Selenium in Cancer Prevention: a Review of the Evidence and Mechanism of Action

Margaret P Rayman

Division of Nutrition, Dietetics and Food
School of Biomedical and Molecular Sciences
University of Surrey
Guildford GU2 7XH
UK

Tel: 01483 686447
Fax: 01483 686481
M.Rayman@surrey.ac.uk

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Synopsis

Selenium (Se) is an unusual trace element in having its own codon in mRNA that specifies its insertion into selenoproteins as selenocysteine (Sec), by means of a mechanism requiring a large Sec-insertion complex. This exacting insertion machinery for selenoprotein production has implications for our Se requirements for cancer prevention. If Se may protect against cancer, an adequate intake of Se is desirable. However, the level of intake in Europe and some parts of the world is not adequate for full expression of protective selenoproteins. The evidence for Se as a cancer preventive agent includes that from geographic, animal, prospective and intervention studies. Newly-published prospective studies on oesophageal, gastric-cardia and lung cancer have reinforced previous evidence which is particularly strong for prostate cancer. Interventions with Se have shown benefit in reducing the risk of cancer incidence and mortality in all cancers combined, and specifically in liver, prostate, colorectal and lung cancers. The effect seemed to be strongest in those with the lowest Se status. As the level of Se that appears to be required for optimal effect is higher than that previously understood to be required to maximise the activity of selenoenzymes, the question has been raised as to whether selenoproteins are involved in the anti-cancer process. However, recent evidence showing an association between Se, reduction of DNA damage and oxidative stress together with data showing an effect of selenoprotein genotype on cancer risk implies that selenoproteins are indeed implicated. The likelihood of simultaneous and consecutive effects at different cancer stages still allows an important role for anti-cancer Se metabolites such as methyl selenol formed from γ-glutamyl-selenomethyl-selenocysteine and selenomethyl-selenocysteine, components identified in certain plants and Se-yeast that have anti-cancer effects. There is some evidence that Se may affect not only cancer risk but also progression and metastasis. Current primary and secondary prevention trials of Se are underway in the USA including the SELECT prostate cancer trial, though a large European trial is still desirable given the likelihood of a stronger effect in populations of lower Se status.
Selenium (Se) is an essential trace element like no other. Its unique redox chemistry has been exploited by biological systems since the advent of dioxygen in the earth's environment created a requirement for a two-electron detoxification system for dealing with peroxides (da Silva & Williams, 2001). Its crucial role is underlined by the fact that it is the only trace element to be specified in the genetic code (Prof. RJP Williams, personal communication, 1999) - as selenocysteine (Sec), the 21st amino acid - which when incorporated into selenoproteins, protects tissues and membranes from oxidative stress and controls cell redox status (Rayman, 2000). As we shall see later, Sec is "dramatically different from the other 20 amino acids in the mode of its incorporation and basic biosynthetic steps" (Hatfield & Gladyshev, 2002) and this complex insertion machinery for selenoprotein production has implications for our Se requirements for cancer prevention.

Evidence is accruing, some of which will be presented below, that the level of intake of Se affects the risk of cancer and may even inhibit its spread from a primary tumour. Since UK deaths from cancer in adults now outnumber those from ischaemic heart disease and stroke and around one in three people in Europe will be diagnosed with cancer during their lifetime (CancerStats, 2004a, b), it is timely to consider the potential of Se for cancer reduction.

The nature of the Se species involved in anti-cancer processes is still a matter of speculation and much ongoing experimental work. Whether the selenoproteins are crucial to the anti-cancer effects requires some understanding of the biosynthetic machinery involved and of the function of some of the selenoproteins most likely to be relevant to cancer. These issues will be addressed in the following section.

**Selenoproteins**

*Biothesynthesis*

Unlike the other 20 amino acids, Sec is biosynthesised on its own tRNA, Sec tRNA^[Ser][Sec]^, from selenophosphate as the Se source. Sec tRNA^[Ser][Sec]^ has many unusual features including its long length (Hatfield & Gladyshev, 2002). The insertion of Sec is specified by the UGA codon in mRNA. However, as UGA is also a stop codon, the presence of a stem-loop structure in mRNA - a SECIS (Sec Insertion Sequence) element - downstream from UGA in the 3′-mRNA-untranslated region, is also required for UGA to be read as selenocysteine. SECIS elements function by recruiting additional factors including the SECIS-binding protein, the Sec-specific elongation factor and Sec tRNA^[Ser][Sec]^, to form the large Sec insertion complex required for the synthesis of selenoproteins and known as the selenosome (Berry *et al.* 1991, 1993; Hatfield & Gladyshev, 2002). The human selenoproteome consists of 25 selenoproteins (Kryukov *et al.* 2003).

**Some selenoproteins of particular relevance to cancer**

The functions of many of the 25 human selenoproteins are as yet unknown though they generally participate in antioxidant and anabolic processes (Hatfield & Gladyshev, 2002). Selenoproteins that may be relevant to cancer risk are described in Table 1 and include a
number from the glutathione peroxidase family (GPx), the 15kDa selenoprotein (Sep15), selenoprotein P (SelP), and the thioredoxin reductases (TR) though a beneficial role of the TRs in cancer prevention is questionable.

**Selenium intakes and status of adults in different countries**

If Se may protect against cancer, an adequate intake of Se is desirable. Whether the intake of Se is adequate is however questionable in much of Europe and some other parts of the world. Mean intake levels in a number of countries (Combs, 2001; Rayman 2004) are shown in Figure 1 which also indicates the range of Se intake believed to be required for optimal activity of plasma GPx (Thomson et al. 1993, Duffield et al. 1999). It is clear from this figure that the level of intake in Europe and some parts of China is not adequate for full expression of GPx. [According to Combs (2001), the same may be true of other parts of the world, as there is little or no information on Se intake or status for most of Africa, South America and central and south Asia.] Furthermore, an updated study of Se requirements by Burk’s group in collaboration with Chinese colleagues (Xia et al. 2005), has shown that full expression of selenoprotein P requires a greater selenium intake than does full expression of plasma GPx. Thus it is even more likely that current intakes are inadequate for optimizing protective effects of the selenoproteins. Indeed there is evidence that will be outlined below, that levels of Se intake that are supra-nutritional may be required to reduce cancer risk (Combs, 2001; Rayman, 2002).

**Evidence for an effect of Se on cancer risk**

The evidence for selenium as a cancer preventive agent has been reviewed ably by a number of people including Combs and Grey (1998), Ip (1998), Combs and Lü (2001), Knekt (2002) Whanger (2004) and Combs (2005). It includes findings from in vitro, animal, geographic (ecological) and prospective studies and from interventions with Se. Such evidence will be summarised and updated below though in vitro studies and those on Se-compounds that cannot arise from food sources will only be referred to in passing: interested readers are referred to the references above. Case-control studies will be excluded as it is not possible to distinguish between selenium concentration as an indicator of cancer risk and that which is a consequence of the disease process (Overvad 1998).

