Adaptive dysfunction of selenoproteins from the perspective of the triage theory: why modest selenium deficiency may increase risk of diseases of aging

Joyce C. McCann[1] and Bruce N. Ames[1]

Nutrition and Metabolism Center, Children's Hospital Oakland Research Institute, Oakland, California, USA

ABSTRACT **The triage theory proposes that modest deficiency of any vitamin or mineral (V/M) could increase age-related diseases. V/M-dependent proteins required for short-term survival and/or reproduction (*i.e.*, "essential") are predicted to be protected on V/M deficiency over other "nonessential" V/M-dependent proteins needed only for long-term health. The result is accumulation of insidious damage, increasing disease risk. We successfully tested the theory against published evidence on vitamin K. Here, we review about half of the 25 known mammalian selenoproteins; all of those with mouse knockout or human mutant phenotypes that could be used as criteria for a classification of essential or nonessential. Five selenoproteins (Gpx4, Txnrd1, Txnrd2, Dio3, and Sepp1) were classified as essential and 7 (Gpx1, Gpx 2, Gpx 3, Dio1, Dio2, Msrb1, and SelN) nonessential. On modest selenium (Se) deficiency, nonessential selenoprotein activities and concentrations are preferentially lost, with one exception (Dio1 in the thyroid, which we predict is conditionally essential). Mechanisms include the requirement of a special form of tRNA sensitive to Se deficiency for translation of nonessential selenoprotein mRNAs except Dio1. The same set of age-related diseases and conditions, including cancer, heart disease, and immune dysfunction, are prospectively associated with modest Se deficiency and also with genetic dysfunction of nonessential selenoproteins, suggesting that Se deficiency could be a causal factor, a possibility strengthened by mechanistic evidence. Modest Se deficiency is common in many parts of the world; optimal intake could prevent future disease.—McCann, J. C., Ames, B. N. Adaptive dysfunction of selenoproteins from the perspective of the triage theory: why modest selenium deficiency may increase risk of diseases of aging. *FASEB J.* 25, 1793–1814 (2011). www.fasebj.org**

Key Words: nutrition · micronutrients · cancer · heart disease · infection

EACH OF THE ~30 KNOWN vitamins and minerals (V/Ms) used in metabolism are required for life. The triage theory (1–3) adapts basic principles of the "disposable soma" evolutionary theory of aging (4) to highlight the potential importance of V/M biology in diseases of aging, such as cancer, heart disease, osteoporosis, and dementia. Specifically, the triage theory posits that when the dietary availability of a V/M is moderately inadequate, nature ensures that V/M-dependent functions that are essential from an evolutionary perspective (*i.e.*, required for short-term survival and/or reproduction) are protected at the expense of those that are less essential (*i.e.*, whose lack does not have acute short-term negative consequences but may have long-term insidious effects that increase risk of diseases associated with aging). The triage theory does not imply that any particular V/M deficiency is the only cause of an age-related disease but rather that it is a contributing factor along with the sum of all contributing causal factors.

If the theory is correct, it has important implications for public health. For example, it is common for metabolic pathways to include at least one enzyme that requires one or more V/Ms for activity, and most people are moderately deficient in one or more V/Ms (1, 2, 5). Modest deficiency occurs not only in poor countries but also in the United States (especially in the poor, obese, and elderly), caused in part by consumption of calorie-rich V/M-poor unbalanced diets (1). Societal concern is low because modest V/M deficiency is not accompanied by overt clinical symptoms. However, the triage theory proposes that insidious changes are occurring that, over the long term, lead to increased risk of diseases of aging.

We are analyzing published evidence relevant to the functional spectrum of individual V/Ms to test 3 predictions of the theory. Prediction 1: essential V/M-

[1] Nutrition and Metabolism Center, Children's Hospital Oakland Research Institute (CHORI), 5700 Martin Luthur King Jr. Way, Oakland, CA 94609, USA. E-mail: B.N.A., bames@chori.org; J.C.M., jmccann@chori.org

doi: 10.1096/fj.11-180885

This article includes supplemental data. Please visit *http://www.fasebj.org* to obtain this information.

dependent proteins are more resistant to V/M deficiency than those that are nonessential. Prediction 2: modest V/M deficiency is prospectively associated with diseases of aging. Prediction 3: decreased functionality of nonessential V/M-dependent proteins contributes causally to increased risk of diseases of aging.

These predictions are not testable unless V/M-dependent proteins can first be categorized as "essential" or "nonessential." Since, from the perspective of the triage theory, an essential V/M-dependent protein is defined as "required for survival to reproductive age or required to reproduce," mouse knockout (KO) phenotypes provide a convenient (though imperfect) tool for classification. Proteins with a KO phenotype indicating either prenatal lethality or postnatal loss of fertility can be classified essential, and proteins with KOs that survive and are fertile can be classified nonessential. Homozygous human mutants resulting in complete loss of a protein are, of course, most directly relevant to essentiality in humans but such mutant phenotypes are often not available. See several reviews for detailed discussion of selenoprotein (SP) KOs (6–9).

For an illustration and further discussion of the general approach, we have taken to test the triage theory, see our recently published analysis of vitamin K (vitK; ref. 3), results of which strongly supported predictions 2 and 3. Some results were also supportive of prediction 1, although only limited evidence was available. The SP evidence base is much more extensive than that for vitK, allowing a more adequate test.

BACKGROUND

In mammals, the great majority of dietary selenium (Se) is incorporated as selenocysteine during translation at the active site of 25 SPs in humans (24 in rodents). This process is highly complex and still incompletely understood. It depends on the reinterpretation of UGA on SP mRNAs, normally a stop codon, as selenocysteine. Factors required to accomplish this "recoding" include an unusual mRNA stem-loop structure present in all SP mRNAs called the selenocysteine insertion sequence (SECIS) element, an elongation factor (EFSec), SECIS-binding protein 2 (SBP2) that binds to the SECIS element and promotes selenocysteine insertion from Sec $tRNA^{[Ser]Sec}$ (selenocysteine tRNA) into the growing peptide chain, and other factors. For a thorough discussion of these and other aspects of SP biosynthesis, see several reviews in refs. 10–12. The substitution of Se for sulfur at the catalytic site has a major effect on enzyme potency (13) and can increase efficiency by >100-fold (14, 15). SPs are required for life, as demonstrated by the lethality of mouse KOs unable to incorporate Se into SPs (16), and by severe impairment if SP synthesis is knocked out in specific tissues (*e.g.*, refs. 17, 18). About a dozen SPs are well characterized, most of which are enzymes involved in redox regulatory pathways and maintenance of optimal redox status. For an overview of the biology of SPs, see several reviews (11, 19–22).

This review focuses on 12 SPs with known mouse or human mutants with phenotypes that permit a classification of essential or nonessential. These 12 SPs include representatives of all three major classes of SPs [4 glutathione peroxidases (Gpx1, Gpx2, Gpx3, and Gpx4), 2 of the 3 thioredoxin reductases (Txnrd1 and Txnrd2), and all 3 deiodinases (Dio1, Dio2, and Dio3)], as well as the Se transport protein selenoprotein P (Sepp1), the methionine-*R*-sulfoxide reductase selenoprotein R (Msrb1 or SelR), and selenoprotein N (SelN), a redox-active SP involved in muscle development and calcium homeostasis. The nomenclature used in this review, major tissue expression profiles, subcellular locations, primary functions, and mouse KO or human homozygote phenotypes of these 12 SPs are in **Table 1** (14, 20, 21, 23–76).

SP hierarchies

The protection against Se deficiency of some tissues and SPs at the expense of others has been of great interest in Se biology for >2 decades (*e.g.*, ref. 77). There are at least 2 layers of hierarchies, as discussed in many reviews (6, 10, 11, 78–84). The first layer involves the preferential distribution of Se to, and retention by, some tissues (brain, endocrine, and reproductive organs) over others (*e.g.*, liver; refs. 85, 86). A broad spectrum of both essential and nonessential SPs are present in these favored tissues and also in all other tissues.

A second layer of hierarchies involves the protection against Se deficiency of some SPs over others within specific tissues. Within-tissue hierarchies can be observed both *in vivo* and *in vitro* by measuring SP concentrations or enzyme activities (79), mRNA transcripts (10, 87), or SP translation rates (78). This analysis will focus on within-tissue hierarchies of SP activities or concentrations measured *in vivo* where the sensitivities of essential and nonessential SPs to Se deficiency are directly compared. While these hierarchies have been widely discussed, to our knowledge this review is the first to systematically analyze all available evidence. Differential effects of Se availability on within-tissue mRNA expression, stability, or SP translation rates provide critical evidence required to understand the highly complicated array of homeostatic mechanisms that, together, effect the end result. An important literature on these various mechanisms exists and is rapidly growing; several representative citations are listed (10, 18, 78, 82, 88–95). How these mechanisms combine to maintain within-tissue hierarchies is briefly considered in Discussion.

Overlapping enzyme activities of 3 major SP enzyme classes

Testing the first prediction of the triage theory involves comparing the within-tissue relative sensitivities of es-

sential and nonessential SP activities to Se deficiency, as indicated above. Within each of the 3 major SP enzyme classes (glutathione peroxidases, thioredoxin reductases, and deiodinases), the activities of within-class isoforms overlap, at least to some extent (Table 1). For example, all 4 glutathione peroxidases reduce hydrogen peroxide (H_2O_2) and free fatty acid hydroperoxides using glutathione (GSH) as reductant, and all are thus detected when H_2O_2 is the substrate. Only Gpx4 can be individually identified because it is the only Gpx that reduces complex hydroperoxides in phospholipids and low-density lipoproteins (23, 24). Thus, all comparisons among different glutathione peroxidases using enzymatic assays are limited to a comparison of Gpx4 activity to overall Gpx activity. A lack of specificity also pertains to the activity of the thioredoxin reductases and, to some extent, to the several deiodinases (Table 1). Without some additional means of distinguishing between these within-class isoforms, their overlapping enzyme activities would make it impossible to rank them according to their sensitivities to Se deficiency, a major goal of this review. Fortunately, the different tissue and subcellular distributions of these SPs (Table 1), combined with other isoform-specific properties, such as different substrate affinities or sensitivities to inhibitors, as in the case of Dio1 and Dio2, make it possible to infer their relative sensitivities to Se deficiency from enzyme assay results in most cases.

MATERIALS AND METHODS

Standard search methods were employed to retrieve published information, including PubMed, Google, and the ISI Web of Knowledge Cited Reference Search tool (http://pcs.isiknowledge.com). Expert reviews were an important resource. Together with published resources, web-based data repositories were also utilized, particularly the Mouse Genomic Informatics (MGI) database (http://www.informatics.jax.org), which was used to search for mouse KO mutants. To facilitate discussion of the large amount of data describing the relative sensitivities of essential and nonessential SPs to modest and severe Se deficiency, several quantitative measures were used to summarize results, permitting visual display (**Figs. 1–4**). Results summarized quantitatively were from experiments with the same basic design. Different groups of subjects (usually rodents) were fed diets variously deficient in Se for an extended period of time (≥1 mo). At termination, enzyme activities (occasionally protein concentrations) were measured for at least 2 SPs in the same tissue, one essential and one nonessential. SP enzyme activities were recorded from published data in tables or estimated from results presented in figures. For each experiment, percentage of activity compared to that on a Se-replete diet was determined at two dietary concentrations of Se, modest and severe deficiency. For modest deficiency, percentage activity (relative to a replete diet) at the highest dietary concentration at which the activity of the most sensitive SP decreased significantly below activity on a replete diet was used to represent sensitivity to modest Se deficiency. Two-sample *t* tests were used to determine statistical significance when *P* values were not reported. Standard deviations (SDs) were estimated from results displayed in published figures when necessary. Results were not included if SDs were not provided. For severe deficiency, percentage activity (relative to a replete diet) at the lowest dietary concentration of Se employed, which in all experiments summarized represented a severe level of deficiency, was used to represent sensitivity to severe Se deficiency. These measures are illustrated by an example in Supplemental Material, and results of calculations are presented in Supplemental Table S1. When the same SPs were compared in the same tissue in >1 experiment, means ± SD are displayed. Since quantitative measures used are not precise (as they are dependent on the particular diets employed, times of sampling, *etc.*) and since the means ± SD plotted are across experiments that are not strict replicates, it was expected that variances would be considerably greater than had it been possible to use more precise measures and experiments with more closely replicated designs.

RESULTS

Categorizing SPs according to their essentiality

Mouse KO phenotypes for 11 SPs are shown in Table 1. Five KOs were either embryonic lethal (Gpx4, Dio3, Txnrd1, and Txnrd2) or offspring had severely reduced fertility (Sepp1 and Dio3). These 5 SPs were classified essential, a classification logically consistent with their functions.

Gpx4

One isoform of Gpx4, cytosolic Gpx4 (cGpx4), is essential for embryonic development (46) and another, mitochondrial Gpx4 (mGpx4), for fertility (45, 96, 97). The lethality of the cGpx4 KO is consistent with its unique function in protecting against damage from lipid peroxidation and participating in critical lipid peroxide signaling pathways (98–100). Severe infertility of mGpx4-KO offspring is also not surprising, given the fact that mGpx4 plays an essential structural role in spermatozoa (45, 101).

Txnrd1 and Txnrd2

These SPs are required for embryogenesis, presumably because of the extensive involvement of thioredoxin in many signal transduction pathways (52, 53, 102). However, they may be less essential postnatally (48). This suggestion is supported by observations that postnatal and prenatal functions may not be the same (48, 103, 104), that significant loss of Txnrd1 activity in hemizygotes (105) or complete loss in liver hepatocytes (106) is well tolerated, and that Txnrd2 may at least partially compensate for loss of Txnrd1 (53, 107).

Dio3

Partial lethality in Dio3 KOs is not surprising, since Dio3 is highly expressed during fetal development (64) and protects against high T_3 concentrations known to be toxic to the fetus (57). Mechanisms explaining the severe reduction in fertility in Dio3-KO offspring are less certain, but the testes in these animals are

TABLE 1. *Basic properties of SPs discussed in this review*

SPs and selected references	Primary tissue expression[a]	Major subcellular location	Major function	Mouse KO phenotype
Glutathione peroxidases (Gpx; refs. 23–25)			Reduction of H_2O_2 and some soluble hydroperoxides; use GSH as a reducing substrate	
Gpx1 (26–28)	Ubiquitous, predominant[b]	Cytosol		Nonlethal
Gpx2 (27, 30–32)	Major intestinal epithelial Gpx; induced in other epithelial cells (lung, skin, breast; refs. 33, 34)	ER (35)		Nonlethal
Gpx3 (36, 37)	Secreted into ECM: plasma from kidney, thyroid colloidal lumen from thyrocytes[a]	Plasma		Nonlethal
Gpx4 (40–47)	Testis (highly concentrated)	Cytoplasm (cGpx4), mitochondria (mGpx4), and nucleus (nGpx4)	Additional functions unique to Gpx4 include reduction of hydroperoxides in membrane phospholipids and a critical structural role in spermatozoa	cGpx4: embryonic lethal; mGpx4: nonlethal, severe male infertility; nGpx4: nonlethal
Thioredoxin reductases (Txnrd; refs. 48–51)			Reduction of thioredoxin, necessary for many important cellular functions; use NADPH as a reducing substrate	
Txnrd1 (50, 52–54)	Ubiquitous (55)	Major form cytosolic		Embryonic lethal
Txnrd2 (102)	Prostate, testis, liver, lung, kidney, breast, cerebral cortex (55)	Major form mitochondrial, some cytoplasmic splice variants		Embryonic lethal
Deiodinases (Dio; refs. 29, 38, 56–60)				
Dio1 (59, 61)	Thyroid, liver, kidney, pituitary, ovary	Plasma membrane	Broad specificity ($rT_3 \rightarrow T_2 > T_4 \rightarrow T_3$, $T_4 \rightarrow rT_3 = T_3 \rightarrow T_2$); may act primarily to scavenge iodide; $T_4 \rightarrow T_3$ and $T_4 \rightarrow rT_3$ activities sensitive to the inhibitor PTU[c]	Nonlethal
Dio2 (38, 59, 62)	Pituitary, brain, ear, BAT, reproductive tissues, human thyroid, muscle, heart	ER membrane	Important for T_3 (active thyroid hormone) production in peripheral tissues ($T_4 \rightarrow T_3 > rT_3 \rightarrow T_2$); $T_4 \rightarrow T_3$ activity insensitive to the inhibitor PTU[c]	Nonlethal
Dio3 (38, 59, 63–65)	Fetal tissues and placenta; skin, brain, reproductive tissues; not expressed in thyroid	Plasma membrane	Important for inactivation of T_3 ($T_3 \rightarrow T_2 > T_4 \rightarrow rT_3$); $T_4 \rightarrow rT_3$ activity is insensitive to the inhibitor PTU[c]	Partial lethality, severe infertility in both sexes

(*continued on next page*)

TABLE 1. *(continued)*

SPs and selected references	Primary tissue expression[a]	Major subcellular location	Major function	Mouse KO phenotype
Sepp1 (21, 66–69)	Secreted from liver	Plasma (accounts for most plasma Se)	Transports Se to brain, testis, and kidney; some enzymatic redox properties	Severe infertility in KO males; partial postnatal lethality in KOs and in offspring of female KOs
Msrb1 (14, 70–74)	Heart, liver, muscle, kidney (human); liver, kidney, prostate, testis (mouse)	Cytosol and nucleus	Reduces protein methionine-*R*-sulfoxides using thioredoxin; contains zinc	Nonlethal
SelN (75, 76)	Skeletal muscle, brain, lung placenta.	ER membrane	Required for muscle development; oxidative stress protection and redox regulation involving calcium homeostasis	No mouse KO; nonlethal and fertile human homozygote

BAT, brown adipose tissue; cGpx4, cytoplasmic isoform of Gpx4; CNS, central nervous system; ECM, extracellular matrix; ER, endoplasmic reticulum; GSH, glutathione; mGpx4, mitochondrial isoform of Gpx4; nGpx4, nuclear isoform of Gpx4; PTU, 6N-propylthiouracil. [a]All SPs listed are also expressed at lower levels in at least some other tissues, some more broadly than others. For example, in rodents, Gpx2 was formerly thought to be exclusively expressed in the intestine (23) but recently has been detected in the lung (34). On the other hand, Gpx3 is primarily expressed in the kidney but is also expressed to some extent in a number of other tissues, including the liver, heart, lung, and thyroid, and is secreted into extracellular spaces, such as the colloidal lumen of the thyroid (20, 25, 38); and Gpx4, while highly concentrated in the testis, is ubiquitously expressed in other tissues, although at relatively low levels (39). [b]Predominant except in the testis, intestine, and thyroid, where there is also substantial expression of Gpx4, Gpx2, and Gpx3 (29), respectively. [c]Symbol > indicates different degrees of substrate preference.

unusually small, and Dio3 is known to be expressed in parts of the neonatal brain involved in sexual differentiation (59).

Sepp1

The severe infertility of male KO offspring (67–69) is consistent with the role of Sepp1 in transporting Se to the testis (66).

