} foliar-applied, on soils of low sulphur (S) concentrations (2-5 mg kg^{-1}), no Se toxicity symptoms were observed. In pot trials of four weeks' duration, the critical plant level for Se toxicity was around 325 mg kg^{-1} DW, a level attained by addition of 2.6 mg Se kg^{-1} to the growth medium as selenate. Solution concentrations above 10 mg Se l^{-1} inhibited early root growth of wheat in laboratory studies, with greater inhibition by selenite than selenate. For selenite, Se concentrations around 70 mg l^{-1} were required to inhibit germination, while for selenate germination % was unaffected by a solution concentration of 150 mg Se l^{-1}. Leaf S concentration and content of wheat increased three-fold with the addition of 1 mg Se kg^{-1} as selenate to the growth medium. This effect is probably due to the induction of the S deficiency response of the main sulphate transporter. This study found wheat to be more Se-tolerant than did earlier studies of tobacco, soybeans and rice. We conclude that Se phytotoxicity in wheat will not be observed at the range of Se application rates that would be used to increase grain Se for human consumption (4-200 g Se ha^{-1} as selenate, which would result in soil and shoot levels well below those seen in the above studies), even when – as is common in Australia – soil S concentration and grain yield are low.

Keywords: phytotoxicity, selenium, sulphur, wheat (*Triticum aestivum* L.).

Introduction

Higher plant species vary widely in Se uptake and accumulation in shoots and other edible parts, and also in tolerance to high Se concentrations in solution, soil or shoots (Marschner, 1995). Primary Se-accumulators such as *Astragalus bisulcatus* L. may contain as much as 15,000 mg Se kg^{-1} DW, which is toxic to livestock (Beath et al., 1937). It is likely that this ability to accumulate and tolerate high concentrations of Se has evolved as a defence against insect herbivores (Hanson et al., 2003; Pickering et al., 2003). On the other hand, tobacco and soybeans are Se-sensitive and can be affected by concentrations as low as 1 mg Se kg^{-1} in culture media (Martin & Trelease, 1938). Toxic plant tissue levels of Se are generally above 5 mg kg^{-1} (Reilly, 1996), but among agricultural crops the phytotoxicity of Se is variable (Mikkelson et al., 1989a).

Studies of Se phytotoxicity in wheat have also reported varied results. One study reported an inhibition of plant growth at a shoot concentration of 4.9 mg Se kg^{-1} in wheat grown for 45 days, and even the relatively low soil concentration of 0.2 mg Se kg^{-1} as selenate produced harmful effects, including growth reduction and chlorosis (Tripathi & Misra, 1974). However, a five-day study found that a solution containing 20 mg Se l^{-1} as selenate did not affect germination in wheat, whilst the early growth of wheat and lucerne was slightly reduced (Lintschinger et al., 2000). Of all the plant species tested by Carlson et al. (1989), wheat was the least sensitive to Se, with no effect on germination at up to 32 mg Se l^{-1} in culture solution, as selenite or selenate, while reduction in root growth occurred above 2 mg Se l^{-1}. Selenite is generally more toxic to plants than selenate (Smith & Watkinson, 1984; Carlson et al., 1989). Se phytotoxicity can be reduced by increased levels of sulphate or phosphate in the soil or culture solution and increased S in the roots and shoots (Bollard, 1983).

It is important not to over-fertilise cereal crops with micronutrients because of possible toxicity effects and reduced quantity and quality of grain yield (Rengel et al., 1999). Although it is unlikely that phytotoxic effects would occur at the range of selenate applications that would normally be used to biofortify wheat with Se (i.e. 10-200 g ha^{-1}) (Yläranta, 1983; Lyons et al., 2004), the variable findings of earlier studies indicate that further research is needed to clarify the threshold of toxicity of Se in wheat. Furthermore, studies of phytotoxicity of Se in wheat grown in Australia, where abiotic stressors including low soil fertility and low moisture levels usually result in grain yields lower than those commonly found in North America and Europe, have not been previously reported. Importantly, the low S and P status of many Australian soils may produce different outcomes for plant nutrition and toxicity when Se is added as a fertiliser, compared to many other countries.

Materials and methods

This study included field trials, soil pot trials conducted in a glasshouse, and *in vitro* trials. Several trials involved very young plants, as it has been shown for wheat and corn that they are more susceptible than mature plants to Se toxicity (Rosenfeld & Beath, 1964).

Field trials

Field trials were conducted at two South Australian sites, Charlick and Minnipa, in 2002, where Se was applied as sodium selenate at rates from 0-120 g Se ha^{-1} either to the soil at seeding or as a foliar spray after flowering. At Charlick, 70 km south of Adelaide, a 2x5x2 factorial trial with a split-split-plot design and four replications was conducted. Two commercial bread wheat varieties, Krichauff and Kukri were each randomly allocated to one of two whole plots within each replicate. The whole plots were then divided into five sub-plots and randomly assigned Se treatments of 0, 4, 12, 40 and 120 g ha^{-1} applied as sodium selenate. These sub-plots were further divided into sub-sub-plots and randomly allocated with soil or foliar Se treatments. Growing season (April to October) rainfall was 211 mm, and mean grain yield was 1.8 t ha^{-1}. The final harvested area per plot was 3.5 m^2. At Minnipa, 400 km north-west of Adelaide, a 5x2 factorial trial with a split-plot design and four replications was conducted, using var. Krichauff. Selenium application rates (the same as for Charlick) were randomly allocated to the five whole plots within each replicate. The whole plots were divided into two sub-plots and randomly allocated with soil or foliar Se treatments. Growing season rainfall was 219 mm, and mean grain yield was 1.4 t ha^{-1}. These trials are described fully elsewhere

(Lyons et al., 2004). The sites differed in soil type and in characteristics that determine Se availability (Table 1).

Table 1. Comparison of surface soil characteristics at two South Australian sites

	Charlick	Minnipa
Soil texture	Clay loam	Sandy loam
pH (H_2O)	6.6	8.6
Conductivity	0.05 dS m^{-1}	0.11 dS m^{-1}
S[a]	4.4 mg kg^{-1}	2.4 mg kg^{-1}
Fe (DTPA)	66 mg kg^{-1}	7 mg kg^{-1}
Reactive Fe[b]	773 mg kg^{-1}	293 mg kg^{-1}
P (Colwell)	57 mg kg^{-1}	22 mg kg^{-1}
Organic C[c]	13,300 mg kg^{-1}	7,800 mg kg^{-1}
Se[d]	<200 µg kg^{-1}	<200 µg kg^{-1}

[a] Extractable S determined using calcium hydrogen phosphate.
[b] Reactive Fe determined by ammonium oxalate extraction. It includes ferrihydrite, magnetite and organically-bound Fe.
[c] Organic C determined by sulphuric acid/dichromate method. The amount of chromic ions is proportional to the organic C oxidised, and is measured colorimetrically at 600 nm.
[d] Total soil Se determined by inductively coupled plasma mass spectrometry (ICP-MS) after digestion with nitric/perchloric acid.

