Proceedings of the Nutrition Society (2003), **62**, 393–397 DOI:10.1079/PNS2003254

The Summer Meeting of the Nutrition Society was held at the University of Leeds on 2–5 July 2002

Micronutrient Group Symposium on 'Micronutrient supplementation: when and why?'

Selenium supplementation: does soil supplementation help and why?

John R. Arthur
Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, Scotland, UK

There are now concerns that dietary Se intake is inadequate for the population in the UK and parts of Europe. Many different methods can be proposed to deal with this problem. Experience from Finland suggests that the addition of Se to fertiliser is a safe and effective means of increasing the intake of the micronutrient in the human population. However, careful consideration needs to be given to the potential consequences of increasing Se intake. It is important to understand the biochemical and physiological changes that may occur with any increase in Se intake within the UK population. Se is an essential component of at least twenty functional proteins within mammals. These proteins are essential for a range of metabolic functions, including antioxidant activity, thyroid hormone synthesis and immune function. Thus, any increase in Se intake has the potential to influence in a wide range of factors that may impinge on the incidence of chronic disease. Treatment of soil with Se-supplemented fertiliser will certainly increase total Se in food products derived from areas where this treatment is in place. Consumption of such foods will increase Se status in many populations where the existing intake does not meet requirements. If the increases in Se intake are not toxic the overall consequences have the potential to be beneficial.

Selenium intake: Selenium status: Selenium biochemistry: Selenium-supplemented fertiliser

Se has been recognised since the 1950s as essential for normal health and development in a wide range of animals (Combs & Combs, 1986; Arthur & Beckett, 1994; Makela *et al*. 1995; Hansen & Deguchi, 1996; Van Metre & Callan, 2001). Much of this knowledge resulted from the study of diseases, notably white muscle disease or nutritional myopathy, in some areas where forage crops contained inadequate levels of Se. In general, Se-responsive diseases in farm animals occurred when there was a concurrent vitamin E deficiency. The discovery that Se was an essential component of the peroxide-metabolising enzyme glutathione peroxidase led to the hypothesis that these deficiency diseases were a result of uncontrolled oxidation in the tissues of the animals (Combs & Combs, 1986). Whilst such hypotheses provided an excellent stimulus to research in the areas of Se and vitamin E deficiency, not all effects of Se deficiency could be explained by oxidative mechanisms. Thus, many changes, both enzymic and functional in severely-Se-deficient animals, which occurred when vitamin E levels were adequate, led to the search for other non-peroxidase functions for Se (Burk, 1983; Reiter & Wendel, 1983; Arthur *et al*. 1987*a,b*). These studies, coupled with more recent molecular and bioinformatic studies, have indicated that there are between twenty and thirty selenoproteins expressed in mammals. These selenoproteins may arise from twenty-five genes identified by computer-searching of the human genome, with alternate splicing resulting in the expression of more than twenty-five selenoproteins (Lescure *et al*. 1999; Hatfield & Gladyshev, 2002; Kryukov & Gladyshev, 2002; Kryukov *et al*. 2002).

The essentiality of Se for human health was first recognised when the element was shown to prevent Keshan disease, a cardiomyopathy that occurred in China, particularly in adolescents and mothers (Levander & Beck, 1997). Since then, some studies have shown that Se supplementation may be effective in the protection against certain types of cancer, albeit at levels that may be considered

Corresponding author: Professor John R. Arthur, fax +44 1224 716629, email j.arthur@rowett.ac.uk

supranutritional (Combs, 2001*a*; Duffield-Lillico *et al*. 2002).