Six SP KOs did not exhibit any embryonic or postnatal lethality or infertility (Gpx1, Gpx2, Gpx3, Dio1, Dio2, and Msrb1). These SPs were classified nonessential, as was SelN, based on the human homozygote mutant phenotype (Table 1). This classification is also consistent with the known functions of these SPs.

Gpx1, Gpx2, and Gpx3

The glutathione peroxidases are part of an elaborate partially overlapping system that maintains redox balance in the cell (108, 109). While loss of Gpx activity would be expected to somewhat perturb this balance, the absence of acute effects in KOs is not surprising.

Dio1 and Dio2

Dio1 and Dio2 are not required for the biosynthesis of T_4 and T_3, which are independently produced in the thyroid and released into systemic circulation (110, 111). Schneider *et al.* (61) observed significant increases in both T_4 and T_3 in the Dio1-KO mouse thyroid, suggesting that *de novo* synthesis of T_3 by the thyroid is increased in Dio1 KOs, leading to the observed normal serum T_3 levels (60, 61). T_3 levels are also normal in Dio1/Dio2 double KOs (58).

Msrb1 (SelR)

The nonessentiality of Msrb1 is also most likely due to redundancy, since it is not the only Msrb present in mammalian cells. Msrb1 is the predominant Msrb in the cytosol and nucleus (Table 1), and the other 2 Msrb enzymes (which are not SPs) are primarily targeted to the mitochondria (Msrb2) and the endoplasmic reticulum (Msrb3) (14, 72). Despite these differences in subcellular location, enough Msrb activity most likely remains in Msrb1 KOs to avoid lethality.

SelN

SelN was classified nonessential based on observations that human homozygotes with undetectable concentrations of SelN in fibroblasts and skeletal muscle have normal reproductive capacity, despite suffering from a muscle disorder (112, 113). The precise function of SelN is not known, although it appears to be important in muscle development and to play a role in protection against oxidants, redox signaling, and calcium homeostasis (75, 76).

The remainder of this review is focused on this subset of 12 SPs (selected recent reviews of each class of SP are cited): 4 glutathione peroxidases (Gpx1, Gpx 2, Gpx 3,

and Gpx 4; refs. 23–25, 47); 3 iodothyronine deiodinases (Dio1, Dio 2, and Dio 3; refs. 38, 56); 2 thioredoxin reductases (Txnrd1 and Txnrd 2; refs. 48, 114); selenoprotein P (Sepp1; refs. 21, 66); methionine sulfoxide reductase B1 (Msrb1 or SelR; ref. (14); and selenoprotein N (SelN; refs. 75, 76).

Prediction 1: are essential SPs more resistant than nonessential SPs to Se deficiency?

The relative sensitivities to Se deficiency of essential compared with nonessential SP activities or concentrations *in vivo* within individual tissues are discussed below. Results were assembled from 37 research reports, all of which we are aware (32, 62, 82, 115–148). Ten of the 12 SPs classified above as essential or nonessential are examined in the 37 reports: Gpx1, Gpx2, Gpx3, Gpx4, Txnrd1, Txnrd2, Dio1, Dio2, Sepp1, and Msrb1. Results are summarized in Figs. 1–4. Plotted points are results of single experiments or, when >1 experiment in a particular tissue was available, averages and SDs.

Experiments conducted over a prolonged period of time (≥1 mo)

Gpx4 vs. *overall Gpx activity (primarily Gpx1)* Figure 1 (82, 115, 116, 118–120, 123–126, 144) illustrates the greater resistance of the essential SP Gpx4 compared with overall Gpx activity (primarily Gpx1) after both modest and severe Se deficiency in all rodent tissues examined: liver, kidney, thyroid, muscle, heart, lung, and testis. Despite the fact that the quantitative measures we used to describe sensitivity to deficiency are imprecise, as discussed in Supplemental Material, differences between Gpx4 and overall Gpx activity are significant in almost all cases where statistical comparisons were possible (*i.e.*, where ≥2 experiments were available): modest deficiency (liver, $P=0.033$; heart, $P=0.024$); severe deficiency (liver, $P=0.0001$; kidney, $P=0.0007$; lung, $P=0.045$). The striking resistance of Gpx4 to severe Se deficiency compared with nonessential Gpx activity is highlighted by the observation that in liver the loss of Gpx4 activity on severe Se deficiency lasting for 28 d (116, 118, 124) or 1 yr (115) was approximately the same.

Txnrd1 vs. *Gpx4 and overall Gpx activity* Figure 2 (82, 117, 118, 124, 135, 137, 140, 142, 145, 147, 148) paints a somewhat different picture for Txnrd1, which, as discussed above, is essential if its loss occurs during development but is of uncertain essentiality if loss occurs postnatally. Txnrd1 is similar to Gpx4 in being clearly more resistant to modest Se deficiency in rodent liver compared with Gpx1 (Fig. 2*A*). In contrast, after very severe Se deficiency (basal diets <0.01 μg Se/g), Txnrd1 is no longer as resistant as Gpx4 in liver (Gpx4 *vs.* Txnrd1, $P<0.01$) but appears still somewhat more resistant than Gpx1 (Gpx1 *vs.* Txnrd1, $P=0.052$). When basal diets used were of intermediate severity, Txnrd1 was still only modestly more resistant ($P=0.052$) than

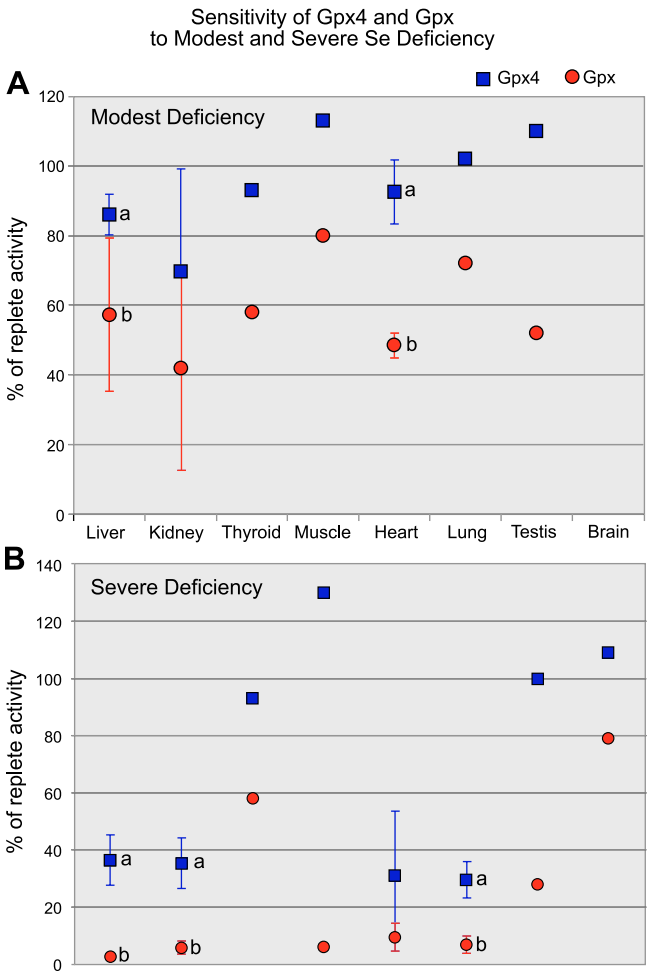

Figure 1. Sensitivity of Gpx4 and overall Gpx activities to modest and severe Se deficiency in multiple tissues. Essential SPs are represented by squares and nonessential SPs by circles: blue squares, Gpx4; red circles, overall Gpx activity (see text). When >1 experimental result was available in a given tissue, means ± SD are plotted. Within a given tissue, means that do not share a common letter (*a*, *b*) are significantly different ($P<0.05$). Points plotted without SDs represent results of single experiments. Citations from which results were retrieved are as noted. Results from Lei *et al.* (116) were not plotted because Gpx4 and Gpx1 were not separated before assay (see text). *A*) Modest deficiency: liver ($P=0.033$; refs. 82, 115, 116, 118, 120, 124); kidney ($P=0.31$; refs. 115, 116, 118); thyroid (120); muscle (118); heart ($P=0.024$; refs. 116, 120); lung (116); testis (119). *B*) Severe deficiency: liver ($P<0.0001$; refs. 82, 115, 116, 118, 120, 123–126); kidney ($P=0.0007$; refs. 115, 116, 118, 126); thyroid (120); muscle (118); heart ($P=0.19$; refs. 116, 120, 126); lung ($P=0.045$; refs. 116, 126); testis (119); brain (144).

Gpx1 (>0.01 μg Se/g), as shown in the second plotted point in Fig. 2*B*. Only a limited number of studies were available for each of 3 other tissues (kidney, brain, and intestine) that compared Txnrd1 to overall Gpx activity. Furthermore, basal diets of each pair of studies were not of the same degree of severity, precluding a meaningful statistical test.

Dio1 or Dio2 vs. *Gpx4 and overall Gpx activity* Figure 3 (120, 121, 143, 144, 146) illustrates the unusual response to severe Se deficiency of the nonessential

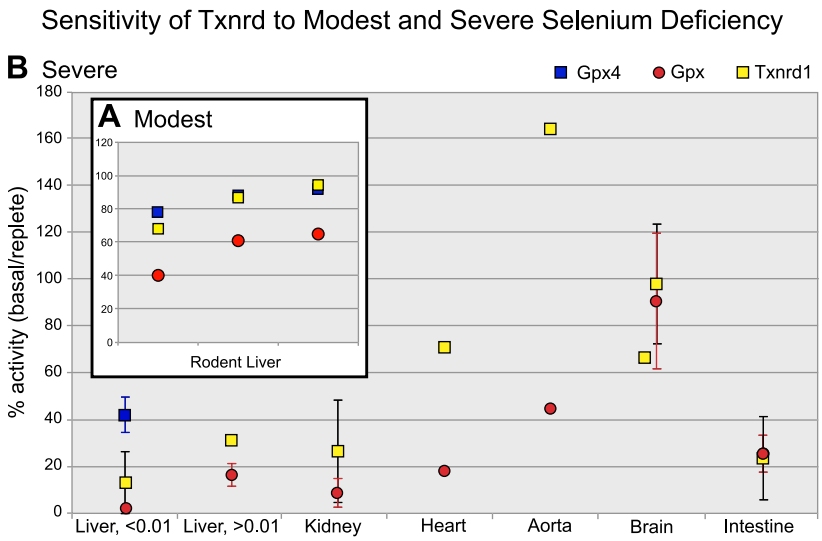

Figure 2. Sensitivity of Txnrd1 compared with Gpx4 and overall Gpx activity to modest and severe Se deficiency in multiple tissues. Blue squares, Gpx4; red circles, overall Gpx activity; yellow squares, Txnrd1. See Fig. 1 for additional explanation. *A*) Modest deficiency: Txnrd1 *vs.* Gpx (P=0.064); all 3 points plotted are from results in liver and are, from left to right, Sunde *et al.* (82), Barnes *et al.* (118), and Hadley and Sunde (124). *B*) Severe deficiency: liver (basal diets, <0.01 μg Se/g: Gpx4 *vs.* Txnrd1, P<0.01; Gpx *vs.* Txnrd1, P=0.052; refs. 82, 117, 118, 124, 140, 148); liver (basal diets, >0.01 μg Se/g: Txnrd1 *vs.* Gpx, P=0.052; refs. 137, 145); kidney (Txnrd1 *vs.* Gpx, P=0.38; refs. 117, 145); heart (145); aorta (135); brain (Txnrd1 *vs.* Gpx, P=0.39; refs. 117, 145, 148); intestine (P=n.s.; refs. 137, 147).

deiodinase Dio1 in the thyroid. Dio1 was of intermediate sensitivity in the liver (120, 121, 143, 146) compared with Gpx4 (Gpx4 *vs.* Dio1, P<0.01) and Gpx activities (Dio1 *vs.* Gpx, P<0.01) but was at least as resistant to severe deficiency as Gpx4 in the thyroid (Dio1 *vs.* Gpx4, P=0.34; refs. 120, 143, 146). Results were similar in the only study that examined responses of Dio1 to modest Se deficiency (120). Dio1 may also be more protected relative to Gpx4 after severe Se deficiency in the pituitary (144). Dio2 was below the detection limit in both Se-deficient and replete animals in the pituitary (144).

Sepp1 vs. *Gpx3* Figure 4 (117, 129–134, 136) illustrates the comparative sensitivities to Se deficiency of Sepp1 and Gpx3 in plasma from rodents and humans. As predicted, Sepp1 is more resistant than Gpx3 to both modest and severe Se deficiency in rodents. Despite the severe loss of both Sepp1 and Gpx3, the difference in percentage loss between Sepp1 and Gpx3 across the 4 rodent experiments in Fig. 4 is significant (P=0.0056). Results also suggest there could be a species difference between rats and humans in the relative sensitivities of these 2 SPs to both modest and more severe Se deficiency. In all human experiments shown, Gpx3 is more resistant to Se deficiency than Sepp1. Differences in percentage loss of Sepp1 and Gpx3 across the 4 human studies plotted are not significant, due to the fact that basal Se intakes were quite different among the human studies [10 μg Se/d (132), ≅28 μg Se/d (131), and ≅40 μg/d (133, 134)]. The severity of deficiency was considerably greater in the rodent experiments than in the human studies [>95% reduction in rodents compared with a replete diet and 50–75% reduction below recommended intakes (*e.g.*, refs. 149, 150) in the human studies], explaining the greater loss of both proteins on severe deficiency in rodents.

Gpx2 Averaged effects of severe (0.002 μg Se/g; ref. 147) and moderately severe (0.086 μg Se/g; ref. 137) Se deficiency on overall Gpx activity in the mouse intestine compared with Txnrd1 are shown in Fig. 2*B*, suggesting similar sensitivities to deficiency of overall Gpx activity and Txnrd1. These results suggest that overall Gpx activity in the intestine is somewhat more resistant to Se deficiency than in the liver. *In vivo* evidence that this greater resistance is due to Gpx2 was presented by Brigelius-Flohe *et al.* (32), who showed that Gpx2 protein was synthesized much more rapidly than Gpx1 after resupplementation of severely Se-deficient rats. However, in the same study (32), both Gpx2 and Gpx1 were only marginally synthesized in the rodent duodenum and ileum after at least 13 wk of Se

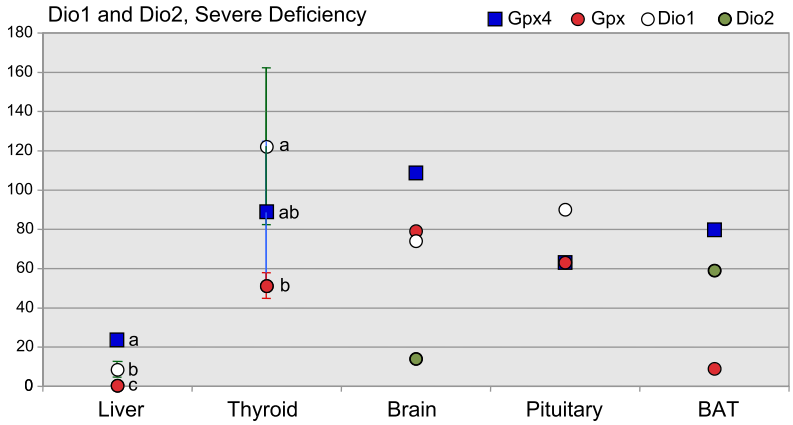

Figure 3. Sensitivity of Dio1 and or Dio2 compared with Gpx4 and overall Gpx activity to severe Se deficiency in multiple tissues. Blue squares, Gpx4; red circles, overall Gpx activity; white circles, Dio1; green dotted circles, Dio2; BAT, brown adipose tissue (144). See Fig. 1 for additional explanation. Liver (Gpx4 *vs.* Dio1/2, P=0.0022; Dio1/2 *vs.* Gpx, P=0.0066; refs. 120, 121, 143, 146); thyroid (Gpx4 *vs.* Dio1/2, P=0.34; Gpx4 *vs.* Gpx, P=0.015; Dio1/2 *vs.* Gpx, P=0.039; refs. 120, 143, 146); brain (144); pituitary (Dio2 below detection limits; ref 144).

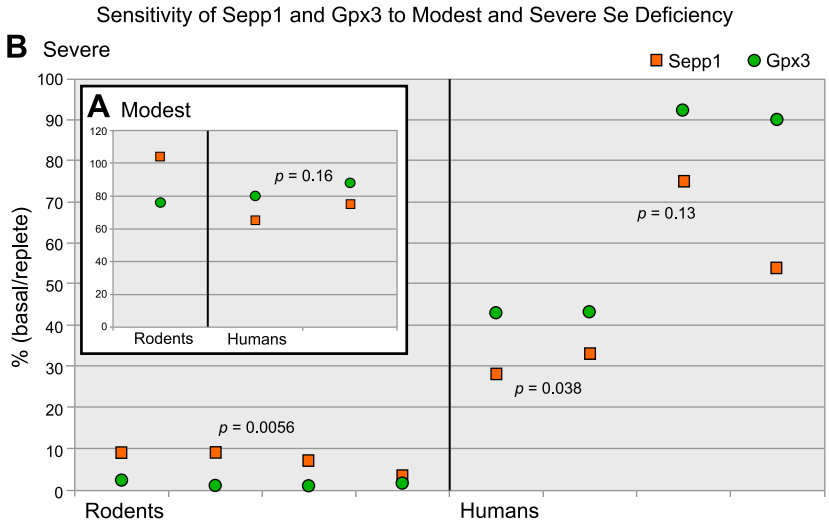

Figure 4. Sensitivity of plasma Sepp1 compared with Gpx3 in rodents and humans consuming diets variously deficient in Se. Yellow squares, Sepp1; green circles Gpx3. *A*) Modest deficiency. Left panel: rodents (136). Right panel: humans (Sepp1 *vs.* Gpx3, P=0.16). Left to right: Xia *et al.* (selenomethionine diet; ref. 132) and Xia *et al.* (selenite diet; ref. 132). *B*) Severe deficiency. Left panel: rodents, very severe deficiency (Sepp1 *vs.* Gpx3, P=0.0056; left to right: refs. 117, 129, 130, 136). Right panel: humans; lane 1, relatively severe deficiency (Sepp1 *vs.* Gpx3, P=0.038; selenomethionine and selenite; ref. 132); relatively less severe deficiency, lanes 2–4 (Sepp1 *vs.* Gpx3, P=0.13; left to right: refs. 131, 133, 134). See text for further explanation.

deficiency (32), suggesting similar sensitivities of both of these nonessential SPs to prolonged severe Se deficiency.

Msrb1 Msrb1 activity was compared with Gpx1 and Txnrd1 in mouse liver under Se-deficient and replete conditions (140, 141). Percentage activities (basal/replete) were as follows: Novoselov *et al.* (141): Msrb1 (10%), Gpx1 (9.8%); and Uthus and Moskovitz (140): Msrb1 (13%), Gpx1 (1.9%), Txnrd1 (35%); suggesting that Msrb1 is sensitive to severe Se deficiency, falling somewhere between Gpx1 and Txnrd1. No experiments were identified that examined the sensitivity of Msrb1 to modest Se deficiency. Protein concentrations of Msrb1 in replete and severely deficient mice were also measured in kidney and brain but were not compared with other SPs (140).

