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Part--Selenoproteins and Cardiovascular Stress

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Abstract

Dietary selenium (Se) is an essential micronutrient that exerts its biological effects through its incorporation into selenoproteins. This family of proteins contains several antioxidant enzymes such as the glutathione peroxidases, redox-regulating enzymes such as thioredoxin reductases, a methionine sulfoxide reductase, and others. In this review, we summarize the current understanding of the roles these selenoproteins play in protecting the cardiovascular system from different types of stress including ischemia-reperfusion, homocysteine dysregulation, myocardial hypertrophy, doxorubicin toxicity, Keshan disease, and others.

Keywords

cardiovascular; heart; oxidative stress; selenium; selenoproteins

Introduction

Selenium (Se) is a dietary trace element essential for various aspects of human health, including the development and function of the cardiovascular system (1, 2). This micronutrient is acquired through the consumption of various foods including grains, fish, and meat (3). Se content in most foods depends on the Se content of the soil where plants or animal feeds are grown, and most seafoods are replete in Se. Human Se deficiency is rare, but does occur in regions of China and Russia and, to a lesser extent in parts of Europe, where soil is Se-deficient and foods grown locally are the predominant sources of nutrition. Se content in soil may vary within the U.S., but most individuals in the U.S. meet or exceed the recommended dietary intake of 55 µg/day and sodium selenite or selenomethionine supplements have been used to boost Se levels (1, 4). Evidence suggests that dietary Se levels affect cardiac function in mice (5), but the effects of dietary Se levels on human cardiovascular health are less clear and the limited clinical trial data available to date do not support the notion that providing Se supplements to healthy adults prevents the occurrence of major cardiovascular disease (6–8).

A better understanding of the effects of Se intake on cardiovascular disease requires research focused on the mechanisms by which Se influences stress on the cardiovascular system. This likely will rely on a clear understanding of the roles that individual selenoproteins play in

cardiovascular health. Unlike other essential trace elements such as zinc and iron, Se is incorporated directly into proteins as an amino acid, selenocysteine (Sec) (9). Translation of selenoproteins is similar to generalized protein translation in that it consists of three main steps: initiation, elongation, and termination. The special feature of selenoprotein translation lies in the recoding of UGA from a stop codon to a Sec insertion codon. That is, the translational machinery within the cell typically reads the UGA codon as a termination signal, releasing the nascent polypeptide from the ribosome. During translation of selenoproteins, the machinery is redirected to insert Sec at UGA codons instead of terminating polypeptide synthesis (10). Most selenoprotein mRNAs, with the exception of selenoprotein P, contain a single UGA codon encoding a single Sec residue per polypeptide chain. The incorporation of Sec requires special factors to synthesize this amino acid, recognize the site at which it is to be inserted, and carry out the insertion process (11). The recoding process in eukaryotes involves specific secondary structure in the mRNA, a unique tRNA, an RNA binding protein (SBP2), and a specialized elongation factor (EFsec). Other protein factors have been implicated in selenoprotein biosynthesis and their translational regulation is complex and is regulated by more than just dietary Se intake (12).

The Selenoprotein Family

Twenty-five selenoproteins have been identified in humans, with all but one of these existing as Sec-containing proteins in rodents (13). As shown in Table I, these proteins exhibit a wide variety of tissue distributions and functions (14). The first mammalian selenoprotein to be identified was glutathione peroxidase (GPX)1 (15, 16), an antioxidant enzyme that detoxifies intracellular hydrogen peroxide (H_2O_2). This enzyme is one member of the important group of peroxide detoxifying GPX enzymes (GPX1-4). In addition to the GPXs, thioredoxin reductases (Txnrd1-3) have been well characterized as enzymes that regenerate reduced thioredoxin and thereby control the redox tone of cells. Sel R, also called methionine sulfoxide reductase (Msr) B1, along with nonselenoprotein Msr A, B2 and B3 proteins comprise an important antioxidant family of enzymes that reduce proteins that have been reversibly oxidized on methionine groups. Sel R is widely distributed throughout various tissue and cell types (17), and may be particularly important in some types of cardiac stress as described in more detail below. Collectively, the antioxidant selenoproteins are of particular interest in relation to the “radical theory of aging” (18). One of the hallmarks of aging is the accumulation of oxidized proteins and macromolecules, either through decreased antioxidant activity, or increased oxidative stress (19). It has been shown that along with the increased accumulation of oxidized proteins, the expression of antioxidant selenoproteins falls (19). The Msr enzymes are important in *Drosophila* and in mice for lengthened lifespan (20). Paradoxically, another study showed that GPX4^{+/-} mice had increased lifespan compared to GPX4^{+/+} mice possibly due to greater sensitivity to apoptotic signals (21). GPX1 and GPX2 deletion in mice has no obvious effect on lifespan (22). Thus, the relationship between antioxidants and longevity is complex and there may exist redundancy in antioxidant systems that allow loss of function for some selenoproteins to be compensated by other enzymatic systems.

