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Selenium

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Introduction

The nutrient selenium possesses many attributes that excite the public and the research community. These include anticarcinogenic activity, a role in reproduction, toxicity, its apparent activity to protect against oxidant damage or aging, protection in animals against nutritional forms of muscular dystrophy, and even as a nutrient to be considered in treatment strategies for AIDS. Selenium also possesses many unique and novel nutritional, biochemical, and molecular biology properties that continue to make it an exciting target for nutrition research. This research increasingly provides molecular details that help explain the role of selenium in promoting health and preventing disease. This chapter will review the current status of our present knowledge of selenium both in areas of public interest and in topics of research interest. Additional information can be obtained from other symposia and reviews.¹⁻³

Selenium essentiality was first discovered in 1957 when Schwarz and Foltz⁴ showed that traces of dietary selenium prevented liver necrosis in rats fed a diet also deficient in vitamin E. This led to the demonstration that selenium is a nutritionally essential trace element for animals. Widespread use of selenium supplementation in animal feeds eliminated a number of animal diseases attributable to selenium or vitamin E deficiency.⁵ In the 1960s and 1970s, epidemiological data and animal research began to demonstrate that selenium also possesses anticarcinogenic activity.⁶ Using biochemistry, the first selenium-containing enzyme, glutathione peroxidase-1 (GPX1), was discovered in 1973⁷; 30 years later, bioinformatics and molecular biology have revealed that the human genome encodes 25 selenoproteins.³ These recent discoveries have the promise to uncover the full range of roles for selenium in health and disease, and in the process, perhaps reveal key new players in long-term health.

Deficiency Diseases

Selenium Deficiency in Animals

The foundation for our knowledge of selenium nutrition lies in animal experiments. In the laboratory, rats fed selenium-deficient diets develop liver necrosis if these diets are also deficient in vitamin E and sulfur amino acids.⁵ This degenerative liver disease is distinct from fatty liver and liver cirrhosis, and in the past resulted in death within 21 to 28 days. In 1969, selenium was shown to be unconditionally essential for rats and chickens in diets containing adequate levels of vitamin E and the sulfur amino acids. The specific disease associated with selenium deficiency depends on the species; in contrast to rats, which develop primarily liver necrosis during combined selenium and vitamin E deficiency, the mouse develops a multiple necrotic degeneration of skeletal muscle, heart, kidney, liver, and pancreas. Reproductive failure also occurs in males of both rodent species due to defective sperm production. New mouse knockout models with deleted selenoprotein genes are now revealing critical roles for Se in neural function⁸ and in gastrointestinal disease.⁹

The nature of selenium deficiency in production animals provides examples of selenium deficiency diseases that might be useful in characterizing selenium's full role in human health.⁵ Swine develop a cardiac condition called mulberry heart, lambs develop a nutritional muscular dystrophy called white muscle disease, and turkeys develop a gizzard myopathy. Cattle also develop a nutritional myopathy affecting skeletal and heart muscle. Reproductive problems associated with dietary selenium deficiency in cattle also include retained placenta in cows and reproductive failure in bulls. These conditions usually require concomitant vitamin E deficiency. Chickens develop one of several deficiency diseases depending on dietary selenium, vitamin E, and the sulfur amino acids. A degeneration of capillary beds called exudative diathesis is prevented either by selenium or vitamin E, but a pan-

creatic atrophy is only prevented by dietary selenium when vitamin E is at normal levels. Super levels of vitamin E and other antioxidants, however, will prevent pancreatic atrophy.¹⁰ These laboratory animal diseases can also develop in animals fed practical rations produced from selenium-deficient areas.

Several additional observations emerge from these animal studies. The laboratory selenium deficiency diseases reported in the 1950s and 1960s, however, cannot be reproduced today, probably because commercially produced animals now have adequate selenium stores. Second-generation selenium-deficient animals, however, still grow at half the rate of their selenium-supplemented littermates,¹¹ clearly indicating that selenium deficiency in a diet otherwise adequate in nutrients has impact. These animals now often thrive into old age, suggesting that something else is different in the laboratory setting of the past versus what exists today; one such factor may be disease. Second, the differences in disease signs in different species elicited by selenium deficiency indicate that there is species-to-species variation in selenium's protective role relative to other protective mechanisms. A fuller understanding of these alternative mechanisms (e.g., why capillary beds are exclusively sensitive in the chicken but well protected in the rat) may provide clues for disease resistance in humans. Last, the minimum dietary selenium requirements necessary to prevent selenium deficiency disease is remarkably constant across a wide range of species, suggesting that common molecular regulatory mechanisms are shared between these species.¹²

Selenium Deficiency in Humans

Selenium deficiency in humans, known as Keshan disease, still occurs naturally in China as an endemic cardiomyopathy that is localized primarily in peasant populations in certain hilly and mountainous regions in China with low soil selenium.¹³ This disease was eliminated in the 1970s by an aggressive selenium supplementation program after a large study involving over 46,000 subjects clearly demonstrated that selenium supplementation would protect against the disease. The average daily un-supplemented selenium intake for women in these affected areas of China was estimated to be 12 $\mu\text{g Se/d}$ (see Figure 1). This disease does not occur in the United States, where Se intakes are 5 to 15 times higher, and it is also unknown in New Zealand, another world area with low soil selenium, where intakes are approximately 30 $\mu\text{g Se/d}$.¹⁴

Human selenium deficiency can also occur clinically. The first report in 1979 was in a New Zealand patient undergoing total parental nutrition (TPN).¹⁵ The patient lived in a rural area with low-selenium soils in which endemic white muscle disease in sheep was controlled by selenium dosing. Following surgery and TPN, she developed dry flaky skin and bilateral muscular discomfort and muscle pain. Plasma selenium had dropped to 0.11 $\mu\text{mol Se/L}$ (9 $\mu\text{g Se/L}$) versus 0.32 $\mu\text{mol Se/L}$ (25 $\mu\text{g Se/L}$)

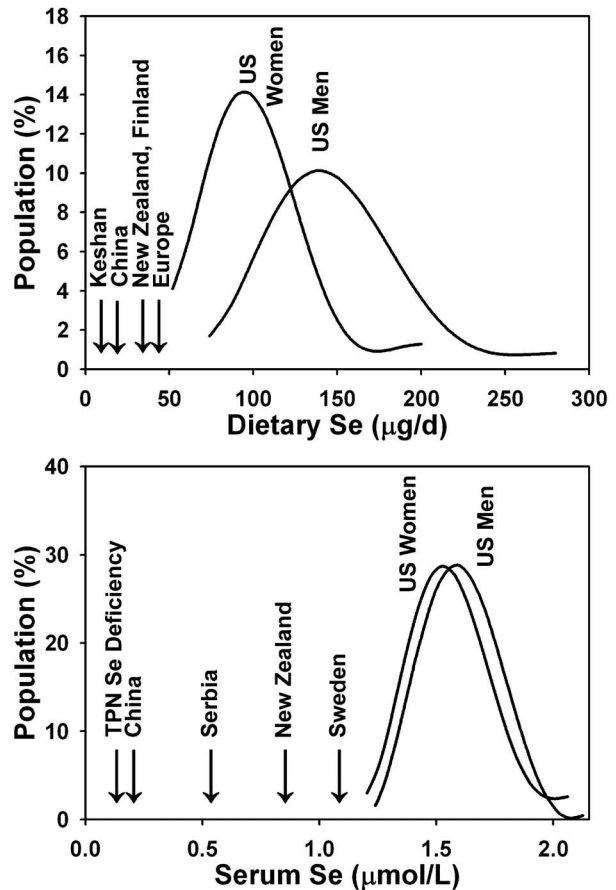


Figure 1. U.S. Dietary Selenium Intakes and Serum Concentrations. The distribution of U.S. selenium dietary intakes (top) and serum selenium concentrations (bottom) for adults (31–50 years old). Arrows show reported levels in other countries, as discussed in the text. Data calculated from (2).

L) immediately before the start of TPN (Figure 1). The patient was then infused intravenously with a 100 $\mu\text{g Se/d}$. Within the next week, muscle pain disappeared and she returned to full mobility. Similar TPN-induced cases of muscle pain and cardiomyopathy leading to death have been reported in the United States. These cases are associated with very low plasma and red blood cell selenium and GPX1 activity, with elevated plasma marker enzymes indicative of tissue damage, and often with white nail beds.