Short-term effects of Se deficiency or resupplementation

These experiments (117, 126, 130, 131, 133, 134, 136, 138, 139, 146) are highly varied in experimental design, ranging from intraperitoneal injection of Se-deficient rodents (117, 130, 146) to human Se-supplementation trials in Se-deficient areas of China (133, 134, 138, 139) or New Zealand (131).

Most results are consistent with the prediction that essential SPs decrease more slowly on initiation of Se deficiency and increase more rapidly on Se resupplementation than nonessential SPs. For example, the essential SP Gpx4 decreased more slowly than overall Gpx activity in the mouse liver, heart, and kidney (although not in the lung) after initiating Se deficiency (126), and it increased more rapidly after Se resupplementation in the only tissues examined, the liver and thyroid (146). Also, the essential SP Txnrd1 responded more quickly to resupplementation than Gpx1 in rat liver (117). Only 1 rat experiment compared the rate of loss of Sepp1 and Gpx3 after initiation of Se deficiency, and results suggested that both proteins rapidly declined (136). However, Sepp1 responded more quickly to Se resupplementation after intraperitoneal injection in rodents (117) and after Se supplementation in all human trials of Se-deficient subjects (131, 133, 134, 138, 139).

Summary

The prediction that essential SPs are more resistant than nonessential SPs to Se deficiency was borne out in all tissues where direct comparisons between essential and nonessential SPs were possible, except the thyroid. The resistance of the activity of Dio1 in the thyroid to Se deficiency (Fig. 3) is inconsistent with the prediction of the triage theory based on the nonlethality of its KO. This reaction to Se deficiency suggests that Dio1 could be conditionally essential (see Discussion). It is difficult to evaluate Gpx2 because its sensitivity to modest Se deficiency has not been determined, and it has not been directly compared with an essential SP. Evidence comparing Gpx2 and Gpx1 (both nonessential) protein concentrations in the intestine is somewhat conflicting. Gpx2 protein increased much more rapidly than Gpx1 in the intestine on Se resupplementation of severely deficient rodents (32), but in the same study, both enzymes were only marginally synthesized on severe Se deficiency.

Predictions 2 and 3: does decreased functionality of nonessential SPs increase risk of diseases of aging, and is there evidence of a causal relationship?

The triage theory predicts that decreased functionality of nonessential SPs due to modest Se deficiency will have insidious consequences that over the long term will increase the risk of diseases associated with aging. It is extremely difficult, if not impossible, to test this prediction directly in long-term randomized controlled trials, as we have discussed (151). This section draws on prospective epidemiological studies and other genetic and mechanistic evidence to ask whether the same diseases or conditions of aging that are prospectively

associated with modest Se deficiency are also linked to phenotypes of nonessential SP mouse KOs or human mutants, and whether plausible mechanisms suggest a causal connection between these same SPs and diseases. Cross-sectional epidemiological studies are not emphasized because, as we (3) and others (*e.g.*, ref. 152) have discussed, it is often not clear how to make inferences about predisease conditions from ongoing pathology where normal cell biology is perturbed. Examples are Txnrd1 (153, 154), Gpx2 (79), and other glutathione-metabolizing enzymes (155), which are protective before the initiation of cancer but tumor promoting after initiation.

The same age-related diseases are linked epidemiologically to modest Se deficiency and to phenotypes of mouse KOs and human polymorphisms of nonessential SPs

Table 2 (14, 26, 33, 34, 71, 76, 79, 112, 113, 147, 152, 156–231) illustrates that some human diseases and age-related conditions are prospectively associated with modest Se deficiency and that characteristics of these diseases also describe phenotypes of some mouse KOs and other rodent and human genetic variants of nonessential SPs. Diseases linked only to severe Se deficiency, such as Keshan and Kashin-Beck diseases, are included in Table 2 in italics. Replicated prospective results report associations between relatively modest Se deficiency and increased mortality (158–160), cancer (166, 167, 169), DNA damage (171–173), heart disease (175, 180–182), and reduced resistance to infection (190, 191). The relationship between Se availability and type 2 diabetes is complex and is considered further in Discussion.

Similarities are striking between these diseases and phenotypes of nonessential SP mouse KOs or human polymorphisms. Note that almost all nonessential SP KOs, SNPs, or other genetic variants are linked to >1 Se-deficiency-associated disease or condition: Dio2 to osteoarthritis (178, 200), bone mineral density (201) or bone fracture (198), emotional and intellectual mental status (205–207), and possibly hypertension and insulin resistance (178, 216); Gpx1 to increased senescence (156, 157), cancer (26, 163, 164), DNA damage (170), heart disease (174, 175), resistance to infection (186, 187), and sensitivity to neurotoxicity (175, 204); Gpx2 to cancer (33, 79) and reduced resistance to antigen-induced airway inflammation (34); and Gpx3 to cancer (164, 165), stroke (176), and cerebral venous thrombosis (177). Note that all 3 nonessential glutathione peroxidases are linked to cancer.

Space does not permit a detailed discussion of the strengths and weaknesses of evidence cited in Table 2. Certainly, the evidence is of varying quality and consistency. For this reason, more weight was given to evidence reported in >1 study. Expert reviews discussing some of this evidence are highlighted in Table 2, and some additional information has been included in the table footnotes. The intent of Table 2 is not to imply that relationships are necessarily fully established or that causal linkages have been clearly demonstrated but to point out that the large body of evidence linking genetic loss of nonessential SP activities to phenotypes similar to those of diseases or conditions of aging associated with modest Se deficiency adds credibility to the dietary Se epidemiological results and suggests that causal linkages between dysfunction of nonessential SPs and diseases of aging is possible. Plausible mechanisms linking nonessential SPs to these diseases are considered in Discussion.

Essential SP SNPs are also linked to diseases of aging

SNPs in a number of SPs classified as essential are also linked to age-related diseases [*e.g.*, Gpx4 to colorectal (232, 233) and breast (234) cancer; Dio3 to osteoarthritis (235); Txnrd1 to colorectal cancer (236) and familial ALS (237); and Sepp1 to prostate (163, 238) and colorectal (233, 236) cancer], as are some other SPs for which mouse KOs have not yet been described (*e.g.*, SelS and Sel 15-kDa to colorectal cancer; ref. 239). Such linkages are not inconsistent with the triage theory, which predicts only that modest Se deficiency is more likely to impair nonessential SPs than essential SPs. It is also important to point out that causal factors other than genetic and nutritional may also interfere with the functionality of some SPs leading to disease, such as impairment of Sepp1 synthesis in the colon due to proinflammatory cytokines, possibly increasing the risk of inflammatory mediated colorectal cancer (240). For broader discussion of relationships between Se, SPs, and disease the reader is referred to many excellent reviews (8, 9, 19, 21, 79, 152, 154, 169, 175, 184, 187, 241–247).

DISCUSSION

The triage theory, grounded in a major evolutionary theory of aging (4), proposes an explanation for why modest V/M deficiencies may increase risk of diseases associated with aging (1–3). In this review, 2 predictions of the theory were tested against published evidence for 5 essential (Gpx4, Dio3, Txnrd1, Txnrd2, and Sepp1) and 7 nonessential (Gpx1, Gpx2, Gpx3, Dio1, Dio2, Msrb1, and SelN) SPs, categorized based primarily on their mouse KO phenotypes (Table 1). Results of the analysis are largely supportive of the theory, suggesting that, among all SPs, dysfunction of those that are nonessential is likely to be the major contributor to increased disease risk due to Se deficiency. Strengths and weaknesses of the analysis are discussed below.

Prediction 1: are essential SPs more resistant than nonessential SPs to Se deficiency?

This prediction was largely borne out, except for Dio1 in the thyroid (discussed separately below). The analysis was focused only on *in vivo* studies that directly

TABLE 2. *Selenium deficiency and genetic impairment of nonessential SPs are linked to the same set of diseases or conditions associated with aging*

Genetic loss		Dietary loss of Se (humans, except as indicated)[a]
Mice[b]	Humans	
Aging/mortality		
Gpx1 KO: increased senescence (156, 157)		Increased mortality (158–160)
Cancer		
Gpx2 KO: increased sensitivity to UV-induced croton oil-promoted SCC (33, 79-R)[c] Gpx1/Gpx2 DKO: sensitive to microflora-associated intestinal cancer (161) I^6A transgenic mice: Increased sensitivity to chemically induced precancerous lesions in the colon (147) and to prostate cancer in a cancer-prone mouse transgenic line (162)[d]	Gpx1 SNP: prostate cancer (163); various cancers (26-R, 164-R) Gpx1 LOH: various cancers (164-R) Gpx3 HYP: Barrett's esophagus, a precancerous condition (164-R, 165)	Increased cancer risk (166, 167, 169-R)
DNA damage		
Gpx1 KD: increased UV-induced micronuclei (170)[e]		Increased DNA damage (Se status correlations; refs. 171-R–173-R)
Heart-related		
Gpx1 HET: heart abnormalities (174, 175-R)	Gpx3 SNP: arterial ischemic stroke (176) and CVT (177)[f] Dio2 SNP: mixed results on hypertension (178-R) Dio1 SNPs: decreased circulating concentrations of the active thyroid hormone T_3 (179-R, 178-R)[g]	Increased risk of heart disease (180), hypertension (181), or mortality in SAP and ACS patients (175-R, 182-R) Increased risk of cardiovascular events in heart disease patients (RBC Gpx1 activity; refs. 183, 184-R) *Keshan disease* (a cardiomyopathy; ref. 185-R)
Immune-related		
Gpx1 KO: increased virally induced myocarditis (186, 187-R) Gpx2 KO: increased sensitivity to antigen-induced airway inflammation (34) Selenocysteine tRNA targeted KO in T cells: impaired T-cell, but normal macrophage, function (188)[h] SelK KO: immune cell defects (189)		Reduced resistance to infection (primarily viral; humans: refs. 190, 191-R; mice: ref. 192) *Agent-dependent reduced innate and adaptive immune responses* (humans: ref. 191-R; mice: refs. 193, 194)
Muscle-related		
	Dio1 SNP: age-related muscle weakness (195) SelN HOMO: myopathy related to muscular dystrophy (76-R, 112, 113)	*Muscle weakness/muscular dystrophy-like symptoms* (196-R, 197-R)
Bone-related		
Dio2 KO: increased sensitivity to bone fracture (198) Selenocysteine tRNA targeted KO in osteo-chondroprogenitor cells: phenotype similar to Kashin-Beck disease (199)	Dio2 SNP: osteoarthritis (178-R, 200); bone mineral density (201) Gpx1 SNP: Kashin-Beck disease (202)	*Kashin-Beck disease* (an osteoarthropathy; ref. 203)

(*continued on next page*)

TABLE 2. *(continued)*

Genetic loss		Dietary loss of Se (humans, except as indicated)[a]
Mice[b]	Humans	
Brain-related		
Gpx1 KO: increased sensitivity to damage in a Parkinson's model (175-R, 204)[i]	Dio2 SNP: mental retardation (plus iodine deficiency; ref. 205) Dio2: psychological well being (206, 207-R)	Poor cognitive function; association in a longitudinal study (208), and a dose-dependent association in a cross-sectional study (209) *Mental retardation (Myxedematous cretinism) with combined iodine and Se deficiency* (210-R)
Diabetes-related		
Gpx1 KO: less fat-induced insulin resistance (211) Gpx1 overexpression: increased insulin resistance (217, 218) Sepp1 knockdown: increased insulin sensitivity (219) Overexpression of I^6A^- mutant selenocysteine tRNA: glucose intolerance and diabetes-like phenotype (220)	Dio2 SNP: insulin resistance, mixed results (178-R); type-2 diabetes (216)	*Humans*: mixed results on dysglycemia (212) and type 2 diabetes (213) in 2 prospective studies[j] *Rodents*: increased insulin sensitivity from severe dietary Se deficiency (214, 215)

ACS, acute coronary syndrome; CVT, cerebral venous thrombosis; DKO, double knockout; ESC, esophageal squamous cell carcinoma; GCA, gastric cardia adenocarcinoma; Het, heterozygote; HYP, hypermethylation; KD, knockdown; KO, knockout; LOH, loss of heterozygosity; R, review; SAP, stable angina pectoris; SCC, squamous cell carcinoma; Se, selenium; SNP, single nucleotide polymorphism. [a]Prospective studies are cited unless otherwise indicated. Associations with severe Se deficiency are indicated in italics. Studies that reported associations with plasma biomarkers of modest Se deficiency are indicated. [b]Msrb1 is not included in the table, but increased oxidative stress in KOs (71, 14, 152) suggests that increased risk for stress-induced diseases in Msrb1 SNPs, when identified, would not be surprising. [c]Several human Gpx2 polymorphisms have been identified (221–223), but no associations were reported in the only study of which we are aware that compared the frequency of Gpx2 polymorphisms in cancer patients and healthy controls (223). [d]I^6A transgenic mice lack a modified form of selenocysteine transfer RNA required by several nonessential SPs, including Gpx1, Gpx3, and Msrb1, for their synthesis (224). [e]Overexpression of Gpx1 (by transfection) increased resistance to UV-induced micronuclei (225). [f]In 3 of 4 families with familial childhood stroke, plasma Gpx3 activity was reduced by ~50% in family members examined who had experienced a stroke but not in family members who had not (226), providing additional evidence pointing to an involvement of Gpx3. [g]Relatively small variations in T3 are known to be linked to various age-related conditions, including atherosclerosis and decreased BMD (179). [h]This KO results in loss of all SPs, both essential and nonessential. [i]In the same model, mice overexpressing Gpx1 had reduced sensitivity to damage (227). [j]As explained in the text, results of Se supplementation trials using high doses of Se (*e.g.*, refs. 228, 229) and cross-sectional studies (*e.g.*, refs. 230, 231) are not included in the table. Se and diabetes are considered further in Discussion.

compared nonessential and essential SP activities or concentrations. Results are illustrated in Figs. 1–4. As widely observed, the essential SP Gpx4 is clearly more resistant to prolonged Se deficiency (both modest and severe) than all other SPs to which it has been compared (82, 115, 116, 118–120, 123–126, 144) (with the exception of Dio1 in the thyroid; refs. 120, 143, 146), whereas the nonessential SP Gpx1 is most sensitive to Se deficiency in all tissues (82, 115, 116, 118–120, 123–126, 144) (Figs. 1–3). Txnrd1, essential prenatally but possibly less so postnatally (see Results), appears to be similar to Gpx4 in resistance to prolonged modest Se deficiency in the liver but more similar in sensitivity to Gpx1 on severe deficiency in the liver (82, 118, 124). Additional results for Txnrd1 are presented in Fig. 2.

In rodent plasma, Sepp1 is more resistant than its nonessential counterpart Gpx3 (117, 129, 130, 136), although the reverse appears to be the case in humans (131–134) (Fig. 4). Additional evidence that Sepp1 is more sensitive to modest Se deficiency than Gpx3 in humans includes a recent study comparing these 2 SPs in vegetarians (believed to be modestly Se deficient) and omnivores (believed to be Se replete), in which Gpx3 and serum Se were similar in both groups, but Sepp1 was lower in vegetarians (248). Since the classification of essentiality of Sepp1 was based on severely reduced male fertility in mouse KOs (67–69), it remains to be seen whether a similar classification of essentiality would apply to humans. It would be of interest to determine whether any known Sepp1 SNPs (163, 249) are associated with reduced fertility in humans. To our knowledge, possible differences between rodents and humans in the relative sensitivities of Sepp1 and Gpx3 in plasma have not heretofore been pointed out and should be examined further.

Limited information on the sensitivities of Msrb1 and Dio2 enzyme activities to modest or severe Se deficiency suggests that both are relatively sensitive to severe Se deficiency [Msrb1 in liver (140, 141) and Dio2 in the brain (144); see Results: Prediction 1 and Fig. 3]. It is difficult to evaluate Gpx2 within the defined framework of this analysis because its sensitivity to modest Se deficiency has not been determined, and it has not

been directly compared with an essential SP (refs. 32, 137, 147; see text for further discussion).

Why is the nonessential SP Dio1 resistant to Se deficiency in the thyroid?

The relative resistance to Se deficiency of Dio1 in the thyroid (refs. 120, 143, 146; Fig. 3) appears inconsistent with predictions of the triage theory based on its classification as nonessential (58, 61). We suggest that this apparent inconsistency is because Dio1 may be an example of a conditionally essential SP, one that is nonessential when Se intake is adequate but becomes conditionally essential in the thyroid on Se deficiency. Evidence supporting this possibility is briefly summarized below.

Under normal dietary conditions, the liver is the primary source of plasma T_3 (the active thyroid hormone) due to hepatic deiodination of T_4 by Dio1, which also contributes to normal T_3 output from the thyroid in rodents and humans (250, 251). On Se deficiency, the primary source of plasma T_3 shifts from the liver to the thyroid, at least in rats (110), and there is an increase in Dio1 thyroidal activity (250), with the result that plasma concentrations of T_3 are roughly maintained despite the significant reduction in Dio1 activity in the liver (refs. 120, 121, 143, 146; Fig. 3). For further discussion, see several reviews (38, 57, 61, 250).

It is not obvious, however, why Dio1 should become essential in the thyroid on Se deficiency since the thyroid compensates remarkably well for the complete loss of Dio1 and Dio2 in double-KO mice by increasing Tg-mediated *de novo* synthesis of T_3 (58, 60, 61). It is suggested that the additional requirement for Dio1 on Se deficiency may be due to the different stress profiles presented to the thyroid by genetic loss of Dio1/2 in mouse KOs and Se deficiency. In Dio1/Dio2 KOs, one or both of these enzymes are absent. On Se deficiency, in addition to the depression of both Dio1 and Dio2 (uniquely capable of generating T_3 from T_4) in extrathyroidal tissues (Fig. 3; refs. 120, 121, 143, 144, 146), Gpx activity in the thyroid is also significantly depressed (Fig. 3; refs. 120, 143, 146). This depression leads to increased H_2O_2 in the thyroid (252) and to increased apoptosis (250, 253). Since H_2O_2 is the rate-limiting step in Tg-mediated T_4/T_3 synthesis (254), its increase could be the trigger leading to increased thyroidal T_4/T_3 production on Se deficiency, as suggested by Golstein *et al.* (255) >20 yr ago. Perhaps Dio1 is required to maintain an appropriate T_4/T_3 balance under these conditions. Dio1 increases in the thyroid on iodine deficiency as well, presumably also contributing to increased output of T_3 (61).

Further experiments are required to fully understand the different mechanisms whereby the thyroid maintains homeostasis in Dio1/Dio2 KOs and on Se deficiency and to determine whether Dio1 is essential for homeostasis on Se deficiency. It would be of interest to test whether Dio1 KOs are able to maintain thyroid hormone homeostasis on Se deficiency. If Dio1 is conditionally essential on Se deficiency, the prediction would be that they would not be able to do so.