**Animal studies**

Extensive experimental evidence indicates that selenium supplementation reduces the incidence of cancer in animals (Combs & Gray 1998; Combs & Lü 2001; Medina, & Morrison 1988). However, it is difficult to generalise from such studies to the human situation, as animal studies have generally used doses at least ten times greater than those required to prevent clinical signs of deficiency, which, on a per unit body-weight basis, are considerably higher than most human Se intakes. However, it is worth describing a supplementation study on male beagle dogs, a species that develops spontaneous prostate cancer, as the lower dose given is reasonable for humans. Supplementation of the diet of
sexually intact elderly male dogs with selenium as selenomethionine or high-selenium yeast at 3 or 6 µg/kg body weight per day for seven months, significantly reduced DNA damage and significantly upregulated epithelial-cell apoptosis in their prostates while no such effects were seen in unsupplemented dogs (Waters et al. 2003). It appears that selenium sensitises prostate epithelial cells so that cells with extensive DNA damage undergo apoptosis in vivo.

Geographic (ecological) studies

Since as early as the 1960s, geographic studies have shown a consistent trend for populations with low selenium intakes to have higher cancer mortality rates (Shamberger & Frost 1969; Schrauzer et al. 1977; Clark et al. 1991). In one such study (Schrauzer et al. 1977), significant inverse correlations were observed between apparent dietary selenium intakes estimated from food-consumption data in 27 countries, and age-corrected mortality for a number of cancers, including that of the prostate. However, the value of evidence from this type of study is not rated very highly by epidemiologists.

Prospective and nested case-control studies

Knekt (2002) has tabulated the results of prospective studies of Se and cancer published up to the end of 1998. The following categories were included:- all cancers; lung cancer; colorectal, gastrointestinal and stomach; prostate cancer; female cancers and miscellaneous cancers that included cancers of the liver, bladder, mouth, pharynx, oesophagus and malignant melanoma. Of approximately 72 table entries, 50 showed a lower risk associated with higher Se intake or status though only in 18 studies (25%) that included all cancers, cancers of the bladder, lung, ovary, prostate, stomach and thyroid, was the risk significantly reduced.

More recent evidence that Se status can influence mortality from all cancers combined has recently been found in a cohort of 1389 male and female volunteers recruited in the Etude du Vieillissement Artériel (EVA) study (Akbaraly et al. in press). Mean baseline plasma Se in the cohort was 86 µg/L, similar to levels in much of Europe. During the 9-year follow-up, 101 subjects died, 45 of them from cancer. The risk of mortality from cancer was increased four-fold in subjects in the bottom quartile of baseline plasma Se compared to those in the top quartile (Relative Risk, RR = 4.06; 95% Confidence Interval, CI 1.51; 10.92, p = 0.006).

The strongest evidence for a beneficial effect of Se from prospective studies appears to relate to lung cancer, oesophageal and gastric-cardia cancers and most notably to prostate cancer. The risk of colorectal adenoma, a pre-cancerous condition, also seems to be affected.

Lung Cancer

A recent meta-analysis of existing epidemiological evidence from 16 studies showed a significantly decreased risk of lung cancer (summary RR 0.74) associated with higher Se exposure (Zhuo et al. 2004; Table 3). The effects occurred primarily in populations of low Se exposure (defined as serum Se < 100 µg/L or intake < 55 µg/d). In studies carried out in high-Se areas (defined as serum Se > 100 µg/L or intake > 55 µg/d), protective effects appeared on moving from the lowest to the second-lowest Se category but increasing Se
exposure thereafter appeared to have little further effect, suggesting the existence of a threshold effect.

Oesophageal cancer and gastric cardia cancer

In a nested study from the Nutrition Intervention Trial in Linxian, China, significant inverse associations were found between baseline serum Se concentration as a continuous variable and death from oesophageal squamous cell carcinoma (RR 0.83; 95% CI 0.71, 0.98) and gastric cardia cancer (RR 0.75; 95% CI 0.59, 0.95) in 1103 subjects randomly-selected from the larger trial cohort and followed for 15 years (Wei et al. 2004). When the subjects were classified by quartile of baseline Se, those in the highest quartile had a 65% significant reduction in the risk of death from oesophageal squamous cell carcinoma (RR 0.35; 95% CI 0.16, 0.81) and a 69% significant reduction in the risk of death from gastric cardia cancer (RR 0.31; 95% CI 0.11, 0.87) when compared with those in the lowest quartile. The mean population serum Se concentration in the cohort, at 73 µg/L, was relatively low. The authors have suggested that population-wide Se supplementation in regions of China with low serum Se and high rates of these cancers merits serious consideration (Wei et al. 2004).

Prostate Cancer

Results of large prospective studies of prostate cancer are shown in Table 2 (Knekt et al. 1990, Yoshizawa et al. 1998; Nomura et al. 2000; Helzlsouer et al. 2000; Goodman et al. 2001; Brooks et al. 2001, van den Brandt et al. 2003, Li et al. 2004). Those published in 2003 and 2004 are large studies having 540 (van den Brandt et al. 2003) and 586 (Li et al. 2004) cases. Of the eight prospective studies listed, seven show a reduced risk of prostate cancer overall for the highest versus lowest category of Se status, the risk being significantly-reduced in five. When analysis is confined to subjects with advanced prostate cancer or baseline prostate specific antigen (PSA) > 4, six of the eight prospective studies show a significant reduction in prostate cancer in those in the highest category of Se status.

Though the study of Knekt and colleagues (1990) in Finland showed no relation between serum Se concentration and prostate cancer risk, as pointed out by Platz and Helzlsouer (2001), participants had circulating levels almost three-times lower than in the other studies (around 50 vs. 150 µg/L). Thus it may be possible that the concentration of selenium in this cohort was below the threshold where Se can exert a protective effect on prostate cancer risk. This possibility is given credence by the study of Nomura et al. (2000) that showed the protective effect (Odds Ratio, OR 0.5) mainly in persons with serum Se >147 µg/L with ORs close to 1 in lower quartiles of plasma Se.

In a number of these studies (Yoshizawa et al. 1998; Nomura et al. 2000; van den Brandt et al. 2003, Li et al. 2004), the protective effect of Se was stronger for advanced prostate cancer i.e. disease that has spread beyond the prostate, than for localised disease. Furthermore, when data from the Physicians’ Health Study were analysed according to baseline PSA level, the protective effect was significant for all prostate cancers (both localised and advanced disease) but only in those with baseline PSA > 4 (Li et al. 2004), again suggesting a major effect of Se on prostate cancer progression rather than initiation.

Two studies suggested that smoking modifies the effect of Se: the Netherlands Cohort Study showed by far the strongest effect of Se in ex-smokers (van den Brandt et al. 2003) while the inverse association between Se and prostate cancer was mainly present in current or past cigarette smokers in the study of Nomura and colleagues (2000).
Colorectal adenoma

Colorectal adenoma is closely associated with subsequent development of colorectal cancer (Weingarten et al. 2005). Jacobs and colleagues (2004) carried out a pooled analysis of data from three studies that could be considered as prospective studies of Se and risk of colorectal adenoma. The Wheat Bran Fiber Trial, the Polyp Prevention Trial and the Polyp Prevention Study were 3-4-year interventions in subjects that had recently undergone adenoma removal, 1763 of whom had baseline serum or plasma Se measured. None of the trials affected the risk of adenoma recurrence. Analysis of pooled data showed that those with baseline serum or plasma Se in the highest quartile (median 150 µg/L), when compared with those in the lowest quartile (median 113 µg/L), had significantly lower risk of adenoma recurrence (OR 0.66; 95% CI 0.50, 0.87). These results support previous findings that are suggestive of a beneficial effect of higher Se status on colorectal cancer risk (Jacobs et al. 2004).

