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REVIEW

Assessment of requirements for selenium and adequacy of selenium status: a review

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Objective: The intent of this review is to evaluate the scientific evidence for the assessment of adequacy of selenium status and of the requirements for selenium. From this evidence, attempts have been made to define levels of plasma selenium and dietary selenium intake, which could be used for the assessment of deficiency or adequacy of selenium status.
Method: The first section briefly reviews the methods for assessment of selenium status. The second section outlines the requirements for selenium based on a number of criteria, and how these have been translated into recommended intakes of selenium. In the final section, levels of plasma selenium and dietary intake based on different criteria of adequacy have been proposed.
Results and conclusion: The minimum requirement for selenium is that which prevents the deficiency disease, Keshan disease. The recommended intakes of selenium have been calculated from the requirement for optimum plasma glutathione peroxidase (GPx) activity that must, because of the hierarchy of selenoproteins, also take account of the amounts needed for normal levels of other biologically necessary selenium compounds. Whether optimal health depends upon maximization of GPx or other selenoproteins, however, has yet to be resolved, and the consequences of less-than-maximal GPx activities or mRNA levels need investigation. Intakes, higher than recommended intakes, and plasma selenium concentrations that might be protective for cancer or result in other additional health benefits have been proposed. There is an urgent need for more large-scale trials to assess any such beneficial effects and to provide further data on which to base more reliable estimates for intakes and plasma selenium levels that are protective.
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Introduction

Selenium exerts its biological effect through several selenoproteins of which there may be more than 30 in mammalian systems. These selenoproteins include a number of glutathione peroxidases (GPx) including cellular GPx (GPx1) and phospholipid hydroperoxide GPx (PHGPx; GPx4), iodothyronine 5′-deiodinases (IDI), sperm capsule selenoprotein and thioredoxin reductase (Burk & Levander, 1999; Holben & Smith, 1999). Thus, selenium functions in the body as an antioxidant, in thyroid hormone metabolism, redox reactions, reproduction and immune function (Rayman, 2000). Selenium deficiency in humans is rare, but is seen as Keshan disease, an endemic cardiomyopathy that occurs during preadolescent or adolescent years (Keshan Disease Research Group, 1979a, b) and as an endemic osteoarthritis, Kashin–Beck disease (Levander, 1987), both of which occur in low selenium areas of China. Low selenium status has also been associated with a number of chronic diseases such as cancer (Ip, 1998; Combs, 1999), cardiovascular disease (Nève, 1996; Huttunen, 1997), asthma and many others (Rayman, 2000; Brown & Arthur, 2001). Of these, evidence for a protective effect of selenium is strongest against some cancers.

Assessment of selenium requirements has been based on intakes that maximize activities of the selenoenzyme GPx, the criterion used in the recent update of the US and Canadian Dietary Reference Intakes (DRIs) (Standing Committee on the Evaluation of Dietary Reference Intakes, 2000). There is still much debate, however, whether it is necessary

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This review is dedicated to the memory of Professor Marion Robinson, my teacher and postgraduate supervisor, who later became my mentor, colleague and friend. Marion, a pioneer in research on selenium nutrition, died in Dunedin, New Zealand, on 25 February 2003.
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for maximal levels of the enzyme for optimal function, and whether increased intakes will provide protection against chronic disease. This review focuses on available evidence for the assessment of adequacy of selenium status and of requirements for selenium.

Biochemical tests for nutritional status of selenium

It is useful, first, to outline the measurements used to assess the nutritional status of selenium and their limitations, which have been reviewed in detail by Nève (1991). Blood selenium concentration is generally considered a useful measure of both selenium status and intake, but other tissues such as hair and nails are also used (Nève, 1991; Burk & Levander, 1999; Sheehan & Halls, 1999). Plasma or serum selenium reflects short-term status, and erythrocyte selenium reflects longer-term status, due to the incorporation of selenium during synthesis of these cells. There are, however, no accepted 'normal' reference ranges because of variations in selenium status from country to country. Toenails are often used as a measure of long-term selenium status (Longnecker *et al*, 1996; Mannisto *et al*, 2000). Hair selenium has been related to long-term selenium intake (Yang *et al*, 1989b), but selenium-containing shampoos limit the suitability of hair samples. Daily urinary excretion is closely associated with plasma selenium and dietary intake in low selenium populations (Griffiths & Thomson, 1974), and, therefore, can be used to assess selenium status reflecting recent dietary intake. Balance studies show that over a wide range of intakes, urinary excretion accounts for 50–60% of the total amount excreted (Robinson *et al*, 1973), and therefore, total dietary intake can be estimated as twice the daily urinary excretion (Thomson, 1998).

Tissue concentrations may be misleading as measures of selenium status, as they do not accurately reflect the functional activity, which can vary with the form of selenium ingested (Thomson *et al*, 1993; Nève, 1995). If selenomethionine is a major dietary form, then the tissue content may be high because it is nonspecifically incorporated into proteins as selenomethionine in the place of methionine (Behne *et al*, 1991). In blood components, selenium from organic forms but not inorganic forms, is incorporated nonspecifically into haemoglobin in erythrocytes and into albumin in plasma (Butler *et al*, 1991; Burk *et al*, 2001). A 'true' measure of selenium status should reflect the amount of selenium that is available for activity of the functional selenoproteins. Measurement of individual selenoproteins, therefore, provides more accurate and useful information than does total selenium alone (Patching & Gardiner, 1999). Even then, determination of the concentration of only one selenoprotein may be insufficient and misleading because of the hierarchy of the importance of selenoproteins as outlined below.