Roles for other selenoproteins are emerging, with many implicated in regulating cellular processes through redox mechanisms. For example, a subfamily of selenoproteins,

designated Rdx, contain primary sequence motifs and a thioredoxin-like fold that reflect a possible redox function that may use catalytic Sec to form transient mixed disulfides with substrate proteins (23). This subfamily includes SelW, SelV, SelT and SelH. Sel H appears to localize to the nucleus and bind to DNA in a redox-sensitive manner (24) and exhibits nucleolar oxidoreductase activity (25). Sel T has been suggested to function as an endoplasmic reticulum (ER) protein involved in Ca^{2+} mobilization (26) and glucose metabolism (27). Other selenoproteins localized to the ER include Sel M and Sep15, which may function as oxidoreductases in the ER lumen (28–30). Sel K and Sel S are localized to the ER membrane and have been suggested to be involved in shuttling misfolded proteins out of the ER (31). Our laboratory has shown a separate role for Sel K in regulating receptor-mediated Ca^{2+} flux in immune cells (32, 33). Recently, we have shown that Sel K is required for the expression and function of the scavenger receptor, CD36, in macrophages (34). This in turn influences foam cell formation and atherogenesis. However, Sel K is not expressed in mouse heart (32) and it remains to be determined if it plays any role in cardiac stress.

While work continues on defining molecular functions for selenoproteins, the requirement of selenoproteins for life has been demonstrated in knockout mice lacking the gene (*trsp*) encoding the tRNA required for co-translational insertion of Sec into all selenoproteins. Knockout of the *trsp* gene resulted in early embryonic death (35). Thus far, knockouts of specific selenoproteins have included embryonic lethal phenotypes for GPX4, Txnrd1, and Txnrd2 (36). GPX4 knockout mice die at an early embryonic stage (day 7.5), reflecting the crucial role this selenoprotein plays in protecting nearly all tissues from oxidative damage to phospholipids (37). Examination of Txnrd1 deficient mice showed that this selenoprotein plays an essential role during embryogenesis in most developing tissues except the heart (38). In Txnrd2-knockout mice, embryonic lethality at day 13 is a consequence of hematopoietic and cardiac defects, with cardiac-specific ablation resulting in a fatal dilated cardiomyopathy (39). However, the absence of Txnrd2 in humans leads to glucocorticoid deficiency and no cardiac defects have been reported (40). Non-lethal phenotypes for other selenoproteins include GPX1 (41) and GPX2 (42), thyroid hormone deiodinase 1 (43) and 2 (44), selenoprotein P (45, 46), Sel K (32), Sel R (47), Sel M (48), Sel N (49), and Sep15 (50). None of these knockout models of selenoproteins result in cardiovascular-specific phenotypes or spontaneous cardiac stress, but this does not rule out roles for some selenoproteins in cardiac tissues as they adapt to stress.