Mechanisms used to protect essential SPs; comparison to vitK-dependent proteins

Homeostatic mechanisms used by the body to protect essential SPs and vitK-dependent proteins against deficiency are quite different, though both appear to take advantage of the fact that the liver is among the first organs to receive V/Ms. The liver is the first organ to receive water-soluble V/Ms (such as dietary forms of Se) *via* the portal vein. Lipid-soluble V/Ms (such as vitK) are in chylomicrons, which enter the left subclavian vein through the thoracic duct from the lymphatic system, then pass through the lung and heart, and then to the liver (256). The solution found by evolution to preferentially protect essential vitK-dependent proteins appears to be relatively simple, at least as compared with Se. All essential vitK-dependent proteins are predominantly synthesized and activated by vitK in the liver, and all nonessential VitK-dependent proteins are synthesized and activated in extrahepatic tissues, as we previously discussed (3). This separation sets up a dichotomy that takes advantage of the preferential distribution of the primary dietary form of vitK (phylloquinone) to the liver to preserve essential function (coagulation) when vitK is limiting.

Mechanisms responsible for preferentially protecting SPs are much more complicated than for vitK and have been discussed in many reviews (10, 18, 78, 82, 88–95, 257, 258). Unlike vitK-dependent proteins, both essential and nonessential SPs are synthesized in many tissues throughout the body, those high in the first layer of hierarchies (brain, testis, and endocrine organs) and all others as well. Therefore, a dual strategy is required: to preferentially direct Se to preferred organs and to preferentially protect essential SPs within individual cells in all tissues (second layer of hierarchies). Below, mechanisms currently considered to be involved in maintaining the first and second level of SP hierarchies are briefly summarized.

First layer of hierarchies The liver plays an important role in distributing Se to preferred tissues and protecting it in those tissues against deficiency. The final step in the metabolism of the major dietary form of Se (selenomethionine) to selenide, the species utilized in the biosynthesis of all SPs, primarily occurs in the liver, at least in mice (259). So does the synthesis of Sepp1, which contains many more selenocysteine residues than any other SP (10 residues in rodents and humans) (66, 95). Sepp1 helps maintain the first layer of hierarchies by distributing Se efficiently to preferred organs, particularly the testis and brain (80, 260). The supply of Se to the brain and testis may also be reinforced by the additional synthesis of Sepp1 in these organs, which may function as a Se reservoir in those tissues in times of Se scarcity, as recently reviewed (78).

Second layer of hierarchies The second layer of SP hierarchies is managed during translation of SP mRNAs

by the interaction of several incompletely understood mechanisms (10, 12, 78, 90, 257, 261–263). For the most part, these mechanisms favor the translation of essential over nonessential SP mRNAs. For example, several protein factors required for translation of SP mRNAs, including SBP2 (90, 92, 264), eukaryotic initiation factor 4a isoform 3 (elF4a3), sensitive to Se status (262), and possibly nucleolin (90, 263), all discriminate in binding affinity between different SP mRNAs, usually such that translation of essential SP RNAs is favored. Structural characteristics of specific SECIS elements, required for reinterpretation of the UGA termination codon as selenocysteine, also modulate translation efficiency in favor of essential SPs (9, 92, 265, 266).

A critical mechanism appears to involve the requirement of a special methylated form of Sec $tRNA^{[Ser]Sec}$, termed Um34, for translation of most nonessential SP mRNAs, whereas most essential SP mRNAs are translated using the non-Um34 form (10, 11, 18, 93, 224, 267, 268). Since methylation, the final step in the biosynthesis of Um34, is impaired by Se deficiency (11), unavailability of Um34 leaves nonessential mRNAs exposed to increased nonsense-mediated decay (NMD; refs. 10, 82, 258, 269), a general mechanism used by cells to remove incompletely translated mRNAs (270).

It is of great interest relative to this review that the comparative sensitivities of SP mRNAs to NMD induced in cells lacking Um34 (see Table 2 in Hatfield *et al.*; ref. 10) largely parallel the relative resistance to Se deficiency of the activities or concentrations of the subgroup of essential and nonessential SPs reviewed here. Specifically, mRNAs of the essential SPs Gpx4, Txnrd1, and Sepp1 are more resistant to NMD than mRNAs of the nonessential SPs Gpx1, Gpx3, Msrb1, and Gpx2 (10). Interestingly, the relative resistance to NMD of Dio1 mRNA also parallels the resistance of Dio1 activity to Se deficiency in the thyroid (Fig. 3).

Predictions 2 and 3: does decreased functionality of nonessential SPs increase risk of diseases of aging, and is there evidence of a causal relationship?

These predictions were also largely supported by evidence that age-related diseases or conditions prospectively associated with modest Se deficiency are similar to rodent and human phenotypes resulting from partial or complete genetic loss of nonessential SPs (Table 2). For example, numerous epidemiological studies suggest that Se deficiency is prospectively linked to increased cancer risk (166, 168, 169), and various mutants in mice (33, 79, 161) and humans (26, 163–165) are also linked to increased cancer risk. Other diseases or conditions prospectively associated with modest Se deficiency that are similar to nonessential SP mutant phenotypes (see Table 2 for citations on mutant phenotypes) include increased DNA damage (171–173), increased cardiovascular disease and related conditions (175, 180–182), reduced resistance to infection (primarily viral; refs. 190, 191), and poor cognitive function (208). The similar disease-related nonessential SP mutant phenotypes [DNA damage (170); cancer (26, 33, 79, 147, 161–165); cardiovascular-related (174–179); infection-related (34, 186–189); and brain-related (175, 204–207)] strengthen the epidemiological associations linking Se deficiency to these diseases and conditions and suggest that loss of function of nonessential SPs resulting from modest Se deficiency could conceivably be a disease risk factor. This conclusion is strengthened by evidence pointing to several plausible mechanisms, discussed briefly below.

Mechanistic linkages between nonessential SPs and disease

Mechanistic linkages between nonessential SPs and disease have not been fully elucidated, but evidence points to a variety of plausible mechanisms, as discussed in numerous reviews (8, 22, 79, 175, 184, 241, 271). Several examples are briefly summarized below.

Gpx1 may protect against peroxide-mediated DNA damage possibly leading to cancer not only by direct inactivation of mutagenic oxidants, but by induction of GADD45, which is known to be involved in the regulation of DNA repair (164, 170).

Gpx2 is the only Gpx known to be induced by Nrf2 (272, 273). It was formerly thought to be expressed only in the intestine but is now known to be expressed in other epithelial cells as well, such as in the lung (33, 34). The anti-inflammatory properties of Gpx2 have been demonstrated (274), and it was recently suggested that Gpx2 may protect against inflammatory-induced cancer through a mechanism whereby its removal of hydrogen peroxides in the endoplasmic reticulum (required for COX-2 activity) prevents the subsequent release of high concentrations of prostaglandin E2 (35, 79). It seems reasonable that a similar mechanism could be involved in the increased sensitivity to antigen-induced inflammation reported in Gpx2-KO mice (34).

Gpx3 is strongly induced by inhibition of proteasomes in endothelial cells (275), which are responsible for degrading oxidized proteins. Destruction of oxidants by induced Gpx3 may protect against cardiovascular disease by preserving nitric oxide, which plays an important role in cardiovascular function, and also by preventing the oxidation of fibrinogen, known to promote fibrin thrombus formation (177).

Dio2 is linked to an array of diseases or conditions, including osteoarthritis and other bone-related conditions (178, 198, 200, 201), cognitive dysfunction (205–207), and symptoms of type-2 diabetes (178, 216) (Table 2). The importance of tissue-specific expression of Dio2 in ensuring a proper balance of T_4 and T_3 in various peripheral tissues for normal function is now well established (57, 276) and provides a general rationale for why a broad spectrum of effects might result from reduced Dio2 activity. For example, normal bone development and function requires maintaining a proper T_4/T_3 ratio, in which bone-specific expression of Dio2 plays an important part (277). In muscle, glucose uptake is mediated by the insulin-sensitive

glucose transporter 4 (GLUT4), which is upregulated by T_3, which in turn is regulated by muscle-specific expression of Dio2 (278, 279). This mechanism could possibly explain the linkage of Dio2 SNPs to type 2 diabetes (216). For further discussion of mechanisms that link SPs to disease, see several recent reviews (8, 22, 47, 79, 164, 175).

Se, insulin resistance, and type 2 diabetes

The degree to which the triage theory is supported by evidence linking Se and SPs to insulin resistance and type 2 diabetes is less clear than for the other diseases and conditions discussed above. Experimental results are mixed and confusing, as discussed by many experts (*e.g.*, refs. 175, 220, 247, 250, 280). Predictions of the triage theory are supported by some results and contradicted by others, as briefly discussed below.

On the one hand, there appears to be consensus that intakes of Se significantly above recommended levels (228, 229), or conditions that unusually increase SPs (such as overexpression of Gpx1; refs. 217, 218), can negatively affect glucose metabolism (*i.e.*, increase insulin resistance or type 2 diabetes). Most relevant to the triage theory prediction, however, is evidence relevant to effects on insulin resistance or type 2 diabetes of Se deficiency or reduced SP activity. This evidence is difficult to interpret. Increases in insulin sensitivity reported in rodents as a result of severely decreasing dietary Se (214) or some SPs [Gpx1 (211) or Sepp1 (219)] appear to conflict with recent evidence in mice that severely reducing nonessential SPs increases insulin resistance (220) and that modest Se deficiency in humans is prospectively associated with dysglycemia (212). While it is not clear how to interpret these apparently discrepant results, it is difficult to understand how severe disruption of important antioxidant functions of SPs could have a positive health effect, particularly since high levels of oxidative stress are known to cause insulin resistance (281, 282). Additional evidence clarifying mechanisms is needed. This is an active area of research; see several reviews that discuss possible mechanisms compatible with both protective and exacerbative roles for Se and SPs in diabetes (175, 220, 247, 250, 280).

Implications for public health

The fact that Sepp1 is more sensitive to Se deficiency than Gpx3 in human plasma, illustrated here in Fig. 4 (131–134), has important implications for estimating the percentage of the population that is modestly Se deficient, as previously discussed (132, 149). Since the current RDA (55 μg/d, roughly corresponding to 100 μg/L plasma Se; ref. 283) is based on the sensitivity of Gpx3 in plasma (150, 284), Sepp1 is expected to be at suboptimal levels, even in some individuals meeting current Se intake recommendations. Based on these findings, it recently was suggested that recommended Se intake levels should be raised from 55 to 75 μg/d (132, 149). Higher intakes of almost 100 μg/d were also reported to optimize Sepp1 (285), although results are not inconsistent with Xia *et al.* (132, 149), since intermediate intakes between 50 and 100 μg/d were not examined.

Even using the current RDA as an intake target, modest Se deficiency is quite widespread, including, for example, in most of Western Europe (286, 287). Modest Se deficiency is not generally considered to be of concern among healthy adults in the United States, where intakes range from 60 to 220 μg/d; only a small percentage of the population has Se intakes below the current RDA (288, 289), though Se status declines somewhat in the healthy elderly (290, 291) and greatly declines in subjects who are institutionalized or in poor health (290, 292). If recommended Se intake levels are raised to 75 μg/d, or perhaps even higher, an increased, but still relatively small, percentage of the U.S. population would be considered modestly deficient.

CONCLUSIONS

In this review and in our previous analysis of vitK (3), predictions of the triage theory (1, 2) were largely borne out. If the theory is generally predictive of how the body manages modest V/M shortages for the thousands of V/M-dependent proteins, it has important implications for public health. The most obvious value of the theory is that it provides a rationale for why a particular class of V/M-dependent proteins (*i.e.*, those that are nonessential) may not be fully functional even at modest levels of V/M deficiency not accompanied by any obvious clinical signs. The value of this insight is that it suggests a strategy for identifying sensitive biomarkers of V/M deficiency and candidate proteins mechanistically linked to disease. An important limitation of broadly applying the approach followed here is that mouse KOs are not necessarily reliable predictors of essentiality in humans (*e.g.*, Sepp1 in this analysis) because of many species differences, some known (*e.g.*, refs. 293, 294) and some as yet unknown. The most fruitful strategy is likely to be a combined approach, one that takes advantage of insights gained from mouse KO phenotypes (and human homozygous mutants when available) but that also employs more direct means to assess protein or mRNA hierarchies in people (*e.g.*, refs. 9, 295). FJ

Helpful comments on earlier sections or drafts of this manuscript are greatly appreciated from Tony Allison, Regina Brigelius-Flohé, Raymond Burk, Marcus Conrad, Dolph Hatfield, Harold Helbock, Arne Holmgren, David Killilea, Ashutosh Lal, Margaret Rayman, Mark Shigenaga, Helmut Sies, Holger Steinbrenner, and Jung Suh. Special thanks to Jung Suh for many helpful discussions. The authors greatly appreciate administrative assistance and help with preparation of the figures from Teresa Klask.

REFERENCES

1. Ames, B. N. (2006) Low micronutrient intake may accelerate the degenerative diseases of aging through allocation of scarce micronutrients by triage. *Proc. Natl. Acad. Sci. U. S. A.* **103,** 17589–17594
2. Ames, B. N. (2010) Prevention of mutation, cancer, and other age-associated diseases by optimizing micronutrient intake. *J. Nucleic Acids* **2010,** 725071
3. McCann, J. C., and Ames, B. N. (2009) Vitamin K, an example of triage theory: is micronutrient inadequacy linked to diseases of aging? *Am. J. Clin. Nutr.* **90,** 889–907
4. Kirkwood, T. B. (2008) Understanding ageing from an evolutionary perspective. *J. Intern. Med.* **263,** 117–127
5. Mosfegh, A., Goldman, J., Ahuja, J., Rhodes, D., and LaComb, R. (2009) *What We Eat in America, NHANES 2005–2006: Usual Nutrient Intakes from Food and Water Compared with 1997 Dietary Reference Intakes for Vitamin D, Calcium, Phosphorus, and Magnesium.* U.S. Department of Agriculture, Agricultural Research Service, Washington, DC
6. Brigelius-Flohe, R. (2006) Glutathione peroxidases and redox-regulated transcription factors. *Biol. Chem.* **387,** 1329–1335
7. Conrad, M. (2009) Transgenic mouse models for the vital selenoenzymes cytosolic thioredoxin reductase, mitochondrial thioredoxin reductase and glutathione peroxidase 4. *Biochim. Biophys. Acta* **1790,** 1575–1585
8. Conrad, M., and Schweizer, U. (2010) Unveiling the molecular mechanisms behind selenium-related diseases through knockout mouse studies. *Antioxid. Redox Signal.* **12,** 851–865
9. Schomburg, L. (2010) Genetics and phenomics of selenoenzymes–how to identify an impaired biosynthesis? *Mol. Cell. Endocrinol.* **322,** 114–124
10. Hatfield, D. L., Carlson, B. A., Xu, X. M., Mix, H., and Gladyshev, V. N. (2006) Selenocysteine incorporation machinery and the role of selenoproteins in development and health. *Prog. Nucleic Acids Res. Mol. Biol.* **81,** 97–142
11. Hatfield, D. L., and Gladyshev, V. N. (2002) How selenium has altered our understanding of the genetic code. *Mol. Cell. Biol.* **22,** 3565–3576
12. Hoffmann, P. R., and Berry, M. J. (2005) Selenoprotein synthesis: a unique translational mechanism used by a diverse family of proteins. *Thyroid* **15,** 769–775
13. Jacob, C., Giles, G. I., Giles, N. M., and Sies, H. (2003) Sulfur and selenium: the role of oxidation state in protein structure and function. *Angew. Chem. Int. Ed. Engl.* **42,** 4742–4758
14. Lee, B. C., Dikiy, A., Kim, H. Y., and Gladyshev, V. N. (2009) Functions and evolution of selenoprotein methionine sulfoxide reductases. *Biochim. Biophys. Acta* **1790,** 1471–1477
15. Arnér, E. S. (2010) Selenoproteins - what unique properties can arise with selenocysteine in place of cysteine? *Exp. Cell Res.* **316,** 1296–1303
16. Bosl, M. R., Takaku, K., Oshima, M., Nishimura, S., and Taketo, M. M. (1997) Early embryonic lethality caused by targeted disruption of the mouse selenocysteine tRNA gene (Trsp). *Proc. Natl. Acad. Sci. U. S. A.* **94,** 5531–5534
17. Sengupta, A., Lichti, U. F., Carlson, B. A., Ryscavage, A. O., Gladyshev, V. N., Yuspa, S. H., and Hatfield, D. L. (2010) Selenoproteins are essential for proper keratinocyte function and skin development. *PLoS One* **5,** e12249
18. Carlson, B. A., Yoo, M. H., Tsuji, P. A., Gladyshev, V. N., and Hatfield, D. L. (2009) Mouse models targeting selenocysteine tRNA expression for elucidating the role of selenoproteins in health and development. *Molecules* **14,** 3509–3527
19. Reeves, M. A., and Hoffmann, P. R. (2009) The human selenoproteome: recent insights into functions and regulation. *Cell. Mol. Life Sci.* **66,** 2457–2478
20. Gromer, S., Eubel, J. K., Lee, B. L., and Jacob, J. (2005) Human selenoproteins at a glance. *Cell. Mol. Life Sci.* **62,** 2414–2437
21. Steinbrenner, H., and Sies, H. (2009) Protection against reactive oxygen species by selenoproteins. *Biochim. Biophys. Acta* **1790,** 1478–1485
22. Lu, J., and Holmgren, A. (2009) Selenoproteins. *J. Biol. Chem.* **284,** 723–727
23. Brigelius-Flohe, R. (1999) Tissue-specific functions of individual glutathione peroxidases. *Free Radic. Biol. Med.* **27,** 951–965
24. Arthur, J. R. (2000) The glutathione peroxidases. *Cell. Mol. Life Sci.* **57,** 1825–1835
25. Drevet, J. R. (2006) The antioxidant glutathione peroxidase family and spermatozoa: a complex story. *Mol. Cell. Endocrinol.* **250,** 70–79
26. Lei, X. G., Cheng, W. H., and McClung, J. P. (2007) Metabolic regulation and function of glutathione peroxidase-1. *Annu. Rev. Nutr.* **27,** 41–61
27. Ho, Y. S., Magnenat, J. L., Bronson, R. T., Cao, J., Gargano, M., Sugawara, M., and Funk, C. D. (1997) Mice deficient in cellular glutathione peroxidase develop normally and show no increased sensitivity to hyperoxia. *J. Biol. Chem.* **272,** 16644–16651
28. De Haan, J. B., Bladier, C., Griffiths, P., Kelner, M., O'Shea, R. D., Cheung, N. S., Bronson, R. T., Silvestro, M. J., Wild, S., Zheng, S. S., Beart, P. M., Hertzog, P. J., and Kola, I. (1998) Mice with a homozygous null mutation for the most abundant glutathione peroxidase, Gpx1, show increased susceptibility to the oxidative stress-inducing agents paraquat and hydrogen peroxide. *J. Biol. Chem.* **273,** 22528–22536
29. Schmutzler, C., Mentrup, B., Schomburg, L., Hoang-Vu, C., Herzog, V., and Köhrle, J. (2007) Selenoproteins of the thyroid gland: expression, localization and possible function of glutathione peroxidase 3. *Biol. Chem.* **388,** 1053–1059
30. Wingler, K., and Brigelius-Flohe, R. (1999) Gastrointestinal glutathione peroxidase. *Biofactors* **10,** 245–249
31. Chu, F. F., Doroshow, J. H., and Esworthy, R. S. (1993) Expression, characterization, and tissue distribution of a new cellular selenium-dependent glutathione peroxidase, GSHPx-GI. *J. Biol. Chem.* **268,** 2571–2576
32. Brigelius-Flohé, R., Müller, C., Menard, J., Florian, S., Schmehl, K., and Wingler, K. (2001) Functions of GI-GPx: lessons from selenium-dependent expression and intracellular localization. *Biofactors* **14,** 101–106
33. Walshe, J., Serewko-Auret, M. M., Teakle, N., Cameron, S., Minto, K., Smith, L., Burcham, P. C., Russell, T., Strutton, G., Griffin, A., Chu, F. F., Esworthy, S., Reeve, V., and Saunders, N. A. (2007) Inactivation of glutathione peroxidase activity contributes to UV-induced squamous cell carcinoma formation. *Cancer Res.* **67,** 4751–4758
34. Dittrich, A. M., Meyer, H. A., Krokowski, M., Quarcoo, D., Ahrens, B., Kube, S. M., Witzenrath, M., Esworthy, R. S., Chu, F. F., and Hamelmann, E. (2010) Glutathione peroxidase-2 protects from allergen-induced airway inflammation in mice. *Eur. Respir. J.* **35,** 1148–1154
35. Banning, A., Florian, S., Deubel, S., Thalmann, S., Muller-Schmehl, K., Jacobasch, G., and Brigelius-Flohe, R. (2008) GPx2 counteracts PGE(2) production by dampening COX-2 and mPGES-1 expression in human colon cancer cells. *Antioxid. Redox Signal.* **10,** 1491–1500
36. Comhair, S. A., and Erzurum, S. C. (2005) The regulation and role of extracellular glutathione peroxidase. *Antioxid. Redox Signal.* **7,** 72–79
37. Olson, G. E., Whitin, J. C., Hill, K. E., Winfrey, V. P., Motley, A. K., Austin, L. M., Deal, J., Cohen, H. J., and Burk, R. F. (2010) Extracellular glutathione peroxidase (GPx3) binds specifically to basement membranes of mouse renal cortex tubule cells. *Am. J. Physiol. Renal Physiol.* **298,** F1244–F1253
38. Köhrle, J., and Gärtner, R. (2009) Selenium and thyroid. *Best Pract. Res. Clin. Endocrinol. Metab.* **23,** 815–827
39. Baek, I. J., Seo, D. S., Yon, J. M., Lee, S. R., Jin, Y., Nahm, S. S., Jeong, J. H., Choo, Y. K., Kang, J. K., Lee, B. J., Yun, Y. W., and Nam, S. Y. (2007) Tissue expression and cellular localization of phospholipid hydroperoxide glutathione peroxidase (PHGPx) mRNA in male mice. *J. Mol. Histol.* **38,** 237–244
40. Conrad, M., Schneider, M., Seiler, A., and Bornkamm, G. W. (2007) Physiological role of phospholipid hydroperoxide glutathione peroxidase in mammals. *Biol. Chem.* **388,** 1019–1025
41. Savaskan, N. E., Ufer, C., Kuhn, H., and Borchert, A. (2007) Molecular biology of glutathione peroxidase 4: from genomic structure to developmental expression and neural function. *Biol. Chem.* **388,** 1007–1017
42. Yant, L. J., Ran, Q., Rao, L., Van Remmen, H., Shibatani, T., Belter, J. G., Motta, L., Richardson, A., and Prolla, T. A. (2003) The selenoprotein GPX4 is essential for mouse development and protects from radiation and oxidative damage insults. *Free Radic. Biol. Med.* **34,** 496–502