Glasshouse trials

Two trials were conducted in the glasshouse, using wheat. The growth medium used was University of California (UC) potting mix (Barker et al., 1998), Waite Campus version (University of Adelaide). This comprises 400 litres of coarse washed sand, 300 litres of peatmoss, 700 g calcium hydroxide, 480 g calcium carbonate and 600 g *Nitrophoska* 15:4:12 (which contains, *inter alia,* 5.3% sulphates), with pH (H_2O) of 5.5.

Trial 1
The growth medium was sieved (3 mm aperture) to remove pebbles and weighed into 2.5 kg lots. Sodium selenate derived from a 0.1 M stock solution was added with enough pure water (*Milli-Q*) to make a total of 300 ml water per pot (19 cm diameter x 17 cm depth) to achieve 12% (w/w) water content. The selenate solution was mixed with the growth medium by shaking vigorously in a plastic container. Selenium was applied at the following rates: 0, 1, 2, 4, 8, 16, 32, 64 and 132 mg kg^{-1}. There were two replications (18 pots total). Wheat seed (bread wheat cultivar Janz, that contained a low Se concentration of 5 µg kg^{-1}) was pre-germinated on filter paper and planted at 1.5 cm depth, 12 seeds per pot in late June, 2001. Pots were watered to weight regularly with pure water, and at each watering were shifted randomly on the bench. After six days, the four smallest plants per pot were culled, leaving eight plants per pot. Whole tops were harvested at 22 days and fresh weights recorded, then dried for 48 hours at 80^{0}C, and analysed for Se and mineral nutrients. Mean air temperatures throughout the trial were 18^{0}C day and 10^{0}C night, with mean relative humidity of 65%.

Trial 2
This trial was conducted with UC growth medium, Janz wheat (eight plants per pot) and two replications, as in the first trial, but used larger pots (21 cm diameter x 17.5 cm depth) containing 4 kg soil. Selenium application rates included several additional lower levels (0, 0.01, 0.1 and 0.5 mg Se kg^{-1}) and excluded the highest levels used in the first trial (32

mg kg^{-1} and above). The trial was planted in late August, and whole plants (including roots) were harvested after 30 days. The youngest emerged leaves (YEL) and the dead leaves (from the 4, 8 and 16 mg kg^{-1} treatments) were separated from the remainder of the tops, and, after drying for 48 hours at 80^0C, were analysed separately. Mean air temperatures throughout the trial were 23^0C day and 13^0C night, with mean relative humidity of 60%.

In vitro trials

Trials were conducted to determine the effect of different concentrations of selenate and selenite in solution on germination and root and shoot growth over two days post-germination, using seed of the bread wheat cultivar Janz, which had a low grain Se concentration of 5 μg kg^{-1}. These trials used 15 cm Whatman ashless filter paper in 15 cm plastic Petri dishes, 20 ml of solution (using Milli-Q water; this volume ensuring the seeds had sufficient contact with the solution to enable imbibition and germination without being submerged and thus subject to lack of oxygen) and 20 seeds. Seeds were placed on the wet filter paper, and lids placed on the Petri dishes, which were then transferred to an incubator at 24^0C in darkness. After 48 hours the Petri dishes were removed and placed in a freezer, with upper and lower surfaces exposed to ensure rapid cessation of growth. Once frozen, Petri dishes were removed singly and thawed. Total root length (usually from a radicle and two lateral roots) and shoot length for each plant were measured with a ruler.

The trials used 12 concentrations of either selenate or selenite, ranging from 0 to 363 mg Se l^{-1}, with three replications. The selenite trial was conducted at 24^0C, but in the selenate trial, the incubator malfunctioned soon after commencement and the temperature climbed to 28^0C, so the two trials, although indicating the threshold of toxicity for each Se form, cannot be directly compared for growth.

Chemical analysis

Grain samples from the field trials and leaf samples from the pot assays were analysed for Se using hydride-inductively coupled plasma optical emission spectrometry (ICP-OES), based on the method of Tracy & Moller (1990), and for 12 mineral nutrients (Fe, Zn, Mg, P, S, Mn, B, Cu, Mo, Ca, Na, and K, using ICP-OES at the University of Adelaide's Waite Analytical Services laboratory, South Australia. The reference plant material used was NIST durum wheat flour (8436) with a certified Se concentration of 1.23 mg kg^{-1} for grain samples, and NIST peach leaves (1547) with a certified Se concentration of 0.12 mg kg^{-1} for leaf samples.

Statistical analysis

Analyses of variance were conducted using *Genstat* 6th Edition (Lawes Agricultural Trust, Rothamsted Experimental Station, UK).

Results and discussion

Field trials

In the field trials there was no effect of Se (either soil- or foliar-applied at rates of up to 120 g Se ha^{-1} as selenate) on grain yield (Table 2).

Table 2. Wheat grain selenium concentration and yield at two South Australian field sites in 2002. Selenium applied as selenate. Means of four replications. Cultivar: Krichauff.

	Charlick				Minnipa			
	Soil		Foliar		Soil		Foliar	
Se rate ($g\ ha^{-1}$)	Grain Se conc. ($\mu g\ kg^{-1}$)	Yield ($t\ ha^{-1}$)	Grain Se conc. ($\mu g\ kg^{-1}$)	Yield ($t\ ha^{-1}$)	Grain Se conc. ($\mu g\ kg^{-1}$)	Yield ($t\ ha^{-1}$)	Grain Se conc. ($\mu g\ kg^{-1}$)	Yield ($t\ ha^{-1}$)
0	57	1.89	65	1.89	600	1.41	650	1.44
4	205	1.82	145	1.80	1050	1.48	888	1.43
12	355	1.82	354	1.80	940	1.41	1,130	1.48
40	2,125	1.83	597	1.76	2,730	1.40	1,780	1.42
120	8,325	1.79	1,240	1.88	11,950	1.46	3,580	1.49

SE of differences in means: Charlick: 395 (grain Se); 0.14 (yield).
Minnipa: 600 (grain Se); 0.07 (yield).
LSD: Charlick: 790 (grain Se); 0.28 (yield).
Minnipa: 1,220 (grain Se); 0.14 (yield).

Furthermore, in pilot trials that used up to 500 g Se ha^{-1} soil-applied or up to 330 g Se ha^{-1} foliar-applied in soils with relatively low S concentrations (2-5 mg kg^{-1}) (Lyons et al., 2004) and field trials in Finland that used up to 500 g Se ha^{-1} (Yläranta, 1983), no Se toxicity symptoms were observed. At low soil S, more Se is taken up by the plants, and at low shoot S, Se is more toxic (Bollard, 1983; Mikkelsen & Wan, 1990); hence, as no Se toxicity was evident at a low-S site (which also had low Fe, low C and high pH; all soil characteristics that increase Se availability) like Minnipa, it would be unlikely to occur, using similar Se application rates, on most soils found in the Australian wheatbelt. It is of interest to note that, although the soils at both sites were found by ICP-MS to have similar total Se concentration (<200 $\mu g\ kg^{-1}$), there was a ten-fold difference in available Se as estimated by Se concentration in the grain of wheat grown at these sites: Charlick 60 $\mu g\ kg^{-1}$; Minnipa 625 $\mu g\ kg^{-1}$.

