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REVIEW

Selenium, immune function and resistance to viral infections

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Abstract

Selenium (Se) is an essential micronutrient that, through its incorporation into selenoproteins, plays a pivotal role in maintaining optimal health. Insufficient intake of Se enhances predisposition to diseases associated with oxidative stress to cells and tissues while supplementation above the recommended levels has been shown to confer health benefits such as enhanced immune competence and resistance to viral infections and in animal models and human studies. Recent studies have also shown that different sources of Se differ in their bioavailability and bioactivity and that Se-enriched milk may be a superior source of Se. In this paper, we briefly describe the nature of selenoproteins, sources of Se in diets and the known mechanisms by which Se/selenoproteins regulate redox balance, augment immune function and mediate resistance to viral infections.

Key words: dairy, immune function, selenium, selenoprotein, viral infection.

INTRODUCTION

The trace element selenium (Se) is of fundamental importance to human health. It is an essential component of several vital metabolic pathways, the antioxidant defence system and the functioning of the immune system. Se status therefore has critical public health implications. Se deficiencies are associated with the high prevalence of chronic diseases such as cancers and cardiovascular disease, increased risk of viral infections, male infertility, decrease in thyroid function and increased incidence of neurological and inflammatory disorders.[1]

Selenium is present in soil and enters the food-chain through incorporation into plant proteins. However, the soils in some regions of the world (including UK, New Zealand and North-East China) contain insufficient or low amounts of Se and this is related to Se insufficiency in some population groups. Although overt Se deficiencies are rare, there is evidence that less-overt Se deficiencies can have adverse consequences for disease susceptibility and the maintenance of optimal health.[1] Worldwide, between 0.5 and 1 billion people are estimated to have inadequate intake of Se.[2]

The current recommended daily allowance (RDA)/recommended daily intake (RDI) levels for Se are based on Se intake required for maximal plasma Glutathione Peroxidase (GPX) activity. In Australia, the RDIs for adult men and women are 85 and 70 μg Se/day, respectively. This level appears to be adequate for preventing Se deficiency in a majority of people but not optimum for promoting health or preventing disease. Results of several recent studies have shown that Se intake significantly above the recommended dietary requirements is needed to maintain health and reduce the risk of disease.[3,4]

Selenium exerts its biological effects through a wide array of selenoproteins/enzymes and some low-molecular-weight Se compounds. Se is incorporated into the poly peptide chain, at the active site, as part of the amino acid selenocysteine (21st amino acid), to form selenoproteins. To date, more than 30 selenoproteins have been identified. However, the biological function of most of these selenoproteins remains unknown.

SELENOPROTEINS

Two broad groups of Se-containing proteins are recognised. These are true selenoproteins where selenocysteine is specifically incorporated within one or more peptide chains (including the Se-binding proteins where the Se is also associated with the protein) and Se-containing proteins where seleno-methionine is non-specifically incorporated within peptide chains by the displacement of methionine from its tRNA by seleno-methionine.[5]

The selenoproteins represent a class of redox-active proteins containing the 21st proteinogenic amino acid, selenocysteine, the Se homologue of cysteine.[6] The codon for this amino acid (UGA) is usually interpreted as the stop codon during translation; however, under the right conditions it can

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be reinterpreted as the incorporation signal for selenocysteine. The reinterpretation requires factors including a selenocysteine insertion sequence, specific translation factors, a selenocysteine-loaded tRNA and the biosynthetic machinery to synthesise and charge this tRNA with selenocysteine.[5]

The sources of Se for the production of selenoproteins can be organic or inorganic so that dietary selenocysteine, seleno-methionine, selenite and selenate all can contribute to the intracellular Se pool.[7] Other low-molecular-weight Se compounds, such as methyl-selenol and methyl-selenocysteine, have been identified as components of the intracellular Se pool and, with seleno-methionine, have been found to be efficient anti-tumorigenic agents in animal studies and *in vitro* models.[8,9]

The genetic machinery required for selenoprotein biosynthesis does not exist in yeast and terrestrial plants and Se incorporation into yeast and plant tissues appears to be non-specific, that is, where Se displaces sulphur in biochemical processes to largely produce seleno-methionine.[10] The genetic machinery for selenoprotein synthesis does exist in archaea, bacteria, protozoa and higher animals, although there are marked differences in the structure of selenoproteins between these groups.[6]