43. Imai, H., Hirao, F., Sakamoto, T., Sekine, K., Mizukura, Y., Saito, M., Kitamoto, T., Hayasaka, M., Hanaoka, K., and Nakagawa, Y. (2003) Early embryonic lethality caused by targeted disruption of the mouse PHGPx gene. *Biochem. Biophys. Res. Commun.* **305,** 278–286
44. Conrad, M., Moreno, S. G., Sinowatz, F., Ursini, F., Kolle, S., Roveri, A., Brielmeier, M., Wurst, W., Maiorino, M., and Bornkamm, G. W. (2005) The nuclear form of phospholipid hydroperoxide glutathione peroxidase is a protein thiol peroxidase contributing to sperm chromatin stability. *Mol. Cell. Biol.* **25,** 7637–7644
45. Schneider, M., Förster, H., Boersma, A., Seiler, A., Wehnes, H., Sinowatz, F., Neumüller, C., Deutsch, M. J., Walch, A., Hrabé de Angelis, M., Wurst, W., Ursini, F., Roveri, A., Maleszewski, M., Maiorino, M., and Conrad, M. (2009) Mitochondrial glutathione peroxidase 4 disruption causes male infertility. *FASEB J.* **23,** 3233–3242
46. Liang, H., Yoo, S. E., Na, R., Walter, C. A., Richardson, A., and Ran, Q. (2009) Short form glutathione peroxidase 4 is the essential isoform required for survival and somatic mitochondrial functions. *J. Biol. Chem.* **284,** 30836–30844
47. Conrad, M. C. (2011) Mouse models for glutathione peroxidase 4 (GPx4). In *Selenium: Its Molecular Biology and Role in Human Health*, 3rd Ed. (Hatfield, D., Gladyshev, V., and Berry, M., eds), Springer, New York. In press
48. Arnér, E. S. (2009) Focus on mammalian thioredoxin reductases - important selenoproteins with versatile functions. *Biochim. Biophys. Acta* **1790,** 495–526
49. Gromer, S., Urig, S., and Becker, K. (2004) The thioredoxin system–from science to clinic. *Med. Res. Rev.* **24,** 40–89
50. Soerensen, J., Jakupoglu, C., Beck, H., Förster, H., Schmidt, J., Schmahl, W., Schweizer, U., Conrad, M., and Brielmeier, M. (2008) The role of thioredoxin reductases in brain development. *PLoS One* **3,** e1813
51. Berndt, C., Lillig, C. H., and Holmgren, A. (2007) Thiol-based mechanisms of the thioredoxin and glutaredoxin systems: implications for diseases in the cardiovascular system. *Am. J. Physiol. Heart Circ. Physiol.* **292,** H1227–H1236
52. Bondareva, A. A., Capecchi, M. R., Iverson, S. V., Li, Y., Lopez, N. I., Lucas, O., Merrill, G. F., Prigge, J. R., Siders, A. M., Wakamiya, M., Wallin, S. L., and Schmidt, E. E. (2007) Effects of thioredoxin reductase-1 deletion on embryogenesis and transcriptome. *Free Radic. Biol. Med.* **43,** 911–923
53. Jakupoglu, C., Przemeck, G. K., Schneider, M., Moreno, S. G., Mayr, N., Hatzopoulos, A. K., de Angelis, M. H., Wurst, W., Bornkamm, G. W., Brielmeier, M., and Conrad, M. (2005) Cytoplasmic thioredoxin reductase is essential for embryogenesis but dispensable for cardiac development. *Mol. Cell. Biol.* **25,** 1980–1988
54. Turanov, A. A., Kehr, S., Marino, S. M., Yoo, M. H., Carlson, B. A., Hatfield, D. L., and Gladyshev, V. N. (2010) Mammalian thioredoxin reductase 1: roles in redox homoeostasis and characterization of cellular targets. *Biochem. J.* **430,** 285–293
55. Dammeyer, P., and Arnér, E. S. (2010) Human protein atlas of redox systems–what can be learnt? *Biochim. Biophys. Acta* **1810,** 111–38
56. Schweizer, U., Weitzel, J. M., and Schomburg, L. (2008) Think globally: act locally. New insights into the local regulation of thyroid hormone availability challenge long accepted dogmas. *Mol. Cell. Endocrinol.* **289,** 1–9
57. Bianco, A. C., Salvatore, D., Gereben, B., Berry, M. J., and Larsen, P. R. (2002) Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocr. Rev.* **23,** 38–89
58. Galton, V. A., Schneider, M. J., Clark, A. S., and St Germain, D. L. (2009) Life without thyroxine to 3,5,3′-triiodothyronine conversion: studies in mice devoid of the 5′-deiodinases. *Endocrinology* **150,** 2957–2963
59. St Germain, D. L., Hernandez, A., Schneider, M. J., and Galton, V. A. (2005) Insights into the role of deiodinases from studies of genetically modified animals. *Thyroid* **15,** 905–916
60. Gereben, B., Zavacki, A. M., Ribich, S., Kim, B. W., Huang, S. A., Simonides, W. S., Zeold, A., and Bianco, A. C. (2008) Cellular and molecular basis of deiodinase-regulated thyroid hormone signaling. *Endocr. Rev.* **29,** 898
61. Schneider, M. J., Fiering, S. N., Thai, B., Wu, S. Y., St Germain, E., Parlow, A. F., St Germain, D. L., and Galton, V. A. (2006) Targeted disruption of the type 1 selenodeiodinase gene (Dio1) results in marked changes in thyroid hormone economy in mice. *Endocrinology* **147,** 580–589
62. Schneider, M. J., Fiering, S. N., Pallud, S. E., Parlow, A. F., St Germain, D. L., and Galton, V. A. (2001) Targeted disruption of the type 2 selenodeiodinase gene (DIO2) results in a phenotype of pituitary resistance to T4. *Mol. Endocrinol.* **15,** 2137–2148
63. Hernandez, A. (2005) Structure and function of the type 3 deiodinase gene. *Thyroid* **15,** 865–874
64. Hernandez, A., Martinez, M. E., Fiering, S., Galton, V. A., and St Germain, D. (2006) Type 3 deiodinase is critical for the maturation and function of the thyroid axis. *J. Clin. Invest.* **116,** 476–484
65. Wagner, M. S., Wajner, S. M., and Maia, A. L. (2009) Is there a role for thyroid hormone on spermatogenesis? *Microsc. Res. Tech.* **72,** 796–808
66. Burk, R. F., and Hill, K. E. (2009) Selenoprotein P-expression, functions, and roles in mammals. *Biochim. Biophys. Acta* **1790,** 1441–1447
67. Hill, K. E., Zhou, J., McMahan, W. J., Motley, A. K., Atkins, J. F., Gesteland, R. F., and Burk, R. F. (2003) Deletion of selenoprotein P alters distribution of selenium in the mouse. *J. Biol. Chem.* **278,** 13640–13646
68. Schomburg, L., Schweizer, U., Holtmann, B., Flohé, L., Sendtner, M., and Köhrle, J. (2003) Gene disruption discloses role of selenoprotein P in selenium delivery to target tissues. *Biochem. J.* **370,** 397–402
69. Olson, G. E., Winfrey, V. P., Nagdas, S. K., Hill, K. E., and Burk, R. F. (2005) Selenoprotein P is required for mouse sperm development. *Biol. Reprod.* **73,** 201–211
70. Aachmann, F. L., Sal, L. S., Kim, H. Y., Marino, S. M., Gladyshev, V. N., and Dikiy, A. (2010) Insights into function, catalytic mechanism and fold evolution of selenoprotein methionine sulfoxide reductase B1 through structural analysis. *J. Biol. Chem.* **285,** 33315–33323
71. Fomenko, D. E., Novoselov, S. V., Natarajan, S. K., Lee, B. C., Koc, A., Carlson, B. A., Lee, T. H., Kim, H. Y., Hatfield, D. L., and Gladyshev, V. N. (2009) MsrB1 (methionine-R-sulfoxide reductase 1) knock-out mice: roles of MsrB1 in redox regulation and identification of a novel selenoprotein form. *J. Biol. Chem.* **284,** 5986–5993
72. Hansel, A., Heinemann, S. H., and Hoshi, T. (2005) Heterogeneity and function of mammalian MSRs: enzymes for repair, protection and regulation. *Biochim. Biophys. Acta* **1703,** 239–247
73. Stadtman, E. R., Moskovitz, J., and Levine, R. L. (2003) Oxidation of methionine residues of proteins: biological consequences. *Antioxid. Redox Signal.* **5,** 577–582
74. Novoselov, S. V., Kim, H. Y., Hua, D., Lee, B. C., Astle, C. M., Harrison, D. E., Friguet, B., Moustafa, M. E., Carlson, B. A., Hatfield, D. L., and Gladyshev, V. N. (2010) Regulation of selenoproteins and methionine sulfoxide reductases A and B1 by age, calorie restriction, and dietary selenium in mice. *Antioxid. Redox Signal.* **12,** 829–838
75. Lescure, A., Rederstorff, M., Krol, A., Guicheney, P., and Allamand, V. (2009) Selenoprotein function and muscle disease. *Biochim. Biophys. Acta* **1790,** 1569–1574
76. Arbogast, S., and Ferreiro, A. (2010) Selenoproteins and protection against oxidative stress: selenoprotein n as a novel player at the crossroads of redox signaling and calcium homeostasis. *Antioxid. Redox Signal.* **12,** 893–904
77. Hill, K. E., Burk, R. F., and Lane, J. M. (1987) Effect of selenium depletion and repletion on plasma glutathione and glutathione-dependent enzymes in the rat. *J. Nutr.* **117,** 99–104
78. Schomburg, L., and Schweizer, U. (2009) Hierarchical regulation of selenoprotein expression and sex-specific effects of selenium. *Biochim. Biophys. Acta* **1790,** 1453–1462
79. Brigelius-Flohé, R., and Kipp, A. (2009) Glutathione peroxidases in different stages of carcinogenesis. *Biochim. Biophys. Acta* **1790,** 1555–1568
80. Burk, R. F., and Hill, K. E. (2005) Selenoprotein P: an extracellular protein with unique physical characteristics and a role in selenium homeostasis. *Annu. Rev. Nutr.* **25,** 215–235
81. Thomson, C. D. (2004) Assessment of requirements for selenium and adequacy of selenium status: a review. *Eur. J. Clin. Nutr.* **58,** 391–402