The close relationships between plasma GPx (GPx3) and red cell GPx (GPx1) activities with total selenium concentrations is useful for assessment in people with relatively low status, but not once the maximal activity of the enzyme is reached at blood selenium levels above 1.27 μmol/l (100 μg/l) (Rea *et al*, 1979; Nève, 1991). Furthermore, it is often difficult to compare results from different laboratories because of variations in methodology. GPx is also used to assess the effect of supplementation with different forms of selenium, but, again, responds only in subjects with low selenium status. Moreover, the extent of the response depends on the initial level of activity (Brown *et al*, 2000). On the other hand, platelet GPx appears to be a more sensitive indicator of increasing selenium intake, showing increases in activity within 1–2 weeks of commencing supplementation (Levander *et al*, 1983; Thomson *et al*, 1985; Nève *et al*, 1988; Alfthan *et al*, 1991; Van der Torre *et al*, 1991; Thomson *et al*, 1993). This might be related to the shorter lifespan of 8–14 days of platelets, compared to 120 days for erythrocytes (Nève, 1995). As distinct from plasma and erythrocyte GPx, there appears to be a difference in the bioavailability of different forms of selenium for platelet GPx. Inorganic selenate or selenite produced a more rapid response than high-selenium yeast and plateaued at higher levels of activity (Levander *et al*, 1983; Alfthan *et al*, 1991; Thomson *et al*, 1993). Thus, modifications in platelet GPx activity are dependent on the chemical form of the selenium supplement, and therefore platelet GPx activity appears to be more sensitive to chemical forms of selenium administered than either erythrocyte or plasma GPx.

More recently, measurement of selenoprotein P has been shown to be a valuable biochemical marker for selenium status (Marchaluk *et al*, 1995; Persson-Moschos *et al*, 1995; Hill *et al*, 1996; Hill & Burk, 1997), and there is a potential for measurement of other enzymes as functional markers. Brown *et al* (2000) have used PHGPx (GPx4) as well as GPx1 to evaluate the effect of selenium supplementation on blood cells. Other workers have suggested that the ratio of thyroxine (T_4) to tri-iodothyronine (T_3) in plasma may give an indication of IDI activities in inaccessible tissues (Olivieri *et al*, 1995; Arthur, 1999). It is necessary to recognize that the conclusions drawn from the measurement of one selenoprotein may not uniformly apply to all related biological functions of selenium because of differences in responses of tissues and selenoproteins to various levels of selenium. Selenium deficiency leads to reduced levels of selenoproteins, but there is preferential incorporation of selenium into some selenoproteins (Behne *et al*, 1989; Patching & Gardiner, 1999). It has not yet been established, however, how measurements of one selenoprotein relate to other biochemical functions (Arthur, 1999).We may conclude that there is unlikely be any single indicator of functional selenium status, but rather a series of markers that apply to specific problems associated with suboptimal selenium status (Thomson, 1998; Arthur, 1999). At present, plasma or serum selenium is still the favoured measure of selenium status for comparison among countries.

Selenium requirements and recommended dietary intakes

Minimum requirement for prevention of Keshan disease

The discovery of Keshan disease, the selenium-responsive cardiomyopathy endemic in certain areas of China (Keshan Disease Research Group, 1979a, b), made it possible to compare dietary intakes in geographical areas in which deficiency occurs with those areas without deficiency. Selenium intakes, estimated from analysis of foods and according to quantities recorded, were 7.7 and 6.6 μg/day in endemic and 19.1 and 13.3 μg/day in nonendemic areas for adult male and female subjects, respectively (Yang *et al*, 1987). Additional results from China indicate that Keshan disease in children does not occur in areas where selenium intakes of adults are 20 μg/day or more (Yang & Xia, 1995; Standing Committee on the Evaluation of Dietary Reference Intakes, 2000). This intake has become generally regarded as the minimum needed for good health (Levander & Whanger, 1996) (Table 2). The World Health Organization (WHO) (WHO/FAO/IAEA, 1996) calculated their basal requirement (the intake needed to prevent pathologically and clinically relevant signs of dietary inadequacy) from a minimum requirement to prevent Keshan disease of 19 μg of selenium per day, with a correction for body weight, giving a value of 21 μg/day for men and 16 μg/day for women.