The human seleno-genome contains 25 genes that can be translated into proteins with distinct functions. These functions include protection from oxidative stress as well as modulation of inflammatory response, removal of damaging or signalling peroxides, reduction of oxidised proteins and membranes, regulation of redox signalling, transport of Se between active sites of biosynthesis within cells and between tissues, synthesis of selenocysteine and as structural proteins.[5]

For example, all three thyroid hormone de-iodinases are selenoproteins as are the three known thioredoxin reductases. The former are responsible for activating the inactive form of thyroid hormone (T4) by removal of iodine while the latter are involved in both intracellular and extracellular metabolism and also in the regulation of biosynthesis of deoxynuceotides.[11] Selenoproteins are required for normal brain and eye function and in spermiogenesis where they also have a structural role.[11–13] Se-binding proteins, such as selenoprotein P, may also play a role in regulating processes of tumorigenesis beyond protection from oxidative damage as the concentrations of these proteins are reported to be lower across a wide range of cancers and in cancer cell lines.[14]

SELENIUM AND OXIDATIVE STRESS

Most of the selenoproteins that have been functionally characterised exhibit enzymatic redox activity mediated via the amino acid selenocysteine.[5] The evolutionary advantage to an organism of using selenocysteine, which supplies a selenol group, over cysteine, which provides a thiol group, is the difference in their reactivity. In particular, selenol is more highly polarised and has a lower pKa when compared with thiol, which results in selenol form being fully ionised at normal physiological pH and active at much lower pH.[15] This can lead to a one or more order of magnitude increase in the catalytic rate constant for the Se-containing enzymes compared with the sulphur equivalent during pH dependant redox processes and therefore better protection of the cell from oxidative stress.[15]

The redox activities of selenoproteins can be further characterised as being either primarily involved in signalling within cells (e.g. the thioredoxin reductases) or in protection of cells against oxidative damage (e.g. the glutathione peroxidases and selenoproteins K, R and W).[7] The thioredoxin reductases indirectly regulate cellular activities such as cell proliferation, cell death and immune response activation.[5] They also participate in controlling selenoprotein biosynthesis and can directly reduce lipid hydroperoxides and hydrogen peroxides. In mammals, five out of a total of seven identified distinct glutathione peroxidases contain Se as selenocysteine (GPx1–4 and GPx6) with the other two containing sulphur as cysteine in the active site.[5] They are responsible for catalysing the reduction of hydrogen peroxide and organic hydroperoxides to water or the corresponding alcohol, respectively, thus protecting cells from oxidative damage.[16] Selenoprotein K provides an antioxidant function in the heart,[17] but is also present in skeletal muscle, pancreas, liver and placenta.[18] Selenoprotein R catalyses the reduction of oxidised methionine and is required for the repair of oxidatively damaged proteins.[19] The functions of selenoprotein W have not been determined; however, it is highly expressed in proliferating myoblasts[20] and low concentrations of this protein in muscle tissue are associated with white muscle disease in Se-deficient sheep and cattle.[5]

MILK AND DAIRY AS A SOURCE OF SELENIUM

Recent studies have highlighted the benefits of milk enriched with Se as a unique source of Se that is more bioavailable and bioactive, compared with inorganic forms such as sodium selenite, or organic forms such as Se-enriched yeast.[21,22] In addition, milk is also a rich source of macro- and micronutrients with immunomodulatory and antibacterial and antiviral properties.[23] As a result, there is increasing interest in the use of dairy as a source of Se in human diets. The production of a Se-enriched milk requires that cows be fed a source of organic Se as the concentration of Se in cow's milk increases linearly in response to feeding supplements of Sel-Plex fed to cows at 18–22 mg/day,[24,25] but appears to be unresponsive to supplements of inorganic Se.[25]

Most of the Se in milk produced by feeding Sel-Plex (predominately seleno-methionine) to cows is likely to be in the form of seleno-methionine as a result of its non-specific incorporation into milk proteins. The evidence for this, however, is circumstantial as there appear to be no reports of milk Se speciation using recent reliable analytical techniques. Milk is a rich source of methionine and therefore, true milk protein derived from cows fed Sel-Plex can become highly enriched with Se (20–40 times that of standard products).[22,26] Increases in concentrations of low-molecular-weight Se compounds and/or peptides containing selenocysteine and/or selenoproteins in milk in response to