Selenium deficiency in humans is often associated with other conditions. With Keshan disease, selenium supplementation is but one factor. An accompanying infection, perhaps viral, is associated with the development of actual Keshan disease.¹³ Coxsackie virus has been isolated from Keshan disease patients, and recent animal experiments (see below) suggest that the virulence of a viral infection may be influenced by selenium status. Selenium and iodine deficiency also exacerbate each other. In Zaire, a combined selenium and iodine deficiency contributes to the etiology of endemic myxedematous cretinism characterized by both thyroid enlargement and reduced intelligence. Administration of selenium alone appears to ag-

gravate this disease by restoring selenium-dependent diiodinase activity, which in turn fosters increased synthesis and use of thyroxine and iodine, leading to exacerbated iodine deficiency.¹⁶ This illustrates the danger of restoring one nutrient but not the entire diet, and it also illustrates the impact of combined nutrient deficiencies at the organism level.

A regional disease of unknown origin, called Kashin-Beck disease, continues to affect eight million individuals in regions of northern China consuming corn-based diets. This endemic disease of the cartilage occurs in preadolescent and adolescent children and is hypothesized to be associated with selenium deficiency. Unlike Keshan disease, however, selenium supplementation does not eliminate Kashin-Beck disease. Alternative hypotheses include mycotoxins, mineral imbalances, contamination of drinking water, and iodine deficiency.¹⁷

Chemical Forms

Selenium is present in food and in the body in both inorganic and organic forms. Plants absorb inorganic selenium from the soil and metabolize selenium as though it was sulfur to form the amino acid selenomethionine (Figure 2), which has the selenium atom replacing the sulfur atom in methionine. Plants readily incorporate selenomethionine into proteins in place of methionine, and thus selenomethionine is the major form of selenium found in most plants.¹⁸ A few species of plants specifically accumulate selenium as analogs of intermediates in sulfur amino acid metabolism, such as selenocystathionine and methylselenocysteine.

Inorganic forms of selenium, selenite (SeO_3^{2-}) and

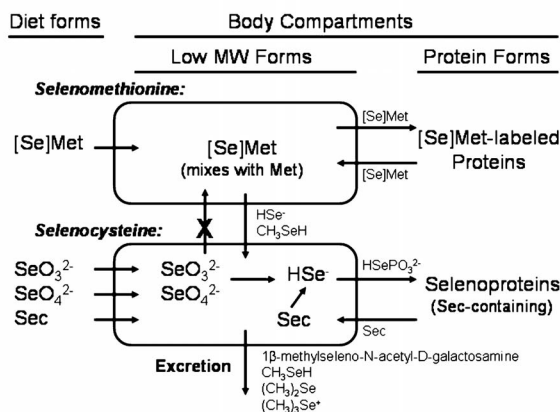


Figure 2. Two compartments of selenium metabolism. Entry points for dietary forms of selenium and the low molecular weight (MW) and protein forms of selenium are shown for the selenomethionine (top) and selenocysteine (bottom) compartments. Common compounds in these pools include selenomethionine ([Se]Met), selenocysteine (Sec), selenite (SeO_3^{2-}), selenate (SeO_4^{2-}), selenide (HSe^-) and selenophosphate (HSePO_3^{2-}). Excretory forms are 1β-methylseleno-N-acetyl-galactosamine, methyl selenol (CH_3SeH), dimethyl selenide ($(\text{CH}_3)_2\text{Se}$) and trimethyl selenonium ion ($(\text{CH}_3)_3\text{Se}^+$).

selenate (SeO_4^{2-}), are often used to supplement animal feed, and these forms of selenium are used in food supplements as well. Alternatively, selenium supplements from health food stores and some vitamin pills can contain selenium as selenized yeast; selenomethionine is the major form of selenium in selenized yeast. Most importantly, humans, animals, and many microorganisms also possess a unique metabolic pathway (see below) that specifically synthesizes selenocysteine, the selenium-containing analog of cysteine, for incorporation into selenoproteins. Selenocysteine is sometimes called the 21st amino acid, “Sec” is the three-letter code commonly used for selenocysteine, and “U” is the one-letter code for selenocysteine in protein sequences. Catabolism of selenomethionine or selenocysteine releases reduced inorganic selenium (as selenide, HSe^-), which can be reincorporated into selenoproteins, or can be methylated to form the excretory forms methyl selenol, or CH_3SeH ; dimethyl selenide, or $(\text{CH}_3)_2\text{Se}$; trimethyl selenonium ion, or $(\text{CH}_3)_3\text{Se}^+$; and 1β-methylseleno-N-acetyl-D-galactosamine ($\text{CH}_3\text{Se-GalN}$).

Analytical Methods

Analytical methods commonly used today for selenium are precise and accurate, largely because of refined instrumentation, and technical expertise is required. The apparent large spread in published tissue and food selenium concentrations, in contrast, arises because of wide differences in selenium status due to geographical differences in soil selenium. Four methods are commonly used for selenium analysis. Fluorometric determination of selenium requires exacting chemical separations,¹⁹ whereas neutron activation analysis is limited to collaboration with scientists at research reactors.²⁰ Atomic absorption spectroscopy using either hydride generation²¹ or graphite furnace²² is now the most common method for routine analysis. Improved instrumentation and availability suggests that inductively coupled plasma-mass spectroscopy²³ will increasingly provide reliable analysis of selenium in combination with analysis of other elements.

Body Selenium

Estimates of total selenium content of humans, determined from cadavers, range between 13.0 and 20.3 mg. Metabolic stable isotope methodology models using US subjects predict that total body selenium asymptotically approaches 30 mg.²⁴ Individuals living in New Zealand or China with considerably lower selenium intakes thus will have a much lower total body burden of selenium. Muscle, liver, blood, and kidneys contain 61% of the estimated total body selenium in humans; if skeleton is included, this increases to 91.5%.²⁵

The NHANES III survey found the mean serum selenium concentration of young adults (19–30 years of age) in the United States is 1.61 and 1.57 $\mu\text{mol Se/L}$

(127 and 124 $\mu\text{g/L}$) for males and females, respectively.² These values are quite similar to most earlier reports for North America. Tabulations of adult European serum or plasma selenium concentrations determined since 1990 range from 1.09 $\mu\text{mol Se/L}$ (86 $\mu\text{g/L}$) in Sweden, France, and Italy, to 0.55 $\mu\text{mol Se/L}$ (43 $\mu\text{g/L}$) in Serbia.²⁶ Adult concentrations in New Zealand in a recent group of subjects were reported to be 0.79 to 0.88 $\mu\text{mol Se/L}$ (62–69 $\mu\text{g/L}$).²⁷ In contrast, subjects in low-selenium areas in China have plasma selenium concentrations of 0.14 to 0.20 $\mu\text{mol Se/L}$ (11–16 $\mu\text{g/L}$).^{13,28} A new tabulation of milk selenium concentrations indicates that human milk from mothers in Canada and the United States averages 0.19 to 0.25 $\mu\text{mol Se/L}$ (15–20 $\mu\text{g/L}$), with selenium content in colostrum more than double, ranging from 0.42 to 1.02 $\mu\text{mol Se/L}$ (33–80 $\mu\text{g Se/L}$).²

Selenium Metabolism

Body Compartments

The two major selenium compartments in the body are the unregulated selenomethionine compartment and the well-regulated selenocysteine/inorganic selenium compartment (Figure 2). These compartments reflect underlying metabolism and storage of selenium, and profoundly influence the selenium we find in food and measure in tissues. The key difference between these two compartments is that the selenocysteine compartment is homeostatically regulated by selenium status, whereas the selenomethionine compartment is not.

The selenomethionine compartment expands and contracts in proportion to selenomethionine intake. This is because selenomethionine cannot be synthesized from inorganic selenium by higher animals. In addition, mammalian enzymes do not differentiate between selenomethionine and methionine.¹² Dietary selenomethionine originating from plants, from animals fed selenomethionine, and from supplements such as selenized yeast mixes with the methionine pool and is incorporated into protein as a methionine analog according to protein needs, which are unrelated to selenium status.²⁹ The selenium in these selenomethionine-labeled proteins (Figure 2) is unavailable until the proteins turn over. Thus, individuals consuming foods with high selenomethionine content will have elevated tissue selenium levels that reflect elevated dietary selenomethionine. Tourists from New Zealand visiting the United States have profoundly increased levels of tissue selenium but not selenium-dependent enzymes, and these levels of selenium return to New Zealand levels upon their returned to their homeland.¹⁴

The selenocysteine compartment consists of the selenium in selenoproteins plus low molecular weight, inorganic forms of selenium (Figure 2). The selenium in mammalian selenoproteins is always present as selenocysteine. This compartment constitutes the biochemically active pool of selenium in the body, and regulation of the

selenoproteins in this compartment appears to account for selenium homeostasis across a wide range of selenium intakes.

Exchange between these two compartments is only one-way. Catabolism of selenomethionine releases selenide via the transsulfuration pathway or methyl selenol via the decarboxylase pathway.¹² These low-molecular-weight species then become part of the selenocysteine compartment.