Physiological requirement for selenium

Maximization of plasma GPx. One criterion for the assessment of physiological requirement of a nutrient is the intake needed for maximization of an enzyme or other known biochemical functions. For selenium, the only functional protein for which there are sufficient data from humans is GPx. Therefore, the main criterion for estimating recommended intakes, including the estimated average requirement (EAR) and recommended dietary allowance (RDA) of the recent US and Canadian DRIs for selenium (Standing Committee on the Evaluation of Dietary Reference Intakes, 2000) and other countries, recommendations (Thomson & Paterson, 2001), was maximization of plasma GPx. Prior to the recent revision of the US and Canadian DRIs, the physiological requirement was estimated from data from one intervention study with Chinese men, in which a supplement of 30 μg Se/day as selenomethionine plus the usual dietary intake of 11 μg/day was sufficient to maximize plasma GPx (Yang *et al*, 1987). Thus, 41 μg/day was taken as the physiological requirement of selenium for this function. From the Chinese data, an intake of 40 μg/day was then adjusted for a weight factor to account for higher body weight of US residents, giving a physiological requirement of 53 μg/day. Additional data from a recent similar New Zealand study (Duffield *et al*, 1999) led to modification of this value, which became the EAR for the 2000 US and Canadian DRIs (Standing Committee on the Evaluation of Dietary Reference Intakes, 2000). The US and Canadian DRI Committee carried out an independent analysis of this study data, which showed that selenium supplementation increased plasma GPx activity in nearly all supplemented individuals, but increases at the lowest level tested (10 μg/day) could not be statistically differentiated from the increase at the highest level tested (40 μg/day). Thus, the DRI Committee chose a conservative physiological requirement of 38 μg/day (28 μg from food + 10 μg from the lowest level supplemented) with no adjustment for weight. This value was in fact considerably lower than that (68 μg/day) recommended by the authors of the New Zealand study (Duffield *et al*, 1999). The physiological requirements derived from the Chinese (52 μg/day) and New Zealand (38 μg/day) studies were averaged to give an EAR of 45 μg/day (Table 2), and from this the US RDA was calculated by adding twice the coefficient of variation of 10% to give 55 μg/day (Standing Committee on the Evaluation of Dietary Reference Intakes, 2000). Nutrient Reference Values (NRVs) are currently under development in Australia and New Zealand. A technical report with proposed NRVs has been prepared. This report includes an EAR of 52 μg/day (average of 52 μg/day from the Chinese study and 38 and 68 μg/day from the two interpretations of the New Zealand data), giving a reference nutrient intake (RNI) of 60 μg/day for male and 55 μg/day for female subjects (Thomson & Paterson, 2001). Other countries have also used the criterion of maximization of plasma GPx yet, dietary standards proposed throughout the world differ considerably (Thomson & Paterson, 2001) (Table 1). Although not considered in the estimation of the US RDAs,

Table 1 Recommended intakes of selenium for adults (μg/day) around the world

	Australia (Truswell et al, 1990) RDI	*USA and Canada (Standing Committee on the Evaluation of Dietary Reference Intakes, 2000) RDA*	*United Kingdom (Department of Health, 1991) RNI*	*World Health Organization (WHO/FAO/IAEA, 1996) NR*	*Europe (Scientific Committee for Food, 1993) PRI*	*Germany, Austria, Switzerland (German Nutrition Society et al, 2000) RNI*
Men	85	55	75	40	55	30–70
Women	70	55	60	30	55	30–70

RDI, recommended dietary intake; RDA, recommended dietary allowance; RNI, reference nutrient intake; NR, normative requirement estimate; PRI, population reference intake.

maximization of whole blood GPx in the New Zealand study occurred at the same supplemental intake as for plasma GPx, and selenoprotein P levels in plasma were also maximal at similar levels (Duffield *et al*, 1999).

Whether optimal health depends upon maximization of GPx activity has yet to be resolved. In setting their Normative Requirement (intake sufficient to maintain a desirable body store or reserve), the WHO Expert Consultation (WHO/FAO/IAEA, 1996) also used the data from the Chinese intervention study (Yang *et al*, 1987), but considered the dietary intake needed to achieve two-thirds of maximum activity of GPx. This was an arbitrary level based on the observation that abnormalities in the ability of blood cells to metabolize hydrogen peroxide (H_2O_2) were apparent only when GPx activity in cells declined to one-quarter or less of normal (WHO/FAO/IAEA, 1996). The intake to achieve two-thirds of maximum GPx (24.3 μg) was adjusted for weight (65 kg males; 55 kg females), and an individual variability of dietary selenium intake of 16% resulting in a value of 40 μg/day for men and 30 μg/day for women.

In rats, GPx1 mRNA levels reach a plateau at half the dietary selenium concentration necessary for plateau levels of GPx (Sunde *et al*, 1997). Thus, the physiological requirement might be set at a lower level that gives maximal mRNA rather than maximal activity of the enzyme itself. Several selenoproteins other than GPx, such as selenoprotein P, the IDIs, PHGPx and thioredoxin reductase, could be used as end points for determining selenium requirements. Maximal activity of some of these proteins, however, occurs at dietary selenium intakes less than those needed for maximal GPx activity (Levander & Whanger, 1996) due to the hierarchy of importance of selenoproteins in tissues (Behne *et al*, 1995). Hence, dietary requirements based on these selenoproteins would likely lead to recommended intakes lower than current values. The results of the recent New Zealand study showed greater proportional increases in selenoprotein P than GPx with every level of supplementation. Maximal activities of selenoprotein P were reached at supplemental intakes a little lower than for GPx (Duffield *et al*, 1999). A critical question that requires further attention is whether maximal levels of this protein, or other selenoproteins, are desirable for optimal health.

In the same New Zealand study, a small decrease in T_4 levels with selenium supplementation suggested that a dietary intake a little higher than the baseline selenium intake of 30 μg/day might be necessary for optimal activities of the deiodinases. Below 30 μg/day there might be changes in the ratio of T_4/T_3. Therefore, lower intakes than necessary for maximal levels of GPx activity may satisfy requirements for the deiodinases (Duffield *et al*, 1999) (Table 3).