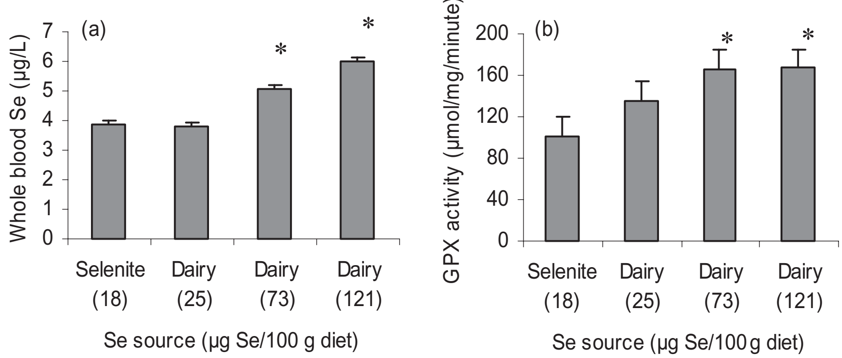

Figure 1 Whole blood Se concentration (a) and plasma glutathione peroxidase activity (b) in mice fed either a standard diet of mouse chow (control) or a modified diet containing Se from milk protein concentrate (*$P < 0.05$ with respect to selenite.[28]

feeding Sel-Plex cannot be ruled out given there is increased free radical scavenging activity and antioxidant status of milk from cows fed Se-enriched yeast.[27]

In animal models, supplementation with Se-enriched milk protein has been found to be effective in enhancing whole Se and GPX activity in a dose-dependent manner (Figure 1).[28]

SELENIUM AND IMMUNOMODULATION

The primary function of the immune system is to protect the body against invasion by pathogenic organisms and the development of malignancies. These protective effects are mediated by innate (non-specific) and adaptive (specific) immune systems. The major effectors of the innate immune system include polymorphonuclear cells (mainly neutrophils), mononuclear phagocytes (monocytes/macrophages) and natural killer cells and the complement system. On the other hand, T (cell-mediated) and B (antibody-mediated) cells represent the key effectors of adaptive immunity. An effective host immune response requires a coordinated interaction between various components of the innate and acquired immune systems. Deficiencies or defects in the functioning of any component of the immune system, enhance predisposition to infectious diseases, cancers and immunoinflammatory disorders.

Adequate nutritional status (macro- and micronutrients) is critical for optimal functioning of the immune system, and nutritional insufficiency, excess or unbalanced intake can have negative impacts on immune competence and resistance to pathogenic organisms and tumours. The most important micronutrients include vitamins and trace minerals. Vitamins form parts of many enzymes, whereas microelements function as enzyme cofactors. Thus, their deficiency can influence a range of physiological functions, including the immune system.

Selenium status is known to influence the functioning of all components of the immune system and its ability to respond to infections and cancers; Se is found in significant amounts in immune tissues such as spleen, lymph nodes and liver.[29]

Selenium deficiency has been reported to reduce the production of free radicals and killing capacity of neutrophils,[30] T cell counts,[31] IL-2R affinity and expression on T cells,[32,33] proliferation and differentiation of T cells,[32,34,35] lymphocyte toxicity,[32,36] NK cell activity,[37] serum IgG and IgM concentrations and antibody responses.[38,39] Reduced percentages of CD8+ cytotoxic T cells, CD2+ T cells, panB cells and NK cells, and impaired responsiveness of thymocytes to ConA stimulation in neonatal rats nursed by mothers fed low-Se diets, have also been reported.[40]