Intervention studies including randomised controlled trials

Chinese trials

NCI sponsored trials in China for the prevention of oesophageal and gastric cancer observed a reduction in total cancer mortality and a significantly reduced incidence of oesophageal and gastric-cardia cancers in the intervention arm containing Se, β-carotene, and vitamin E (Blot et al 1993; Mark et al. 2000). Though Se was not a single agent in these trials, it is likely to have been the most effective component particularly in the light of subsequent studies (Wei et al. 2004). [As one of a number of agents in an Indian trial, Se also aided remission of pre-cancerous lesions of the oral cavity (Krishnaswamy et al. 1995; Prasad et al. 1995).]

Hepatocellular carcinoma (HCC) is highly prevalent in China. In the Qidong county, near Shanghai, its incidence is particularly high. In this region around 15% of adults carry the Hepatitis B surface antigen and these people are 200 times more likely to develop HCC. In a study where 226 Hepatitis B antigen carriers were randomised to either 200 µg of Se-yeast or placebo, no case of HCC occurred in the supplemented group after four years, while seven subjects in the placebo group had developed HCC (Yu et al. 1997). However, as full details of the methodology of this study are not available, it is difficult to assess whether its protocol was sufficiently well-controlled or robust to be confident in its conclusions.

A recent systematic review and meta-analysis of antioxidant supplements for the prevention of gastrointestinal cancers has assessed the evidence for an effect of Se (Bjelakovic et al. 2004). Data from three Chinese trials were included two of which used Se-yeast (Yu et al. 1997), while one used sodium selenite (Li et al. 2000). Bjelakovic and colleagues concluded that, in contrast to other antioxidant nutrients, Se showed a significant beneficial effect, reducing the risk of HCC by 50% (RR 0.50; 95% CI 0.35, 0.71).

The Nutritional Prevention of Cancer (NPC) Trial and follow-up analyses

The strongest evidence of the efficacy of Se as an anti-cancer agent, particularly for prostate cancer, is provided by the Nutritional Prevention of Cancer (NPC) trial, carried out by Clark and co-workers (Clark et al. 1996; Clark et al. 1998). In 1312 subjects with a history of non-melanoma skin cancer who were randomised to placebo or 200 µg Se/day (as Se-enriched yeast), after 4½ years of treatment and 6½ years of follow-up, there was no effect on the primary end-point of non-melanoma skin cancer. However, those receiving Se showed significant secondary end-point effects of 50% lower total cancer mortality and 37% lower
total cancer incidence with fewer cancers of the prostate, colon/rectum and lung (Table 4). Follow-up analyses to the end of the blinded treatment period - a further 25 months - showed a somewhat-reduced significant effect on total cancer but while the protective effect on prostate cancer remained highly-significant, the effect on lung and colorectal cancers no longer reached significance (Duffield-Lillico et al. 2002; Table 4).

Although follow-up analyses confirmed initial findings that Se supplementation was not statistically significantly associated with the incidence of basal-cell carcinoma (Cox proportional hazards model, Hazard Ratio, HR 1.09; 95% CI 0.94, 1.26), the extended treatment period raised the elevated risk of squamous-cell carcinoma and total non-melanoma skin cancer to statistically-significant levels (HR 1.25; 95% CI 1.03, 1.51 and HR 1.17; 95% CI 1.02, 1.34 respectively) (Duffield-Lillico et al. 2003a). However there are a number of reassuring factors that are relevant here:- firstly, when a treatment lag of two years following randomisation was introduced, thus excluding lesions already in the course of development, the significant effect disappeared; secondly, when subjects were divided into tertiles according to baseline Se status, those in the bottom tertile (see above), whose status resembled that found in Europe, did not have an increased risk of squamous-cell carcinoma (HR 0.87; 95% CI 0.62, 1.22). Finally it must be remembered that the subjects in the NPC Trial were all skin-cancer patients whose skin had sustained heavy sun-damage (Duffield-Lillico et al. 2003a).

**The Nutritional Prevention of Cancer (NPC) Trial sub-group analyses**

The protective effect of Se was confined to men both in the initial and follow-up analyses, though the fact that there were many fewer women than men (319 vs. 931) must be taken into consideration (Clark et al. 1996; Duffield-Lillico et al. 2002). As seen in some of the prospective studies quoted above, the protective effect of Se was stronger in former smokers (Duffield-Lillico et al. 2002).

Analysis of treatment effect in the NPC trial by initial plasma Se status, showed that the strongest treatment effect was observed in subjects in the lowest tertile of plasma Se at baseline i.e. those whose plasma Se concentration was <106 µg/L at entry to the trial (Duffield-Lillico et al. 2002). Se supplementation reduced total cancer incidence in this tertile by 49% (HR 0.51; 95% CI 0.32, 0.81) (Duffield-Lillico et al. 2002) and prostate cancer incidence by 86% (HR = 0.14; 95% CI 0.03, 0.61) (Duffield-Lillico et al. 2003b) in the follow-up analyses. Most UK and European populations would fall into this tertile.

A significant interaction between baseline plasma Se and treatment was detected such that those in the top tertile (>121.6 µg/L) that were supplemented with Se had a significantly increased risk of total cancer (HR 1.88; 95% CI 1.15, 3.05; P=0.01) (Duffield-Lillico et al. 2002). Though this is a sub-group analysis of a secondary end-point analysis and must therefore be regarded with caution, it does raise queries about the advisability of supplementing individuals of already-adequate status (say 120 µg/L or more) with Se.

**Insights from the evidence presented**

What lessons can we learn from the NPC Trial? It would appear that plasma Se should reach around 120 µg/L to optimise the anti-cancer effect of Se. This is higher than the level previously understood to be required to maximise the activity or concentration of
selenoenzymes such as GPx (Thomson et al. 1993; Duffield et al. 1999) though we have recently had to revise our ideas upwards on this as a result of new findings on requirements for selenoprotein P (Xia et al. 2005). Does this mean that the selenoenzymes are not relevant to the anti-cancer effects of Se or do some individuals have a higher Se requirement, perhaps as a result of single-nucleotide polymorphisms (SNPs) in their selenoprotein genes? This issue will be addressed as part of a general consideration of possible mechanisms by which Se may reduce cancer risk.

Se anticancer mechanisms

A number of mechanisms have been suggested to explain the anti-cancer effects of Se. These are summarised in Table 5 together with explanatory references. Though there is fairly general acceptance that methyl selenol is involved in the anti-cancer effects of Se at supra-nutritional doses as explained below, evidence is accruing, some from effects of functional selenoprotein polymorphisms, that the selenoenzymes do play a role, particularly at nutritional levels of intake. Se in selenoproteins can reduce oxidative stress and limit DNA damage both of which have been linked to cancer risk. Some of these anti-cancer processes or pathways are discussed more fully below.