Requirement for the prevention of chronic disease

Traditionally, a recommended dietary intake aims to specify an intake that prevents occurrence of a deficiency state in a majority of people in a specific reference group (Solomons & Ruz, 1998). Recently, however, evolution towards recognition of an 'optimal nutrition' has renewed interest in the possible health effects of nutrients in larger than recommended intakes, including 'the promotion of growth, maintenance of good health and the reduction of other disease' not caused by nutritional deficiencies (Solomons & Ruz, 1998). Possible beneficial effects of higher intakes of selenium include a chemopreventive action against certain cancers and an antioxidant protection against cardiovascular disease (Rayman, 2000), which may occur at intakes substantially greater than those sufficient to prevent deficiency or to support maximal activity of selenoenzymes (Combs, 1996). In the US and Canadian DRIs, possible additional health effects of higher intakes of selenium were not considered in the estimation of the new RDA, because the evidence is at present inconclusive and inclusion of this effect in recommended intakes seemed premature (Standing Committee on the Evaluation of Dietary Reference Intakes, 2000).

There is a growing body of evidence, however, suggesting that intakes of selenium above the normal nutritional range confer further benefit, and therefore it may no longer be appropriate to rely on GPx as a marker to indicate optimal selenium intake (Rayman, 2002). Evidence for the role of selenium as an anticarcinogenic agent comes from *in vitro* and animal studies (Ip, 1998), from case-controlled prospective studies with human subjects (Clark, 1985; Yoshizawa *et al*, 1998; Nomura *et al*, 2000), and from a few intervention studies in humans (Blot *et al*, 1993; Clark *et al*, 1996). Results from the Nutritional Prevention of Cancer (NPC) trial in the US, in which supplements of 200 μg Se/day were given to subjects with baseline dietary intakes of around 90 μg/day (Clark *et al*, 1998; Duffield-Lillico *et al*, 2000), suggest that intakes of selenium above those needed to maximize selenoproteins have an anticancer effect. In particular, such intakes are considerably greater than those required to maximize plasma GPx (Clark *et al*, 1996). There is an urgent need for more large-scale trials to confirm such beneficial effects. In another study, intakes of 200 μg Se/day in a selenium-replete group of volunteers greatly magnified the production of cytotoxic T-cells and natural killer cells, thus enhancing cancer-protective capacity (Kiremidjian-Schumacher *et al*, 1994).

There is insufficient evidence at present to estimate reliably a single intake value to produce plasma selenium concentrations that are protective for certain cancers or result in other additional health benefits. Nevertheless, some progress towards such an estimate has been made. In the NPC trial, participants in the lowest tertile of baseline plasma selenium concentration (<1.34 (106 μg) μmol/l) had a statistically significant treatment effect ($RR = 0.08$; $P < 0.002$), as did the participants in the middle tertile (1.34–1.53 (106–121 μg) μmol/l) ($RR = 0.30$; $P < 0.03$) (Clark *et al*, 1998; Duffield-Lillico *et al*, 2000). Combs *et al* (2001) subsequently estimated the daily dietary intake of selenium associated with a plasma selenium concentration of

Table 2 Estimates of requirements for selenium (μg/day) based on data currently available

Minimum requirement for prevention of Keshan disease	20
Physiological requirement (EAR) for maximal GPx and selenoprotein P	45–50
Requirement for IDIs	30
Protection against some cancers	120

IDI, iodothyronine 5′ deiodinases; GPx, glutathione peroxidase.

1.5 μmol/l (120 μg/l) using the formula $\log Y = 1.623 \log X + 3.433$ (X, plasma level in μg/l; Y, daily intake in μg/day) derived from the extensive data of Yang *et al* (1989a). Correction for differences in mean body weights between subjects in the two trials gave 1.5 μg Se per kg body weight/day (ie 96 μg/day for females; 120 μg/day for males).

In another study, Japanese-American men had less chance of prostate cancer if baseline plasma selenium concentration was greater than 1.86 μmol/l (147 μg/l) (Nomura *et al*, 2000). An estimated intake range for the cancer-protective effect using results from both US studies may be calculated using the model derived by Longnecker *et al* (1996) for predicting selenium intake from serum selenium. Using the serum selenium concentrations of 1.34, 1.53 and 1.86 μmol/l (106, 121,147 μg/l) at which there appeared to be a protective effect in the US studies, we can predict cancer-protective intakes of around 75–125 μg/day (Thomson & Paterson, 2001), consistent with Combs's estimate of 120 μg/day. Further dose–control trials are needed to strengthen such conclusions and to determine more precisely the minimum intakes for a protective effect.

Factors affecting selenium requirements

Bio-availability of different forms of selenium

Utilization of a nutrient, in addition to absorption, involves transformation to a biochemically active form. For selenium, this is assessed by monitoring changes in tissue selenoproteins such as GPx and the level at which GPx activity plateaus. Animal studies show a wide variation in the bioavailability of selenium from different foods relative to sodium selenite. In rats the bioavailability from mushrooms, tuna, wheat, beef kidney and Brazil nuts is 5, 57, 83, 97 and 124%, respectively (Levander & Burk, 1990). Human studies show differences among various forms such as selenate, wheat and yeast (Meltzer *et al*, 1993). Such evidence depends to some extent upon the method used to measure bioavailability (Thomson, 1998). The forms of selenium present in foods include selenomethionine (plant and animal), accounting for half of the dietary selenium with a bioavailability of greater than 90% (Thomson & Robinson, 1986); selenocysteine (animal), also with high bioavailability (Standing Committee on the Evaluation of Dietary Reference Intakes, 2000); and inorganic forms such as selenate and selenite (supplements) with bioavailability exceeding 50% (Thomson & Robinson, 1986).