On the other hand, Se supplementation has been shown to have a beneficial effect. In experimental animal models, Se supplementation has been found to increase T cell proliferative responses, lymphokine-activated killer cell activity, NK cell activity, delayed-type hypersensitivity responses and responsiveness to vaccines.[41] In adult human subjects with relatively low Se levels (<1.2 μmol/L), supplementation with Se (100 μg/day) enhanced plasma Se concentrations and lymphocyte cytosolic GPX and phospholipid GPX activities, and augmented a number of host immune responses (increased IFN-g production, T cell proliferation to antigen stimulation and the percentage of total T cells, especially T helper cells).[42] Se-supplemented subjects also exhibited rapid clearance of the poliovirus following immunisation with a live attenuated polio vaccine. In Se-adequate subjects, Se supplementation (above the recommended levels) has been shown to increase lymphocyte proliferation responses to mitogen stimulation,[35,43] upregulate expression of IL-2 receptors,[35] improve cytotoxic lymphocyte-mediated tumour cytotoxicity and NK cell activity[31] and increase percentages of cytotoxic and activated T cells, and antibody responses to vaccines.[44] Studies in our laboratory have also shown that supplementation with Se-enriched milk protein is effective in enhancing lymphocyte proliferative responses to ConA and vaccine antigens in Se-sufficient mice (Figure 2).[45]

Dietary supplementation with Se has also been shown to restore age-related decline in lymphocyte proliferative responses to mitogen stimulation (through upregulation of IL-2 receptors) in mice[46] and NK cell function in the elderly.[37,47]

The exact mechanisms by which Se enhances immune function are not fully known. It is likely that Se exerts its affect by altering the redox status of the cells[41] or by meeting the increased requirements for selenoproteins of the activated immune cells. Upregulation of selenophosphate

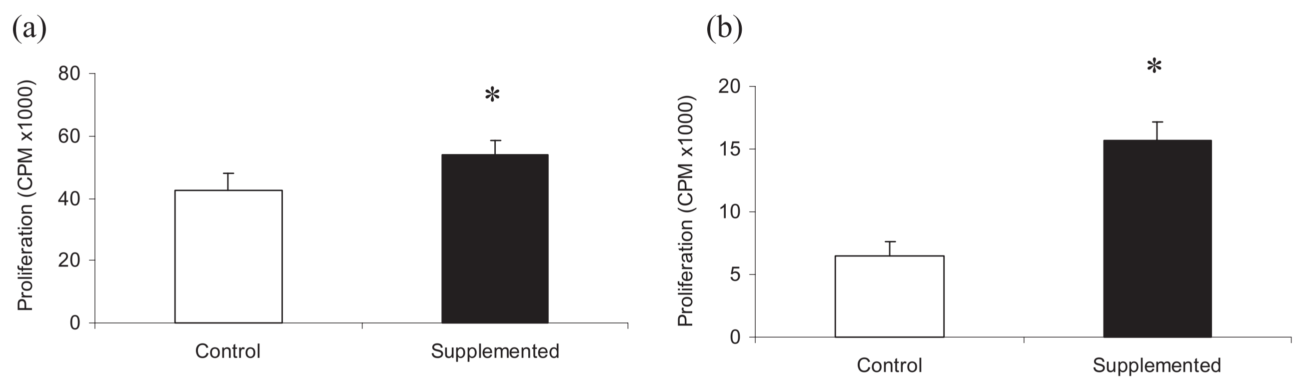

Figure 2 ConA- (a) and influenza antigen-induced (b) proliferative responses of spleen lymphocytes from mice fed Se-sufficient (control; 18 μg/100 g feed) or high-Se diets (supplemented; 121 μg/100 g feed) for 49 days (*$P < 0.05$). Diets were prepared using normal casein or Se-enriched casein.[45]

synthetase activity, directed towards the synthesis of selenocysteine—a key constituent of selenoproteins, in activated T cells[48] and upregulation of several protein biosynthesis genes in the lymphocytes of subjects given Se supplementation for six weeks, has been previously reported.[49] It is also likely that Se exerts its stimulatory effects through upregulation of the IL-2 R expression on activated lymphocytes and NK cells;[31] IL-2 is known to play an important role in the growth, activation and functioning of immune effector cells.