82. Sunde, R. A., Raines, A. M., Barnes, K. M., and Evenson, J. K. (2009) Selenium status highly regulates selenoprotein mRNA levels for only a subset of the selenoproteins in the selenoproteome. *Biosci. Rep.* **29,** 329–338
83. Berry, M. J. (2005) Insights into the hierarchy of selenium incorporation. *Nat. Genet.* **37,** 1162–1162
84. Driscoll, D. M., and Copeland, P. R. (2003) Mechanism and regulation of selenoprotein synthesis. *Annu. Rev. Nutr.* **23,** 17–40
85. Burk, R. F., Brown, D. G., Seely, R. J., and Scaief, C. C., 3rd. (1972) Influence of dietary and injected selenium on whole-blody retention, route of excretion, and tissue retention of 75SeO3 2- in the rat. *J. Nutr.* **102,** 1049–1055
86. Behne, D., Hilmert, H., Scheid, S., Gessner, H., and Elger, W. (1988) Evidence for specific selenium target tissues and new biologically important selenoproteins. *Biochim. Biophys. Acta* **966,** 12–21
87. Kipp, A., Banning, A., van Schothorst, E. M., Méplan, C., Schomburg, L., Evelo, C., Coort, S., Gaj, S., Keijer, J., Hesketh, J., and Brigelius-Flohé, R. (2009) Four selenoproteins, protein biosynthesis, and Wnt signalling are particularly sensitive to limited selenium intake in mouse colon. *Mol. Nutr. Food Res.* **53,** 1561–1572
88. Wingler, K., Bocher, M., Flohé, L., Kollmus, H., and Brigelius-Flohe, R. (1999) mRNA stability and selenocysteine insertion sequence efficiency rank gastrointestinal glutathione peroxidase high in the hierarchy of selenoproteins. *Eur. J. Biochem.* **259,** 149–157
89. Bermano, G., Arthur, J. R., and Hesketh, J. E. (1996) Selective control of cytosolic glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase mRNA stability by selenium supply. *FEBS Lett.* **387,** 157–160
90. Squires, J. E., Stoytchev, I., Forry, E. P., and Berry, M. J. (2007) SBP2 binding affinity is a major determinant in differential selenoprotein mRNA translation and sensitivity to nonsense-mediated decay. *Mol. Cell. Biol.* **27,** 7848–7855
91. Dumitrescu, A., Di Cosmo, C., Liao, X. H., Weiss, R., and Refetoff, S. (2010) The syndrome of inherited partial SBP2 deficiency in humans. *Antioxid. Redox Signal.* **12,** 905–920
92. Low, S. C., Grundner-Culemann, E., Harney, J. W., and Berry, M. J. (2000) SECIS-SBP2 interactions dictate selenocysteine incorporation efficiency and selenoprotein hierarchy. *EMBO J.* **19,** 6882–6890
93. Moustafa, M. E., Carlson, B. A., El-Saadani, M. A., Kryukov, G. V., Sun, Q. A., Harney, J. W., Hill, K. E., Combs, G. F., Feigenbaum, L., Mansur, D. B., Burk, R. F., Berry, M. J., Diamond, A. M., Lee, B. J., Gladyshev, V. N., and Hatfield, D. L. (2001) Selective inhibition of selenocysteine tRNA maturation and selenoprotein synthesis in transgenic mice expressing isopentenyladenosine-deficient selenocysteine tRNA. *Mol. Cell. Biol.* **21,** 3840–3852
94. Fang, W., Goldberg, M. L., Pohl, N. M., Bi, X., Tong, C., Xiong, B., Koh, T. J., Diamond, A. M., and Yang, W. (2010) Functional and physical interaction between the selenium-binding protein 1 (sbp1) and the glutathione peroxidase 1 selenoprotein. *Carcinogenesis* **31,** 1360–1366
95. Speckmann, B., Walter, P. L., Alili, L., Reinehr, R., Sies, H., Klotz, L. O., and Steinbrenner, H. (2008) Selenoprotein P expression is controlled through interaction of the coactivator PGC-1alpha with FoxO1a and hepatocyte nuclear factor 4alpha transcription factors. *Hepatology* **48,** 1998–2006
96. Boitani, C., and Puglisi, R. (2008) Selenium, a key element in spermatogenesis and male fertility. In *Molecular Mechanisms in Spermatogenesis* (Cheng, C. Y., ed) Chap. 5, pp. 1–9, Landes Bioscience & Springer Science + Business Media, Austin, TX, USA
97. Vernet, P., Aitken, R. J., and Drevet, J. R. (2004) Antioxidant strategies in the epididymis. *Mol. Cell. Endocrinol.* **216,** 31–39
98. Seiler, A., Schneider, M., Förster, H., Roth, S., Wirth, E. K., Culmsee, C., Plesnila, N., Kremmer, E., Rådmark, O., Wurst, W., Bornkamm, G. W., Schweizer, U., and Conrad, M. (2008) Glutathione peroxidase 4 senses and translates oxidative stress into 12/15-lipoxygenase dependent- and AIF-mediated cell death. *Cell. Metab.* **8,** 237–248
99. Imai, H., and Nakagawa, Y. (2003) Biological significance of phospholipid hydroperoxide glutathione peroxidase (PHGPx, GPx4) in mammalian cells. *Free Radic. Biol. Med.* **34,** 145–169
100. Schnurr, K., Belkner, J., Ursini, F., Schewe, T., and Kühn, H. (1996) The selenoenzyme phospholipid hydroperoxide glutathione peroxidase controls the activity of the 15-lipoxygenase with complex substrates and preserves the specificity of the oxygenation products. *J. Biol. Chem.* **271,** 4653–4658
101. Ursini, F., Heim, S., Kiess, M., Maiorino, M., Roveri, A., Wissing, J., and Flohé, L. (1999) Dual function of the selenoprotein PHGPx during sperm maturation. *Science* **285,** 1393–1396
102. Conrad, M., Jakupoglu, C., Moreno, S. G., Lippl, S., Banjac, A., Schneider, M., Beck, H., Hatzopoulos, A. K., Just, U., Sinowatz, F., Schmahl, W., Chien, K. R., Wurst, W., Bornkamm, G. W., and Brielmeier, M. (2004) Essential role for mitochondrial thioredoxin reductase in hematopoiesis, heart development, and heart function. *Mol. Cell. Biol.* **24,** 9414–9423
103. Matsui, M., Oshima, M., Oshima, H., Takaku, K., Maruyama, T., Yodoi, J., and Taketo, M. M. (1996) Early embryonic lethality caused by targeted disruption of the mouse thioredoxin gene. *Dev. Biol.* **178,** 179–185
104. Nonn, L., Williams, R. R., Erickson, R. P., and Powis, G. (2003) The absence of mitochondrial thioredoxin 2 causes massive apoptosis, exencephaly, and early embryonic lethality in homozygous mice. *Mol. Cell. Biol.* **23,** 916–922
105. Kiermayer, C., Michalke, B., Schmidt, J., and Brielmeier, M. (2007) Effect of selenium on thioredoxin reductase activity in Txnrd1 or Txnrd2 hemizygous mice. *Biol. Chem.* **388,** 1091–1097
106. Rollins, M. F., van der Heide, D. M., Weisend, C. M., Kundert, J. A., Comstock, K. M., Suvorova, E. S., Capecchi, M. R., Merrill, G. F., and Schmidt, E. E. (2010) Hepatocytes lacking thioredoxin reductase 1 have normal replicative potential during development and regeneration. *J. Cell Sci.* **123,** 2402–2412
107. Turanov, A. A., Su, D., and Gladyshev, V. N. (2006) Characterization of alternative cytosolic forms and cellular targets of mouse mitochondrial thioredoxin reductase. *J. Biol. Chem.* **281,** 22953–22963
108. Winterbourn, C. C., and Hampton, M. B. (2008) Thiol chemistry and specificity in redox signaling. *Free. Radic. Biol. Med.* **45,** 549–561
109. Jones, D. P. (2006) Redefining oxidative stress. *Antioxid. Redox Signal.* **8,** 1865–1879
110. Chanoine, J. P., Braverman, L. E., Farwell, A. P., Safran, M., Alex, S., Dubord, S., and Leonard, J. L. (1993) The thyroid gland is a major source of circulating T3 in the rat. *J. Clin. Invest.* **91,** 2709–2713
111. Sherwood, L. S. (2006) *Fundamentals of Physiology: A Human Perspective*, 3rd Ed., Thomson Brooks/Cole, Pacific Grove, CA, USA
112. Allamand, V., Richard, P., Lescure, A., Ledeuil, C., Desjardin, D., Petit, N., Gartioux, C., Ferreiro, A., Krol, A., Pellegrini, N., Urtizberea, J. A., and Guicheney, P. (2006) A single homozygous point mutation in a 3′untranslated region motif of selenoprotein N mRNA causes SEPN1-related myopathy. *EMBO Rep.* **7,** 450–454
113. Maiti, B., Arbogast, S., Allamand, V., Moyle, M. W., Anderson, C. B., Richard, P., Guicheney, P., Ferreiro, A., Flanigan, K. M., and Howard, M. T. (2009) A mutation in the SEPN1 selenocysteine redefinition element (SRE) reduces selenocysteine incorporation and leads to SEPN1-related myopathy. *Hum. Mutat.* **30,** 411–416
114. Holmgren, A., and Lu, J. (2010) Thioredoxin and thioredoxin reductase: current research with special reference to human disease. *Biochem. Biophys. Res. Commun.* **396,** 120–124
115. Sunde, R. A., and Thompson, K. M. (2009) Dietary selenium requirements based on tissue selenium concentration and glutathione peroxidase activities in old female rats. *J. Trace Elem. Med. Biol.* **23,** 132–137
116. Lei, X. G., Evenson, J. K., Thompson, K. M., and Sunde, R. A. (1995) Glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase are differentially regulated in rats by dietary selenium. *J. Nutr.* **125,** 1438–1446
117. Hill, K. E., McCollum, G. W., Boeglin, M. E., and Burk, R. F. (1997) Thioredoxin reductase activity is decreased by selenium deficiency. *Biochem. Biophys. Res. Commun.* **234,** 293–295
118. Barnes, K. M., Evenson, J. K., Raines, A. M., and Sunde, R. A. (2009) Transcript analysis of the selenoproteome indicates that dietary selenium requirements of rats based on selenium-regulated selenoprotein mRNA levels are uniformly less than

those based on glutathione peroxidase activity. *J. Nutr.* **139,** 199–206
119. Schriever, S. C., Barnes, K. M., Evenson, J. K., Raines, A. M., and Sunde, R. A. (2009) Selenium requirements are higher for glutathione peroxidase-1 mRNA than gpx1 activity in rat testis. *Exp. Biol. Med. (Maywood)* **234,** 513–521
120. Bermano, G., Nicol, F., Dyer, J. A., Sunde, R. A., Beckett, G. J., Arthur, J. R., and Hesketh, J. E. (1995) Tissue-specific regulation of selenoenzyme gene expression during selenium deficiency in rats. *Biochem. J.* **311**, 425–430
121. Beckett, G. J., Nicol, F., Rae, P. W., Beech, S., Guo, Y., and Arthur, J. R. (1993) Effects of combined iodine and selenium deficiency on thyroid hormone metabolism in rats. *Am. J. Clin. Nutr.* **57,** 240S–243S
122. Crosley, L. K., Méplan, C., Nicol, F., Rundlöf, A. K., Arnér, E. S., Hesketh, J. E., and Arthur, J. R. (2007) Differential regulation of expression of cytosolic and mitochondrial thioredoxin reductase in rat liver and kidney. *Arch. Biochem. Biophys.* **459,** 178–188
123. Weiss Sachdev, S., and Sunde, R. A. (2001) Selenium regulation of transcript abundance and translational efficiency of glutathione peroxidase-1 and -4 in rat liver. *Biochem. J.* **357,** 851–858
124. Hadley, K. B., and Sunde, R. A. (2001) Selenium regulation of thioredoxin reductase activity and mRNA levels in rat liver. *J. Nutr. Biochem.* **12,** 693–702
125. Sunde, R. A., Evenson, J. K., Thompson, K. M., and Sachdev, S. W. (2005) Dietary selenium requirements based on glutathione peroxidase-1 activity and mRNA levels and other Se-dependent parameters are not increased by pregnancy and lactation in rats. *J. Nutr.* **135,** 2144–2150
126. Weitzel, F., Ursini, F., and Wendel, A. (1990) Phospholipid hydroperoxide glutathione peroxidase in various mouse organs during selenium deficiency and repletion. *Biochim. Biophys. Acta* **1036,** 88–94
127. Nakayama, A., Hill, K. E., Austin, L. M., Motley, A. K., and Burk, R. F. (2007) All regions of mouse brain are dependent on selenoprotein P for maintenance of selenium. *J. Nutr.* **137,** 690–693
128. Hill, K. E., Zhou, J., McMahan, W. J., Motley, A. K., and Burk, R. F. (2004) Neurological dysfunction occurs in mice with targeted deletion of the selenoprotein P gene. *J. Nutr.* **134,** 157–161
129. Chittum, H. S., Hill, K. E., Carlson, B. A., Lee, B. J., Burk, R. F., and Hatfield, D. L. (1997) Replenishment of selenium deficient rats with selenium results in redistribution of the selenocysteine tRNA population in a tissue specific manner. *Biochim. Biophys. Acta* **1359,** 25–34
130. Burk, R. F., Hill, K. E., Read, R., and Bellew, T. (1991) Response of rat selenoprotein P to selenium administration and fate of its selenium. *Am. J. Physiol.* **261,** E26–E30
131. Duffield, A. J., Thomson, C. D., Hill, K. E., and Williams, S. (1999) An estimation of selenium requirements for New Zealanders. *Am. J. Clin. Nutr.* **70,** 896–903
132. Xia, Y., Hill, K. E., Byrne, D. W., Xu, J., and Burk, R. F. (2005) Effectiveness of selenium supplements in a low-selenium area of China. *Am. J. Clin. Nutr.* **81,** 829–834
133. Persson-Moschos, M., Alfthan, G., and Akesson, B. (1998) Plasma selenoprotein P levels of healthy males in different selenium status after oral supplementation with different forms of selenium. *Eur. J. Clin. Nutr.* **52,** 363–367
134. Levander, O. A., Alfthan, G., Arvilommi, H., Gref, C. G., Huttunen, J. K., Kataja, M., Koivistoinen, P., and Pikkarainen, J. (1983) Bioavailability of selenium to Finnish men as assessed by platelet glutathione peroxidase activity and other blood parameters. *Am. J. Clin. Nutr.* **37,** 887–897
135. Wu, Q., Huang, K., and Xu, H. (2003) Effects of long-term selenium deficiency on glutathione peroxidase and thioredoxin reductase activities and expressions in rat aorta. *J. Inorg. Biochem.* **94,** 301–306
136. Yang, J. G., Hill, K. E., and Burk, R. F. (1989) Dietary selenium intake controls rat plasma selenoprotein P concentration. *J. Nutr.* **119,** 1010–1012
137. Hrdina, J., Banning, A., Kipp, A., Loh, G., Blaut, M., and Brigelius-Flohé, R. (2009) The gastrointestinal microbiota affects the selenium status and selenoprotein expression in mice. *J. Nutr. Biochem.* **20,** 638–648
138. Xia, Y. M., Hill, K. E., and Burk, R. F. (1989) Biochemical studies of a selenium-deficient population in China: measurement of selenium, glutathione peroxidase and other oxidant defense indices in blood. *J. Nutr.* **119,** 1318–1326
139. Hill, K. E., Xia, Y., Akesson, B., Boeglin, M. E., and Burk, R. F. (1996) Selenoprotein P concentration in plasma is an index of selenium status in selenium-deficient and selenium-supplemented Chinese subjects. *J. Nutr.* **126,** 138–145
140. Uthus, E. O., and Moskovitz, J. (2007) Specific activity of methionine sulfoxide reductase in CD-1 mice is significantly affected by dietary selenium but not zinc. *Biol. Trace. Elem. Res.* **115,** 265–276
141. Novoselov, S. V., Calvisi, D. F., Labunskyy, V. M., Factor, V. M., Carlson, B. A., Fomenko, D. E., Moustafa, M. E., Hatfield, D. L., and Gladyshev, V. N. (2005) Selenoprotein deficiency and high levels of selenium compounds can effectively inhibit hepatocarcinogenesis in transgenic mice. *Oncogene* **24,** 8003–8011
142. Venardos, K., Harrison, G., Headrick, J., and Perkins, A. (2004) Effects of dietary selenium on glutathione peroxidase and thioredoxin reductase activity and recovery from cardiac ischemia-reperfusion. *J. Trace Elem. Med. Biol.* **18,** 81–88
143. Mitchell, J. H., Nicol, F., Beckett, G. J., and Arthur, J. R. (1996) Selenoenzyme expression in thyroid and liver of second generation selenium- and iodine-deficient rats. *J. Mol. Endocrinol.* **16,** 259–267
144. Mitchell, J. H., Nicol, F., Beckett, G. J., and Arthur, J. R. (1997) Selenium and iodine deficiencies: effects on brain and brown adipose tissue selenoenzyme activity and expression. *J. Endocrinol.* **155,** 255–263
145. Wu, Q., and Huang, K. (2004) Effect of long-term SE deficiency on the antioxidant capacities of rat vascular tissue. *Biol. Trace Elem. Res.* **98,** 73–84
146. Bermano, G., Nicol, F., Dyer, J. A., Sunde, R. A., Beckett, G. J., Arthur, J. R., and Hesketh, J. E. (1996) Selenoprotein gene expression during selenium-repletion of selenium-deficient rats. *Biol. Trace Elem. Res.* **51,** 211–223
147. Irons, R., Carlson, B. A., Hatfield, D. L., and Davis, C. D. (2006) Both selenoproteins and low molecular weight selenocompounds reduce colon cancer risk in mice with genetically impaired selenoprotein expression. *J. Nutr.* **136,** 1311–1317
148. Lu, J., Zhong, L., Lönn, M. E., Burk, R. F., Hill, K. E., and Holmgren, A. (2009) Penultimate selenocysteine residue replaced by cysteine in thioredoxin reductase from selenium-deficient rat liver. *FASEB J.* **23,** 2394–2402
149. Xia, Y., Hill, K. E., Li, P., Xu, J., Zhou, D., Motley, A. K., Wang, L., Byrne, D. W., and Burk, R. F. (2010) Optimization of selenoprotein P and other plasma selenium biomarkers for the assessment of the selenium nutritional requirement: a placebo-controlled double-blind study of selenomethionine supplementation in selenium-deficient Chinese subjects. *Am. J. Clin. Nutr.* **92,** 525–531
150. NAS/DRI (2000) *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*, National Academy Press, Washington, DC
151. Ames, B. N., McCann, J. C., Stampfer, M. J., and Willett, W. C. (2007) Evidence-based decision making on micronutrients and chronic disease: long-term randomized controlled trials are not enough. *Am. J. Clin. Nutr.* **86,** 522–523
152. Brigelius-Flohe, R. (2008) Selenium compounds and selenoproteins in cancer. *Chem. Biodivers.* **5,** 389–395
153. Yoo, M. H., Carlson, B. A., Tsuji, P., Irons, R., Gladyshev, V. N., and Hatfield, D. L. (2010) Alteration of thioredoxin reductase 1 levels in elucidating cancer etiology. *Methods Enzymol.* **474,** 255–275
154. Hatfield, D. L., Yoo, M. H., Carlson, B. A., and Gladyshev, V. N. (2009) Selenoproteins that function in cancer prevention and promotion. *Biochim. Biophys. Acta* **1790,** 1541–1545
155. Mandal, P. K., Schneider, M., Kölle, P., Kuhlencordt, P., Förster, H., Beck, H., Bornkamm, G. W., and Conrad, M. (2010) Loss of thioredoxin reductase 1 renders tumors highly susceptible to pharmacologic glutathione deprivation. *Cancer Res.* **70,** 9505–9514
156. de Haan, J. B., Bladier, C., Lotfi-Miri, M., Taylor, J., Hutchinson, P., Crack, P. J., Hertzog, P., and Kola, I. (2004) Fibroblasts derived from Gpx1 knockout mice display senescent-like features and are susceptible to H2O2-mediated cell death. *Free Radic. Biol. Med.* **36,** 53–64
157. Wolf, N., Penn, P., Pendergrass, W., Van Remmen, H., Bartke, A., Rabinovitch, P., and Martin, G. M. (2005) Age-related