Methyl selenol and its precursors

The in vivo production of small-molecular weight Se metabolites such as methyl selenol (CH₃SeH) that have potent anti-cancer properties has been inferred from work carried out by a number of research groups (Ip, 1998; Ip et al. 2000, 2002; Jiang et al. 1999; Davis & Finley, 2003; Spallholz et al. 2004; Whanger, 2004). The metabolism of dietary forms of Se is shown in Figure 2 (adapted from Combs, 2001 and Rayman, 2004) from which it can be seen that methyl selenol can be formed by the methylation of hydrogen selenide as part of the Se excretory pathway. There is also some evidence that methyl selenol can be formed directly from selenomethionine (SeMet) either by the action of a γ-lyase, also known as methioninase (Nakamuro et al. 1997; Spallholz et al. 2004; Wang et al. 2002) or by an α,γ-elimination reaction (Okuno et al. 2005). Alternatively it can be formed from a storage form of Se, namely γ-glutamyl-Se-methyl-Se-cysteine (γ-glutamyl-SeMeSeCys), that is present in plants of the Brassica and Allium families (Ip et al. 2000; Kotrebai et al. 2000; Whanger, 2004) and probably accounts for the anti-tumour effects of Se-enriched broccoli and garlic (Ip et al. 2000; Davis & Finlay 2003). Metabolism removes the γ-glutamyl group to give Se-methyl-Se-cysteine (SeMeSeCys) which is acted upon by a β-lyase to give methyl selenol directly (Ip et al. 2000; Combs, 2001). There is a suggestion that the β-lyase is present at a higher level in cancer cells than normal cells, ensuring greater exposure of the tumour cells to the anti-cancer agent (Spallholz et al. 2004).

Speciation studies have been carried out on Se-enriched yeast (Se-yeast), the form of Se shown to be effective in most human interventions. These have shown the presence of small amounts of both γ-glutamyl-SeMeSeCys and SeMeSeCys, dependent on the method of extraction, inferring that methyl selenol may be produced directly from the Se-yeast without the necessity of conversion from SeMet, its major Se constituent (Goenaga Infante et al. 2000).
As SeMeSeCys was more than twice as effective as SeMet in reducing mammary tumours in rats (Whanger, 2004), even these small amounts may be important.

Precursors of methyl selenol, typically methyl seleninic acid (CH$_3$SeO$_2$H) in experimental in vitro systems, have been shown to block progression of the cell cycle, induce apoptosis of cancer cells and inhibit the formation of new blood vessels, without which tumours cannot grow or metastasise (Ip, 1998; Jiang et al. 1999; Ip et al. 2000; Davis & Finley, 2003; Whanger, 2004). Processes by which these effects are achieved may involve redox cycling linked to oxidative-stress-induced apoptosis as described by Spallholz and colleagues (2004) and include changes in the expression of genes that control the cell-cycle checkpoint, regulate signalling pathways and caspase-mediated apoptosis (Dong et al. 2003). For instance, SeMeSeCys activates caspase-3 in mouse mammary epithelial tumor cells in vitro (Unni et al. 2001) while methyl seleninic acid is known to activate initiator caspases-1, 8, 10, and 12 (Zu & Ip, 2003). Apoptosis induced by methyl seleninic acid in DU-145 and PC-3 human prostate cancer cells is principally initiated by caspase-8 and involves cell detachment as a pre-requisite (Jiang et al. 2001; Zu & Ip, 2003). Caspase-12, an endoplasmic reticulum (ER)-resident caspase essential for ER stress-induced apoptosis, is also activated during apoptosis induced by methyl seleninic acid in PC-3 cells, suggesting a possible role for ER stress in apoptosis induced by methyl selenol (Zu & Ip, 2003).

**Reduction of DNA damage**

Evidence that Se can reduce DNA damage comes from studies in dogs and humans. In a canine model of prostate cancer, 49 elderly male beagle dogs, physiologically equivalent to 62-69 year old men and similarly subject to prostate cancer, received nutritionally adequate or supra-nutritional levels of dietary Se as SeMet or Se-yeast for 7 months (Waters et al. 2005). DNA damage in the prostate was measured by the alkaline comet assay while Se was measured in toenails. The percentage of prostate cells with extensive DNA damage fell with increased Se exposure up to a level of 0.8-0.9 µg/g, as measured in dog toenails. Above 1.0 µg/g toenails, damage began to rise, demonstrating the typical U-shaped response to a nutrient that is toxic at high levels. Though the authors claim to have supplemented the dogs over the range of intake seen in US men, the baseline maintenance diet, at 0.3 ppm Se, gave an intake in the control group of 6 µg/kg body-weight, already equivalent to a high human intake i.e. 450 µg/d for a 75 kg man. The highest supplement level was an additional 6 µg Se/kg body-weight, equivalent to a total daily intake of 900 µg/d for a 75 kg man. It is therefore not surprising that the upward arm of the U was breached.

In a New Zealand study of men aged 50-75 y at risk of prostate cancer (PSA > 4), the comet assay showed a significant inverse relationship with overall accumulated DNA damage (p = 0.02) in blood leukocytes from those with serum Se below the mean (Karunasinghe et al. 2004). As mean serum Se was measured as 98 ± 17 µg/L, this suggests that serum levels above 98 µg/L are required for the prevention of DNA damage in New Zealand men.

Women born with a BRCA1 mutation carry a lifetime risk of breast cancer of 80% and a lifetime risk of ovarian cancer of 40% (Kowalska et al. 2005). The BRCA1 gene product is involved in maintaining the integrity of the human genome and helps repair double-strand breaks. When blood lymphocytes from BRCA1 carriers are exposed to bleomycin, a known mutagen that induces double-strand breaks, an increased frequency of chromosome breaks
per cell occurs i.e. 0.58 in BRCA1 carriers vs. 0.39 in non-carriers (Kowalska et al. 2005). In 32 female BRCA1 carriers supplemented with Se (276 µg/d as sodium selenite) for 1-3 months, the frequency of chromosome breaks was significantly reduced from 0.63 per cell before supplementation with Se to 0.40 per cell after supplementation with Se, bringing it to the level in non-carrier controls. Thus Se may have the potential to reduce breast-cancer risk in these women.

Reduction of oxidative stress

That the ability of Se in selenoproteins to reduce oxidative stress is relevant to its anti-cancer effects is suggested by the modification of these effects by other antioxidant nutrients. Thus Se intake or status becomes more important when the concentration of other antioxidants or activity of other antioxidant enzymes is low. The strongest effect of Se on cancer risk has been shown among those with the lowest levels of dietary antioxidant vitamins and carotenoids (Willett et al. 1983; Kok et al. 1987; Salonen et al. 1985; Knekt et al. 1990; van den Brandt et al. 1993, 2003; Yu et al. 1999) and particularly at low α-tocopherol concentrations (Combs & Gray, 1998). In the study of Yoshizawa and colleagues (1998) summarised in Table 2, the inverse association between Se status and advanced prostate cancer was slightly stronger after excluding men with an intake of vitamin E that exceeded 30 IU/d, mostly from supplementary sources (OR = 0.29 vs. 0.35). Data as yet unpublished from the NPC Trial show that the effect of Se supplementation on prostate cancer risk only reached significance in subjects in the bottom half of α-tocopherol status (p = 0.03 vs. 0.31 in the top half; Dr. Mary Reid, personal communication, 2005).