Absorption

Selenium homeostasis is clearly not regulated by absorption. Animals and humans readily digest proteins containing selenomethionine, and absorb the selenomethionine intact. Selenite, selenate, and selenomethionine are highly available, and selenium from selenocysteine-containing selenoproteins is also highly available. Numerous studies demonstrate absorption rates well above 50%; in one recent series of studies with large doses,²⁴ selenite and selenomethionine absorption from 200 μg selenium doses was 84% and 98%, respectively, illustrating that these high rates are real. Differences in availability of selenomethionine versus selenite have generally been reported to be small compared with day-to-day and individual-to-individual intake, but a recent study reported that selenomethionine was twice as available as selenite when given as daily single-tablet supplements to treat extremely selenium-deficient Chinese subjects.³⁰ Exceptionally low selenium availability (<10%) has been found for selenium in high-mercury tuna and in mushrooms, apparently due to complexed forms that are not available.

The enzymes/transporters responsible for absorption or movement of selenium across membranes are unknown. Selenomethionine is actively transported by the same systems that transport methionine.

Metabolism

The intracellular metabolism of selenium is unique relative to other mineral nutrients because this trace “metal” bonds covalently to carbon. In addition, novel metabolic pathways are necessary to convert simple dietary forms of selenium into the selenocysteine moiety found in selenoproteins.

The central metabolism of selenium occurs within the selenocysteine compartment.¹² The conversion of dietary selenate and selenite to selenide (HSe^-) proceeds via a reductive pathway that now appears to be catalyzed by the selenoenzyme thioredoxin reductase.¹ Glutathione and glutathione reductase may also possibly catalyze this reduction. This reduction usually occurs in intestinal cells or in red blood cells, but also is readily accomplished in other tissues. Selenium released from selenomethionine catabolism also enters this pool as selenide (Figure 2).

Synthesis of selenocysteine occurs during protein synthesis, involves several unusual intermediates, and requires at least five unique gene products (four proteins/

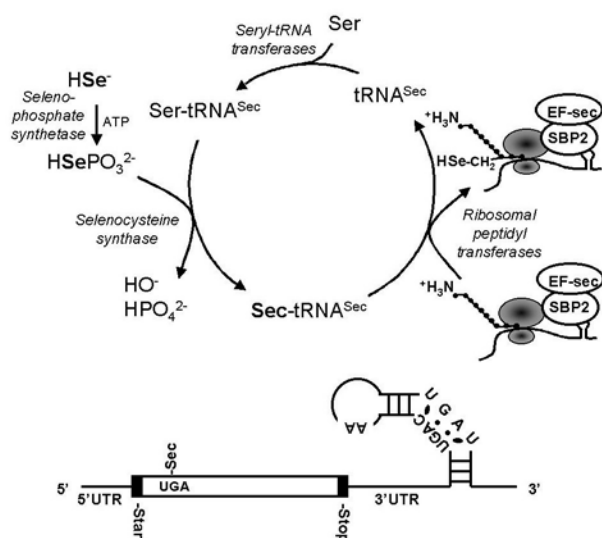


Figure 3. Selenocysteine (Sec) synthesis and incorporation during protein synthesis (top) and diagram of a typical selenoprotein gene (bottom). Top: Sec is synthesized co-translationally from serine (Ser) and selenide (HSe^-) while esterified to tRNA^{Sec} by the indicated enzyme activities. Bottom: diagram of typical selenoprotein mRNA showing the UGA codon in the coding region (open box) and the SECIS in the 3'UTR. Also shown are the consensus sequences in the SECIS stem-loop, including the loop AA and a typical non-Watson-Crick base-pair motif (UGAC/UGAU) that causes a 90-degree bend in the stem.

enzymes and a unique tRNA, $\text{tRNA}^{\text{Sec}}_{\text{UGA}}$ (Figure 3). In addition, each selenoprotein mRNA must contain two specific mRNA elements (a novel use of the UGA codon plus a unique SECIS element) for selenium incorporation to occur.³¹ Selenoproteins are found in all three kingdoms (prokaryotic, eukaryotic, and archaea), and the mechanism of selenocysteine synthesis and incorporation is generally the same in all three kingdoms.³² Much of what we know about mammalian selenoprotein synthesis was first characterized in bacteria.³³

Synthesis of selenocysteine (Figure 3) starts with selenide and the formation of selenophosphate (HSePO_3^{2-}), which is the activated selenium compound used in the synthesis of selenoproteins. This reaction is catalyzed by selenophosphate synthetase using ATP.³⁴ Intact selenocysteine from the diet or from selenomethionine catabolism is not used for synthesis of selenoproteins. Instead, the amino acid serine provides the carbon skeleton for selenocysteine.³⁵ Serine, from the same cellular pool used for protein synthesis, is esterified to the 3' terminal adenosine of $\text{tRNA}^{\text{Sec}}_{\text{UGA}}$ to form $\text{Ser-tRNA}^{\text{Sec}}_{\text{UGA}}$,³⁶ in a reaction catalyzed by the regular seryl-tRNA synthetases. Next, the unique enzyme, selenocysteine synthase,³⁷ replaces the serine-OH with $-\text{SeH}$ from selenophosphate to form selenocysteine- $\text{tRNA}^{\text{Sec}}_{\text{UGA}}$. Thus the synthesis of all selenocysteine occurs while the serine is esterified to the $\text{tRNA}^{\text{Sec}}_{\text{UGA}}$.

Selenocysteine is degraded by a selenium-specific enzyme, selenocysteine lyase,³⁸ which directly releases elemental selenium. This selenium is then reduced non-

enzymatically to selenide by glutathione or other thiols. There is recent evidence that selenocysteine lyase may directly transfer selenium to selenophosphate synthase to facilitate reutilization.³⁸ This may enhance homeostatic selenium retention in selenium deficiency.

Excretion

In rats, selenide is converted in liver to a selenosugar, 1 β -methylseleno-N-acetyl-D-galactosamine ($\text{CH}_3\text{Se-GalN}$), which is the major urinary form under low to adequate selenium conditions.³⁹ Selenide can also be methylated using S-adenosylmethionine (SAM) by either microsomal or cytosolic methyl transferases to form methyl selenol, trimethyl selenonium ion, and dimethyl selenide.¹² Both the size of the dose and the selenium status of an animal influence the form and amount of urinary selenium excretion. Trimethyl selenonium constitutes only a small fraction of urinary selenium in rats with low selenium status, whereas it is the major urinary selenium form in animals ingesting super-nutritional levels of selenium.²⁵ When pharmacological doses of selenium are injected into rats, selenium is expired in the breath as dimethyl selenide; 50% of selenite selenium and 35% of selenomethionine selenium are expired as dimethyl selenide in the first 24 hours after rats are injected with 5 mg Se/kg body weight.

In humans, $\text{CH}_3\text{Se-GalN}$ is likely to be the major urinary form of selenium under deficient and adequate conditions.³⁹ Following the administration of 200 μg of selenium to selenium-adequate adult humans, 17% and 11% of the selenium from tracer selenite and tracer selenomethionine, respectively, appears in the urine over the following 12 days; between 7% and 17% of urinary selenium is present as trimethyl selenonium ion.²⁴ Dimethyl selenide with a garlic-like odor may be detected in the breath of people ingesting high levels of selenium. Thus, under physiological conditions, selenium homeostasis is clearly not regulated by absorption, but, rather, urinary excretion is likely to be important for homeostasis.

Molecular Biology

In all three kingdoms, the genes for selenoproteins employ a novel use of TGA, usually a termination codon, to encode selenocysteine.⁴⁰ These TGA codons reside within exons of the gene DNA, and thus result in in-frame UGA codons in the selenoprotein mRNA (Figure 3). Because UGA in mRNA routinely and unambiguously serves as a termination codon for many proteins, an additional RNA element in the 3' untranslated region (3'UTR) of eukaryotic mRNAs is necessary for UGA-encoded selenocysteine incorporation. This element is called a eukaryotic SECIS element (for selenocysteine insertion sequence).³¹ A single SECIS is used in most selenoproteins, but two SECIS elements are present in the 3'UTR of plasma selenoprotein P (SELP). The consensus SECIS element is a stem-loop structure with 10 to 19 unpaired bases including an AA sequence in the

loop and a quartet of non-Watson and Crick base pairs, which results in over a 90-degree kink in the stem-loop.⁴¹ Two additional forms of SECIS loops have been identified, and the specific secondary structure of the SECIS elements found in different selenoproteins may affect the rate of selenocysteine insertion.⁴²

Two unique selenocysteine-specific elongation factors—SBP2 and EFSec—are also necessary for incorporation of UGA-encoded selenocysteine. EFSec is very specific for selenocysteine-tRNA^{Sec},⁴³ and SBP2 binds the SECIS on the mRNA as well as EFSec and GTP.⁴⁴ These factors are thought to increase the concentration of the selenocysteine-tRNA on the mRNA.