Selenium addition to fertilizer

The indications that Se may be essential for human health, allied to the common occurrence of Se-responsive disorders in farm livestock, prompted Finnish authorities to instigate a programme of augmenting Se status. The strategy adopted was to add Se as sodium selenate to fertilisers used for both grain and forage production (Varo *et al*. 1993; Aro *et al*. 1995; Makela *et al*. 1995). Similar strategies to increase the Se status of plants have been implemented in areas of Australia and New Zealand. In both Europe and Australasia this methodology was adopted to protect animal health and improve production, which were experiencing deterioration as the result of widespread Se deficiency. Initially, all applications of Se to fertiliser were very carefully monitored, since as well as being essential for health, Se has the potential to be toxic. When Se was added to fertilisers in Finland in 1984 there was a rapid rise in both the Se content of the crops and the foodstuffs derived from animals consuming the crops. In addition, human Se status, which was monitored by determination of plasma Se concentrations, also rose. Both the Se content of foodstuff and the human Se status reached a plateau within 2 years of the introduction of Se-supplemented fertilisers. Thereafter, there were still slight rises in Se concentrations after the initial sharp increases, and in 1990 the Se content of fertiliser used for grain production was decreased from 12 to 6 mg/kg. This action resulted in an immediate fall in the Se contents of both grain and human plasma (Varo *et al*. 1993).

The increases in Se content of Finnish meat and rye resulting from the addition of Se to fertiliser in 1984 varied from 3-fold to 10-fold. This improvement was reflected in the levels in foodstuffs, e.g. the Se contents of wheat bread, potato and meat (pork and beef) increased by approximately 3-, 10- and 3-fold respectively, as did the Se concentration of whole milk.

The plasma Se concentrations of the Finnish population, which were used as an indicator of Se status, increased after 1978 when high-Se wheat was imported into the country. At this time the average plasma Se concentration increased from approximately 0·7 μmol to approximately 1·0 μmol. These levels remained stable between 1980 and 1985, until Se was introduced into fertiliser. Thereafter, the plasma Se content increased to approximately 1·4 μmol/l. After the addition of Se to fertiliser there were similar increases in the plasma Se concentrations of both male and female and old and young members of the population. In addition, the increases in plasma Se were similar in different regions of the country, again in both young and old subjects. The Se content of tissues taken from accident victims also showed increases of between 50 and 100 % after the introduction of Se-supplemented fertiliser for crops (Varo *et al*. 1993).

Potential implications of selenium supplementation

The populations of the UK and many other European countries now have plasma Se concentrations that are comparable with levels that were prevalent in Finland before Se was added to fertiliser (Rayman, 1997, 2000, 2002; Shortt *et al*. 1997; Brown *et al*. 2000). There is now much biochemical and nutritional information that can be used as the basis for informed decisions as to whether it would be beneficial to increase dietary Se intake in order to increase the Se status of the populations of these countries, possibly by the use of Se-containing fertilisers as in Finland.

Before consideration of the potential biochemical changes that could be achieved by increasing the Se status of the UK population, it is important to consider the range of biochemistry and functions of Se in man, since it is now clear that Se is essential for the maintenance of human health through its participation in a wide range of biochemical processes.

Selenium biochemistry and selenoproteins

The majority of Se in human diets is present as selenomethionine in plant materials and as both selenomethionine and selenocysteine in animal products (Combs, 2001*b*). In the selenoenzymes that mediate functions of Se, the active sites contain the amino acid, selenocysteine. At physiological pH the selenol group of selenocysteine is >99 % ionised and is able, therefore, to function as a very efficient redox catalyst. Inorganic forms of Se such as selenite or selenate are also present in the diet and, although generally less abundant than the organic amino acids, they are more direct precursors of selenocysteine in enzymes (Arthur, 2000; McKenzie *et al*. 2002*a,b*). Paradoxically, selenocysteine and selenomethionine have to be degraded to an inorganic form of Se, chemically similar to selenide, which is then used in combination with serine in the synthesis of the selenoproteins. The complex nature of Se metabolism and the mechanisms by which Se is incorporated into selenoproteins have probably evolved to deal with the potential for Se compounds to participate in spontaneous chemical reactions. For example, selenite can react very rapidly and directly with glutathione to form selenodiglutathione, which may further reduce Se to a form that can be incorporated into selenoproteins or react non-specifically with other thiol groups (McKenzie *et al*. 2002*b*). Thus, it is probably essential that Se is handled mainly as a relatively unreactive organic form before its incorporation into the catalytically-reactive selenoproteins.