A further indication of a link between the antioxidant capacity of Se and cancer risk is seen in the modification of that Se-dependent risk by a polymorphism in manganese superoxide dismutase (MnSOD), the primary antioxidant enzyme in mitochondria. MnSOD has a valine (V) to alanine (A) polymorphism at codon 16 in the mitochondrial targeting sequence that affects the structure of the protein. The relationship between prostate cancer, the MnSOD polymorphism and baseline plasma Se concentration was investigated in 567 cases and 764 controls nested within the prospective Physicians’ Health Study (Li et al. 2005). Though there was little overall association between MnSOD polymorphism and prostate cancer risk, in men with the A/A genotype, high Se status (4th vs. 1st quartile) was associated with a significantly lower risk (RR 0.3; 95% CI 0.2, 0.7, p for trend = 0.002). For clinically-aggressive prostate cancer, the relative risk was even more reduced (RR 0.2; 95% CI 0.1, 0.5, p for trend < 0.001). In contrast, in men with one or two V alleles, the relative risk in the 4th compared to the 1st quartile was less affected by Se status (RR 0.6; 95% CI 0.4, 1.0 and RR 0.7; 95% CI 0.4, 1.2) for total and clinically-aggressive prostate cancer respectively (Li et al. 2005). The interdependence of MnSOD, Se status and prostate-cancer risk implies a role for the antioxidant selenoenzymes.

Evidence for a role of selenoproteins in cancer prevention from selenoprotein genotype data

It had been thought that selenoenzymes were not involved in anti-cancer mechanisms because the amount of Se supplemented that reduced cancer risk (200µg/d) was significantly greater than the amount then believed to be needed to optimise selenoenzyme activity (Combs & Gray, 1998). However, it has recently become clear that optimal expression of some
selenoproteins, notably selenoprotein P, requires a higher amount, as yet undetermined, of dietary Se (Xia et al. 2005), and furthermore that a substantial number of individuals may have a higher requirement for Se for efficient synthesis of selenoproteins as explained below.

People differ substantially in their ability to increase selenoprotein activity in response to additional dietary Se (Brown et al. 2000). This inter-individual variation in selenoprotein expression levels may be accounted for by SNPs in selenoprotein genes that determine the efficiency with which individuals can incorporate selenium into selenoproteins (Hu et al. 2001; Hu & Diamond, 2003; Kumaraswamy et al. 2000; Ratnasinghe et al. 2000). Thus requirements for dietary selenium for optimal protection against cancer may be much higher in individuals carrying particular functional selenoprotein SNPs such as those described below.

**Cytosolic glutathione peroxidase, GPx1**

Recent studies have reported a link between cancer risk and polymorphisms in the cytosolic glutathione peroxidase selenoprotein (GPx1) gene at Proline/Leucine 198 (P/L198). Possession of the L198 allele was associated with an increased risk of lung cancer in Caucasians but not among ethnic Chinese who do not appear to show this polymorphism (Ratnasinghe et al. 2000). Possession of the L198 allele also conferred an increased risk of bladder cancer (see Table 6) and that risk was further raised in men that had one or two A alleles at codon 9 (apparently identical to codon 16, as described above) in exon 2 of MnSOD (Ichimura et al. 2004). In the 213 bladder cancer patients, when compared with the P/P genotype, the P/L genotype was significantly associated with advanced tumour stage: OR 2.58 (95% CI 1.07, 6.18, p = 0.034) for tumour stage T2-4 vs. Ta+1 (Ichimura et al. 2004). By contrast, in a case-control study of 399 cases of incident, invasive breast cancer and 372 controls, no association between breast cancer and GPx1 L/P198 was found (Knight et al. 2004). However, the allele of GPx1 containing four GCG repeats was significantly associated with breast-cancer risk in pre-menopausal women (OR 1.55; 95% CI 1.04, 2.30, for carriers vs. non-carriers). Importantly, GPx1 with the L-allele was less responsive to stimulation of its enzyme activity by Se supplementation than was GPx1 with the P-allele (Hu & Diamond, 2003).

Studies showing selective loss of the P198-allele of the GPx1 gene during tumour development, as detected by loss of heterozygosity at this locus, implicate GPx1 in the risk and development of tumours. In DNA from breast-cancer tissue, the L/L genotype was almost twice as common as in DNA from cancer-free individuals while the P/L genotype was underrepresented, indicating loss of heterozygosity at this locus in breast-tumour development (Hu & Diamond, 2003). Similarly, DNA samples from head and neck tumours exhibited fewer heterozygotes and an increased frequency of the L/L genotype compared with DNA from the cancer-free population (Hu et al. 2004).

**15kDa selenoprotein, Sep15**

The 15kDa selenoprotein (Sep15) is expressed at high levels in normal liver and prostate but at reduced levels in the corresponding malignant organs (Behne et al. 1997). It is located in the ER, tightly complexed to UDP-glucose:glycoprotein glucosyltransferase (UGTR), an enzyme involved in the quality control of protein folding (Korotkov et al. 2001). (This may be of interest as some forms of Se appear to activate ER stress-induced apoptosis as mentioned above.) The Sep15 gene lies on chromosome 1p22.3 at a locus commonly deleted or mutated in human cancers (Kumaraswamy et al. 2000; Kryukov et al. 2003). Two SNPs at positions 811 (C/T) and 1125 (G/A) that are in strong allelic association have been studied.
in the 3'-UTR of the Sep15 gene: G/A1125 lies within a functional SECIS element (Kumaraswamy et al. 2000). The T811-A1125 variant was more effective in supporting UGA readthrough than the C811-G1125 variant, but was less responsive to the addition of Se to the culture medium (Kumaraswamy et al. 2002; Hu et al. 2001). Thus the identity of the nucleotides at 811 and 1125 influences the function of the Sep15 SECIS element in a Se-dependent manner (Kumaraswamy et al. 2000). Individuals possessing one or other of these haplotypes may therefore differ in the efficiency with which they can make Sep15 and in how well they can use dietary Se.

The frequency of the T811/A1125 haplotype is 0.25 in Caucasians and 0.57 in African Americans, who have a higher incidence of prostate cancer (Hu et al. 2001). If lower levels of the Sep15 gene product predispose cells to malignant transformation in the human population, then those carrying a particular Sep15 gene polymorphism may be at a greater risk of cancer and might require significantly higher selenium intake for protection. Furthermore, among African Americans (but not Caucasians) there was a difference in allele frequencies in DNA from breast or head and neck tumours compared with DNA from cancer-free controls. The authors suggest that this difference is likely to be due largely to loss of heterozygosity at the Sep15 locus (Hu et al. 2001; Diwadkar-Navsariwala & Diamond, 2004).

Additional evidence for an effect of this polymorphism on cancer risk comes from a study of Apostolou and colleagues (2004) which showed that the A1125 variant of Sep15 was less responsive to the apoptotic and growth-inhibitory effects of Se than the G1125 variant. The Sep15 gene was shown to be downregulated in 60% of malignant-mesothelioma cell lines and tumour specimens in this study.