Interactions of selenium with other nutrients and drugs

Nutrient reference values are estimated on the assumption that diets are adequate in other nutrients. A number of factors, however, may affect the requirement for selenium. In particular, because of its role in GPx, selenium is likely to interact with any other nutrient that affects the antioxidant/pro-oxidant balance of the cell. Indeed, lipid peroxidation is inhibited by PHGPx only if sufficient vitamin E is present in the membranes, which indicates a synergism between the two antioxidants (Roveri *et al*, 1994).

Interaction between selenium and vitamin E is observed in the aetiology of many deficiency diseases in animals and pure selenium deficiency is rare. Deficiency may only occur when low selenium status is linked with additional stress such as exposure to toxic chemicals, infections or trauma. Such stress may be heightened by vitamin E deficiency. Selenium and vitamin E deficiencies have recently been shown to potentiate cardiotoxicity of myocarditic strains of coxsackie virus (Beck & Levander, 2000), due to a viral mutant and the resultant changed phenotype expression from avirulent to virulent (Beck, 1997). Combined selenium and vitamin E deficiency has also resulted in unfavourable lipid profiles in animal models (Mazur *et al*, 1996). A protective effect of selenium and vitamin E against lipid peroxidation has been observed and a possible mechanism of action through GPx or selenoprotein P has been proposed (Awad *et al*, 1994; Burk *et al*, 1995; Nolan *et al*, 1995). Results of the NPC trial (Clark *et al*, 1996) suggest an involvement of vitamin E, in that subjects ranking below the median plasma α-tocopherol concentration at entry to the study showed higher rates of subsequent carcinomas and greater apparent protective effects of selenium supplementation during the course of the trial (Combs *et al*, 2001). These observations suggest that under normal physiological conditions, a low GPx activity may be compensated by other components of the antioxidative system such as vitamins E and C, but that the protective effects of GPx are of particular importance when the organism is exposed to additional stress factors. On the other hand, diets low in vitamin E may increase the requirement for selenium. There are no data, however, on which to base quantitative estimates of such requirements in relation to vitamin E for humans.

Selenium has strong interactions with heavy metals such as cadmium, silver and mercury in marine foods and may protect against the toxic effects of these metals (Magos & Webb, 1980). On the other hand, binding of selenium to metals may reduce the bioavailability of selenium from foods (Rayman, 2000).

Two classes of drugs commonly used in the treatment of arthritis, mercaptocarboxylic acids (eg, penicillamine) and gold compounds (eg, aurothioglucose) are potent inhibitors of GPx purified from animal tissue (Combs & Combs, 1986).

Other drugs (ie xenobiotic agents) have been shown to decrease GPx activities in animal models (Combs & Combs, 1986). More recently, organic gold compounds used to treat some autoimmune diseases have been shown to be inhibitors of purified mammalian thioredoxin reductase (Hill *et al*, 1997).

Effect of lifestyle and other factors on requirements

Because of selenium's antioxidant role, lifestyle factors involving oxidative stress such as smoking, high PUFA intake and strenuous exercise may increase the overall requirement for selenium and should be considered in dietary assessment.

Smoking. There is growing evidence that smoking increases oxidative stress and therefore requirements for antioxidants (Thomas, 1995). The selenium status of smokers is lower than that of nonsmokers. This may be partly due to the effect of a less than adequate diet and selenium intake (Lloyd *et al*, 1983; Duthie *et al*, 1993). Although no quantitative value can be put on the influence of smoking, smokers and health advisors should be aware that smoking might increase the requirements for antioxidants including selenium.

Exercise. It has been suggested that for some nutrients the recommended nutrient intakes are unlikely to be sufficient for athletes with high volumes of training. In particular, there is increasing evidence that intense exercise increases oxidative stress and may raise requirements for antioxidants such as vitamin E (Kanter, 1998). There is no evidence yet, however, that such exercise results in an increased requirement for selenium. Studies in this area are desirable.

Assessment of deficiency or adequacy of selenium status

Having established requirements for selenium for the prevention of Keshan disease and for the maximization of selenoproteins, and possible intakes necessary for the prevention of some cancers, how may we assess adequacy from simple biochemical tests? Universal normal ranges of plasma or serum selenium levels have not been set because of the dramatic variability in blood selenium levels according to geographical location. Factors involved are soil selenium levels and, to a lesser extent, the source of certain specific imported foods such as wheat. Using similar criteria and arguments outlined for estimating selenium requirements, it may be possible to estimate cutoff levels for plasma selenium concentrations for assessing the adequacy of selenium status (Table 3).

Table 3 Assessment of adequacy of selenium status: estimates of cutoff concentrations for plasma selenium based on currently available data

	Se concentration (μmol/l)
Prevention of Keshan disease	>0.25
Optimal activity of IDIs	>0.82
Maximization of plasma GPx, selenoprotein P	>1.00–1.20
Protection against some cancers	>1.50

IDI, iodothyronine 5′ deiodinases; GPx, glutathione peroxidase.

Blood selenium concentrations above which Keshan disease is not seen

Blood selenium concentrations measured in areas of China where Keshan disease is endemic are around 0.25 μmol/l (21 μg/l) in comparison with 1.2 μmol/l (95 μg/l) in nonendemic areas, and most samples from affected areas were below 0.25 μmol/l (Yang *et al*, 1984). In the same endemic area, however, no significant differences were found between affected and normal children and, therefore, it is likely that other factors in addition to blood selenium levels, determine whether Keshan disease occurs in individuals. It seems that blood selenium levels below 0.25 μmol/l indicate an increased risk for Keshan disease, whereas those above are protective.