As indicated previously, at least twenty-five selenoprotein genes have been identified by computer-searching in the human genome. However, labelling with ^{75}Se both *in vivo* and in cell culture indicates that there are more selenoproteins produced in mammalian systems (Evenson & Sunde, 1988; Behne & Kyriakopoulos, 2001; Miller *et al*. 2002). Furthermore, ^{75}Se labelling of different cell types and of whole animals indicates very tissue-specific expression of the selenoproteins. For example, glutathione peroxidases account for most of the labelling in keratinocytes, whereas in several endothelial cell types >60 % of the ^{75}Se label is found in thioredoxin reductase (cytosolic; Rafferty *et al*. 1998; Miller *et al*. 2002). Examination of ^{75}Se-labelling patterns or determination of selenoprotein mRNA expression in different tissues may be used to assess the principal functions of Se. For example, in Se and I deficiency selenoprotein synthesis is favoured in the thyroid gland, thus

https://doi.org/10.1079/PNS2003254 Published online by Cambridge University Press

preserving thyroid hormone metabolism (Bermano *et al*. 1995; Arthur *et al*. 1999). Thus, the determination of an increased plasma Se concentration in any population that has received Se supplements indicates there have been potential changes in more than thirty selenoproteins in different organs in the body. Furthermore, these changes will not have been consistent from organ to organ and may not even have been consistent from individual to individual.

It is important that different cell types from human tissue may have a different distribution of selenoproteins from those of other animal species. Thus, when investigating the role of Se in human metabolism it is essential to thoroughly validate the model system being used. For example, the selenoprotein profiles of bovine and porcine endothelial cells do not resemble those of human endothelial cells (Miller *et al*. 2002). However, the distribution of selenoproteins in human and rat thyroid are similar (Arthur *et al*. 1999). This relationship makes the rat a potential suitable model for study of the effects of Se deficiency on human thyroid hormone metabolism.

Selenium: specific issues in human biology

Differences in responses to Se supplementation may, in part, be explained by single nucleotide polymorphisms. In the stem loop structure of the glutathione peroxidase-4 gene there is a polymorphism that can be associated with changes in lipid metabolism in lymphocytes. This polymorphism is distributed in the population in accordance with normal genetic variation (Hardy Weinberg distribution; Hedrick, 2000). The influence on lipid metabolism is associated with changes in 5-lipoxygenase activity and, thus, perhaps with inflammatory responses (Villette *et al*. 2002). It is surprising, however, that the polymorphism is not directly related to glutathione peroxidase-4 activity, although the absence of a direct relationship may reflect inadequacies in the enzyme measurements, which direct determination of glutathione peroxidase proteins may resolve. Searches of the human genome have revealed the potential for many single nucleotide polymorphisms in Se-containing proteins, and investigation of these polymorphisms may shed further light on individual variation in response to Se deficiency or supplementation. In general, selenoproteins have great potential to influence lipid metabolism, particularly that of polyunsaturated fatty acids (Schnurr *et al*. 1996; Marinho *et al*. 1997; Hurst *et al*. 2001). Both the cyclooxygenase and lipoxygenase pathways rely on peroxides as intermediates for enzyme activity. Regulation of the levels of peroxides within the cell and direct degradation of peroxides provide mechanisms whereby Se-containing proteins can exert their effects. The ability of Se intake in the normal nutritional range to modulate glutathione peroxidase activities indicates that this role constitutes a plausible mechanism for its effects on inflammation. Thus, the effects of selenoproteins on lipid metabolism will directly influence immune function, particularly if it relies for activity on derivatives of arachidonate metabolism. Consistent with these theoretical mechanisms Se supplementation of normal subjects in the UK has led to changes that stimulate the immune system. After supplementation with 100 μg Se as sodium selenite/d, normal subjects showed improved responses of lymphocytes to a challenge with poliovirus. In particular, aspects of the cell immune system producing cytokines and T-cell responses were improved. In addition, poliovirus was removed from the system more quickly in the Se-supplemented subjects than in those receiving placebo treatment. The Se-supplemented subjects also had fewer mutations in residual poliovirus recovered from the faeces (Broome *et al*. 2002). In addition to effects on whole-body metabolism, Se supplementation has potential beneficial effects that can be demonstrated by the use of cell culture techniques. Endothelial cells provide a model for the study of factors that may be involved in the pathogenesis of cardiovascular disease. The cell culture medium used to grow endothelial cells can be inadequate for the supply of Se necessary to maximise selenoprotein activity. Thus, impaired selenoprotein expression can be associated with increased susceptibility of the cells to challenge with either organic hydroperoxides or oxidised lipoproteins (Anema *et al*. 1999; Miller *et al*. 2001; Lewin *et al*. 2002).