Glasshouse trials

Trial 1

In the first pot trial, a wide range of Se applications was tested, up to the high level of 132 mg Se kg^{-1} as selenate. Selenate's mobility and solubility was reflected in the high shoot Se concentrations, even at 1 mg kg^{-1}, the lowest level of applied Se (see Table 3). This trial showed that a Se level above 2 mg kg^{-1} applied as selenate (which produced around 200 mg Se kg^{-1} in shoots) inhibited growth. In contrast, another study using soil-applied selenate and wheat grown for 45 days found growth inhibition above the relatively low plant tissue Se concentration of 5 mg kg^{-1} (Tripathi & Misra, 1974). Soil and shoot S levels were not reported by Tripathi and Misra (1974), but if these were very low it could explain the high Se susceptibility of the plants in that study. Caution should be exercised, however, in comparing our results using an artificial growth medium with those from a soil substrate.

Table 3. Effect of applied soil selenium on plant yield and concentration of selenium and sulphur in shoots of wheat grown for 22 days in UC growth medium treated with sodium selenate. Mean (SE) of two replicates

Applied Se (mg kg^{-1} soil)	Shoot FW[a] (mg/plant)	Shoot Se conc. (mg kg^{-1} [b])	Shoot S conc. (mg kg^{-1} [b])	Se toxicity symptoms
0	730 (40)	0.1 (0.01)	5200 (100)	None
1	720 (10)	85 (8)	9300 (500)	None
2	700 (40)	209 (11)	10900 (100)	None
4	520 (40)	685 (105)	16150 (950)	Stunting, mainly of 2nd youngest leaf; bleached 2nd and 3rd youngest leaves in some plants; leaf tip necrosis and exudate.
8	280 (10)	1465 (45)	21250 (1750)	Stunting; wilting; chlorosis; tip necrosis and exudate; bleached 2nd and 3rd youngest leaves in most plants.
16	200 (10)	1440 (10)	9850 (350)	Stunting; wilting and tip necrosis and exudate (but less than the 8 mg/kg plants); chlorosis; complete bleaching of the 2nd and 3rd youngest leaves.
32	100 (0)	1745 (95)	6000 (400)	Severe stunting, wilting, chlorosis and bleaching.
64	40 (0)	1810 (80)	3650 (50)	Severe stunting, leaf tip exudation and chlorosis; some bleaching on lower part of leaves.
132	20 (0)	870 (80)	2900 (200)	Plants 2 cm tall; most dark green.

[a] Fresh weight
[b] Dry weight

Visual symptoms of Se toxicity in our trials, such as growth reduction and bleached leaves, were similar to those reported in other studies (Tripathi & Misra, 1974; Smith & Watkinson, 1984). Selenium phytotoxicity appears to involve several mechanisms. Sulphate and selenate compete for various enzymes in the S assimilation pathway, and incorporation of selenocysteine into protein instead of cysteine alters disulphide bridge formation, which reduces enzyme activity (Brown & Shrift, 1982). A similar effect was observed for selenomethionine as a substitute for methionine (Eustice et al., 1981). For example, the bleaching induced by excess Se (Smith & Watkinson, 1984) may be due to inhibition of porphobilinogen synthetase, an enzyme required for chlorophyll biosynthesis (Padmaja et al., 1989). Furthermore, both selenate and selenite inhibit the reduction of nitrate in leaves (Aslam et al., 1990), and selenate may inhibit glutathione biosynthesis (De Kok & Kuiper, 1986). More recently, it has been found that, while Se acts as an antioxidant and inhibits lipid peroxidation at low concentrations, it has the opposite effect at higher concentrations and can increase lipid peroxidation products (Hartikainen et al., 2000).

Trial 2

The second trial used lower levels of Se and again found growth inhibition due to Se above 2 mg kg^{-1} as selenate (230 mg Se kg^{-1} in plant tissue) (Table 4 and Figure 1). The

plants were bigger in this trial as it was conducted in conditions of higher temperature and light intensity than the first trial, and the plants were grown for eight days longer.

Table 4. Plant yield and concentration of selenium and sulphur in shoots of wheat grown for 30 days in UC mix growth medium treated with selenium as sodium selenate. Mean (SE) of two replicates

Applied Se (mg kg^{-1} soil)	Shoot dry wt (mg/plant)	Shoot Se conc. (mg kg^{-1})	Shoot S conc. (mg kg^{-1})
0	677 (7)	0.1 (0.02)	3500 (0)
0.01	748 (11)	0.34 (0.02)	3750 (50)
0.1	617 (3)	3.1 (0.02)	4150 (150)
0.5	730 (9)	29 (1)	7450 (250)
1	692 (42)	75 (4)	9200 (0)
2	674 (25)	230 (10)	11350 (150)
4	504 (3)	500 (30)	12600 (300)
8	221 (25)	875 (35)	14400 (100)
16	50 (8)	2250 (250)	17350 (1850)

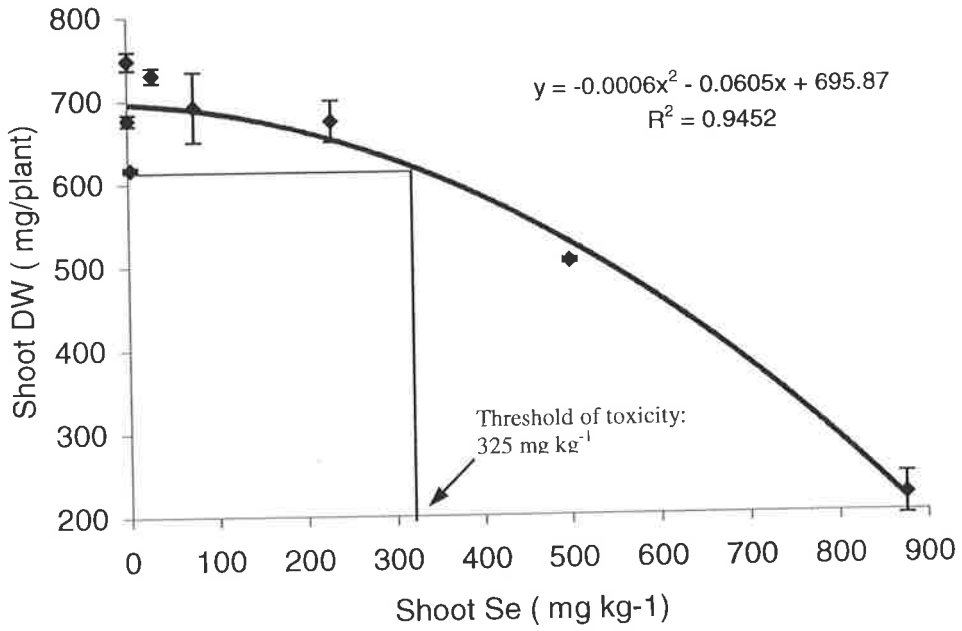

Figure 1. Effect of applied sodium selenate on selenium concentration and growth of shoots of wheat grown for 30 days. Mean (SE) of two replicates. The threshold of toxicity is defined as the concentration of the nutrient in the shoot above which a 10% reduction in growth can be expected (Andrew & Hegarty, 1969).