cataract progression in five mouse models for anti-oxidant protection or hormonal influence. *Exp. Eye Res.* **81,** 276–285
158. Bleys, J., Navas-Acien, A., and Guallar, E. (2008) Serum selenium levels and all-cause, cancer, and cardiovascular mortality among US adults. *Arch. Intern. Med.* **168,** 404–410
159. Ray, A. L., Semba, R. D., Walston, J., Ferrucci, L., Cappola, A. R., Ricks, M. O., Xue, Q. L., and Fried, L. P. (2006) Low serum selenium and total carotenoids predict mortality among older women living in the community: the women's health and aging studies. *J. Nutr.* **136,** 172–176
160. Akbaraly, N. T., Arnaud, J., Hininger-Favier, I., Gourlet, V., Roussel, A. M., and Berr, C. (2005) Selenium and mortality in the elderly: results from the EVA study. *Clin. Chem.* **51,** 2117–2123
161. Chu, F. F., Esworthy, R. S., Chu, P. G., Longmate, J. A., Huycke, M. M., Wilczynski, S., and Doroshow, J. H. (2004) Bacteria-induced intestinal cancer in mice with disrupted Gpx1 and Gpx2 genes. *Cancer Res.* **64,** 962–968
162. Diwadkar-Navsariwala, V., Prins, G. S., Swanson, S. M., Birch, L. A., Ray, V. H., Hedayat, S., Lantvit, D. L., and Diamond, A. M. (2006) Selenoprotein deficiency accelerates prostate carcinogenesis in a transgenic model. *Proc. Natl. Acad. Sci. U. S. A.* **103,** 8179–8184
163. Steinbrecher, A., Meplan, C., Hesketh, J., Schomburg, L., Endermann, T., Jansen, E., Akesson, B., Rohrmann, S., and Linseisen, J. (2010) Effects of selenium status and polymorphisms in selenoprotein genes on prostate cancer risk in a prospective study of European men. *Cancer Epidemiol. Biomarkers Prev.* **19,** 2958–2968
164. Zhuo, P., and Diamond, A. M. (2009) Molecular mechanisms by which selenoproteins affect cancer risk and progression. *Biochim. Biophys. Acta* **1790,** 1546–1554
165. Lee, O. J., Schneider-Stock, R., McChesney, P. A., Kuester, D., Roessner, A., Vieth, M., Moskaluk, C. A., and El-Rifai, W. (2005) Hypermethylation and loss of expression of glutathione peroxidase-3 in Barrett's tumorigenesis. *Neoplasia* **7,** 854–861
166. Steevens, J., van den Brandt, P. A., Goldbohm, R. A., and Schouten, L. J. (2009) Selenium status and the risk of esophageal and gastric cancer subtypes: the Netherlands cohort study. *Gastroenterology* **138,** 1704–1713
167. Wei, W. Q., Abnet, C. C., Qiao, Y. L., Dawsey, S. M., Dong, Z. W., Sun, X. D., Fan, J. H., Gunter, E. W., Taylor, P. R., and Mark, S. D. (2004) Prospective study of serum selenium concentrations and esophageal and gastric cardia cancer, heart disease, stroke, and total death. *Am. J. Clin. Nutr.* **79,** 80–85
168. Amaral, A. F., Cantor, K. P., Silverman, D. T., and Malats, N. (2010) Selenium and bladder cancer risk: a meta-analysis. *Cancer Epidemiol. Biomarkers. Prev.* **19,** 2407–2415
169. Whanger, P. D. (2004) Selenium and its relationship to cancer: an update. *Br. J. Nutr.* **91,** 11–28
170. Baliga, M. S., Diwadkar-Navsariwala, V., Koh, T., Fayad, R., Fantuzzi, G., and Diamond, A. M. (2008) Selenoprotein deficiency enhances radiation-induced micronuclei formation. *Mol. Nutr. Food Res.* **52,** 1300–1304
171. Dusinská, M., Vallová, B., Ursínyová, M., Hladíková, V., Smolková, B., Wsólová, L., Raslová, K., and Collins, A. R. (2002) DNA damage and antioxidants; fluctuations through the year in a central European population group. *Food. Chem. Toxicol.* **40,** 1119–1123
172. Karunasinghe, N., Ryan, J., Tuckey, J., Masters, J., Jamieson, M., Clarke, L. C., Marshall, J. R., and Ferguson, L. R. (2004) DNA stability and serum selenium levels in a high-risk group for prostate cancer. *Cancer Epidemiol. Biomarkers Prev.* **13,** 391–397
173. Wasson, G. R., McKelvey-Martin, V. J., and Downes, C. S. (2008) The use of the comet assay in the study of human nutrition and cancer. *Mutagenesis* **23,** 153–162
174. Forgione, M. A., Cap, A., Liao, R., Moldovan, N. I., Eberhardt, R. T., Lim, C. C., Jones, J., Goldschmidt-Clermont, P. J., and Loscalzo, J. (2002) Heterozygous cellular glutathione peroxidase deficiency in the mouse: abnormalities in vascular and cardiac function and structure. *Circulation* **106,** 1154–1158
175. Bellinger, F. P., Raman, A. V., Reeves, M. A., and Berry, M. J. (2009) Regulation and function of selenoproteins in human disease. *Biochem. J.* **422,** 11–22
176. Voetsch, B., Jin, R. C., Bierl, C., Benke, K. S., Kenet, G., Simioni, P., Ottaviano, F., Damasceno, B. P., Annichino-Bizacchi, J. M., Handy, D. E., and Loscalzo, J. (2007) Promoter polymorphisms in the plasma glutathione peroxidase (GPx-3) gene: a novel risk factor for arterial ischemic stroke among young adults and children. *Stroke* **38,** 41–49
177. Voetsch, B., Jin, R. C., Bierl, C., Deus-Silva, L., Camargo, E. C., Annichino-Bizacchi, J. M., Handy, D. E., and Loscalzo, J. (2008) Role of promoter polymorphisms in the plasma glutathione peroxidase (GPx-3) gene as a risk factor for cerebral venous thrombosis. *Stroke* **39,** 303–307
178. Dayan, C. M., and Panicker, V. (2009) Novel insights into thyroid hormones from the study of common genetic variation. *Nat. Rev. Endocrinol.* **5,** 211–218
179. Peeters, R. P., van der Deure, W. M., and Visser, T. J. (2006) Genetic variation in thyroid hormone pathway genes; polymorphisms in the TSH receptor and the iodothyronine deiodinases. *Eur. J. Endocrinol.* **155,** 655–662
180. Flores-Mateo, G., Navas-Acien, A., Pastor-Barriuso, R., and Guallar, E. (2006) Selenium and coronary heart disease: a meta-analysis. *Am. J. Clin. Nutr.* **84,** 762–773
181. Nawrot, T. S., Staessen, J. A., Roels, H. A., Den Hond, E., Thijs, L., Fagard, R. H., Dominiczak, A. F., and Struijker-Boudier, H. A. (2007) Blood pressure and blood selenium: a cross-sectional and longitudinal population study. *Eur. Heart. J.* **28,** 628–633
182. Lubos, E., Sinning, C. R., Schnabel, R. B., Wild, P. S., Zeller, T., Rupprecht, H. J., Bickel, C., Lackner, K. J., Peetz, D., Loscalzo, J., Münzel, T., and Blankenberg, S. (2009) Serum selenium and prognosis in cardiovascular disease: results from the AtheroGene study. *Atherosclerosis* **209,** 271–277
183. Blankenberg, S., Rupprecht, H. J., Bickel, C., Torzewski, M., Hafner, G., Tiret, L., Smieja, M., Cambien, F., Meyer, J., and Lackner, K. J. (2003) Glutathione peroxidase 1 activity and cardiovascular events in patients with coronary artery disease. *N. Engl. J. Med.* **349,** 1605–1613
184. Rayman, M. P. (2009) Selenoproteins and human health: insights from epidemiological data. *Biochim. Biophys. Acta* **1790,** 1533–1540
185. Gu, B. Q. (1983) Pathology of Keshan disease. A comprehensive review. *Chin. Med. J. (Engl.)* **96,** 251–261
186. Beck, M. A., Esworthy, R. S., Ho, Y. S., and Chu, F. F. (1998) Glutathione peroxidase protects mice from viral-induced myocarditis. *FASEB J.* **12,** 1143–1149
187. Schweizer, U., and Schomburg, L. (2005) New insights into the physiological actions of selenoproteins from genetically modified mice. *IUBMB Life* **57,** 737–744
188. Carlson, B. A., Yoo, M. H., Shrimali, R. K., Irons, R., Gladyshev, V. N., Hatfield, D. L., and Park, J. M. (2010) Role of selenium-containing proteins in T-cell and macrophage function. *Proc. Nutr. Soc.* **69,** 1–11
189. Verma, S., Hoffmann, F. W., Kumar, M., Huang, Z., Roe, K., Nguyen-Wu, E., Hashimoto, A. S., and Hoffmann, P. R. (2011) Selenoprotein K knockout mice exhibit deficient calcium flux in immune cells and impaired immune responses. *J. Immunol.* **186,** 2127–2137
190. Baum, M. K., Shor-Posner, G., Lai, S., Zhang, G., Lai, H., Fletcher, M. A., Sauberlich, H., and Page, J. B. (1997) High risk of HIV-related mortality is associated with selenium deficiency. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol* **15,** 370–374
191. Hoffmann, P. R., and Berry, M. J. (2008) The influence of selenium on immune responses. *Mol. Nutr. Food Res.* **52,** 1273–1280
192. Yu, L., Sun, L., Nan, Y., and Zhu, L. Y. (2010) Protection from H1N1 influenza virus infections in mice by supplementation with selenium: a comparison with selenium-deficient mice. [E-pub ahead of print] *Biol. Trace. Elem. Res.* doi: 10.1007/s12011-010-8726-x
193. Wang, C., Wang, H., Luo, J., Hu, Y., Wei, L., Duan, M., and He, H. (2009) Selenium deficiency impairs host innate immune response and induces susceptibility to *Listeria monocytogenes* infection. *BMC Immunol.* **10,** 55
194. Hoffmann, F. W., Hashimoto, A. C., Shafer, L. A., Dow, S., Berry, M. J., and Hoffmann, P. R. (2010) Dietary selenium modulates activation and differentiation of CD4+ t cells in mice through a mechanism involving cellular free thiols. *J. Nutr.* **140,** 1155–1161
195. Peeters, R. P., van den Beld, A. W., van Toor, H., Uitterlinden, A. G., Janssen, J. A., Lamberts, S. W., and Visser, T. J. (2005) A polymorphism in type I deiodinase is associated with circulat-

ing free insulin-like growth factor I levels and body composition in humans. *J. Clin. Endocrinol. Metab.* **90,** 256–263
196. Lescure, A., Deniziak, M., Rederstorff, M., and Krol, A. (2008) Molecular basis for the role of selenium in muscle development and function. *Chem. Biodivers.* **5,** 408–413
197. Chariot, P., and Bignani, O. (2003) Skeletal muscle disorders associated with selenium deficiency in humans. *Muscle Nerve* **27,** 662–668
198. Bassett, J. H., Boyde, A., Howell, P. G., Bassett, R. H., Galliford, T. M., Archanco, M., Evans, H., Lawson, M. A., Croucher, P., St Germain, D. L., Galton, V. A., and Williams, G. R. (2010) Optimal bone strength and mineralization requires the type 2 iodothyronine deiodinase in osteoblasts. *Proc. Natl. Acad. Sci. U. S. A.* **107,** 7604–7609
199. Downey, C. M., Horton, C. R., Carlson, B. A., Parsons, T. E., Hatfield, D. L., Hallgrímsson, B., and Jirik, F. R. (2009) Osteo-chondroprogenitor-specific deletion of the selenocysteine tRNA gene, Trsp, leads to chondronecrosis and abnormal skeletal development: a putative model for Kashin-Beck disease. *PLoS Genet.* **5,** e1000616
200. Meulenbelt, I., Min, J. L., Bos, S., Riyazi, N., Houwing-Duistermaat, J. J., van der Wijk, H. J., Kroon, H. M., Nakajima, M., Ikegawa, S., Uitterlinden, A. G., van Meurs, J. B., van der Deure, W. M., Visser, T. J., Seymour, A. B., Lakenberg, N., van der Breggen, R., Kremer, D., van Duijn, C. M., Kloppenburg, M., Loughlin, J., and Slagboom, P. E. (2008) Identification of DIO2 as a new susceptibility locus for symptomatic osteoarthritis. *Hum. Mol. Genet.* **17,** 1867–1875
201. Heemstra, K. A., Hoftijzer, H., van der Deure, W. M., Peeters, R. P., Hamdy, N. A., Pereira, A., Corssmit, E. P., Romijn, J. A., Visser, T. J., and Smit, J. W. (2010) The type 2 deiodinase Thr92Ala polymorphism is associated with increased bone turnover and decreased femoral neck bone mineral density. *J. Bone Miner. Res.* **25,** 1385–1391
202. Xiong, Y., Mo, X., Zou, X., Song, R., Sun, W., Lu, W., Chen, Q., Yu, Y., and Zang, W. (2010) Association study between polymorphisms in selenoprotein genes and susceptibility to Kashin-Beck disease. *Osteoarthritis Cartilage* **18,** 817–824
203. Cao, J., Li, S., Shi, Z., Yue, Y., Sun, J., Chen, J., Fu, Q., Hughes, C. E., and Caterson, B. (2008) Articular cartilage metabolism in patients with Kashin-Beck Disease: an endemic osteoarthropathy in China. *Osteoarthritis Cartilage* **16,** 680–688
204. Klivenyi, P., Andreassen, O. A., Ferrante, R. J., Dedeoglu, A., Mueller, G., Lancelot, E., Bogdanov, M., Andersen, J. K., Jiang, D., and Beal, M. F. (2000) Mice deficient in cellular glutathione peroxidase show increased vulnerability to malonate, 3-nitropropionic acid, and 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine. *J. Neurosci.* **20,** 1–7
205. Guo, T. W., Zhang, F. C., Yang, M. S., Gao, X. C., Bian, L., Duan, S. W., Zheng, Z. J., Gao, J. J., Wang, H., Li, R. L., Feng, G. Y., St Clair, D., and He, L. (2004) Positive association of the DIO2 (deiodinase type 2) gene with mental retardation in the iodine-deficient areas of China. *J. Med. Genet.* **41,** 585–590
206. Panicker, V., Saravanan, P., Vaidya, B., Evans, J., Hattersley, A. T., Frayling, T. M., and Dayan, C. M. (2009) Common variation in the DIO2 gene predicts baseline psychological well-being and response to combination thyroxine plus triiodothyronine therapy in hypothyroid patients. *J. Clin. Endocrinol. Metab.* **94,** 1623–1629
207. Bunevicius, R., and Prange, A. J. (2010) Thyroid disease and mental disorders: cause and effect or only comorbidity? *Curr. Opin. Psychiatry* **23,** 363–368
208. Akbaraly, T. N., Akbaraly, N. T., Hininger-Favier, I., Carrière, I., Arnaud, J., Gourlet, V., Roussel, A. M., and Berr, C. (2007) Plasma selenium over time and cognitive decline in the elderly. *Epidemiology* **18,** 52–58
209. Gao, S., Jin, Y., Hall, K. S., Liang, C., Unverzagt, F. W., Ji, R., Murrell, J. R., Cao, J., Shen, J., Ma, F., Matesan, J., Ying, B., Cheng, Y., Bian, J., Li, P., and Hendrie, H. C. (2007) Selenium level and cognitive function in rural elderly Chinese. *Am. J. Epidemiol.* **165,** 955–965
210. Schweizer, U., Bräuer, A. U., Köhrle, J., Nitsch, R., and Savaskan, N. E. (2004) Selenium and brain function: a poorly recognized liaison. *Brain Res. Brain Res. Rev.* **45,** 164–178
211. Loh, K., Deng, H., Fukushima, A., Cai, X., Boivin, B., Galic, S., Bruce, C., Shields, B. J., Skiba, B., Ooms, L. M., Stepto, N., Wu, B., Mitchell, C. A., Tonks, N. K., Watt, M. J., Febbraio, M. A., Crack, P. J., Andrikopoulos, S., and Tiganis, T. (2009) Reactive oxygen species enhance insulin sensitivity. *Cell Metab.* **10,** 260–272
212. Akbaraly, T. N., Arnaud, J., Rayman, M. P., Hininger-Favier, I., Roussel, A. M., Berr, C., and Fontbonne, A. (2010) Plasma selenium and risk of dysglycemia in an elderly French population: Results from the prospective Epidemiology of Vascular Ageing Study. *Nutr. Metab. (Lond.)* **7,** 21
213. Stranges, S., Sieri, S., Vinceti, M., Grioni, S., Guallar, E., Laclaustra, M., Muti, P., Berrino, F., and Krogh, V. (2010) A prospective study of dietary selenium intake and risk of type 2 diabetes. *BMC Public Health* **10,** 564
214. Mueller, A. S., Klomann, S. D., Wolf, N. M., Schneider, S., Schmidt, R., Spielmann, J., Stangl, G., Eder, K., and Pallauf, J. (2008) Redox regulation of protein tyrosine phosphatase 1B by manipulation of dietary selenium affects the triglyceride concentration in rat liver. *J. Nutr.* **138,** 2328–2336
215. Mueller, A. S., Bosse, A. C., Most, E., Klomann, S. D., Schneider, S., and Pallauf, J. (2009) Regulation of the insulin antagonistic protein tyrosine phosphatase 1B by dietary Se studied in growing rats. *J. Nutr. Biochem.* **20,** 235–247
216. Dora, J. M., Machado, W. E., Rheinheimer, J., Crispim, D., and Maia, A. L. (2010) Association of the type 2 deiodinase Thr92Ala polymorphism with type 2 diabetes: case-control study and meta-analysis. *Eur. J. Endocrinol.* **163,** 427–434
217. McClung, J. P., Roneker, C. A., Mu, W., Lisk, D. J., Langlais, P., Liu, F., and Lei, X. G. (2004) Development of insulin resistance and obesity in mice overexpressing cellular glutathione peroxidase. *Proc. Natl. Acad. Sci. U. S. A.* **101,** 8852–8857
218. Wang, X. D., Vatamaniuk, M. Z., Wang, S. K., Roneker, C. A., Simmons, R. A., and Lei, X. G. (2008) Molecular mechanisms for hyperinsulinaemia induced by overproduction of selenium-dependent glutathione peroxidase-1 in mice. *Diabetologia* **51,** 1515–1524
219. Misu, H., Takamura, T., Takayama, H., Hayashi, H., Matsuzawa-Nagata, N., Kurita, S., Ishikura, K., Ando, H., Takeshita, Y., Ota, T., Sakurai, M., Yamashita, T., Mizukoshi, E., Yamashita, T., Honda, M., Miyamoto, K., Kubota, T., Kubota, N., Kadowaki, T., Kim, H. J., Lee, I. K., Minokoshi, Y., Saito, Y., Takahashi, K., Yamada, Y., Takakura, N., and Kaneko, S. (2010) A liver-derived secretory protein, selenoprotein p, causes insulin resistance. *Cell Metab.* **12,** 483–495
220. Labunskyy, V. M., Lee, B. C., Handy, D. E., Loscalzo, J., Hatfield, D. L., and Gladyshev, V. N. (2011) Both maximal expression of selenoproteins and selenoprotein deficiency can promote development of type 2 diabetes-like phenotype in mice. [Epub ahead of print] *Antioxid. Redox Signal.* doi: 10.1089/ars.2010.3526
221. Kelner, M. J., Bagnell, R. D., Montoya, M. A., and Lanham, K. A. (2000) Structural organization of the human gastrointestinal glutathione peroxidase (GPX2) promoter and 3′-nontranscribed region: transcriptional response to exogenous redox agents. *Gene* **248,** 109–116
222. Chu, F. F., Rohan de Silva, H. A., Esworthy, R. S., Boteva, K. K., Walters, C. E., Roses, A., Rao, P. N., and Pettenati, M. J. (1996) Polymorphism and chromosomal localization of the GI-form of human glutathione peroxidase (GPX2) on 14q24.1 by in situ hybridization. *Genomics* **32,** 272–276
223. Al-Taie, O. H., Uceyler, N., Eubner, U., Jakob, F., Mörk, H., Scheurlen, M., Brigelius-Flohe, R., Schöttker, K., Abel, J., Thalheimer, A., Katzenberger, T., Illert, B., Melcher, R., and Köhrle, J. (2004) Expression profiling and genetic alterations of the selenoproteins GI-GPx and SePP in colorectal carcinogenesis. *Nutr. Cancer* **48,** 6–14
224. Carlson, B. A., Xu, X. M., Gladyshev, V. N., and Hatfield, D. L. (2005) Selective rescue of selenoprotein expression in mice lacking a highly specialized methyl group in selenocysteine tRNA. *J. Biol. Chem.* **280,** 5542–5548
225. Baliga, M. S., Wang, H., Zhuo, P., Schwartz, J. L., and Diamond, A. M. (2007) Selenium and GPx-1 overexpression protect mammalian cells against UV-induced DNA damage. *Biol. Trace Elem. Res.* **115,** 227–242
226. Kenet, G., Freedman, J., Shenkman, B., Regina, E., Brok-Simoni, F., Holzman, F., Vavva, F., Brand, N., Michelson, A., Trolliet, M., Loscalzo, J., and Inbal, A. (1999) Plasma glutathione peroxidase deficiency and platelet insensitivity to nitric oxide in children with familial stroke. *Arterioscler. Thromb. Vasc. Biol.* **19,** 2017–2023