The expression of selenoproteins in the heart has been investigated through real-time PCR measurement of mRNA abundance in murine heart tissue that, to a large extent, is similar to other tissues (51). Two of the most abundant selenoprotein mRNAs detected in the heart are GPX3 and GPX4. The high levels of each may reflect important roles for protecting lipids (GPX4) and extracellular matrix (GPX3) from oxidative damage under normal (nonstressed) conditions. Selenoprotein synthesis regulation may differ in heart compared to other tissues. For example, regulation of some of the selenoprotein synthesis machinery, such as tRNA^{Sec}, differs between heart and other tissues (52). A distal upstream enhancer element regulates the transcription of tRNA^{Sec} in liver, kidney, and muscle, but not in heart. Another example is the expression of the family of deiodinase (DIO) enzymes, which consists of three selenoproteins, DIO1-3 (53). DIO1 and DIO2 each convert prohormone T4 into active T3,

both of which are inactivated by DIO3. DIO1 is the predominant form of deiodinase in most tissues, while the heart predominantly expresses DIO2.

The heart is an organ capable of adapting to various types of stress through induced expression of various factors and programmed remodeling, and much can be learned about the roles that selenoproteins play in coping with cardiac stress by comparing expression patterns under physiological versus pathophysiological conditions. This approach will be emphasized in the following sections that discuss different types of cardiac stress. Importantly, it is not necessary for selenoproteins to be expressed in the heart itself to be of importance for proper function of this organ. For example, Sel P and GPX3 are secreted into the blood accounting for 34% and 20% of plasma Se, respectively (54). These selenoproteins may be derived from renal or hepatic sources to supply the heart with Se and antioxidant capacity. It is also worth mentioning that the topic of dietary Se and cardiovascular disease has been extensively covered by others (55, 56), and the remainder of this review will mainly focus on selenoprotein subgroups such as GPXs, Txnrd, and methionine sulfoxide reductases and their roles in protecting against different types of cardiovascular stress.

Glutathione peroxidases regulate oxidative stress in cardiovascular tissues

One group of selenoproteins that clearly have a role in cardiac function are the GPX enzymes. The different GPX enzymes use glutathione (GSH) to detoxify hydroperoxides in intracellular and extracellular spaces as well as lipid peroxides in cellular membranes. In humans, there are five Sec-containing GPX enzymes: cytosolic GPX (GPX1), phospholipid hydroperoxide GPX (GPX4), plasma GPX (GPX3), gastrointestinal GPX (GPX2), and an enzyme restricted to the olfactory system (GPX6) (57). These proteins are also Sec-containing enzymes in mice, except for murine GPX6, which contains cysteine in place of Sec. The reactions catalyzed by the GPX enzymes relevant to cardiovascular physiology are illustrated in Figure 1.

GPX1 is particularly important for detoxifying intracellular reactive oxygen species such as H₂O₂. Heterozygous deficiency of GPX1 leads to endothelial dysfunction, which in turn produces significant structural abnormalities in vascular and cardiac tissues (58). GPX1 also protects against oxidative damage from ischemia-reperfusion involving ROS. Indeed, studies with transgenic GPX1 overexpressing mice as well as mice treated with ebselen (a selenium-containing compound) that upregulated GPX1 were more resistant to myocardial infarction (59, 60), whereas GPX1^{-/-} mice were more susceptible to myocardial ischemia-reperfusion injury through processes that involved the regulation of cardiomyocyte apoptosis (61, 62). The relevance of dietary Se to these findings was highlighted by studies showing that low Se diets fed to rats led to reduced recoveries following myocardial ischemia-reperfusion that was associated with lower expression and enzymatic activity of both GPX-1 and Txnrd1 (63, 64).

GPX1 may also impinge on athero-thrombotic vascular disease by modulating circulating homocysteine levels (65). Treatment of cultured endothelial cells with homocysteine decreases bioavailable nitric oxide (NO) (66, 67) and produces cytotoxicity mediated by