These components assemble in a complex on the ribosome for co-translational selenocysteine incorporation (Figure 3). The SECIS is tethered via the 3'UTR to the mRNA, and the SBP2-GTP-EFSec-Sec-tRNA^{Sec}-mRNA complex is hypothesized to orient the selenocysteine at just the correct position to position the tRNA anti-codon to interact with the approaching UGA.³¹ Peptide bond formation is then catalyzed by the ribosomal peptidyltransferase, resulting in the formation of a peptide bond between selenocysteine and the growing polypeptide chain. The location of the SECIS in the 3'UTR provides necessary spacing for orientation of the selenocysteine insertion complex; too short a distance between the UGA and the SECIS reduces or blocks efficient incorporation of selenocysteine.³¹

Biochemical Functions

The logical role for selenium as a trace element is as a catalytic component in enzymes or proteins, and thus biochemical roles of selenium should arise as a consequence of the biological functions of these proteins. Almost all selenoproteins contain the selenocysteine in a variant of the CxxC redox motif, such as UxxT.⁴⁵ Knocking out the tRNA^{Sec}_{UCA} gene is fatal, demonstrating that one or more selenoproteins are essential.⁴⁶ A newly discovered role for a selenoprotein in sperm is the only known structural role.

The absolute requirement of the molecular biology components (described above) for encoding and synthesizing selenoproteins has now been used to screen the human genome and other genomes for selenoproteins. A bioinformatics-based program called the SECIS-search protocol was developed and used by Vadim Gladyshev³ to screen genomes for conserved SECIS elements. The newly identified SECIS elements were then compared with known SECIS elements in orthologous selenoprotein genes in rodents to refine the list. Next, upstream genomic sequences were screened for open reading frames that contained in-frame TGA codons. Lastly, these predicted human selenoprotein genes were further screened for homologs in other species that contained cysteine rather than selenocysteine. The result is that the entire

Table 1. Human Selenoproteins in the Selenoproteome

AQ1

Abbr.	Selenoprotein
GPX1	Classical glutathione peroxidase (GSH-Px) (47)
GPX2	Gastrointestinal glutathione peroxidase (GPX-GI) (52)
GPX3	Plasma glutathione peroxidase (plasma GPX) (53)
GPX4	Phospholipid hydroperoxide GPX (PHGPX) (54)
GPX6	Glutathione peroxidase-6 (3)
DI1	Iodothyronine 5'-deiodinase-1 (Type I DI) (55)
DI2	Iodothyronine 5'-deiodinase-2 (Type II DI) (56)
DI3	Iodothyronine 5-deiodination-3 (Type III DI) (56)
TRR1	Thioredoxin reductase-1 (57)
TRR2	Thioredoxin reductase-2 (58)
TRR3	Thioredoxin reductase-3 (58)
SPS2	Selenophosphate synthetase-2 (59)
SELP	Plasma selenoprotein P (60)
SELW	Muscle selenoprotein W (61)
SELV	Selenoprotein V (paralog of SELW) (3)
SEP15	15 kD selenoprotein (1)
SELR	Methionine-R-sulfoxide reductase (MsrB1) (62)
SELT	Selenoprotein T (18.8 kD, globular) (63)
SELM	Selenoprotein M (localized to golgi, ER) (64)
SELN	Selenoprotein N (47.5 kD) (65)
SELH	Selenoprotein H (globular) (3)
SELI	Selenoprotein I (phosphotransferase) (3)
SELK	Selenoprotein K (plasma membrane protein) (3)
SELO	Selenoprotein O (globular) (3)
SELS	Selenoprotein S (plasma membrane protein) (3)

human “selenoproteome” has now been identified³ ([Table 1](#)). This is the first complete proteome to be identified for any nutrient, and it illustrates the powerful implications of genome science and bioinformatics.

Glutathione Peroxidase-1

GPX was discovered in 1957 by Mills⁴⁷ in his search for factors that protect against oxidative damage to erythrocytes.⁴⁸ GPX was also the first protein shown to contain selenium⁷ and the first cloned selenoprotein. There are now five identified selenium-containing GPXs, all with the UxxT redox motif. Classical GPX (GPX1) is the major form of selenium in the body, found in all tissues,

and is estimated to account typically for more than 50% of total body selenium. Mitochondrial GPX arises from the GPX1 gene.⁴⁹ The selenocysteine is located at residue 47 of the 201 amino acid (23-kD) polypeptide chain, and selenocysteine is the active moiety in the enzyme reaction. GPX1 is potentially important because it not only destroys hydrogen peroxide, but also works on a number of hydroperoxides that might be produced during oxidant damage in the body. In the past decade, however, GPX1-knockout mice have been produced and have been found to display no effects on growth, reproduction, resistance to disease, etc.,⁵⁰ thus indicating that GPX1 does not have a critical role under normal conditions. It has been postulated that GPX1 functions as a biological selenium buffer that can be used to expand and store selenium before excretion mechanisms are activated.^{12,51}

The work with the GPX1-knockout mouse has demonstrated two laboratory conditions where GPX1 activity is still important. GPX1-knockout mice are far more susceptible to acute paraquat toxicity, demonstrating that GPX1 activity can protect against the peroxides generated from this deleterious chemical.⁶⁶ Secondly a Coxsackie virus is more virulent in GPX1-knockout mice than in wild-type mice, just as in selenium-deficient versus selenium-adequate mice,⁶⁷ indicating a role for peroxidases or peroxides in this process (see below).

Glutathione Peroxidase-2

GPX2 or GPX-GI was initially identified from a human liver cDNA library, but now is found to be the predominant GPX species in rat intestine.⁵² This peroxidase is thought to be important in the protection of the intestine against external peroxides. GPX2-knock out mice are viable, indicating that both GPX1 and GPX2 functions appear redundant under normal conditions,⁶⁸ but double-knockout mice lacking GPX1 and GPX2 develop ileocolitis.⁹

Glutathione Peroxidase-3

Plasma GPX3 arises from a distinct GPX3 gene,⁵³ and is predominantly secreted by human kidney. GPX3 normally accounts for 20% of plasma selenoproteins.⁶⁹ This secreted GPX3 is also the major form of selenium in milk,⁷⁰ and the role of GPX3 in milk may be to ensure that milk has adequate selenium levels. The function of GPX3 in plasma is unclear because of the low levels of circulating plasma GSH; it has been hypothesized that the major protective role of GPX3 may be in the intracellular spaces, especially in the kidney.⁷¹

Glutathione Peroxidase-4

Phospholipid hydroperoxide GPX or GPX4 is unique in several aspects. This enzyme is active as a monomer rather than as a tetramer.⁷² In addition, its more open, active site allows it to react with bulky hydroperoxides that are not substrates for the other peroxidases. GPX4 is also lipophilic and is postulated to roll along membranes and destroy peroxides.

GPX4 has been shown to account for the high concentration of selenium present in sperm and testis.⁷³ This GPX apparently is deposited as precipitated selenoenzyme during spermatogenesis, perhaps in response to elevated hydroperoxide production in the absence of sufficient glutathione to keep it reduced. The impact is that the enzyme GPX4 becomes a critical structural protein necessary for the integrity of sperm mid-piece. This may be the first case where we can identify a specific biochemical lesion that causes a selenium-deficiency disease, in this case sperm mid-piece breakage and male infertility. Knocking out GPX4 in mice is embryonically lethal.⁷⁴

Glutathione Peroxidase-6

GPX6 is an odorant-metabolizing protein, with about 40% amino acid sequence identity to GPX1, and appears to be expressed only in Bowman's gland of the rodent olfactory system. The screening of the human genome found that GPX6 in humans and swine is a selenoprotein, whereas orthologs in mice and rats contain Cys in place of selenocysteine.³

GPX7 is another member of the GPX family that so far has only been found as a Cys homolog. The function of these GPX homologs is especially unclear, as enzymatic activity of Cys as opposed to selenocysteine homologs are expected to differ dramatically.

Non-Selenium GPXs

Several members of the GPX family with unknown function have been cloned and found to have a cysteine codon replacing the UGA codon,¹² including GPX5 in humans, which is androgen-regulated epididymal secretory protein that lacks a functional SECIS. The non-selenium dependent GPX activity found in the liver of selenium-deficient humans and animals is due to several of the glutathione-S-transferases.⁷⁵ Levels of this enzyme increase two-fold in selenium-deficient liver when GPX1 is fully depleted.