**Phospholipid glutathione peroxidase, GPx4**

GPx4 decreases lipid hydroperoxide levels. By so doing, it inhibits the lipoxygenases that metabolise arachidonic acid to generate intermediates that mediate signals for increasing cell proliferation and inhibiting apoptosis (Kim & Milner, 2001). In particular, it inhibits 5-lipoxygenase and reduces the production of 5-hydroxyeicosatetraenoic acid (5-HETE), which is known to stimulate the proliferation of prostate-cancer cells (Ghosh & Myers 1998). Inhibition of 5-lipoxygenase has been shown to trigger massive apoptosis in human prostate cancer cells (Ghosh & Myers, 1998). The C 718 allele of the GPx4 T/C718 SNP, which is close to the SECIS element in the 3'-UTR, has a frequency of 0.45 in Caucasians and is associated with increased levels of lymphocyte 5-lipoxygenase total products (Villette et al. 2002). Thus this polymorphism has functional consequences and may influence the production of 5-HETE and consequently the proliferation or apoptosis of prostate-cancer cells (Villette et al. 2002). Two genetic studies (Wiklund et al. 2003; Hsieh et al. 2001) have shown linkage of the chromosome 19p13.3 region that contains the GPx4 gene to prostate cancer.

**Selenoprotein P, Sel-P**

SNPs have also been identified in selenoprotein P (Sel-P), a selenoprotein believed to be involved both in protection from reactive oxygen and nitrogen species and in the transport of selenium to tissues. Normally, the Sel-P gene is highly expressed in prostatic epithelium but it is down-regulated in a subset of human prostate tumours, mouse tumours and prostate carcinoma cell lines (Calvo et al. 2002). Calvo and colleagues suggest that reduced Sel-P synthesis occurs in a subset of patients resulting in loss of protection from oxidative stress.
Likelihood of simultaneous and consecutive effects at different cancer stages

Given the breadth of evidence for the involvement of forms of Se in various anti-cancer processes, it is likely that Se acts at a number of stages in cancer development and by a number of different mechanisms which may operate simultaneously, or consecutively, involving both small-molecular-weight Se metabolites and selenoproteins. Diwadkar-Navsariwala and colleagues (2004) have proposed a model in which the likelihood of cancer development is linked to reduced levels of one or more protective selenoproteins resulting from:- (i) inadequate dietary Se intake and/or (ii) genetic polymorphisms that result in an increased Se requirement for selenoprotein synthesis and/or (iii) allelic loss of one or two gene copies during tumour development. It may even be that exposure to some forms of Se provokes cellular stress, upregulating protective response systems (such as glutathione-S-transferase) that reduce cancer risk (personal communication, Dr. Vadim Gladyshev, June 2005). Clearly we are far from a full understanding of this very complex area.

Effect of Se on progression and metastasis

There are a few indications that Se can have an effect on cancer progression or metastasis: three examples follow. (i) The effect of Se status on prostate cancer is greater for advanced disease (disease that has spread beyond the prostate) than for primary disease (Nomura et al. 2000; Li et al. 2004; van den Brandt et al. 2003) suggesting an inhibitory effect on tumour spread. (ii) Angiogenesis is required for progression and metastasis. It requires growth factors such as vascular endothelial growth factor (VEGF) and proteolytic degradation of the extracellular matrix by the family of matrix metalloproteinases (MMPs). VEGF expression and protein levels were significantly lowered, as was the activity of MMPs, by methyl selenol precursors (Jiang et al. 1999, 2000, 2004) while selenite inhibited invasion of human fibrosarcoma cells by reducing the expression of MMP-2 and MMP-9 (Yoon et al. 2001). (iii) The tumour stage of bladder cancer is affected by GPx1 genotype, giving indirect evidence that GPx1 is relevant to bladder cancer progression (Ichimura et al. 2004).

Current and future Se-cancer projects

The Selenium and Vitamin E Cancer Prevention Trial (SELECT), sponsored by the National Cancer Institute at a cost of $180m, is a Phase III, randomized, double-blind, placebo-controlled trial designed to test the efficacy of Se (200 µg L-SeMet) and vitamin E (400 mg racemic α-tocopherol), both alone and in combination, in the prevention of prostate cancer (Klein, 2004). The target accrual of 32,400 male volunteers has been achieved and final results are expected in 2013.

In Europe, the possibility of raising even a tenth of the sum made available in the US for SELECT for a similar-scale trial is remote. However, European investigators are still hopeful that a sufficient sum can be raised to carry out a less-expensive web-based trial (PRECISE - PREvention of Cancer by Intervention with Selenium) with Se-yeast in Europe where Se intakes and status are so much lower. As the strongest treatment effect in the NPC Trial was observed in subjects in the lowest tertile of plasma Se at baseline (Duffield-Lillico et al.)
this would greatly increase the chance of seeing an effect. Equally importantly, it would eliminate the possibility of adverse effects in individuals of already-adequate Se status (120 µg/L or more) such as were seen in the top tertile in the NPC Trial (Duffield-Lillico et al. 2002). Furthermore, women as well as men would be included in the European trial.

Se-yeast is currently being used in further prostate cancer studies at the Arizona Cancer Center at doses of 200-800 µg/d, viz. the Negative Biopsy Trial (Stratton et al. 2003a), the Preprostatectomy Trial (Marshall, 2001) and the Watchful Waiting Trial (Stratton et al. 2003b).

There has not yet been a human trial with SeMeSeCys though apparently preparation for such a study in humans by Ip and colleagues is underway (personal communication, Dr. Mary Reid, June 2005). As SeMeSeCys is not a very good precursor for selenoproteins, the results of such a study would be very informative.

My colleagues and I are investigating the effect of functional selenoprotein SNPs on prostate cancer risk using 1400 DNA samples from prostate cancer cases and 800 age- and location-matched controls from the CAPS study in Sweden (Wiklund et al. 2003). We are also extending our careful speciation work (Goenaga-Infante et al. 2004, 2005) to identify low molecular-weight Se species in body tissues and fluids and in Se-enriched yeast and plants.

**Will industry let us find the definitive answer?**

Much time has elapsed during which scientists have spent increasing amounts of time and effort in fund-raising for demanding and meticulous studies to clarify whether Se truly has an effect in reducing cancer risk. Industry has already made up its mind and is not prepared to wait. Apart from Se supplements which have been around for many years, we are now seeing a greater push towards Se-containing functional foods and fertilizers and the selection or breeding of high-Se crop varieties (Broadley et al. 2005). The worry is that population-based studies will become increasingly difficult to carry out under these circumstances so that the answer on Se and cancer in populations may never be definitive unless a European-based trial can be prioritised.

**Acknowledgements**

I should like to acknowledge the help and support I have had from my collaborators in pursuing my work on Se, particularly from Dr. Fiona Green at the University of Surrey and Dr. Heidi Goenaga Infante at the Laboratory of the Government Chemist. Thanks are also due to my funders, Cancer Research UK, the US National Institutes of Health, the UK Prostate Cancer Charitable Trust and Wassen International.