Root weights followed a similar trend to shoot weights. There were no visual symptoms of Se toxicity in plants up to and including the 2 mg Se kg^{-1} soil treatment, and the symptoms in the higher-Se plants were similar to the corresponding plants in Table 1. The critical nutrient concentration for toxicity has been defined as the concentration in the shoots above which a 10% reduction in growth can be expected (Andrew & Hegarty, 1969). The combined results of these two trials suggest that, for wheat grown on a sandy loam soil of pH (H_2O) 5.5 containing normal mineral nutrient levels in the presence of the most available Se form (selenate), the critical level is around 325 mg Se kg^{-1} DM, which is

attained with the application of around 2.6 mg Se kg^{-1} as selenate to the soil. This compares with critical Se toxicity levels for rice of 81 and 160 mg kg^{-1}, for flooded and upland rice, respectively (Mikkelson et al., 1989b). Moreover, in seleniferous regions such as parts of South Dakota, wheat is able to accumulate Se at concentrations of up to 63 mg kg^{-1} in grain without apparent detriment to the plants (Moxon et al., 1943). However, as our glasshouse trials were conducted using UC mix growth medium, further studies are recommended, using typical agricultural soil with varying concentrations of available S and C.

The youngest emerged leaves (YEL) were analysed separately for Se concentration and found to contain the same Se concentration as the remaining leaves for the control and lowest Se treatments (0.01 and 0.1 mg kg^{-1}), but 26% lower for the higher Se treatments. The oldest leaves (which were dead) in the plants at two of the high Se treatments (4 and 8 mg kg^{-1}) were also analysed separately and had 2.5-3 times the Se concentration of the other leaves (excluding the YEL). The oldest leaves, which had experienced greater total transpiration than the younger leaves, accumulated the highest concentrations of Se, up to 2200 mg kg^{-1} in the plants treated with 8 mg Se kg^{-1}.

The plants grown at the 0.01 mg Se kg^{-1} level were around 11% bigger (combined weight of roots and tops) than controls, but there were only two replicates, and a larger trial would be needed to investigate whether this was really a case of growth enhancement by Se. Moreover, a fungicidal effect of higher tissue Se concentrations on powdery mildew (*Erysiphe graminis*) was observed during this trial:

Shoot Se concentration (mg kg^{-1})	Extent of infection
Less than 80	Medium to heavy
200-500	Light
More than 800	None

Selenium sulphide is effective against tinea capitis in humans (Pomeranz & Sabnis, 2002), but little research has been done on Se as a fungicide in plants. However, a study using Indian mustard (*Brassica juncea* L.) found a similar Se fungicidal effect to our study against a leaf pathogen, *Alternaria brassicicola* Schweinitz (Hanson et al., 2003). A further study using Indian mustard found Se in phloem to be lethal to aphids at shoot Se levels as low as 2 mg kg^{-1} (Hanson et al., 2004). It is a question worthy of investigation whether Se might have some protective effects at lower, more common, levels of foliar Se.

In vitro trials

Selenate

Using three replicates of cultivar Janz at 28^0C (compared with 24^0C in the selenite trial) it was found that inhibition of early root and shoot growth commenced at 12 mg Se l^{-1}, but the magnitude of inhibition was not large (a finding similar to that of Lintschinger et al. (2000) in a five-day study), being just 27% for roots and 31% for shoots at the highest Se treatment of 152 mg l^{-1} (Figure 2). The threshold of toxicity for applied Se (the culture solution Se concentration that resulted in a 10% yield reduction from the control level) was 15 mg l^{-1}.

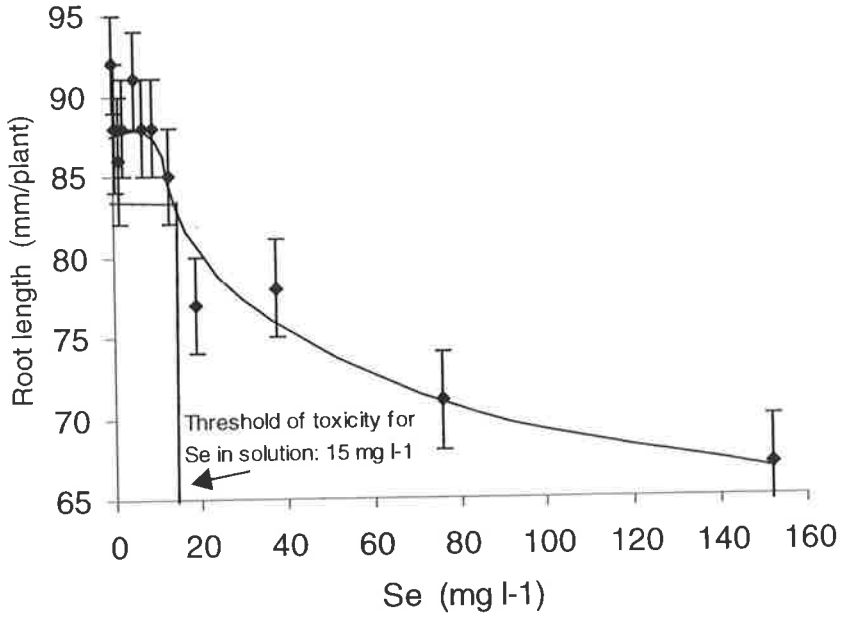

Figure 2. Inhibition of wheat root growth after 48 hours' contact with sodium selenate solution at 28^{0}C. Mean (SE) of three replicates. LSD (0.05) for root length: 10.2 mm.

Neither this trial nor that of Lintschinger et al. (2000) found growth inhibition at the low concentration of 2 mg Se l^{-1}, whether supplied as selenate (or selenite, as reported by Carlson et al (1989) in a 3-day trial). No inhibition of germination was observed in the current trial, even at the 152 mg l^{-1} treatment. This is a higher level than that which reduced germination in our unreplicated preliminary trials conducted at 24^{0}C, so the 4^{0}C higher temperature of the main trial may have increased seed/plant tolerance to Se toxicity. If this were the case, these solution trials may not safely define threshold levels of applied Se in the field where germination temperatures may be less than 10^{0}C. However, another study conducted at high temperatures (28^{0}C day/21^{0}C night, 16/8 hour day/night) found a lower tolerance of wheat for Se than found by our group at 24^{0}C (Carlson et al., 1989). The effect of temperature on Se phytotoxicity warrants further investigation.

Selenite

Using three replicates of cultivar Janz, it was found that inhibition of early root growth commenced at 10 mg Se l^{-1}, and the magnitude of inhibition at all treatment levels was greater than that for selenate. For example, growth was reduced by 50% at 38 mg l^{-1} (Figure 3), compared to a reduction of 15% for selenate at this level.