Iodothyronine Deiodinases

Activation and metabolism of thyroid hormone requires a family of three selenoenzymes, the iodothyronine deiodinases (Table 1). Thyroxine 5'-deiodinase-1 (DI1) in liver is the major enzyme that converts thyroxine (T₄) to triiodothyronine (T₃), and is responsible for the majority of circulating plasma T₃ levels. In selenium deficiency, decreased DI1 activity results in lower T₃ levels, but compensatory feedback raises circulating T₄ levels such that hypothyroidism does not typically occur.⁷⁶ DI1 is a 249-amino acid (27-kD) polypeptide with a UGA encoding selenocysteine at residue 126 in a SxxU motif, and functions as a homodimer with a molecular weight of 55 kD.⁵⁵ Two additional selenium-dependent deiodinases, type II (DI2) and type-III (DI3), are also found in more specialized tissues.⁵⁶ DI2 is found in brain, pituitary, brown adipose tissue, placenta, and skin, and its principal physiological role is local, intracellular production of T₃. DI3 catalyzes the deiodination of the inner ring of T₄ and T₃.

DI3 activity levels are highest in adult brain, skin, and placenta, and in fetal liver, muscle, brain, and central nervous system. The role of DI3 is thought to protect against high levels of T₄ and T₃ by converting them to the inactive rT₃ and T₂, respectively.³¹

Thioredoxin Reductases

Mammalian thioredoxin reductases (TRRs) are a family of three selenium-dependent enzymes that reduce small intracellular molecules, that regulate intracellular redox state, and that may have important roles in antioxidant defense and in control of cell cycling.⁵⁸ Only mammalian TRRs are selenoenzymes. These enzymes transfer reducing equivalents from NADPH through tightly bound FAD to disulfide in the enzyme, and then reduce thioredoxin or other species including selenite. TRRs are 57 kD-subunit (499-amino acid) selenoproteins that contain selenocysteine as the penultimate amino acid in a CU motif.⁵⁷ TRR1 is found in the cytosol and nucleus, whereas TRR2 is found in mitochondria. Selenium deficiency studies in rats have indicated that TRR activity is less affected by selenium deficiency than GPX1 activity, but more affected than plasma selenoprotein-P levels. Loss of TRR activity may be important in the development of the signs and symptoms of selenium deficiency. The discovery that TRR will reduce dehydroascorbate, ascorbate radical, and perhaps vitamin E offers a new potential antioxidant role for selenium.⁷⁷ In addition, TRRs offer a potential biochemical role to explain selenium's anticarcinogenic activity.⁵⁸

Plasma Selenoprotein P

Plasma selenoprotein P (SELP) is the major plasma selenoprotein and normally accounts for about 40% of plasma selenium.⁸ Mature human SELP, secreted primarily from liver, contains 381 amino acids and is glycosylated, resulting in a molecular weight of approximately 57 kD.⁶⁰ Levels of SELP decrease in patients with liver disease, thus accounting for decreased plasma selenium in diseases with reduced liver function. SELP mRNA has 10 open reading frame UGAs and two SECIS stem loops. When selenium is limiting, early termination at the second UGA reduces the selenium content of SELP and results in a smaller circulating protein.

The SELP gene has now been knocked out in mice,⁸ resulting in dramatic decreases in brain and testis selenium concentrations and increases in urinary selenium excretion, which is consistent with the role of SELP as a critical selenium transport protein. Mice lacking SELP develop a lack of coordination leading to paralysis and then death, indicating a critical role for selenium in neurological tissues, and further indicating the importance of delivering selenium to these tissues. Dietary administration of high levels of selenium will prevent these conditions. Knockout mice also have low fertility, and the males cannot be used for breeding even when they are supplemented with high-selenium diets. These studies with

knockout mice thus make it clear that the function of SELP is to deliver selenium to important tissues. In addition, these animals provide models to study critical new roles for selenium in neurological function.

Selenoprotein W

Selenoprotein W (SELW) is small (9.8-kD) selenoprotein found in muscle, which is also postulated to have antioxidant function.⁶¹ The 87-amino acid polypeptide has a UGA-encoded selenocysteine at residue 13, but also can use UGA as a stop codon because the stop-UGA is too close to the SECIS to allow selenocysteine insertion.³¹ The protein was definitively identified after a more than two-decade search for the selenium-dependent factor that would explain white muscle disease in selenium-deficient sheep.⁵ mRNA levels indicate that SELW is abundant in muscle and brain of primate and sheep but not rodents. The role of SELW is unknown, but may involve the tightly bound GSH often isolated with purified SELW, and/or involve the CxU motif in a catalytic role.¹

Selenoprotein V

Selenoprotein V (SELV) is a paralog of SELW that was discovered in the SECIS search of the human genome. Expression of SELV mRNA is restricted to testis, where it occurs in the seminiferous tubules of mouse testis.

Selenoprotein R or Methionine-R-Sulfoxide Reductase

Methionine sulfoxide is produced during oxidative attack of proteins, so methionine-sulfoxide reductases are an essential component in coping with oxidative stress. SELR encoded a small (12-kD) selenoprotein that contains zinc and reduces methionine-R-sulfoxide but not methionine-S-sulfoxide. SELR is now designated MsrB1.⁶² Two other MsrB genes are present in mammalian genomes, but these MsrB genes contain cysteine rather than selenocysteine. An additional human gene, MsrA, encodes a cysteine-containing enzyme that is specific for methionine-S-sulfoxide.

SEP15

A 15-kD selenoprotein, SEP15, was initially purified from human T-cells as a ⁷⁵Se-labeled protein.¹ The gene for SEP15 is apparently universally expressed in mammalian cells, and may be involved in protein folding. The protein is differentially expressed in tumor cells, and single nucleotide polymorphisms in the SEP15 gene suggest that the allelic frequency may vary with susceptibility to cancer.

Selenophosphate Synthetase-2

As discussed above, one of the mammalian selenophosphate synthetases, SPS2, is a selenocysteine-containing selenoprotein. SPS2 may use selenium from selenite reduction, whereas SPS1 appears to be associated with recycling selenium from selenocysteine.⁷⁸

Selenoprotein T

Selenoprotein T, discovered using the SECIS-search program, is a 182-amino acid globular selenoprotein with no known function.⁶³

Selenoprotein M

The discovery of SELM in a mammalian EST database led to the discovery in the 3'UTR of a second SECIS form with an AUGA...CC...GA motif. The N-terminal protein contains a signal peptide that locates the selenoprotein to the Golgi and endoplasmic reticulum, and SELM mRNA is present in multiple organs, especially in brain, kidney, and uterus.⁶⁴

Selenoprotein N

Selenoprotein N is a selenoprotein with no homology to any known protein.⁶⁵

Selenoprotein H

Selenoprotein (SELH) is a new globular selenoprotein with no known function. SELH was shown to be a selenoprotein by transfecting the gene into mammalian cells and demonstrating ⁷⁵Se labeling of the expressed selenoprotein.³

Selenoprotein O

Selenoprotein O (SELO) is another globular selenoprotein identified using the SECIS search program. SELO is the largest human selenoprotein (669 residues), with a selenocysteine located three residues from the C terminus in a CxxU motif. The SECIS also has the AUGA...CC...GA motif.³

Selenoprotein I

Selenoprotein I is another new selenoprotein identified by a SECIS search. SELI is homologous to human and yeast choline/ethanolamine phospho transferases, and has seven putative transmembrane domains.³

Membrane Selenoproteins K and S

Selenoprotein K (SELK) and selenoprotein S (SELS) are newly identified selenoproteins that are unique because the amino acid sequence predicts that they are membrane proteins. GFP fusion constructs of SELK and SELS expressed in mammalian cells localize to the plasma membrane, indicating that these are the first demonstrated membrane selenoproteins. SELK and SELS mRNAs are found in a variety of mouse tissues.³

Selenoprotein S gene expression is increased by glucose deprivation and/or disturbances in the endoplasmic reticulum that generally cause the accumulation of misfolded proteins. Thus, SELS appears to be a novel member of the glucose-regulated protein family. Overexpression of SELS can significantly increase cell tolerance to oxidative stress, suggesting that it may have a role in regulating reactive oxygen species.⁷⁹

The picture emerging from the evaluation of various genomes for selenoproteins reveals that they selenoprot-

eins have a scattered phylogenetic distribution. Methionine-S-sulfoxide reductase (MsrA) occurs as a selenoprotein in *Chlamydomonas reinhardtii*, a green algae, but contains cysteine in vertebrates. A novel selenoprotein family, named SELU, was recently identified in the puffer fish, but mammals, worms, and land plants contain the cysteine homolog. Humans contain three separate genes in the SELU family, each encoding a cysteine-containing protein.⁸⁰ No selenoproteins have yet been found in yeast and land plants, but at least three selenoproteins have been found in insects.⁸¹

A new survey of bacterial genomes found in the Sargasso Sea identified 310 selenoprotein genes in 25 families, including 101 new selenoproteins. The sporadic distribution suggests that the many selenoproteins evolved recently from cysteine-containing homologs. In addition, eukaryotic and bacterial selenoprotein sets partially overlap, suggesting that lateral transfer of selenoprotein genes occurs across wide phylogenetic distances.⁸²

Biochemical Role in Protection Against Viral Infection

A series of exciting studies by Beck⁸³ have demonstrated that a virulent Coxsackie virus B3 (CVB3/20) that induces myocardial lesions in the hearts of mice is more virulent in selenium-deficient than in selenium-supplemented mice. Infection of mice with a benign amyocarditic Coxsackie virus B3 (CVB3/0), which causes no pathology in the hearts of selenium-adequate mice, induces extensive cardiac pathology in selenium-deficient mice. Most interestingly, the virus recovered from the hearts of selenium-deficient mice and inoculated into selenium-adequate mice induces significant heart damage. Characterization of the isolated virus indicates that the avirulent virus has mutated back to the wild-type genotype. The GPX1-knockout mouse also shows increased susceptibility to viral infection.⁸⁴ Notably, the same susceptibility to an avirulent Coxsackie virus also occurs in vitamin E-deficient mice, suggesting that protection is not limited to selenium-dependent proteins. Recent work suggests that selenium-deficient mice will also have increased susceptibility to other viral infections.⁶⁷ Along with Keshan disease, these studies add an anti-viral component to selenium's roles in preventing human disease.