**References**

Akbaraly NT, Arnaud J, Hininger-Favier I, Gourlet V, Roussel A-M & Berr C Selenium and mortality in the elderly: results from the EVA study. *Clinical Chemistry (in press).*


Table 1: Some selenoproteins of particular relevance to cancer

<table>
<thead>
<tr>
<th>Selenoprotein</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione peroxidases</td>
<td><strong>Function</strong>: Antioxidant enzymes: remove hydrogen peroxide, lipid and phospholipid hydroperoxides thereby maintaining membrane integrity, modulating eicosanoid synthesis, modifying inflammation and the likelihood of propagation of further oxidative damage to biomolecules (Spallholz <em>et al.</em> 1990; Diplock, 1994; Sunde, 1997; Allan <em>et al.</em> 1999).</td>
</tr>
<tr>
<td>(particularly GPx1, Cytosolic; GPx2, Gastrointestinal; GPx4, Phospholipid)</td>
<td></td>
</tr>
<tr>
<td>15kDa Selenoprotein (Sep15)</td>
<td>Associated with the endoplasmic reticulum: may be involved in the regulation of protein folding (Korotkov <em>et al.</em> 2001). Gene located in a region often altered in human cancers (Hu <em>et al.</em> 2001). Expressed at high levels in normal liver and prostate but at reduced levels in the corresponding malignant organs: may protect prostate cells against development of carcinoma (Behne <em>et al.</em> 1997).</td>
</tr>
<tr>
<td>Thioredoxin reductases (TR1, TR2, TR3)</td>
<td>NADPH reduction of thioredoxin and other substrates; reduction of nucleotides in DNA synthesis; regeneration of antioxidant systems; maintenance of the intracellular redox state, critical for cell viability and proliferation; regulation of gene expression by redox control of binding of transcription factors to DNA (Allan <em>et al.</em> 1999). More highly expressed in cancer cells than in normal cells and its expression is repressed by p53 (Gladyshev <em>et al.</em> 1998).</td>
</tr>
</tbody>
</table>
Table 2. Large prospective studies of prostate cancer/advanced prostate cancer using tissue indicators of exposure

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>No. of cases</th>
<th>Indicator of exposure</th>
<th>Comparison</th>
<th>RR&lt;sup&gt;1&lt;/sup&gt;</th>
<th>95% confidence interval</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knekt et al. 1990</td>
<td>Finland General population</td>
<td>51</td>
<td>Serum Quintile</td>
<td></td>
<td>1.15</td>
<td>-</td>
<td>0.71</td>
</tr>
<tr>
<td>Yoshizawa et al. 1998</td>
<td>USA Health professionals</td>
<td>181</td>
<td>Toenails Quintile</td>
<td>0.35&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.16-0.78*</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Nomura et al. 2000</td>
<td>USA Hawaii Japanese ancestry</td>
<td>249</td>
<td>Serum Quartile</td>
<td>0.5</td>
<td>0.3-0.9&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non smoker</td>
<td>87</td>
<td></td>
<td>0.8</td>
<td>0.4-1.9</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ex-smoker</td>
<td>86</td>
<td></td>
<td>0.5</td>
<td>0.2-1.1</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Current smoker</td>
<td>76</td>
<td></td>
<td>0.2</td>
<td>0.1-0.8</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Localised disease</td>
<td>120</td>
<td></td>
<td>0.8</td>
<td>0.4-1.8</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Advanced disease</td>
<td>64</td>
<td></td>
<td>0.3&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.1-0.8</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Helzlsouer et al. 2000</td>
<td>USA Washington County</td>
<td>117</td>
<td>Toenails Quintile</td>
<td>0.58</td>
<td>0.29-1.18</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>Goodman et al. 2001</td>
<td>USA CARET Trial asbestos workers/</td>
<td>235</td>
<td>Serum Quartile</td>
<td>1.02</td>
<td>0.7-1.6</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td></td>
<td>current/ex-smokers retinol/β-carotene arm</td>
<td>111</td>
<td></td>
<td>0.75</td>
<td>0.41-1.36</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>placebo arm</td>
<td>124</td>
<td></td>
<td>1.52</td>
<td>0.78-2.79</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>Brooks et al. 2001</td>
<td>USA Baltimore</td>
<td>52</td>
<td>Plasma Quartile</td>
<td>0.24</td>
<td>0.08-0.77&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>van den Brandt et al. 2003</td>
<td>Netherlands Cohort Study</td>
<td>540</td>
<td>Toenails Quintile</td>
<td>0.69</td>
<td>0.48-0.99&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Never smoker</td>
<td>72</td>
<td></td>
<td>1.19</td>
<td>0.48-2.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ex-smoker</td>
<td>300</td>
<td></td>
<td>0.46</td>
<td>0.27-0.79&lt;sup&gt;*&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Current smoker</td>
<td>168</td>
<td></td>
<td>0.97</td>
<td>0.42-2.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Localised disease</td>
<td>189</td>
<td></td>
<td>0.72</td>
<td>0.42-1.24</td>
<td>0.043</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Advanced disease</td>
<td>183</td>
<td></td>
<td>0.62&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.37-1.05</td>
<td>0.020</td>
<td></td>
</tr>
<tr>
<td>Li et al. 2004</td>
<td>USA Physicians' Health Study</td>
<td>586</td>
<td>Plasma Quintile</td>
<td>0.78</td>
<td>0.54-1.13</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Baseline PSA&gt;4</td>
<td>228</td>
<td></td>
<td>0.49</td>
<td>0.28-0.86&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Baseline PSA&lt;4</td>
<td>293</td>
<td></td>
<td>0.77</td>
<td>0.48-1.22</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Localised disease</td>
<td>348</td>
<td></td>
<td>0.97</td>
<td>0.64-1.49</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Advanced disease</td>
<td>171</td>
<td></td>
<td>0.52&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.28-0.98&lt;sup&gt;*&lt;/sup&gt;</td>
<td>&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

*denotes statistically-significant effect
<sup>1</sup>RR relative risk for highest versus lowest category
<sup>2</sup>Advanced disease
<sup>3</sup>Adjusted for BMI at age 21, education and hours since last meal
**Table 3.** Meta-analysis of existing epidemiological evidence from 16 studies of Se and lung cancer (Zhuo et al. 2004)

<table>
<thead>
<tr>
<th></th>
<th>RR high vs. low Se exposure</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td>0.74</td>
<td>0.57, 0.97</td>
</tr>
<tr>
<td>Low Se areas</td>
<td>0.72</td>
<td>0.45, 1.16</td>
</tr>
<tr>
<td>High Se areas</td>
<td>0.86</td>
<td>0.61, 1.22</td>
</tr>
</tbody>
</table>
Table 4. NPC Trial: relative risk (RR) of cancer incidence and mortality in the Se-treated group compared to the placebo group, by follow-up period