Shoot growth was less inhibited by selenite than was root growth, with no inhibition observed until a solution concentration of 24 mg Se l^{-1} was reached, compared with a solution threshold of toxicity for root growth of 11 mg l^{-1}. Inhibition of germination commenced at 76 mg l^{-1}, but was not great, even at the 167 mg l^{-1} treatment (10% reduction). The germination findings for both Se forms agree with other studies (Spencer & Siegel, 1978; Carlson et al, 1989; Lintschinger et al., 2000).

Most studies have shown that selenite, when provided in solution culture, is more toxic than selenate in higher plants (Smith & Watkinson, 1984; Carlson et al., 1989; Läuchli, 1993; Terry et al., 2000), although the reverse is the case for tall fescue (Wu et al., 1988). With selenite a higher proportion is assimilated in the roots (Gissel-Nielsen, 1987), and

selenite undergoes faster conversion to selenoamino acids, which may partly explain its higher toxicity, despite a lower uptake rate (Smith & Watkinson, 1984).

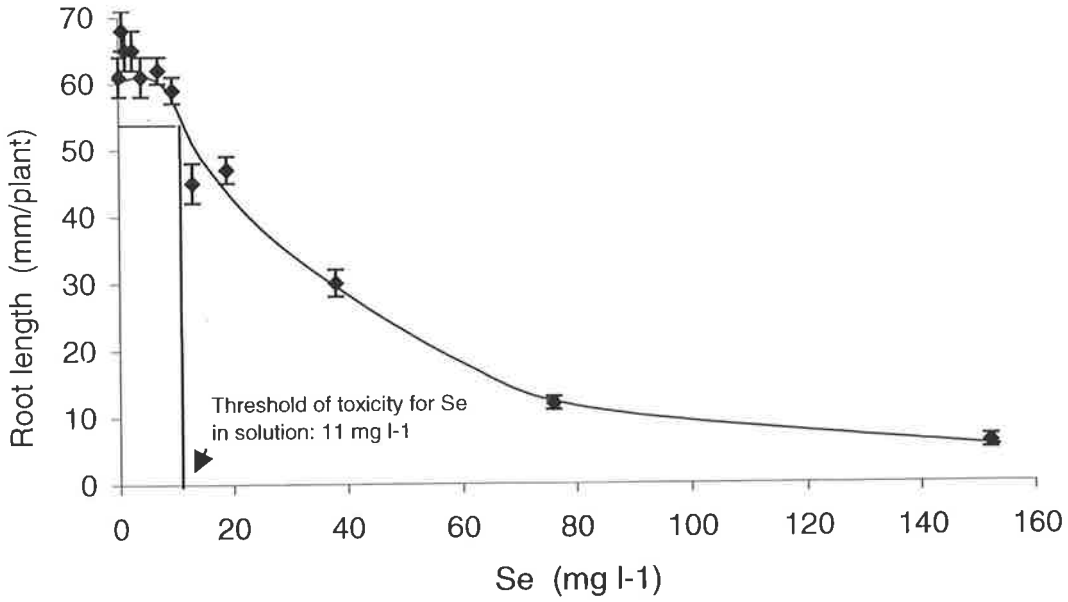

Figure 3. Inhibition of wheat root growth after 48 hours' contact with sodium selenite solution at 24^{0}C. Mean (SE) of three replicates. LSD (0.05) for root length: 8.6 mm.

Increase in leaf S due to selenate

Applied selenate was observed in the glasshouse trials of Se phytotoxicity to increase the concentration and content of S in the leaves of wheat (Tables 3 and 4). For example S concentration and content increased three-fold with the addition of 1 mg Se kg^{-1} as selenate, in our unpublished study using two vetch species, (*Astragalus hamosus* and *A. sinicus* L.), two radish varieties (*Raphanus sativum* L.) and *Arabidopsis thaliana* L. Heynh. This effect was also seen in both field trials, where Se applied as selenate to the soil at seeding at a rate of 120 g ha^{-1} increased foliar S by 100% above the mean S concentration for all the lower Se treatments and the controls at Minnipa, and by 20% at Charlick. By contrast there was no effect on grain S concentration (Lyons et al., 2004). The difference in magnitude of the S concentration increase between the two sites may be due to the lower soil S at Minnipa (2.4 mg kg^{-1}) compared to Charlick (4.4 mg kg^{-1}), as the S-increasing effect of selenate is reduced or absent at higher S levels (Mikkelsen & Wan, 1990). This effect of selenate in increasing foliar S concentration and content has been reported in perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) (Smith & Watkinson, 1984), barley (*Hordeum vulgare* L.) and rice (*Oryza sativa* L.) at low concentrations of sulphate in solution culture (Mikkelsen & Wan, 1990), and in *Arabidopsis* in a short-term study (Yoshimoto et al., 2002). *Arabidopsis* plants grown in the presence of selenate showed patterns of mRNA accumulation for the main sulphate transporter gene similar to those in plants deprived of S. The presence of increased concentrations of selenate in the roots appears to trigger the S deficiency response of the main S transporter (Yoshimoto et al., 2002). However, it also appears that selenate at very high concentrations in the soil (greater than 40 mg Se kg^{-1}) may inhibit the main sulphate transporter (see Table 3).

Conclusions

Trials conducted in the field, glasshouse and laboratory revealed that wheat is more tolerant to high levels of Se in growth media than has been found in studies for other species, including rice (Mikkelsen et al., 1989b), tobacco and soybeans (Martin & Trelease, 1938). No yield reduction was observed in the field at Se applications up to 500 g ha^{-1}. Pot trials suggested that, for wheat grown on a sandy loam substrate of pH (H_2O) 5.5 with normal mineral nutrient levels, a critical shoot level for Se toxicity of around 325 mg kg^{-1} results from the addition of 2.6 mg Se kg^{-1} to soil as sodium selenate, the most soluble Se form. However, growth inhibition of less than 10% may commence at shoot concentrations of 200 mg Se kg^{-1} (obtained by applying 2 mg Se kg^{-1} as selenate). It is important to note that the pot trials were not conducted using soil, and further studies using soil with variable concentrations of available S and C should be conducted to provide definitive results. As shown in the field trials, different grain Se concentrations will result from the same Se applications on different soils.

Leaf S concentration and content of wheat increased three-fold with the addition to growth media of 1 mg Se kg^{-1} as selenate. The effect was probably due to induction of the S deficiency response of the major sulphate transporter.

In laboratory trials which examined germination and early growth in wheat, selenite was more toxic than selenate, and inhibited root growth more than shoot growth. Inhibition of root growth by both Se forms generally commenced at a Se solution concentration of around 10 mg l^{-1}, while inhibition of germination commenced at concentrations around four times higher for selenite and eleven times higher for selenate. In one trial, it appeared that higher temperature may have increased tolerance to selenate, which warrants further investigation.

The combined results of these studies indicate that Se phytotoxicity in wheat will not occur at the range of application rates of sodium selenate that would be used to biofortify wheat in Australia.