A note of caution is warranted here. A number of reports show decreased levels of plasma selenium in patients with AIDS, and the suggestion has been made that nutritional selenium supplementation may be helpful.²⁶ Shisler et al.⁸⁵ found a human skin poxvirus, *Molluscum contagiosum*, that acquired in its genome a cDNA sequence for mammalian GPX1. The discoverers propose that the poxvirus expresses the captured mammalian GPX1 as a counter-measure against the host's anti-virus mechanisms, which include peroxidative stimulation of programmed cell death. This scenario thus suggests that indiscriminate supplementation of AIDS patients with selenium may aid the virus during infection by providing

excess selenium for this pox GPX1, which in turn could block full activation of anti-virus mechanisms.

Mechanism of Selenium Regulation

The 25 selenoproteins are all part of the well-regulated selenocysteine compartment and constitute a high percentage of total body selenium. The underlying mechanisms responsible for tight regulation of this compartment and thus responsible for the uniformity of dietary selenium requirements across species, are emerging in molecular biology studies conducted in laboratory animals and in cultured cells.⁸⁶ The effect of selenium status on multiple biochemical and molecular biology parameters in a single model is best characterized in detail in the rat. Selected response curves (Figure 4) illustrate the differential regulation of selenoprotein expression likely to also underlie human response to selenium status. These curves illustrate where changes in a biomarker such as plasma GPX3 reside in this hierarchy of responses.

In selenium-deficient male rats, liver GPX1 activities and mRNA levels can decrease to 1% and 7% of that of selenium-adequate animals.⁸⁷ In progressive selenium deficiency in rats, there is a coordinated exponential drop in GPX1 mRNA ($t_{1/2} = 3.2$ d), GPX1 activity ($t_{1/2} = 3.3$ d), and GPX1 protein ($t_{1/2} = 5.0$ d).⁸⁸ When young, rapidly growing weanling rats are fed a selenium-deficient diet and supplemented with graded levels of selenium as Na_2SeO_3 for 28 days, liver GPX1 activity and mRNA levels respond sigmoidally to increasing dietary selenium concentration,^{87,89} with GPX1 activity and mRNA reaching plateaus at 0.1 and 0.05 μg Se/g diet, respectively (Figure 4). At concentrations greater than 0.1 μg Se/g diet, selenium status no longer regulates either

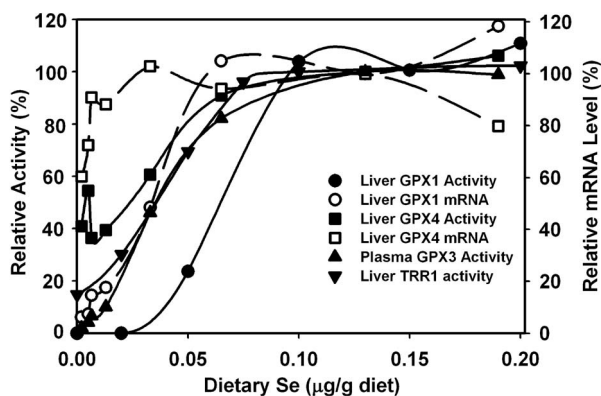


Figure 4. Response curves for GPX1, GPX4, GPX3 and TRR1 in rats. Weanling rats were fed the indicated levels of dietary selenium for 4 weeks. Shown are relative liver GPX1 activity and mRNA, liver GPX4 activity and mRNA, plasma GPX3 activity, and liver TRR1 activity (87,89,93). The hierarchy in terms of dietary selenium necessary to reach plateau levels (lowest to highest) is: GPX4 mRNA < GPX1 mRNA, GPX4 activity, TRR1 activity < GPX3 activity < GPX1 activity.

GPX1 mRNA or GPX1 activity. In contrast, liver GPX4 activity only decreases to 41% of selenium adequate levels and reaches a plateau at 0.05 μg Se/g diet; liver GPX4 mRNA is not significantly affected by dietary selenium. Plasma GPX3 activity in selenium-deficient rats is 7% to 8% of the levels found in selenium-adequate animals, and reaches a plateau at 0.07 μg Se/g diet,⁸⁹ such that when liver GPX1 mRNA is at the plateau, plasma GPX3 activity is about 75% of its plateau. In other studies, rat liver DI1, SELP, and TRR1 activities all decrease to 5% to 10% of selenium-adequate levels, but the corresponding mRNA levels are only modestly affected.⁹⁰⁻⁹³ These experiments clearly show the tight regulation of selenoproteins by selenium status in mammalian species, and also illustrate clear differences in the regulation of individual selenoproteins.

The novel changes in GPX1 mRNA occur because GPX1 mRNA concentration is regulated by mRNA stability; in selenium deficiency, GPX1 mRNA is specifically degraded by a process called non-sense-mediated decay.^{94,95} Decreases in the levels of all selenoproteins in selenium deficiency appear to arise because of reduced protein synthesis when insufficient selenium and thus selenocysteine is available.⁸⁶ In addition, potential effects of age, pregnancy, lactation, and gender may affect selenoprotein transcription unrelated to selenium status,⁹² and should be considered when evaluating selenoprotein markers for the assessment of selenium status. With the sequencing of the human genome and the development of array approaches, mRNA-based evaluation of nutrient status will likely become a common approach, so selenoprotein mRNA levels will likely become useful parameters for assessing human selenium status in the future.

Requirements

The earlier sections in this chapter have discussed a number of direct and indirect measures that could be used to determine selenium status and requirements. Tissue concentration, a measure often used for other nutrients, is particularly unsuitable for selenium because the unregulated selenomethionine compartment reflects selenomethionine intake, not selenium status. In contrast, early studies using biochemical assays in laboratory animals suggest that selenoenzyme expression is highly regulated by selenium status and is very useful for assessing status and requirements.

The current Recommended Dietary Allowance (RDA) requirement for selenium is one of two human dietary requirements for mineral elements that is based on a biochemical parameter² as opposed to diet assessment, balance studies, tissue mineral content, etc. This illustrates the importance of selenium regulation of GPX expression. In 1980, an initial estimated safe and adequate daily dietary intake for humans of 50 to 200 μg Se/d was proposed, based upon extrapolation from animal experiments that used GPX activity to assess selenium status.⁹⁶

Balance studies to determine selenium requirements are of little help, because selenium's homeostatic mechanisms permit human subjects to come to balance between 9 and 200 $\mu\text{g Se/d}$ ^{24,97} by adjusting urinary excretion to match intakes. In 1989, the Food and Nutrition Board of the US Institute of Medicine (FNB) set an RDA of 70 and 55 $\mu\text{g Se/d}$ for North-American males and females, respectively.⁹⁸

2000 RDA

The FNB in 2000² reevaluated plasma GPX3 activity data from a study in Chinese men with very low basal selenium intakes (about 10 $\mu\text{g Se/d}$) who were supplemented with graded levels of selenium. Groups supplemented with 30 $\mu\text{g Se/d}$ or higher (a total of 40 $\mu\text{g Se/d}$) reached plateau levels of plasma GPX3 activity, indicating that 40 $\mu\text{g Se/d}$ was the minimum requirement. Adjusting this value for the standard weight of North-American males, the Board estimated a minimal selenium requirement for maximal plasma GPX3 of 52 $\mu\text{g Se/d}$.²