Conclusions

The importance of Se in man is now recognised through its essentiality for several metabolic functions. As this range of functions has been identified and characterised, the importance of maintaining adequate dietary Se intake has been emphasised. In particular, supplementation trials have shown that some populations may benefit from an increased Se intake that has the potential to improve the efficiency of biochemical pathways dependent on selenoproteins. Potential beneficial effects of Se in the prevention of cancer, improvement in thyroid hormone metabolism, stimulation of immune function and prevention of male infertility all need to be confirmed in further studies.

Dietary Se intake in many populations is inadequate for maximum expression of many of the selenoproteins that occur in human tissues. If it is accepted that these biochemical functions of Se can be related to particular diseases, it is important to increase Se intake as a preventative measure. Experience in Finland suggests that a convenient way of increasing dietary Se intake for the human population is to add Se to fertilisers. The information available from Finnish studies suggests that this measure can be implemented without inducing toxic levels of Se in crops, animals consuming crops or in the human population. However, it is more difficult to associate changes in Se intake in Finland with any improvement in chronic disease or health. This situation is probably due to other changes that occurred in parallel with the increased Se intake. For example, dietary advice, improved medical care and increased levels of exercise could not be dissociated from the changes in dietary Se.

Although there are proven methods for adding Se to fertilisers and thus influencing Se intake in man, the desirability of this course of action may be assessed by smaller supplementation trials within the target population. In answer to the question, 'Se supplementation: does soil supplementation help and why?', Se supplementation of fertiliser will increase forage, food crop and animal Se status. Consumption of such food will certainly increase Se status in the human population that previously had marginal

https://doi.org/10.1079/PNS2003254 Published online by Cambridge University Press

intake. Se supplementation trials that have already been carried out indicate such increases in dietary intake can change the activity of Se-dependent pathways in man. The actual health benefits of such actions can only be predicted after extensive Se-supplementation trials in which the effect of the micronutrient can be separated from any other dietary and medical interventions taking place.

Acknowledgement

J.R.A.'s laboratory is funded by the Scottish Executive Environment and Rural Affairs Department (SEERAD).

References

Anema SM, Walker SW, Howie AF, Arthur JR, Nicol F & Beckett GJ (1999) Thioredoxin reductase is the major selenoprotein expressed in human umbilical-vein endothelial cells and is regulated by protein kinase C. *Biochemical Journal* **342**, 111–117.

Aro A, Alfthan G & Varo P (1995) Effects of supplementation of fertilizers on human selenium status in Finland. *Analyst* **120**, 841–843.

Arthur JR (2000) The glutathione peroxidases. *Cellular and Molecular Life Sciences* **57,** 1825–1835.

Arthur JR & Beckett GJ (1994) New metabolic roles for selenium. *Proceedings of the Nutrition Society* **53**, 615–624.

Arthur JR, Beckett GJ & Mitchell JH (1999) The interactions between selenium and iodine deficiencies in man and animals. *Nutrition Research Reviews* **12**, 55–73.

Arthur JR, Morrice PC, Nicol F, Beddows SE, Boyd R, Hayes JD & Beckett GJ (1987*a*) The effects of selenium and copper deficiencies on glutathione S-transferase and glutathione peroxidase in rat liver. *Biochemical Journal* **248**, 539–544.