Acknowledgements

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4. General Discussion

Selenium: essential for human health

Although not proven to be essential for higher plants, selenium (Se) is an essential micronutrient for human and animal health, with antioxidant, anti-viral and anti-cancer effects (Rayman, 2000, 2002). Cancer prevention appears to require a supra-nutritional Se intake, but in some regions Se is declining in the food chain (Combs, 2001; Frost, 1987), and new strategies are needed to increase its intake. It is estimated that at least a billion people are Se deficient (Combs, 2001), and its deficiency in much of Sub-Saharan Africa has been postulated as a determinant of the rapid spread of HIV/AIDS in this region (Foster, 2003).

Clarifying the Se status of South Australians

Few surveys of Se levels in Australians have been conducted and most have been small. Given the geographical variability in Se intake and blood levels and their sensitivity to changes in the food supply, it is necessary to use local data to accurately monitor Se status. In order to determine the Se status of healthy South Australian residents, a survey of nearly 300 Adelaide blood donors was conducted. The study yielded a mean plasma Se concentration of 103 μg/l, which is above that of most countries, and just above the estimate of 100 μg/l required for maximising the activity of the important antioxidant selenoenzyme, glutathione peroxidase (Rayman, 2000). However, only 8% of men surveyed reached 120 μg/l Se, a plausible target for maximum protection against prostate cancer (Brooks et al, 2001; Duffield-Lillico et al, 2002; Rayman & Clark, 2000), which is the most commonly diagnosed solid tumour and the second leading cause of cancer mortality in Australian men (Meuillet et al, 2004). These findings indicate that many Australian men (given that most previous Australian post-1990 studies found lower Se levels than this study) would be in the responsive range for lower risk of prostate cancer by increasing their Se intake. We conclude that men living in Australia with a plasma Se concentration below 100 μg/l would be likely to benefit from Se supplementation.

The inclusion of data from earlier South Australian surveys conducted by the South Australia Research and Development Institute (to give a total sample size of 834

individuals) resulted in the largest human Se survey yet conducted in Australia, and it showed a 20% decline in the Se status of South Australians sampled from 1977 to 1988. This may be due to changes in dietary composition and/or a decline in the mean Se concentration of South Australian wheat (*Triticum aestivum* L.), given the importance of wheat as a source of dietary Se (Barrett et al, 1989; Watkinson, 1981), and this apparent decline will be discussed below in the context of surveys of Se concentration in wheat grain.

Less-healthy sub-groups of the South Australian population than the groups surveyed in this study, such as heavy smokers (Kafai & Ganji, 2003; Luty-Frackiewicz et al, 2002)), people with low socioeconomic status and the frail elderly (Bates et al, 2002), could be expected to have substantially lower levels of blood Se. Pregnant and lactating women (Cumming et al, 1992; Golubkina & Alfthan, 1999) and infants (Daniels et al, 2000) are also at risk of low Se status.

Strategies to increase selenium intake

Background

Given that the Australian population (and *a fortiori* the populations of the many countries with a lower Se intake) would be likely to benefit from an increased Se intake, how could this be best achieved? Options include increased consumption of higher-Se foods (such as Brazil nuts, cereals, meat and fish) through education; addition of Se to water supplies (as is done with fluoride in Australia); individual supplementation; food (process) fortification; and biofortification, including supplementation of livestock, use of selenium fertilisers, and plant breeding for enhanced selenium accumulation. Each option shall be discussed briefly in the following two sub-sections.

Dietary composition, drinking water, supplementation, food fortification

Globally, wheat is one of the most important dietary sources of Se. The sensitivity of a population's Se status to changes in its source of wheat is illustrated by the United Kingdom, where blood Se levels have declined by around 50% since changing from high-Se Canadian and USA-grown wheat to low-Se European wheat in the mid-1980s (Rayman, 2000). In Australia, it is estimated that nearly half of our Se intake is provided by wheat (Barrett et al, 1989). Brazil nuts provide the most concentrated natural food source of Se, but their Se level is highly variable (Chang et al, 1995). There is currently

no food available to Australians that contains a reliable moderate-to-high (i.e. around 1 mg/kg) Se concentration. Inorganic Se could be added to a population's water supply, but a proportion would be adsorbed on organic and clay particles and become unavailable, and furthermore, organic Se (usually selenomethionine) is preferable to selenate or selenite as a dietary Se form for humans (Reilly, 1996). Se supplements in both inorganic and organic forms are available in Western countries. However, the health-conscious individuals who take them tend to be those least in need of supplements, and studies suggest that dietary sources of Se, vitamin E and beta-carotene are more bioavailable and more effective than supplements (Moyad, 2002). Food or process fortification has been used successfully with folate-enriched breakfast cereals, Fe-enriched milk, iodised salt, carotene- and vitamin E-enriched margarine, as well as selenised salt in Se-deficient regions of China. Continual inputs and rigorous quality control are required, and fortification programs have frequently failed in developing countries due to lack of infrastructure and compliance (Graham & Welch, 1996).

Biofortification

Biofortification with Se may involve supplementation of livestock, fertilisation of food crops or breeding food crop varieties with enhanced Se uptake efficiency to achieve higher Se density in edible parts. Supplementation of livestock with Se is unlikely to be an efficient strategy to increase Se level in the human population. In New Zealand, little increase in the Se content of human foods was observed after the introduction of Se supplementation for farm animals in the 1960s (Thomson & Robinson, 1980). However, addition of selenate to NPK fertilisers for use on crops and pastures in Finland since 1984 has been an effective method to increase the entire population's Se status (Aro et al, 1995). Another option is plant breeding, which represents a self-sustaining Se biofortification strategy. Substantial variability exists within cereal crop varieties for Zn, Fe and other nutrients (Graham et al, 2001), and this may also be the case for Se, but little research has been done. It is considered that the strategies of plant breeding for enhanced Se uptake efficiency (or *genetic* biofortification) and Se fertilisation (or *agronomic* biofortification) are the most desirable methods to increase Se status as they represent a food systems approach that could deliver increased Se to a whole population safely, effectively, efficiently and in the most suitable chemical forms. A food systems paradigm encompasses an agriculture that aims not only at productivity and sustainability, but also at improved nutrition (Welch & Graham, 1999). The remainder of this discussion will

focus on our investigations of these two strategies as they relate to wheat, the most important Se source for the Australian population.

Genetic biofortification of wheat with Se

Wheat-Se concentration is highly variable

To determine whether a breeding approach was feasible to increase the Se concentration of wheat, surveys and field trials were conducted in Australia and Mexico. Diverse genotypes, including ancestral and wild relatives of wheat, landraces, modern commercial bread and durum wheats, doubled haploid populations, synthetic wheats, triticale, rye and barley were analysed. Grain Se concentration varied 144-fold, from 5 μg/kg (grown on a high-Fe lateritic soil on Kangaroo Island, South Australia) to 720 μg/kg (grown on a high pH, low Fe, low S, low organic matter soil at Minnipa, Eyre Peninsula, South Australia). Zn, on the other hand, varied three-fold in the same sample. The grand mean for Se was 155 μg/kg (SE 15), and the median was 100 μg/kg. Most wheat grown in South Australia from 2000-2002 was in the range 70-280 μg Se/kg. While a more extensive, systemmatic survey of the state's entire wheatbelt is needed, this study provisionally places South Australian wheat higher than New Zealand, the UK, most of Europe and China, but lower than Canada and the USA for grain Se concentration.