Blood selenium at which maximal activities of selenoproteins are observed

GPx. In the New Zealand study, plasma selenium needed to achieve full expression of plasma GPx was around 1.14 μmol/l (90 μg/l) (Duffield *et al*, 1999), consistent with the value of 1.2 μmol/l derived in an earlier New Zealand study (Thomson *et al*, 1993), and with the study of Yang *et al* (1987), in which plasma GPx was found to plateau at a whole-blood selenium concentration of 1.13 μmol/l (Alfthan *et al*, 1991). Whole-blood GPx activity in the New Zealand subjects also plateaued at a selenium concentration of around 1.15 μmol/l. Thus, maximal activities of both cellular and intracellular GPx appear to occur at similar blood selenium concentrations (Duffield *et al*, 1999). On the other hand, platelet GPx appears to plateau at higher plasma selenium levels. Nève (1995) has compiled the results of a number of supplementation studies and shown plasma selenium concentrations corresponding to the plateau of platelet GPx activity to be 1.2–1.5 μmol/l (95–115 μg/l) for inorganic forms and 1.4–1.7 μmol/l (110–135 μg/l) for organic forms (selenomethionine, Se-yeast and food). The reasons for this difference are unclear and there is insufficient evidence at present to conclude whether this reflects selenium requirement for platelet enzyme activity or not.

Selenoprotein P. In the New Zealand study, selenoprotein P maximized at selenium concentrations a little lower (1.05–1.10 μmol/l) than those for GPx (Duffield *et al*, 1999). Marchaluk *et al* (1995) reported that selenoprotein P concentrations increased up to a selenium concentration of 1.2–1.5 μmol/l and suggested that 1.2–1.7 μmol/l might be the concentration at which selenoprotein P is maximized in

subjects consuming normal diets (Persson-Moschos *et al*, 1995). On the other hand, in Chinese subjects with low selenium status, who were supplemented with selenate, maximal activity of selenoprotein P was reached at a plasma selenium concentration of 0.9 μmol/l (70 μg/l) (Hill *et al*, 1996). These results collectively indicate that responses of blood selenium concentrations vary with different forms of dietary selenium and, perhaps, among different population groups.

Iodothyronine 5′ deiodinases. As discussed previously, a small decrease in plasma T_4 concentrations with selenium supplementation in the New Zealand study indicated that optimal activity of the deiodinases may be achieved at an intake (30 μg/day) lower than that required for maximal GPx activities (Duffield *et al*, 1999). This intake corresponds to a baseline plasma selenium concentration of 0.82 μmol/l (65 μg/l). This, indeed, is consistent with observations in elderly Italian subjects (Olivieri *et al*, 1995) and in Scottish men with low selenium status (Rayman, 2002). Kvícala *et al* (1995) have shown associations among measures of selenium status and those of thyroid status (TSH, T_4, T_3, thyroid volume) in a population with low selenium status, suggesting that these associations might be used as a biological assessment of the magnitude of selenium deficiency.

We may conclude that a blood selenium concentration of 1.0–1.2 μmol/l (80–95 μg/l) is sufficient for maximization of GPx and selenoprotein P and probably other selenoproteins. The question as to whether deficiency is likely to occur at concentrations below 1.0 μmol/l is unresolved, in part, because we do not yet have good clinical markers for selenium deficiency.

Blood selenium levels in relation to chronic disease

Cancer. As previously outlined, selenium appears to protect against certain cancers at levels higher than those necessary for maximization of GPx and other selenoproteins. Combs (1999) has suggested a two-stage model for chemoprevention, reflecting two types of roles in anticarcinogenesis: that of an essential nutrient providing the catalytic centre of antioxidant enzymes (nutritional dose range), and as a source of metabolites produced from relatively high doses of several forms of the element (supranutritional dose range) that directly affect tumorigenesis.

As mentioned previously, serum selenium concentrations at which there appeared to be a protective effect in two US studies (Clark *et al*, 1998; Duffield-Lillico *et al*, 2000; Nomura *et al*, 2000) were 1.34–1.54 μmol/l (106–147 μg/l). The NPC trial found supplemental selenium (200 μg as Se-enriched yeast) to be associated with significant reductions in cancer risk (lung, prostate, colorectal, total cancer), in subjects with pretreatment plasma selenium below 1.34–1.54 μmol/l. Combs *et al* (2001) concluded that the chemoprotective effect of supplemental selenium was greatest for those subjects who entered the trial with plasma selenium levels in the lower tertiles of the cohort. Subjects with pretrial plasma selenium less than 1.34 μmol/l showed not only the highest rates of subsequent cancer but also the strongest apparent protective effect of selenium supplementation. Subjects entering with plasma levels above 1.53 μmol/l showed no cancer-protective benefits of supplementation. In a nested case–control study of Japanese-American men, Nomura *et al* (2000) found that the lowest risk of prostate cancer was seen in subjects with prediagnostic plasma selenium levels of above 1.86 μmol/l (147 μg/l). The inverse association between serum selenium and prostate cancer was present mainly in smokers and past smokers. This may affect the serum selenium concentration at which protection occurs. Current evidence thus suggests that a plasma level of around 1.50 μmol/l (120 μg/l) may be optimal for cancer protection, at least against some cancers (Combs *et al*, 2001).