The FNB also reevaluated a second study from New Zealand²⁷ that used 52 subjects consuming an average of 28 $\mu\text{g Se/d}$ and supplemented with 0 to 40 $\mu\text{g Se}$ as selenomethionine. The authors concluded that the plateau in plasma GPX3 activity was reached at a total intake (diet plus supplement) of 58 or 68 $\mu\text{g Se/d}$, but the Board's independent analysis of the data² found that plasma GPX3 activity increased for nearly every individual supplemented with selenium. Furthermore, differences between groups in plasma GPX3 activity were small relative to the variations between individual values within the same supplementation group, such that the increase in the lowest supplementation group (38 $\mu\text{g total Se/d}$) was not statistically different from that of the highest supplementation group (68 $\mu\text{g Se/d}$). Thus, the FNB conservatively concluded that these data could only support an Estimated Average Requirement (EAR) of 38 $\mu\text{g Se/d}$. The FNB used an average of these two studies to choose an EAR of 45 $\mu\text{g Se/d}$ for men. Because Keshan disease suggests that women have a greater susceptibility to selenium deficiency, an EAR of 45 $\mu\text{g Se/d}$ was retained for women in spite of smaller body weight. The RDA, based on this average EAR, was calculated to be 55 $\mu\text{g Se/d}$ for North-American men and women. An Adequate Intake (AI) was estimated to be 15 and 20 $\mu\text{g Se/d}$ for babies under 6 and 12 months, respectively, based on typical human milk selenium levels. Estimates for children of other ages were extrapolated from the adult values. Human EAR and RDA during pregnancy were estimated to be 49 and 60 $\mu\text{g Se/d}$, based on calculated fetal selenium deposition of 4 $\mu\text{g Se/d}$, and human EAR and RDA during pregnancy were estimated to be 59 and 70 $\mu\text{g Se/d}$, based on an estimated additional need of 14 $\mu\text{g Se/d}$ for selenium deposition in milk.²

The RDA for selenium in the United States, however, may be high. Comparison of dietary selenium intakes in adult Chinese populations in areas susceptible to Keshan

disease versus areas seemingly protected, suggests a protective level of 21 $\mu\text{g Se/d}$ for 65-kg males and 16 $\mu\text{g/d}$ for 55-kg females.⁹⁹ New Zealand estimates of daily selenium intakes that are not associated with any selenium deficiency symptoms suggest that 33 and 23 $\mu\text{g/d}$ for men and woman, respectively, are adequate.¹⁴ In addition, it is clear that daily selenium consumption in the rest of the world is well below the 2000 RDA recommendations without apparent adverse impact on health.

World Health Organization Requirement

The World Health Organization (WHO) proposed a new approach to evaluate selenium requirements, again based on plasma GPX3 activity as the biochemical parameter, by selecting a normative requirement for selenium calculated by estimating the dietary intake needed to achieve two-thirds of the maximum attainable activity of GPX3⁹⁹: 26 $\mu\text{g Se/d}$ for a 65-kg male. Further adjustment for 16% interindividual variation results in a calculated 40 $\mu\text{g Se/d}$ for adult males and 30 $\mu\text{g Se/d}$ for adult females as lower limits of the safe range of population mean intakes.⁹⁹

These WHO requirements are still below the 2000 RDA recommendations, but are far more in line with typical selenium consumption worldwide. When the authors of the New Zealand study²⁷ used a criterion of 67% of maximal GPX3 activity to establish a requirement, this approach yielded a normative requirement of 39 $\mu\text{g Se/d}$, almost identical to the WHO normative requirement. Additional studies to determine the nature of human selenium response curves, similar to those in Figure 4, should help to link selenium requirements more closely to the underlying homeostatic mechanisms.

Human Requirements Based on SELP

A new study has further carefully evaluated the human selenium requirement in selenium-deficient subjects in China³⁰; 120 subjects were supplemented with graded levels of selenium up to 66 $\mu\text{g Se/d}$ as selenite or selenomethionine for 120 days. These individuals were consuming an average of 9 $\mu\text{g Se/d}$ for women and 11 $\mu\text{g Se/d}$ for men and had plasma selenium values of 0.28 $\mu\text{mol Se/L}$ (22 $\mu\text{g/L}$) or 18% of US levels (Figure 3). Plasma GPX3 values were 40% of US levels, whereas SELP levels were 23% of US levels, clearly indicating that these subjects were selenium deficient. Several interesting aspects arose from this study. First, full expression of plasma GPX3 was achieved with 37 $\mu\text{g Se/d}$ as selenomethionine, which is consistent with earlier estimates, and thus a total intake of 47 $\mu\text{g Se/d}$. Interestingly, selenite supplementation in this study required 66 $\mu\text{g Se/d}$ to achieve plateau levels, clearly indicating a dramatic difference in the bioavailability of inorganic selenite selenium compared with selenomethionine selenium in these subjects when provided in as a single dietary pill per day. Plasma SELP levels were also very low, but continued to increase with increasing dietary selenium at all levels of Se, indi-

cating that apparent US plateau levels could not be achieved for plasma SELP.

Because SELP appears to be a transport form of selenium, it is not clear whether this indicates that selenium supplementation as high as 66 $\mu\text{g Se/d}$ is suboptimal in these subjects, or that these initially selenium-deficient subjects were still not at steady-state, or that the SELP transport protein cannot be saturated at these levels. Whatever the case, it is clear that supplementation in acutely selenium-deficient individuals over 120 days is not sufficient to raise selenium parameters up to those seen in Americans consuming 98 to 145 $\mu\text{g Se/d}$.

Dietary Sources

There is a wide range in selenium content in soils, and thus the selenium content in foods of plant origin varies widely. Cereals and grains range from <0.1 to >0.8 $\mu\text{g Se/g}$, and fruits and vegetables are typically <0.1 $\mu\text{g Se/g}$.⁹⁹ Corn, rice, and soybeans grown in areas of China where Keshan disease is prevalent had 0.005, 0.007, and 0.010 $\mu\text{g Se/g}$, respectively, whereas in a seleniferous area in China they had 8.1, 4.0, and 11.9 $\mu\text{g Se/g}$, respectively.¹⁰⁰ Selenium content of livestock animals will similarly be affected. Organ meats and seafood can range 0.4 to 1.5 $\mu\text{g Se/g}$, muscle meats 0.1 to 0.4 $\mu\text{g Se/g}$, and dairy products <0.1 to 0.3 $\mu\text{g Se/g}$.⁹⁹ Thus, handbook values for selenium in foods reflecting average content should be considered unreliable unless confirmed by actual analysis.² As livestock in the United States are typically supplemented with inorganic selenium, animal products will usually contain substantial selenium as selenoproteins and thus as selenocysteine. Thus, the range in selenium content of animal products reflects variations in selenomethionine content of the feedstuffs.

Dietary supplements and foods today are often supplemented with selenium. Inorganic selenium, generally selenite and sometimes selenate, is used most commonly, but selenized yeast or selenomethionine is also used. Supplementation levels are typically under 100 $\mu\text{g Se/pill}$, such that total selenium intakes will still be <250 $\mu\text{g Se/d}$ based on reported average US intakes. The impact of supplements on daily dietary selenium intake was $<3\%$ in the NHANES III study.²

Drinking water usually has negligible selenium content. Well waters in seleniferous areas in Wyoming and South Dakota can contain much higher levels.¹⁰¹

Vitamin E

Vitamin E and selenium have been inexorably linked since of the discovery that selenium would prevent liver necrosis. Interest remains high because of the links between reactive oxygen species and aging. The overlapping roles of these two nutrients are illustrated by seminal studies comparing the efficacy of selenium and vitamin E in preventing lipid peroxidation, as measured by volatile gas

evolution and formation of F₂-isoprostanes, and in modulating Coxsackie virus virulence (see above). Unfortunately, direct evidence linking vitamin E and selenium to aging remains tenuous. As discussed above, selenium-dependent TRR may be important in vitamin E recycling.⁷⁷

Early studies showed that combined selenium and vitamin E deficiency (double deficiency) results in elevated levels of tissue malondialdehyde, which arises from the free radical attack on polyunsaturated fatty acids. More specific indicators of peroxidation, ethane and pentane evolution in the breath, are due to peroxidative breakdown of Ω -3 or Ω -6 unsaturated fatty acids, respectively. Ethane and pentane evolution is minimized by vitamin E alone, and partially reduced by 0.2 $\mu\text{g Se/g}$ diet alone to 40% of the rate in doubly-deficient rats.¹⁰²

F₂-isoprostanes are an exciting new marker of *in vivo* peroxidation. These prostaglandin F₂-like compounds result from the free radical-catalyzed peroxidation of arachidonic acid *in vivo*. These F₂-isoprostanes are found esterified to phospholipids in tissues and are also found as free F₂-isoprostanes in plasma. Plasma F₂-isoprostanes in rats fed a double vitamin E/selenium-deficient diet are 5-fold higher than animals fed the control diet. Selenium deficiency alone is not associated with the production of F₂-isoprostanes, but the plasma F₂-isoprostane level is twice that of control rats in the vitamin E-deficient alone group. F₂-isoprostanes present in phospholipids in various tissues also show similar results, with selenium deficiency exacerbating vitamin E deficiency in most tissues, whereas selenium deficiency alone is without effect compared with rats adequate in vitamin E and selenium.¹⁰³

Toxicity

Selenium was first known as a toxic element, due to high soil levels resulting in the accumulation of selenium in plants, which then caused chronic and acute toxicity in livestock.⁵ The range of dietary selenium concentrations that are adequate and yet not toxic is very narrow. In rats, while the minimum dietary requirement is 0.1 $\mu\text{g Se/g}$ diet, dietary levels above 2 $\mu\text{g Se/g}$ diet are chronically toxic, resulting in a factor of 20 between the requirement and the onset of toxicity. Inorganic selenium and amino acid forms are readily available and toxic, whereas methylated forms such as trimethyl selenonium chloride and dimethyl selenide are one to three orders of magnitude, respectively, less toxic. The gas hydrogen selenide is the most toxic form of selenium. There is no evidence for the existence of homeostatic mechanisms to decrease selenium uptake under toxic dietary conditions.¹²

The biochemical mechanism underlying selenium toxicity is unknown. In one known case, inorganic selenium inactivates eukaryotic initiation factor-2 α ,¹⁰⁴ but further details are not known.