Grain Se concentrations varied across location and appeared lower than those presented for the 1981 season in South Australia (Babidge, 1990), the most recent published Australian survey before the current study. The Babidge study found large differences between regions and an overall median Se concentration of 170 μg/kg (range 47-316). A possible decline in soil Se levels in the SA wheatbelt in the 1980s may have been due to more intensive cropping (Gissel-Nielsen, 1998), increased use of gypsum (which contains around 20% sulphur [S], a Se uptake competitor) for canola growing and to treat sodic soils, or a combination of factors. This is a plausible explanation for the decline in human Se status in South Australia during the 1980s observed in our human surveys.

Genotypic variation of Se density in modern wheat cultivars is difficult to detect

The genotype x environment studies showed that most of the variation in grain Se concentration was due to available soil Se. Total soil Se was found to be an unsuitable indicator of availability. Although it is commonly stated in the literature that a total soil Se concentration of up to 0.6 mg/kg is considered to be deficient (Gupta & Gupta, 2000),

this may need to be revised as our study found at least a 100-fold variation in available soil Se (as determined by Se concentration in wheat grain, e.g. Kangaroo Island 5 μg/kg, Minnipa 720 μg/kg) in soils testing < 0.2 mg/kg of total Se. The Minnipa site grew the highest-Se wheat in the survey, and could not be considered Se-deficient. South Australian soils are renowned for their micro-spatial variability in available micronutrient concentration (RD Graham, personal communication), and this study shows this to be particularly so for Se. Spatial variation in available soil Se, even within an individual site (for example, at Bordertown, a six-fold variation in grain Se concentration was found between samples from four replicate plots of a single wheat cultivar grown together in one field) was found to be large, making detection of genotypic differences in Se uptake efficiency difficult. In the trials conducted in Mexico, the soil Se variation, while not as large as in South Australia, was substantial.

No significant genotypic variation in grain Se density among modern commercial bread or durum (*T. turgidum* L. var durum) wheat, triticale (*X Triticosecale* Wittmack) or barley (*Hordeum vulgare* L.) varieties was detected in this study. However, the diploid wheat, *Aegilops tauschii* L. and rye (*Secale cereale* L.) were significantly higher in grain Se concentration than other cereals in separate field trials, and in a short-term hydroponic trial rye was significantly higher in foliar Se content than two wheat landraces. While genotypic differences may exist in modern wheat varieties, they are likely to be small in comparison with background soil variation, at least in South Australia and Mexico. Field sites that are spatially very uniform in available soil Se would be needed to allow comparison of grain Se concentration and content in order to assess genotypic variation. Moreover, enhanced uptake efficiency for selenate (the most soluble, mobile Se form), as might be identified in hydroponic studies, may be of limited value in wheat grown on soils of very low available Se, where most Se is present as selenite, selenide and elemental Se (Cary & Allaway, 1969).

Agronomic biofortification of wheat with Se

Soil-applied selenate: a little goes a long way

Fertilisation of wheat with Se, or agronomic biofortification, was investigated for the first time in Australia, as an alternative to breeding to raise grain Se concentration. Se applied as selenate to the soil at seeding was more effective than post-anthesis foliar application on soils of variable pH (6.5-8.5 pH H_2O), Fe (7-66 DTPA Fe), S (2.4-4.4 mg/kg) and

organic carbon (0.8-1.3%) content. This finding, together with the absence of crop damage from pre-sowing application to soil, and the knowledge that Australian cereal crops are usually subjected to late-season moisture and heat stress, which result in rapid reduction of viable leaf area, suggests that soil application would be the preferred method of Se biofortification for a majority of soil types found in the Australian wheatbelt. However, further trials, where foliar Se is applied earlier, for example from late stem elongation (Zadok stage 38) to mid ear emergence (Zadok stage 55) are needed.

The highest conversion efficiency in these trials was obtained with the highest Se application (120 g/ha), so the most efficient way to biofortify may involve treating a relatively small area with a high Se application, then blending this grain with average-Se grain to achieve the desired Se concentration. Moreover, the method is inexpensive, with the selenate likely to cost less than $20/ha to achieve a high grain Se concentration of 10 mg/kg Se on a wide range of soil types (assuming a selenate cost of $50/kg, and grain yields below 2.7 t/ha).

Tolerance of wheat to high soil and solution Se levels

Studies of Se phytotoxicity in wheat grown in Australia, where rainfall and grain yield are usually lower than in Europe, the UK, Canada and the USA, have not been reported previously, and overseas studies have presented varied results. This study used trials conducted in the field, glasshouse and laboratory to assess Se phytotoxicity in wheat. It revealed that wheat is more tolerant to high levels of Se in growth media than has been found in other studies for various other species, including rice (Mikkelsen et al, 1989), tobacco and soybeans (Martin & Trelease, 1938).

In field trials that used rates of up to 120 g/ha Se as sodium selenate, no yield reduction or other adverse effects were observed. Moreover, in unreplicated pilot trials using selenate at up to 500 g/ha Se soil-applied or 330 g/ha Se foliar-applied, no Se toxicity symptoms were seen. These findings are similar to those of a study conducted in Finland using barley grown at higher yields (5 t/ha) (Ylaranta, 1983). The four-week soil pot trials conducted in a glasshouse revealed a critical tissue level for Se toxicity of around 325 mg/kg, a level attained by the addition of 2.6 mg/kg of soil Se as selenate. Petri dish studies of germination and early root growth, where seeds were bathed in Se solution, found no inhibition of germination at up to 150 mg/*l* Se as selenate, or up to 23-39 mg/kg Se as selenite. Inhibition of early root growth commenced at 6-13 mg/kg for both Se

forms, but inhibition was greater from selenite. The germination findings are in agreement with other studies (Carlson et al, 1989; Lintschinger et al, 2000; Spencer & Siegel, 1978), but we found early wheat growth to be more tolerant to high solution Se levels than did Carlson et al (1989) and Tripathi & Misra (1974). In one trial, it appeared that higher temperature may have increased tolerance to selenate, which warrants further investigation.

The significance of the combined results of these trials is to indicate that wheat is quite tolerant to high levels of available Se in soil or solution, and toxicity would not occur at the range of application rates of selenate that would be used to biofortify wheat (10-200 g/ha Se, that would result in soil and tissue Se levels well below those seen in the above studies), even when – as is common in Australia – soil S concentration (which affects Se uptake and toxicity) and grain yield are low.