Arthur JR, Nicol F, Boyne R, Allen KGD, Hayes JD & Beckett GJ (1987*b*) Old and new roles for selenium. In *Trace Substances in Environmental Health*, vol. XXI, pp. 487–498 [DD Hemphill, editor]. Colombia, MO: University of Missouri.

Behne D & Kyriakopoulos A (2001) Mammalian selenium-containing proteins. *Annual Review of Nutrition* **21**, 453–473.

Bermano G, Nicol F, Dyer JA, Sunde RA, Beckett GJ, Arthur JR & Hesketh JE (1995) Tissue-specific regulation of selenoenzyme gene expression during selenium deficiency in rats. *Biochemical Journal* **311**, 425–430.

Broome CS, McArdle F, Kyle JAM, Andrews F, Hart CA, Arthur JR & Jackson MJ (2002). Functional effects of selenium supplementation in healthy UK adults. *Free Radical Biology and Medicine* **33**, Suppl. 1, S261.

Brown KM, Pickard K, Nicol F, Beckett GJ, Duthie GG & Arthur JR (2000) Effects of organic and inorganic selenium supplementation on selenoenzyme activity in blood lymphoctyes, granulocytes, platelets and erythrocytes. *Clinical Science* **98**, 593–599.

Burk RF (1983) Biological activity of selenium. *Annual Review of Nutrition* **3**, 53–70.

Combs GF (2001*a*) Impact of selenium and cancer-prevention findings on the nutrition–health paradigm. *Nutrition and Cancer* **40**, 6–11.

Combs GF (2001*b*) Selenium in global food systems. *British Journal of Nutrition* **85**, 517–547.

Combs GF & Combs SB (1986) *The Role of Selenium in Nutrition*. New York: Academic Press Inc.

Duffield-Lillico AJ, Reid ME, Turnbull BW, Combs GF, Slate EH, Fischbach LA, Marshall JR & Clark LC (2002) Baseline characteristics and the effect of selenium supplementation on cancer incidence in a randomized clinical trial: A summary report of the Nutritional Prevention of Cancer Trial. *Cancer Epidemiology Biomarkers and Prevention* **11**, 630–639.

Evenson JK & Sunde RA (1988) Selenium incorporation into selenoproteins in the Se-adequate and Se-deficient rat. *Proceedings of the Society for Experimental Biology and Medicine* **187**, 169–180.

Hansen JC & Deguchi Y (1996) Selenium and fertility in animals and man – A review. *Acta Veterinaria Scandinavica* **37**, 19–30.

Hatfield DL & Gladyshev VN (2002) How selenium has altered our understanding of the genetic code. *Molecular and Cellular Biology* **22**, 3565–3576.

Hedrick PW (2000) *Genetics of Populations*, 2nd ed. Boston, MA: Jones and Barlett.

Hurst R, Korytowski W, Kriska T, Esworthy RS, Chu FF & Girotti AW (2001) Hyperresistance to cholesterol hydroperoxide-induced peroxidative injury and apoptotic death in a tumor cell line that overexpresses glutathione peroxidase isotype-4. *Free Radical Biology and Medicine* **31**, 1051–1065.

Kryukov GV, Castellano S, Novoselov SV, Lobanov AV, Guigo R & Gladyshev VN (2002) Identity of selenocysteine and terminator UGA codons: Genome-wide search for selenoprotein genes. *FASEB Journal* **16**, 606Abstr.

Kryukov GV & Gladyshev VN (2002) Mammalian selenoprotein gene signature: Identification and functional analysis of selenoprotein genes using bioinformatics methods. *Methods in Enzymology* **347**, 84–100.

Lescure A, Gautheret D, Carbon P & Krol A (1999) Novel selenoproteins identified in silico and in vivo by using a conserved RNA structural motif. *Journal of Biological Chemistry* **274**, 38147–38154.

Levander OA & Beck MA (1997) Interacting nutritional and infectious etiologies of Keshan disease – Insights from Coxsackie virus B-induced myocarditis in mice deficient in selenium or vitamin E. *Biological Trace Element Research* **56**, 5–21.