SELENIUM AND VIRAL INFECTIONS

Selenium deficiency has also been associated with increased incidence, severity (virulence) and/or progression of viral infections such as influenza, HIV and Coxsackie virus. For example, infections with influenza are known to cause significantly greater lung pathology in Se-deficient mice compared with Se-adequate mice; number of inflammatory cells and the pathology score were significantly higher in Se-deficient mice compared with Se-adequate mice.[50] Se-deficient mice were also found to develop a Th-2 type response following influenza infection, whereas the Se-adequate mice displayed a Th-1 type response. Viral titres and influenza-specific antibody responses did not differ between the two groups. It has been suggested that increased severity of viral infections in Se-deficient mice could be the result of increased oxidative stress caused by impaired GPX activity.[51] Excessive inflammation may be the result of viral-induced tissue damage and an increased expression of NF-kB because of increased oxidative stress.[50] Furthermore, it has been shown that benign strains of influenza virus become virulent, by undergoing genetic mutations, when passaged through Se-deficient hosts.[52] Whether it results from impaired host immune responsiveness allowing increased replication rate of virus or increased damage to viral genome from free radicals is not known. Similar observations have also been reported for Coxsackie virus infection by Beck *et al*.[51,53]

Selenium also plays an important role in counteracting the development of virulence and inhibiting HIV progression to AIDS.[1] The progression of HIV infection is accompanied by the progressive loss of CD4+ T cells and plasma Se levels. Baum *et al*.[54] reported that plasma Se is a significantly greater risk factor (by a factor of 16) for mortality in HIV patients than CD4+ T cell counts and that Se-deficient HIV patients have 20 times greater likelihood of dying from HIV-related causes than those with adequate Se levels. Se deficiency was defined as plasma concentrations at or below 85 μg/L, which is around the level required for maximal levels of selenoproteins.[4] A significant and independent relationship between low plasma Se levels and mortality and faster disease progression in HIV-infected children has also been reported.[55] Whether the decline in plasma Se levels results from the hijacking of host Se by HIV, for incorporation into viral selenoproteins,[1] or is causatively related to disease progression, is not known. Recent studies have provided evidence that daily supplementation with Se, in HIV-infected subjects, is effective in suppressing the progression of HIV-1 viral burden, increasing CD-4+ T cell counts,[56] reducing oxidative stress, and increasing T cell proliferation and differentiation and IL-2 production.[57]

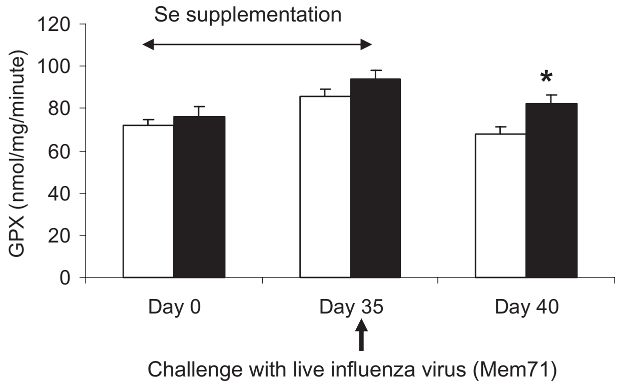

Figure 3 Plasma GPX activity in mice fed adequate (□, 166 μg/kg diet) or high-Se (■, 653 μg/kg diet) diets for seven weeks and then challenged with live influenza virus (Mem71) on day 35 ($*P < 0.001$).[45] Diets were prepared using normal casein or Se-enriched casein.

The exact mechanisms by which Se exerts its antiviral effects are not fully known. However, growing evidence suggests that establishment of viral infection and the progression of viral disease is regulated by the redox state of the host cell.[58] For example, cells of different origins display different permissivity for influenza A virus replication, depending on their intracellular redox power as reflected by glutathione content and Bcl-2 expression; Bcl-2 expressing cells were reported to have higher levels of Glutathione (GSH) and to produce lower amounts of virus than Bcl-2 negative cells.[59] A strong relationship between immune dysfunction in AIDS patients and altered GSH status of T lymphocytes in HIV-infected individuals has been observed.[60] Furthermore, infections with influenza virus in mice are also accompanied by a significant decline in GPX activity and plasma Se levels. Supplementation with casein enriched with Se (653 μg/kg diet) was found to be effective in mitigating the negative impact of infection on GPX activity (Figure 3) and the numbers of CD8 T cells.[45] Thus, it is likely that Se mediates its protective effects through upregulating the antioxidant defences of the cell and by augmenting host immune responses.

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CONFLICT OF INTEREST

No conflict of interest has been declared by H. Gill or G. Walker.

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