227. Bensadoun, J. C., Mirochnitchenko, O., Inouye, M., Aebischer, P., and Zurn, A. D. (1998) Attenuation of 6-OHDA-induced neurotoxicity in glutathione peroxidase transgenic mice. *Eur. J. Neurosci.* **10,** 3231–3236
228. Lippman, S. M., Klein, E. A., Goodman, P. J., Lucia, M. S., Thompson, I. M., Ford, L. G., Parnes, H. L., Minasian, L. M., Gaziano, J. M., Hartline, J. A., Parsons, J. K., Bearden, J. D. 3, Crawford, E. D., Goodman, G. E., Claudio, J., Winquist, E., Cook, E. D., Karp, D. D., Walther, P., Lieber, M. M., Kristal, A. R., Darke, A. K., Arnold, K. B., Ganz, P. A., Santella, R. M., Albanes, D., Taylor, P. R., Probstfield, J. L., Jagpal, T. J., Crowley, J. J., Meyskens, F. L. J., Baker, L. H., and Coltman, C. A. J. (2009) Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). *JAMA* **301,** 39–51
229. Stranges, S., Marshall, J. R., Natarajan, R., Donahue, R. P., Trevisan, M., Combs, G. F., Cappuccio, F. P., Ceriello, A., and Reid, M. E. (2007) Effects of long-term selenium supplementation on the incidence of type 2 diabetes: a randomized trial. *Ann. Intern. Med.* **147,** 217–223
230. Bleys, J., Navas-Acien, A., and Guallar, E. (2007) Serum selenium and diabetes in U.S. adults. *Diabetes Care* **30,** 829–834
231. Czernichow, S., Couthouis, A., Bertrais, S., Vergnaud, A. C., Dauchet, L., Galan, P., and Hercberg, S. (2006) Antioxidant supplementation does not affect fasting plasma glucose in the Supplementation with Antioxidant Vitamins and Minerals (SU.VI.MAX) study in France: association with dietary intake and plasma concentrations. *Am. J. Clin. Nutr.* **84,** 395–399
232. Bermano, G., Pagmantidis, V., Holloway, N., Kadri, S., Mowat, N. A., Shiel, R. S., Arthur, J. R., Mathers, J. C., Daly, A. K., Broom, J., and Hesketh, J. E. (2007) Evidence that a polymorphism within the 3′UTR of glutathione peroxidase 4 is functional and is associated with susceptibility to colorectal cancer. *Genes Nutr.* **2,** 225–232
233. Méplan, C., Hughes, D. J., Pardini, B., Naccarati, A., Soucek, P., Vodickova, L., Hlavatá, I., Vrána, D., Vodicka, P., and Hesketh, J. (2010) Genetic variants in selenoprotein genes increase risk of colorectal cancer. *Carcinogenesis* **31,** 1074–1079
234. Udler, M., Maia, A. T., Cebrian, A., Brown, C., Greenberg, D., Shah, M., Caldas, C., Dunning, A., Easton, D., Ponder, B., and Pharoah, P. (2007) Common germline genetic variation in antioxidant defense genes and survival after diagnosis of breast cancer. *J. Clin. Oncol.* **25,** 3015–3023
235. Meulenbelt, I., Bos, S. D., Chapman, K., van der Breggen, R., Houwing-Duistermaat, J. J., Kremer, D., Kloppenburg, M., Carr, A., Tsezou, A., González, A., Loughlin, J., and Slagboom, P. E. (2010) Meta-analyses of genes modulating intracellular T3 bio-availability reveal a possible role for the DIO3 gene in osteoarthritis susceptibility. *Ann. Rheum. Dis.* **70,** 164–167
236. Peters, U., Chatterjee, N., Hayes, R. B., Schoen, R. E., Wang, Y., Chanock, S. J., and Foster, C. B. (2008) Variation in the selenoenzyme genes and risk of advanced distal colorectal adenoma. *Cancer Epidemiol. Biomarkers Prev.* **17,** 1144–1154
237. Mitchell, J., Morris, A., and de Belleroche, J. (2009) Thioredoxin reductase 1 haplotypes modify familial amyotrophic lateral sclerosis onset. *Free Radic. Biol. Med.* **46,** 202–211
238. Cooper, M. L., Adami, H. O., Grönberg, H., Wiklund, F., Green, F. R., and Rayman, M. P. (2008) Interaction between single nucleotide polymorphisms in selenoprotein P and mitochondrial superoxide dismutase determines prostate cancer risk. *Cancer Res.* **68,** 10171–10177
239. Sutherland, A., Kim, D. H., Relton, C., Ahn, Y. O., and Hesketh, J. (2010) Polymorphisms in the selenoprotein S and 15-kDa selenoprotein genes are associated with altered susceptibility to colorectal cancer. *Genes Nutr.* **5,** 215–223
240. Speckmann, B., Pinto, A., Winter, M., Förster, I., Sies, H., and Steinbrenner, H. (2010) Proinflammatory cytokines down-regulate intestinal selenoprotein P biosynthesis via NOS-2 induction. *Free Radic. Biol. Med.* **49,** 777–785
241. Boosalis, M. G. (2008) The role of selenium in chronic disease. *Nutr. Clin. Pract.* **23,** 152–160
242. Papp, L. V., Holmgren, A., and Khanna, K. K. (2010) Selenium and selenoproteins in health and disease. *Antioxid. Redox Signal.* **12,** 793–795
243. Naithani, R. (2008) Organoselenium compounds in cancer chemoprevention. *Mini Rev. Med. Chem.* **8,** 657–668
244. Rayman, M. P. (2005) Selenium in cancer prevention: a review of the evidence and mechanism of action. *Proc. Nutr. Soc.* **64,** 527–542
245. Selenius, M., Rundlöf, A. K., Olm, E., Fernandes, A. P., and Björnstedt, M. (2010) Selenium and the selenoprotein thioredoxin reductase in the prevention, treatment and diagnostics of cancer. *Antioxid. Redox Signal.* **12,** 867–880
246. Brenneisen, P., Steinbrenner, H., and Sies, H. (2005) Selenium, oxidative stress, and health aspects. *Mol. Aspects Med.* **26,** 256–267
247. Steinbrenner, H. S., Speckmann, B. S., Pinto, A. P., and Sies, H. S. (2011) High selenium intake and increased diabetes risk: experimental evidence for interplay between selenium and carbohyrate metabolism. *J. Clin. Biochem. Nutr.* **48,** 1–6
248. Hoeflich, J., Hollenbach, B., Behrends, T., Hoeg, A., Stosnach, H., and Schomburg, L. (2010) The choice of biomarkers determines the selenium status in young German vegans and vegetarians. *Br. J. Nutr.* **104,** 1601–1604
249. Méplan, C., Nicol, F., Burtle, B. T., Crosley, L. K., Arthur, J. R., Mathers, J. C., and Hesketh, J. E. (2009) Relative abundance of selenoprotein P isoforms in human plasma depends on genotype, Se intake, and cancer status. *Antioxid. Redox Signal.* **11,** 2631–2640
250. Beckett, G. J., and Arthur, J. R. (2005) Selenium and endocrine systems. *J. Endocrinol.* **184,** 455–465
251. Beech, S. G., Walker, S. W., Dorrance, A. M., Arthur, J. R., Nicol, F., Lee, D., and Beckett, G. J. (1993) The role of thyroidal type-I iodothyronine deiodinase in tri-iodothyronine production by human and sheep thyrocytes in primary culture. *J. Endocrinol.* **136,** 361–370
252. Ekholm, R., and Björkman, U. (1997) Glutathione peroxidase degrades intracellular hydrogen peroxide and thereby inhibits intracellular protein iodination in thyroid epithelium. *Endocrinology* **138,** 2871–2878
253. Demelash, A., Karlsson, J. O., Nilsson, M., and Björkman, U. (2004) Selenium has a protective role in caspase-3-dependent apoptosis induced by H2O2 in primary cultured pig thyrocytes. *Eur. J. Endocrinol.* **150,** 841–849
254. Schweizer, U., Chiu, J., and Köhrle, J. (2008) Peroxides and peroxide-degrading enzymes in the thyroid. *Antioxid. Redox Signal.* **10,** 1577–1592
255. Golstein, J., Corvilain, B., Lamy, F., Paquer, D., and Dumont, J. E. (1988) Effects of a selenium deficient diet on thyroid function of normal and perchlorate treated rats. *Acta Endocrinol. (Copenh.)* **118,** 495–502
256. Havel, R. J. (1997) Postprandial lipid metabolism: an overview. *Proc. Nutr. Soc.* **56,** 659–666
257. Donovan, J., and Copeland, P. R. (2010) The efficiency of selenocysteine incorporation is regulated by translation initiation factors. *J. Mol. Biol.* **400,** 659–664
258. Donovan, J., and Copeland, P. R. (2010) Threading the needle: getting selenocysteine into proteins. *Antioxid. Redox Signal.* **12,** 881–892
259. Okuno, T., Ueno, H., and Nakamuro, K. (2006) Cystathionine gamma-lyase contributes to selenomethionine detoxification and cytosolic glutathione peroxidase biosynthesis in mouse liver. *Biol. Trace Elem. Res.* **109,** 155–171
260. Renko, K., Werner, M., Renner-Müller, I., Cooper, T. G., Yeung, C. H., Hollenbach, B., Scharpf, M., Köhrle, J., Schomburg, L., and Schweizer, U. (2008) Hepatic selenoprotein P (SePP) expression restores selenium transport and prevents infertility and motor-incoordination in Sepp-knockout mice. *Biochem. J.* **409,** 741–749
261. Carlson, B., Xu, X. M., Gladyshev, V., and Hatfield, D. (2005) Um34 in selenocysteine tRNA is required for the expression of stress-related selenoproteins in mammals. In *Topics in Current Genetics, Vol. 12: Fine-Tuning of RNA Functions by Modification and Editing* (Grosjean, H., ed) pp. 431–438, Springer, Berlin/New York, doi: 10.1007/b106652
262. Budiman, M. E., Bubenik, J. L., Miniard, A. C., Middleton, L. M., Gerber, C. A., Cash, A., and Driscoll, D. M. (2009) Eukaryotic initiation factor 4a3 is a selenium-regulated RNA-binding protein that selectively inhibits selenocysteine incorporation. *Mol. Cell* **35,** 479–489
263. Miniard, A. C., Middleton, L. M., Budiman, M. E., Gerber, C. A., and Driscoll, D. M. (2010) Nucleolin binds to a subset of selenoprotein mRNAs and regulates their expression. *Nucleic Acids Res.* **38,** 4807–4820
264. Schoenmakers, E., Agostini, M., Mitchell, C., Schoenmakers, N., Papp, L., Rajanayagam, O., Padidela, R., Ceron-Gutierrez,

L., Doffinger, R., Prevosto, C., Luan, J., Montano, S., Lu, J., Castanet, M., Clemons, N., Groeneveld, M., Castets, P., Karbaschi, M., Aitken, S., Dixon, A., Williams, J., Campi, I., Blount, M., Burton, H., Muntoni, F., O'Donovan, D., Dean, A., Warren, A., Brierley, C., Baguley, D., Guicheney, P., Fitzgerald, R., Coles, A., Gaston, H., Todd, P., Holmgren, A., Khanna, K. K., Cooke, M., Semple, R., Halsall, D., Wareham, N., Schwabe, J., Grasso, L., Beck-Peccoz, P., Ogunko, A., Dattani, M., Gurnell, M., and Chatterjee, K. (2010) Mutations in the selenocysteine insertion sequence-binding protein 2 gene lead to a multisystem selenoprotein deficiency disorder in humans. *J. Clin. Invest.* **120,** 4220–4235

265. Squires, J. E., and Berry, M. J. (2008) Eukaryotic selenoprotein synthesis: mechanistic insight incorporating new factors and new functions for old factors. *IUBMB Life* **60,** 232–235
266. Latrèche, L., Jean-Jean, O., Driscoll, D. M., and Chavatte, L. (2009) Novel structural determinants in human SECIS elements modulate the translational recoding of UGA as selenocysteine. *Nucleic Acids Res.* **37,** 5868–5880
267. Carlson, B. A., Moustafa, M. E., Sengupta, A., Schweizer, U., Shrimali, R., Rao, M., Zhong, N., Wang, S., Feigenbaum, L., Lee, B. J., Gladyshev, V. N., and Hatfield, D. L. (2007) Selective restoration of the selenoprotein population in a mouse hepatocyte selenoprotein-less background with different mutant selenocysteine tRNAs lacking Um34. *J. Biol. Chem.* **282,** 32591–32602
268. Diamond, A. M., Choi, I. S., Crain, P. F., Hashizume, T., Pomerantz, S. C., Cruz, R., Steer, C. J., Hill, K. E., Burk, R. F., and McCloskey, J. A. (1993) Dietary selenium affects methylation of the wobble nucleoside in the anticodon of selenocysteine tRNA([Ser]Sec). *J. Biol. Chem.* **268,** 14215–14223
269. Moriarty, P. M., Reddy, C. C., and Maquat, L. E. (1998) Selenium deficiency reduces the abundance of mRNA for Se-dependent glutathione peroxidase 1 by a UGA-dependent mechanism likely to be nonsense codon-mediated decay of cytoplasmic mRNA. *Mol. Cell. Biol.* **18,** 2932–2939
270. Nicholson, P., Yepiskoposyan, H., Metze, S., Zamudio Orozco, R., Kleinschmidt, N., and Mühlemann, O. (2010) Nonsense-mediated mRNA decay in human cells: mechanistic insights, functions beyond quality control and the double-life of NMD factors. *Cell. Mol. Life Sci.* **67,** 677–700
271. Lü, J., and Jiang, C. (2005) Selenium and cancer chemoprevention: hypotheses integrating the actions of selenoproteins and selenium metabolites in epithelial and non-epithelial target cells. *Antioxid. Redox Signal.* **7,** 1715–1727
272. Banning, A., Deubel, S., Kluth, D., Zhou, Z., and Brigelius-Flohé, R. (2005) The GI-GPx gene is a target for Nrf2. *Mol. Cell. Biol.* **25,** 4914–4923
273. Singh, A., Rangasamy, T., Thimmulappa, R. K., Lee, H., Osburn, W. O., Brigelius-Flohé, R., Kensler, T. W., Yamamoto, M., and Biswal, S. (2006) Glutathione peroxidase 2, the major cigarette smoke-inducible isoform of GPX in lungs, is regulated by Nrf2. *Am. J. Respir. Cell Mol. Biol.* **35,** 639–650
274. Chu, F. F., Esworthy, R. S., and Doroshow, J. H. (2004) Role of Se-dependent glutathione peroxidases in gastrointestinal inflammation and Cancer *Free Radic. Biol. Med.* **36,** 1481–1495
275. Westphal, K., Stangl, V., Fähling, M., Dreger, H., Weller, A., Baumann, G., Stangl, K., and Meiners, S. (2009) Human-specific induction of glutathione peroxidase-3 by proteasome inhibition in cardiovascular cells. *Free Radic. Biol. Med.* **47,** 1652–1660
276. Köhrle, J. (2005) Selenium and the control of thyroid hormone metabolism. *Thyroid* **15,** 841–853
277. Gogakos, A. I., Duncan Bassett, J. H., and Williams, G. R. (2010) Thyroid and bone. *Arch. Biochem. Biophys.* **503,** 129–136
278. Torrance, C. J., Devente, J. E., Jones, J. P., and Dohm, G. L. (1997) Effects of thyroid hormone on GLUT4 glucose transporter gene expression and NIDDM in rats. *Endocrinology* **138,** 1204–1214
279. Chidakel, A., Mentuccia, D., and Celi, F. S. (2005) Peripheral metabolism of thyroid hormone and glucose homeostasis. *Thyroid* **15,** 899–903
280. Mueller, A. S., Mueller, K., Wolf, N. M., and Pallauf, J. (2009) Selenium and diabetes: an enigma? *Free Radic. Res.* **43,** 1–31
281. Cheng, Z., Tseng, Y., and White, M. F. (2010) Insulin signaling meets mitochondria in metabolism. *Trends Endocrinol. Metab.* **21,** 589–598
282. Houstis, N., Rosen, E. D., and Lander, E. S. (2006) Reactive oxygen species have a causal role in multiple forms of insulin resistance. *Nature* **440,** 944–948
283. Rayman, M. P. (2000) The importance of selenium to human health. *Lancet* **356,** 233–241
284. Ashton, K., Hooper, L., Harvey, L. J., Hurst, R., Casgrain, A., and Fairweather-Tait, S. J. (2009) Methods of assessment of selenium status in humans: a systematic review. *Am. J. Clin. Nutr.* **89,** 2025S–2039S
285. Hurst, R., Armah, C. N., Dainty, J. R., Hart, D. J., Teucher, B., Goldson, A. J., Broadley, M. R., Motley, A. K., and Fairweather-Tait, S. J. (2010) Establishing optimal selenium status: results of a randomized, double-blind, placebo-controlled trial. *Am. J. Clin. Nutr.* **91,** 923–931
286. Rayman, M. P. (2008) Food-chain selenium and human health: emphasis on intake. *Br. J. Nutr.* **100,** 254–268
287. Alfthan, G. A., Aspila, P. A., Ekholm, P. E., Eurola, M. E., Hartikainen, H. H., Hero, H. H., Hietaniemi, V. H., Root, T. R., Salminen, P. S., Venalainen, E. V., and Aro, A. A. (2010) *Nationwide Supplementation of Sodium Selenate to Commercial Fertilizers. History and 25-Year Results from the Finnish Selenium Monitoring Programme.* FAO Report, Revision 3 (18.6.2010/GA)
288. Laclaustra, M., Navas-Acien, A., Stranges, S., Ordovas, J. M., and Guallar, E. (2009) Serum selenium concentrations and diabetes in U.S. adults: National Health and Nutrition Examination Survey (NHANES) 2003–2004. *Environ. Health Perspect.* **117,** 1409–1413
289. Laclaustra, M., Stranges, S., Navas-Acien, A., Ordovas, J. M., and Guallar, E. (2010) Serum selenium and serum lipids in US adults: National Health and Nutrition Examination Survey (NHANES) 2003–2004. *Atherosclerosis* **210,** 643–648
290. Bates, C. J., Thane, C. W., Prentice, A., and Delves, H. T. (2002) Selenium status and its correlates in a British national diet and nutrition survey: people aged 65 years and over. *J. Trace Elem. Med. Biol.* **16,** 1–8
291. Letsiou, S., Nomikos, T., Panagiotakos, D., Pergantis, S. A., Fragopoulou, E., Antonopoulou, S., Pitsavos, C., and Stefanadis, C. (2009) Serum total selenium status in Greek adults and its relation to age. The ATTICA study cohort. *Biol. Trace Elem. Res.* **128,** 8–17
292. Arnaud, J., Akbaraly, T. N., Akbaraly, N. T., Hininger, I., Roussel, A. M., and Berr, C. (2007) Factors associated with longitudinal plasma selenium decline in the elderly: the EVA study. *J. Nutr. Biochem.* **18,** 482–487
293. Krämer-Guth, A., Nauck, M., Quaschning, T., Pavenstädt, H., Wieland, H., Schollmeyer, P., and Wanner, C. (1996) VLDL and LDL metabolism in human and rat mesangial cells. *Nephron* **74,** 378–385
294. De Grooth, G. J., Klerkx, A. H., Stroes, E. S., Stalenhoef, A. F., Kastelein, J. J., and Kuivenhoven, J. A. (2004) A review of CETP and its relation to atherosclerosis. *J. Lipid. Res.* **45,** 1967–1974
295. Sunde, R. A. (2010) mRNA transcripts as molecular biomarkers in medicine and nutrition. *J. Nutr. Biochem.* **21,** 665–670

Received for publication January 27, 2011.
Accepted for publication February 17, 2011.