Lewin MH, Arthur JR, Riemersma RA, Nicol F, Walker SW, Millar EM, Howie AF & Beckett GJ (2002) Selenium supplementation acting through the induction of thioredoxin reductase and glutathione peroxidase protects the human endothelial cell line EAhy926 from damage by lipid hydroperoxides. *Biochimica et Biophysica Acta* **1593**, 85–92.

McKenzie RC, Arthur JR & Beckett GJ (2002*a*) Selenium and the regulation of cell signalling, growth, and survival: Molecular and mechanistic aspects. *Antioxidants and Redox Signalling* **4**, 339–351.

McKenzie RC, Arthur JR, Miller S, Rafferty TS & Beckett GJ (2002*b*) Selenium and the immune system. In *Nutrition and Immune Function*, pp. 229–250 [PC Calder, CJ Field and HS Hill, editors]. Wallingford, Oxon.: CABI/Nutrition Society.

Makela AL, Wang WC, Hamalainen M, Nanto V, Laihonen P, Kotilainen H, Meng LX & Makela P (1995) Environmental effects of nationwide selenium fertilization in Finland. *Biological Trace Element Research* **47**, 289–298.

Marinho HS, Antunes F & Pinto RE (1997) Role of glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase in the reduction of lysophospholipid hydroperoxides. *Free Radical Biology and Medicine* **22**, 871–883.

Miller S, Walker SW, Arthur JR, Lewin MH, Pickard K, Nicol F, Howie AF & Beckett GJ (2002) Selenoprotein expression in endothelial cells from different human vasculature and species. *Biochimica et Biophysica Acta* **1588**, 85–93.

Miller S, Walker SW, Arthur JR, Nicol F, Pickard K, Lewin MH, Howie AF & Beckett GJ (2001) Selenite protects human endothelial cells from oxidative damage and induces thioredoxin reductase. *Clinical Science* **100**, 543–550.

Rafferty TS, McKenzie RC, Hunter JA, Howie AF, Arthur JR, Nicol F & Beckett GJ (1998) Differential expression of selenoproteins by human skin cells and protection by selenium from

UVB-radiation-induced cell death. *Biochemical Journal* **332**, 231–236.
Rayman MP (1997) Dietary selenium: Time to act – Low bioavailability in Britain and Europe could be contributing to cancers, cardiovascular disease, and subfertility. *British Medical Journal* **314**, 387–388.
Rayman MP (2000) The importance of selenium to human health. *Lancet* **356,** 233–241.
Rayman MP (2002) The argument for increasing selenium intake. *Proceedings of the Nutrition Society* **61**, 203–215.
Reiter R & Wendel A (1983) Selenium and drug-metabolism 1. Multiple modulations of mouse-liver enzymes. *Biochemical Pharmacology* **32**, 3063–3067.
Schnurr K, Belkner J, Ursini F, Schewe T & Kuhn H (1996) The selenoenzyme phospholipid hydroperoxide glutathione peroxidase controls the activity of the 15-lipoxygenase with complex substrates and preserves the specificity of the oxygenation products. *Journal of Biological Chemistry* **271**, 4653–4658.
Shortt C, Duthie GG, Robinson JD, Morrice PC, Nicol F & Arthur JR (1997) Selenium studies of a group of Scottish adults. *European Journal of Clinical Nutrition* **51**, 400–404.
Van Metre DC & Callan RJ (2001) Selenium and vitamin E. *Veterinary Clinics of North America* **17**, 373–386.
Varo P, Alfthan G, Huttunen JK & Aro A (1993) Nationwide selenium supplementation in Finland – Effects on diet, blood and tissue levels, and health. In *Selenium in Biology and Human Health*, pp. 119–218 [RF Burk, editor]. New York: Springer-Verlag.
Villette S, Kyle JAM, Brown KM, Pickard K, Milne JS, Nicol F, Arthur JR & Hesketh JE (2002) A novel single nucleotide polymorphism in the 3′ untranslated region of human glutathione peroxidase 4 influences lipoxygenase metabolism. *Blood Cells Molecules and Diseases* **29**, 174–178.

https://doi.org/10.1079/PNS2003254 Published online by Cambridge University Press