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Follow-up until</th>
<th>RR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All sites</td>
<td>Mortality</td>
<td>Dec 31 1993</td>
<td>0.50</td>
<td>0.31, 0.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Feb 1 1996</td>
<td>0.59</td>
<td>0.39, 0.89</td>
</tr>
<tr>
<td>All sites</td>
<td>Incidence</td>
<td>Dec 31 1993</td>
<td>0.63</td>
<td>0.47, 0.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Feb 1 1996</td>
<td>0.75</td>
<td>0.58, 0.98</td>
</tr>
<tr>
<td>Lung</td>
<td>Incidence</td>
<td>Dec 31 1993</td>
<td>0.54</td>
<td>0.30, 0.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Feb 1 1996</td>
<td>0.70</td>
<td>0.40, 1.21</td>
</tr>
<tr>
<td>Colorectal</td>
<td>Incidence</td>
<td>Dec 31 1993</td>
<td>0.42</td>
<td>0.18, 0.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Feb 1 1996</td>
<td>0.46</td>
<td>0.19, 1.08</td>
</tr>
<tr>
<td>Prostate</td>
<td>Incidence</td>
<td>Dec 31 1993</td>
<td>0.37</td>
<td>0.18, 0.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Feb 1 1996</td>
<td>0.51</td>
<td>0.29, 0.87</td>
</tr>
</tbody>
</table>
### Table 5. Some cellular processes and molecular pathways that may be involved in the anti-cancer effect of selenium

<table>
<thead>
<tr>
<th>Anti-cancer processes or pathways</th>
<th>Selected evidence for Se involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Selenoenzyme mechanisms</strong></td>
<td></td>
</tr>
<tr>
<td>Reduction of DNA damage</td>
<td>Se intake or status affected DNA damage in both human and animal studies (see text, Karunasinghe et al. 2004; Kowalska et al. 2005; Waters et al. 2005). Levels of dietary antioxidant vitamins and carotenoids and SNPs that affect antioxidant selenoproteins modify the effect of Se on cancer risk (see text for references). Selenoenzymes can reduce hydroperoxide intermediates in the cyclooxygenase and lipooxygenase pathways preventing the production of pro-inflammatory prostaglandins and leukotrienes (Rayman, 2000).</td>
</tr>
<tr>
<td>Reduction of oxidative stress</td>
<td></td>
</tr>
<tr>
<td>Reduction of inflammation: inflammation promotes tumour growth (Caruso et al. 2004).</td>
<td></td>
</tr>
<tr>
<td><strong>Induction of Phase II conjugating enzymes:</strong> detoxify carcinogens and reduce DNA adduct formation</td>
<td>Some selenocompounds e.g. methyl selenol, can upregulate phase II conjugating enzymes such as glutathione-S-transferase (GST), increasing detoxification of carcinogens (Ip &amp; Lisk 1997). Carcinogen adducts were reduced in liver and mammary gland of rats fed Se-enriched garlic, mushrooms and selenite (Davis &amp; Finley 2003).</td>
</tr>
<tr>
<td><strong>Enhancement of immune response:</strong> cytotoxic lymphocytes and natural-killer-cells are able to destroy tumour cells</td>
<td>Se supplementation (sodium selenite) enhanced the immune response of volunteers and cancer patients by increasing the numbers of cytotoxic lymphocytes and natural-killer cells (Kiremidjian-Schumacher et al. 1994, 2000).</td>
</tr>
<tr>
<td><strong>Increase in tumour-suppressor protein p53:</strong> inhibits proliferation, stimulates DNA repair and promotes apoptotic death by acting as a transcription factor for several genes, including the damage-inducible gadd genes</td>
<td>SeMet can activate p53 through redox regulation of key p53 cysteine residues. Methyl seleninic acid and sodium selenite modulate p53 activity by phosphorylation (Smith et al. 2004). Selenodiglutathione also induces p53 (Lanfear et al. 1994). Se compounds induced specific patterns of expression of gadd genes (Kaeck et al. 1997).</td>
</tr>
<tr>
<td>Inactivation of protein kinase C (PKC), a signaling receptor that plays a crucial role in tumour promotion by oxidants</td>
<td>Selective inactivation of PKC results from reaction of its catalytic domain with selenometabolites such methyl seleninic acid (formed from membrane-bound methyl selenol and fatty acid hydroperoxides), inhibiting tumour promotion and cell growth (Gopalakrishna &amp; Gumimeda, 2002).</td>
</tr>
<tr>
<td>Alteration in DNA methylation: abnormal methylation patterns are associated with neoplasia and inactivation of tumour-suppressor genes</td>
<td>Se affects the extent of DNA methylation and the activity of DNA methyl transferase (Davis et al. 2000; Davis &amp; Uthus, 2003; Fiala et al. 1998).</td>
</tr>
<tr>
<td>Blockage of the cell cycle: inhibits growth and may allow DNA repair to take place</td>
<td>Methyl selenol precursors can induce cell cycle arrest without single-strand breaks and with or without caspase induction and p53 regulation (Davis &amp; Finley 2003). By contrast, selenite induces DNA single- and double-strand breaks, cell-cycle arrest, reduction in DNA synthesis and cell death, predominantly by necrosis (Medina et al. 2001).</td>
</tr>
<tr>
<td>Induction of apoptosis of cancer cells: generally involves the sequential activation of the caspases, a family of proteases capable of degrading cellular components</td>
<td>Methyl selenol precursors induce DNA double-strand breaks and cell death by apoptosis (Medina et al. 2001) involving the caspase cascade (Unni et al. 2001; Wang et al. 2002; Davis &amp; Finley, 2003).</td>
</tr>
<tr>
<td>Inhibition of angiogenesis: new blood vessels are required for the growth and metastasis of tumours</td>
<td>Methyl selenol reduced microvessel density in chemically-induced rat mammary carcinomas (but not in normal tissue), the expression of vascular endothelial growth factor (VEGF) and matrix metalloproteinases (Jiang et al. 1999). p38 MAPK may be a key upstream mediator for the methyl selenol-specific induction of vascular endothelial caspase-dependent apoptosis (Jiang et al. 2004).</td>
</tr>
</tbody>
</table>
Table 6. Association of GPx proline/leucine 198 (P/L198) allele with cancer risk (odds ratio, OR) and modification of risk by MnSOD genotype

<table>
<thead>
<tr>
<th>Cancer Tissue sampled</th>
<th>SNP genotype</th>
<th>OR (95% CI) compared to P/P</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung Blood</td>
<td>P/L</td>
<td>1.8 (1.2, 2.8)</td>
<td>D Ratnasinghe et al. 2000</td>
</tr>
<tr>
<td></td>
<td>L/L</td>
<td>2.3 (1.3, 3.8)</td>
<td></td>
</tr>
<tr>
<td>Bladder Blood</td>
<td>P/L</td>
<td>2.6 (1.5, 4.8)</td>
<td>Y Ichimura et al. 2004</td>
</tr>
<tr>
<td></td>
<td>+MnSOD V/A+A/A</td>
<td>6.3 (1.3, 31.2)</td>
<td></td>
</tr>
<tr>
<td>Breast Blood</td>
<td>P/L</td>
<td>0.9 (0.7, 1.2)</td>
<td>J Knight et al. 2004</td>
</tr>
<tr>
<td></td>
<td>L/L</td>
<td>0.8 (0.5, 1.3)</td>
<td></td>
</tr>
</tbody>
</table>
Figure captions

Figure 1
Mean Se intake levels (µg/d) in different countries (Combs, 2001; Rayman 2004) and the range of Se intake believed to be required for optimal activity of plasma GPx (Thomson et al. 1993, Duffield et al. 1999)

Figure 2
The metabolism of dietary forms of Se (adapted from Combs, 2001, and Rayman, 2004)