Cardiovascular disease. Evidence for a protective role of selenium against cardiovascular disease (CVD) is conflicting. Although two large studies indicated that selenium was an independent risk factor for myocardial infarction in a low selenium population (Salonen *et al*, 1982; Suadicani *et al*, 1992), other research groups have not found this to be the case (Rayman, 2000). The apparent inconsistency of the evidence may be due to a threshold in the protective effect of selenium. Thus, inverse associations would be observed in populations with low intakes of selenium but not in those with high intakes (Huttunen, 1997). Consistent with this is the observation of Salonen *et al* (1982) of a two-to-three-fold increase in cardiovascular morbidity and mortality for subjects with serum selenium levels less than 0.60 μmol/l (45 μg/l), compared with those with higher selenium levels at the start of the study. In a large prospective study in Denmark, an increased risk of ischaemic heart disease (relative risk 1.55) was observed among subjects with serum selenium below 1.00 μmol/l (80 μg/l) (Suadicani *et al*, 1992), whereas no association was observed between the risk of myocardial infarction and serum selenium in US physicians in which very few case or control subjects had plasma levels below 1.00 μmol/l (Salvini *et al*, 1995). No association between the risk of CVD and serum selenium has been observed in populations with high serum selenium (mean values above 1.27 μmol/l), whereas a modest association has been present in several populations with lower mean values (Huttunen, 1997). Some studies, however, have not shown a clear cardiovascular risk for low selenium levels (Nève, 1996).

Thus, despite significant research, whether or not selenium plays a role in protecting against CVD, remains inconclusive. Any protection is likely to be related to an antioxidant effect through the ability of GPx to combat oxidative modification of lipids and reduce the aggregation of platelets. This would be consistent with an apparent risk threshold of around 1.00 μmol Se/l in plasma that corresponds with that required for maximization of the antioxidant selenoproteins. In some studies, the effect is seen only

in smokers, who are known to have lower blood selenium concentrations than nonsmokers (Kay & Knight, 1979; Thomas, 1995). The status of other antioxidants such as vitamin E might also influence any protective effect.

Viral infection. That adequate selenium is required for protection against viral infection has been demonstrated by Beck (1997) and Beck and Levander (2000), who suggest that nutritional deprivation may be one of many factors that increase the susceptibility of individuals to influenza infection (Beck *et al*, 2001). There is insufficient data, particularly in human subjects, to estimate an optimal dietary intake or an optimal plasma selenium concentration for protection against viral infection. As the protection against viral infection appears to be due to an antioxidant function, the optimum levels required are likely to be those for maximization of selenoproteins, GPx and selenoprotein P. As α-tocopherol has a similar protective effect, the requirement for viral protection is likely to be influenced by vitamin E status and the status of other antioxidants.

Immune function. Adequate selenium is essential for many aspects of immune function. Selenium deficiency depresses the effectiveness of immune cells generally, with diverse specific effects (McKenzie *et al*, 1998). Several supplementation studies indicate that increased intake of selenium may enhance aspects of the immune system. For example, supplementation with 200 μg Se/day as sodium selenite during therapy for squamous cell carcinoma of the head and neck resulted in a significantly enhanced cell-mediated immune response (Kiremidjian-Schumacher & Roy, 2001). This effect was observed in patients with a mean plasma selenium concentration of 1.11 μmol/l (88 μg/l), which is around the level required for maximal levels of selenoproteins. Similarly, Baum *et al* (1997) found that a high risk of HIV-related mortality in drug-abusing adults was associated with selenium deficiency, which they defined as plasma selenium of 1.08 μmol/l (85 μg/l). Low plasma selenium level was an independent predictor of mortality in HIV-positive children and was associated with faster disease progression (Campa *et al*, 1999). It is possible that the immunosuppression observed at baseline in these patients was not entirely related to maximal levels of GPx or selenoprotein P (Kiremidjian-Schumacher & Roy, 2001), and that higher intakes may be necessary for optimal immune function.

It is noteworthy that platelet GPx plateaus at higher plasma selenium concentrations than either plasma or erythrocyte GPx. It is not known at what plasma selenium concentrations GPx activity is at maximum in other cells such as leucocytes. Assuming it is similar to that for platelets, we may infer that this is the level sufficient to optimize immunomodulatory properties of selenium and, perhaps, also some anticarcinogenic properties, which apparently require higher selenium doses and possibly specific chemical forms to exercise full activity (Nève, 1995).

Consequences of selenium intakes or blood selenium concentrations that are inadequate for maximal GPx activities

Evidence to date suggests that increased risk of some cancers is associated with low selenium intakes, but this has yet to be confirmed in comprehensive clinical trials. Any effect is probably only partly related to inadequate selenoprotein levels. The suggested increased risk of CVD may be related to an inadequate level of antioxidant enzymes. There appears to be an enhancement of immune function associated with higher levels than required for maximal GPx, but there is currently little clinical evidence of adverse effects on health due to somewhat lower immune function. It may be that such effects are seen only during periods of additional stresses that make individuals more susceptible to infection.

There is, however, little evidence of other health effects associated with intakes between that required to prevent Keshan disease (20 μg/day) and that required for maximal levels of selenoproteins ($\sim$80 μg/day). Combs (2001) has evaluated country-level data for blood selenium concentrations using the serum or plasma concentration of 0.9 μmol/l (70 μg/l) as the criterion of nutritional adequacy suggested by Nève (1995). This evaluation indicates that nutritional selenium deficiency would appear to affect substantial numbers of people ($>$10%) in most countries for which data were available, and to be highly prevalent (affecting $>$50% of population) in almost half of those countries (Combs, 2001). New Zealanders, however, may exist without signs of deficiency or health effects on intakes of 20–40 μg/day that result in lower than maximal GPx activity (Robinson, 1988), as do residents of other countries with low selenium status (Combs, 2001). Thus, although the limited expression of one or more selenoenzymes would suggest at least a subclinical deficiency of the element, no obvious health problems can be ascribed to the habitual consumption of low intakes, and the associated low plasma selenium concentrations. More studies of the health effects associated with these plasma concentrations between 0.25 and 1.00 μmol/l are desirable, although at present, there is a lack of good biochemical techniques providing early detection of the pathological effects of inadequate selenium intake.