In humans, modest selenium intakes (<800 $\mu\text{g Se/d}$) are clearly not toxic.¹⁰⁵ In seleniferous regions of the

United States (South Dakota and Wyoming), a study evaluating 142 subjects consuming as much as 724 μg Se/d found no evidence of selenium toxicity. Consumption of well water, with inorganic selenium levels as much as 50 times the US drinking water standard of 10 μg Se/L, results in increased urinary selenium but without increased blood selenium content, illustrating that blood selenium cannot be used as a good marker for high selenium intakes.¹⁰¹

There are only a few reports in the literature of fatal or near-fatal acute selenium poisoning, and these are associated with ingestion of gun blueing or sheep drench solutions.² Ingestion of gram quantities of selenium, for which there is no ready antidote, can result in severe gastrointestinal and neurological disturbance, acute respiratory distress syndrome, myocardial infarction, and renal failure. Selenosis, or chronic selenium toxicity, is associated most commonly in humans with changes in nail structure and loss of nails and hair.² With continuous intakes and higher doses of excess selenium, symptoms can include lesions of the skin and nervous system, nausea, weakness and diarrhea, and mottling of the teeth. These toxicity symptoms are present with selenium intakes ranging from 3200 to 6700 μg Se/d. Milder morphological changes in the fingernails also will occur in individuals consuming an average of 1260 μg Se/d.^{105,106}

A study conducted in a seleniferous region of China continues to be used to calculate the no-observed-adverse-effect level (NOAEL) for selenium, using nail morphology as the endpoint. Mildly prolonged prothrombin times and reduced glutathione concentrations were observed in these Chinese subjects, but were not found to be reliable indicators of selenosis. A lowest observed adverse effect was found at 913 μg Se/d, with 853 μg Se/d being the level calculated to not be accompanied by adverse effects.¹⁰⁶ Based on these studies, the FNB has recently selected a NOAEL of 800 μg Se/d.² Using an uncertainty factor of two, this results in a tolerable upper intake level (UL) of 400 μg Se/d for adults, including pregnant and lactating women.

Selenium intoxication due to misformulation of supplements can also occur. In the early 1980s, 13 people were identified who had been taking over-the-counter dietary selenium supplements containing 27.3 mg Se/tablet (182 times higher than the amount specified on the label). One individual took one pill a day for a 2.5-month period in spite of symptoms of selenium toxicity.¹⁰⁷

Selenium is especially toxic to waterfowl. The Kesterson Reservoir, located in the San Joaquin Valley, gained considerable notoriety in the 1980s for environmental selenium toxicity. A high incidence of dead and deformed newborn and adult waterfowl was observed at the reservoir, and selenium was identified as the probable cause. The origin of the selenium was the high-selenium soils that led to an average concentration of 350 μg Se/L in the runoff, and further concentrated in the reservoir.¹⁰⁸ This toxicity is specific to birds, apparently because embryos in the egg have limited ability to excrete the selenium.

Cancer

The association between dietary selenium and cancer protection began in the 1960s and 1970s⁶ with the observation that country-by-country selenium intakes were inversely associated with cancer incidence. There is considerable evidence that selenium supplementation at levels that are chronically toxic (2–5 μg Se/g diet) will decrease the tumor incidence in several animal models.

Excitement about selenium's anti-carcinogenic role rose in the 1980s were due to a retrospective study using prediagnostic serum selenium concentrations.¹⁰⁹ Subjects in the lowest quintile of serum selenium concentration had a cancer risk that was twice as high as those in the highest quintile. Biologically, however, there was little difference in the average serum selenium values in cancer versus non-cancer subjects (129 vs. 136 μg Se/L). A number of additional studies have since also concluded that low selenium status is associated with increased cancer incidence,²⁶ although at least six case-controlled, prospective studies using tissue selenium and breast cancer incidence have provided no evidence for a protective effect of selenium.¹¹⁰ A clear impact of selenium, however, was observed in a prospective study of 33,700 men on selenium status and prostate cancer incidence, with men in the lowest quintile of selenium status (as assessed by toenail selenium) having three times the risk of developing advanced prostate cancer as those in the highest selenium status quintile.¹¹¹ Collectively, these studies suggest that subjects who self-select foods with lower selenium content have a higher risk of developing cancer.

Two recent intervention trials have further drawn attention to selenium and cancer. A randomized trial with 1312 patients with histories of basal cell or squamous cell carcinomas of the skin were supplemented with either an oral supplement of 200 μg Se/d or a placebo.¹¹² Selenium supplementation did not significantly reduce the primary endpoints of incidence of new basal or squamous cell carcinoma of the skin, and actually significantly increased the risk of squamous cell carcinoma (25%) and total non-melanoma skin cancer (17%).¹¹³ In the initial analysis,¹¹² however, selenium treatment was associated with a statistically significant reduction in several secondary endpoints that were not the focus of the study (total and lung cancer mortality, total cancer incidence, colon rectal cancer, and prostate cancer incidence); total cancer incidence was 42% lower in the selenium group ($P < 0.001$).¹¹² Analysis of the complete study,¹¹⁴ however, found that selenium supplementation reduced total cancer and prostate cancer risk but not lung and colorectal cancer incidence, and the cancer-protective effect was confined to males. Furthermore, only subjects with plasma selenium levels in the lowest two tertiles at entry into the trial ($< 1.54 \mu\text{mol}$ Se/L or $< 121.6 \mu\text{g}$ /L) experienced total reduction in cancer incidence, whereas those in the highest tertile showed an elevated incidence.¹¹⁴

Randomized nutrition intervention trials involving

nearly 30,000 participants over 5 years were conducted in a rural county in north central China. These studies found a small but significant reduction in total mortality in subjects receiving a combination of 15 mg β -carotene, 50 μ g Se as selenized yeast, and 30 mg α -tocopherol, whereas no appreciable effects were found for other supplements, which included retinol, zinc, riboflavin, niacin, ascorbate, and molybdenum.¹¹⁵

Collectively, these studies in humans and animals indicate an overall inverse relationship between cancer risk and selenium status. US prospective studies using supplementation of 200 μ g Se/d support a hypothesis that supplemental selenium above the levels needed to maximize selenoprotein levels is beneficial in reducing cancer risk, especially for prostate cancer. This hypothesis is especially intriguing for residents of countries with selenium intakes that will not maximize selenium incorporation into all selenoproteins.²⁶ However, at this time, small sample sizes, other confounding factors, and potential for excess selenium supplementation to raise cancer risk collectively do not allow use of these studies as the basis to set a higher RDA.²

Future Directions

The identification of the complete selenoproteome in humans using molecular biology and bioinformatics approaches shows that a full understanding of selenium's role in health and disease is just around the corner. Continued use of knockout animals and genome-based discovery of inborn errors of selenium metabolism have promise in identifying the specific roles of selenium in protection against human and animal diseases. Identification of the transporters involved in selenium absorption and excretion, and characterization of the regulation of selenium excretion are needed to more fully understand selenium homeostasis. A complete characterization of patterns of regulation of selenoprotein expression in humans by selenium status, including regulation of mRNA levels, is needed to better identify the optimum parameter(s) for use as biomarkers for establishing human selenium requirements and for use in individualized medicine. Similarly, fuller characterization of the pathways activated in selenium toxicity are needed so that good biomarkers of selenium toxicity can be identified. The impact of selenium deficiency and selenium supplementation on viral infection, including AIDS, should be assessed. Lastly, results from ongoing large trials are needed, both in the United States and in other countries, to directly answer the question about cancer and higher levels of selenium supplementation.

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