Selenate increases leaf S concentration

In the Se-wheat fertilisation field trials and the Se phytotoxicity trials it was observed that selenate applied to soil or solution culture increased the concentration of S in the leaves of all species tested: wheat (where S concentration and content increased nearly three-fold with the addition of 1 mg/kg Se as selenate to soil), the legumes *Astragalus hamosus* L. and *A. sinicus* L., radish (*Raphanus sativum* L.) (all grown on soil) and *Arabidopsis thaliana* L. Heynh (grown hydroponically). This effect has been reported in perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) (Smith & Watkinson, 1984), barley and rice (*Oryza sativa* L.), but only at low concentrations of sulphate in solution culture (Mikkelsen & Wan, 1990), and in *Arabidopsis* in a short-term study (Yoshimoto et al, 2002). Plants grown in the presence of selenate showed patterns of mRNA accumulation of the main sulphate transporter gene similar to those in plants deprived of S. The presence of increased concentrations of selenate in the roots appears to trigger the S deficiency response of the main S transporter (Yoshimoto et al, 2002). However, one of our trials showed that selenate at very high concentrations in the soil (greater than 40 mg/kg Se) may inhibit the main sulphate transporter.

S decreases grain Se concentration

The field trial that investigated the effect of different combinations of applied N and S on grain Se concentration found that a relatively low application of 30 kg/ha S decreased grain Se concentration by 16%. This confirms the findings of other studies, although only

one reported a significant Se reduction from a comparable S application in the field (Adams et al, 2002). The effect is due to competitive inhibition by S of Se uptake via the main S transporter (Lauchli, 1993). In future it may be necessary to add Se to gypsum (calcium sulphate, which is applied at rates of up to 10 t/ha to treat sodic soils) and high-S fertilisers like single superphosphate, ammonium sulphate and potassium sulphate, which are used widely in Australia. Moreover, in Australian coastal areas (which comprise a sizeable portion of the low-Se areas identified in five States (Judson & Reuter, 1999)), S deposition from rainfall can be as high as 20 kg/ha/year (Blair et al, 1997), which could be limiting Se uptake by crops grown in these areas.

Grain protein and Se were not associated in this study

No association between late-applied foliar N and grain Se concentration was observed in the field trial at Strathalbyn, a finding supported by an earlier field trial study (Soltanpour et al, 1982). However, a Serbian study found that foliar urea, when applied together with sodium selenite, increased grain Se concentration in wheat (Djujic et al, 1998). The effect was large (up to 110% increase over foliar Se alone when applied at the stem elongation stage) and further trials, using foliar urea and both selenite and selenate, are warranted.

Grain Se concentration and protein were not associated in any of our trials, which included a wide range of both native soil Se and applied Se. Other Se fertilisation studies with cereals have not reported grain protein concentrations and we conclude that agronomic Se biofortification is unlikely to influence grain protein, an important consideration for milling and nutrition. However, late-applied foliar urea was effective at increasing grain protein - a well-known finding -, and also increased grain S concentration, which has also been found previously (Martin, 1997; Maslova, 1998).

Effects of post-harvest processing on wheat-Se

Most wheat-Se gets to the consumer

It is important that post-harvest processing losses of grain Se are minimised, but previous studies are few and findings variable. This was the first reported study to assess Se concentrations at each processing step from harvest to toasting. Moreover, no published studies have examined Se concentration in the embryo of wheat grain. The dissection study, while showing Se concentration to be highest in the embryo, confirmed (along with the milling study) previous findings that Se and S, being protein-bound, are more evenly

distributed throughout the grain when compared to other mineral nutrients, and hence lower proportions are removed in the milling residue. Post-milling processing (in our study baking or toasting) did not affect Se concentration or content.

No genotypic variability in the five cultivars assessed was observed for grain distribution of Se in the dissection and milling studies, in contrast to Cu, Fe, Mn and Zn. The bread wheat cultivar Krichauff, for example, contained three times as much Cu in the embryo (as a proportion of whole grain Cu) than did cultivar Kukri. Although possibly not applicable to Se, this variability could be exploited in breeding for higher proportions of Cu, Fe, Mn and Zn in the endosperm to make white flour more nutritious. Further research could include grain dissection and milling studies of larger numbers of cultivars that have been grown together.

Se biofortified wheat: commercialisation and further research

Although we do not think it is necessary at this stage to adopt Finland's approach to legislate for addition of Se to all NPK fertilisers used in Australia, it would be desirable to have a range of healthy foods available to consumers which contain a guaranteed substantial level of Se. Novel wheat (or other cereal) products that contain enhanced levels of organic Se due to agronomic biofortification could be considered as *functional foods*, which are likely to experience strong consumer demand as evidence builds for Se's crucial role in human health (Combs, 2001; Rayman, 2002).

Commercial application of this research could be undertaken by food companies that would supply selenate to contracted growers to apply at a specified time and rate. Currently (April 2004) a South Australian milling company intends to produce 500 tonnes of high-Se wheat in 2004, and launch a new product in 2005. A Se concentration of around 1 mg/kg would be desirable in a "high-Se" breakfast cereal, flour, pasta or bread, as it is within food regulatory guidelines and, based on average consumption, would significantly increase a consumer's daily Se intake. Furthermore, growers who produce high-Se wheat could receive substantial premiums. For example, it is reported that growers in South Dakota have been paid up to US $1,000 per tonne by European buyers for high-Se durum wheat (RM Welch, personal communication).

Further research that is needed, in addition to that mentioned in the discussion above, includes conducting animal and human trials to compare the bioefficacy of different Se forms (for example, high-Se wheat, sodium selenite, selenomethionine) and to investigate interactions with other nutrients. Se has been shown to affect tissue distribution and have a "normalising" effect on several other mineral nutrients in blood and various organs (Djujic et al, 2000; Farrag, 1999; Giray et al, 2003), but more studies are needed, using animals or human participants deficient or marginal for Fe, Zn, vitamin A (the nutrients targeted by *HarvestPlus*, a Global Challenge Program for biofortification of staple crops in developing countries (CGIAR, 2003)) and iodine. It is important to elucidate interactions between these nutrients in order to ensure efficient delivery in nutrition programs.

Short-term (3-6 months) clinical intervention trials using sensitive biomarkers of genome instability such as the micronucleus and Comet assays may be an efficient way to assess both the optimum intake of Se and its bio-efficacy in terms of antioxidant capacity and protection against cancer, cardiovascular disease (Ames, 1999; Fenech, 2003) and retroviruses. A clinical trial is planned to investigate the bio-efficacy of Se from high-Se wheat compared with low-Se wheat or supplemental selenomethionine in participants with low baseline Se status, using biomarkers of genome instability, immunocompetence, lipid peroxidation and inflammation.

Conclusion

Increasing the Se content of wheat represents a food systems approach that would increase population intake, with consequent likely improvement in public health. While a breeding approach to increase grain Se density in modern bread and durum wheat cultivars may not be feasible, agronomic biofortification using selenate fertiliser is effective, relatively easy to implement and inexpensive. Mounting evidence for Se's important roles in human health, along with widespread sub-optimal Se status, a decline in Se intake in some areas, and a current lack of market competition should ensure the popularity of high-Se cereal products in Western countries. A greater challenge lies in improving the Se status (along with that of other nutrients) of whole populations in developing countries with very low-Se soils, such as Zaire and Burundi. A substantial improvement in human Se status in these countries has the potential to reduce the incidence and prevalence rates of HIV/AIDS and other infectious diseases.

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