There is a clear need for functional measures of nutritional adequacy for assessment of nutritional requirements that are related to clinical or health outcomes. It is difficult to correlate biochemical deficiency with clinical symptoms because selenium has many roles in metabolism. Synergism between different micronutrients (eg that between vitamin E and selenium) pre-empts against consideration of each dietary constituent in isolation (Evans & Halliwell, 2001). Biomarkers of lipid peroxidation or oxidative DNA damage might indicate inadequate antioxidant defence as a result of low selenium status, although these are not necessarily specific to selenium. Unfortunately, there are few reliable measures, but several new biomarkers such as F-2 α-isoprostanes are promising (Bowen & Mobarhan, 1995;

Halliwell, 1999). Evaluation of immune function might also lead to useful biomarkers of nutrient requirements for health benefits of micronutrients (Bronson *et al*, 1999). Like measures of lipid peroxidation, however, these are not specific for selenium inadequacy.

Cohen *et al* (1989) have investigated the effects of selenium deficiency in patients on total parenteral nutrition, examining the effect on GPx and its role in cellular metabolism of H_2O_2. In severely deficient patients (mean plasma selenium 0.24 μmol/l, 19 μg/l), the ability of granulocytes and erythrocytes to metabolize H_2O_2 through the glutathione cycle and the hexose monophosphate shunt was consistently diminished, and was corrected when selenium deficiency was reversed. The abnormality, however, became apparent only when the GPx activity in these cells declined to one-quarter or less of normal (WHO/FAO/IAEA, 1996). It would be of interest to determine to what extent this abnormality occurs in subjects with less severe deficiency, and, perhaps of even greater interest, to determine any clinical effects of the decreased ability to metabolize H_2O_2. Nève (1991) has described other physiological and clinical indices that are influenced by selenium status, but they have limited value in the assessment of selenium adequacy.

Conclusions

The evidence on which requirements for selenium are based is considered by some to be relatively good in comparison with other nutrient elements (Levander & Whanger, 1996). Many gaps, however, remain in our understanding. These result in the need for approximations and extrapolations, sometimes tenuous, in defining requirements for different groups. Further information is especially desirable on the relationship between intakes to maximize GPx activity and those to optimize other selenoproteins, such as selenoprotein P, the IDIs, PHGPx and thioredoxin reductase. Such knowledge could help provide benchmarks in setting selenium requirements. The incorporation of selenium into most selenoproteins has priority over that of plasma and cytosolic GPx. Thus, requirements for selenium based on optimum plasma GPx activity alone should also take account of the amounts needed for normal levels of other biologically necessary selenium compounds. If it should be shown that optimization of GPx is not necessary for optimum health, then the present recommended dietary intakes might indeed be higher than necessary. More data are required on normal levels of selenoproteins other than plasma GPx, and also on the intakes necessary for maximal activity of such proteins.

Little information exists concerning the effects of lifestyle on selenium requirements, other than that smoking increases oxidative stress and therefore requirements for antioxidants (Thomas, 1995). No quantitative value can be placed on the influence of smoking or other lifestyle factors. Thus, such factors cannot currently be used as a basis for refining recommended intakes. Similarly, insufficient data on synergistic or antagonistic effects of other nutrients and of drugs are available to incorporate into the estimation of recommended intakes. Neither do we know how the requirements of selenium are influenced by the status of other antioxidants such as vitamins E and C.

Possible beneficial effects of higher than recommended intakes of selenium have yet to be confirmed. Further intervention trials, using a controlled dose-dependent design, are needed to evaluate fully selenium as a cancer chemopreventive agent. Similarly, intervention studies in humans are necessary to investigate the role of selenium in viral protection and other aspects of the immune function, CVD and other chronic conditions. Compelling epidemiological evidence that is supported by clinical trials and biologically plausible mechanisms, producing specific health benefits, is necessary before such evidence can be used in recommending higher intakes (Anonymous, 1997).

Whether optimal health depends upon maximization of GPx or other selenoproteins has yet to be resolved, and the consequences of less-than-maximal GPx activities or mRNA levels need investigation. There is currently a lack of suitable biochemical techniques to provide early detection of pathological effects of inadequate selenium intake. The US RDA has been reduced to 55 μg/day (Standing Committee on the Evaluation of Dietary Reference Intakes, 2000), yet this recommendation remains higher than intakes in many other countries. An intake of 20 μg/day is apparently protective against Keshan disease, and estimates of intakes that are not associated with any deficiency symptoms in the New Zealand population suggest that 25–40 μg/day is adequate. Intakes below the US RDA are typical for much of the world's population and selenium intakes around half of that value do not appear to be associated with adverse impacts on health. Clearly other factors, besides selenium status, influence susceptibility to cancer and to other chronic diseases reported to be associated with low selenium intakes. While our understanding of the importance of selenium in human nutrition has been significantly heightened in recent years, there remains an urgent need for studies to underpin the refinement of national intake recommendations.

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