H₂O₂ (68). Given the role of GPX1 in detoxifying H₂O₂, it follows that GPX1 might ameliorate homocysteine-related endothelial dysfunction. Indeed, GPX1-deficiency has been shown to exacerbate hyperhomocysteinemia-induced endothelial dysfunction (69). GPX1^{-/-} mice fed high methionine diets, which induces hyperhomocysteinemia, had impaired relaxation in response to acetylcholine compared to GPX1^{+/+} mice. In hyperhomocysteinemia caused by genetic deletion of cystathionine β-synthase, overexpression of GPX1 ameliorates the endothelial dysfunction that is otherwise observed (70). Overall, the adverse effects of homocysteine on endothelial function, mediated at least in part by oxidative inactivation of NO, are countered by GPX1. Given that GPX1 levels are highly susceptible to fluctuations in Se intake (71, 72), this selenoenzyme may represent a key regulator by which dietary Se influences susceptibility to atherosclerosis. In addition to GPX1 affecting the outcome of hyperhomocysteinemia, homocysteine levels may alter levels of GPX1. Interestingly, increased homocysteine in the media of cells decreases GPX1 activity by a mechanism that involves interrupted read-through of the UGA codon that signals Sec insertion during translation (73).

Another example of a role for GPX1 in ameliorating cardiovascular involves the cardiac injury that accompanies treatment with chemotherapeutic agents such as Adriamycin (doxorubicin). Moderate dietary Se supplementation has been shown to counteract Adriamycin-induced cardiotoxicity by preservation of endogenous antioxidants (74, 75). As GPX1 carries out crucial intracellular antioxidant functions, this selenoenzyme seems a logical candidate to mediate the protective effects of Se during Adriamycin-induced cardiotoxicity. Indeed, experiments involving GPX1 overexpressing transgenic mice demonstrated that hearts from these mice were more resistant than nontransgenic hearts to Adriamycin-induced acute dysfunction including effects on contractility, diastolic properties, flow rate, and heart rate (76). Furthermore, GPX1-deficient mice were more susceptible to adriamycin-induced cardiotoxicity (77).

Bioavailable Se is not the only mechanism by which selenoprotein expression and function are regulated in the heart. Selenoproteins are regulated through the NF-E2-related factor 2 (Nrf2), Hypoxia-inducible factor 1α (HIF-1α), and mammalian target of rapamycin (mTOR) signaling pathways as well (78–80). Nrf2 is a major antioxidant response transcription factor that binds antioxidant response elements (AREs) in promoters of GPX1 and Txnrd1 genes along with other non-selenoprotein antioxidant factors. Prior to oxidative challenge, Nrf2 is retained in the cytoplasm by Keap1, which turnover of Nrf2 through proteasomal degradation (81). Increased ROS promotes oxidation of cysteine residues on Keap1 that leads to its ubiquitination, allowing Nrf2 to translocate to the nucleus and bind to AREs (78). Low dose proteasome inhibition in cardiac myocytes stabilized Nrf2 and led to ARE-mediated upregulation of antioxidant proteins, thereby protecting against both ROS challenge in cells and ischemic injury in rat ischemia reperfusion models (82).

HIF and its relationship with heart disease, particularly with ischemic injury, are well characterized (79, 83). More recently HIF-1α signaling was shown to be protective for stem cell populations (84). It has become clear that bone marrow stem cells reside predominantly in low oxygen environments and long-term stem cells must adapt through the expression of HIF-1α. Interestingly, there are also pockets of stem cells in the heart epicardium and

subepicardium that are maintained primarily in a hypoxic state (85). Even in hypoxic environments, HIF-1 α signaling is dependent on ROS (86), with mitochondrial ROS playing a particularly important role (87). The sensitivity of HIF1 α signaling to ROS suggest that selenoproteins have an impact and this was directly demonstrated in studies showing the absence of Txnrd1 resulted in impairment of p53 and HIF-1 α function (88). Degradation of HIF-1 α is catalyzed by prolyl hydroxylases and oxygen (89), and these processes may require Txnrd1 activity in cardiac stem cells for proper homeostasis in their hypoxic states. HIF-2 α (Epas1) has also been shown to regulate antioxidant activity including the expression of GPX1. HIF-2 α KO mice suffer from greater oxidative stress due to low levels of antioxidant enzymes including GPX1 (90). A relationship between mTOR and GPX1 was uncovered in leukemia patients receiving imatinib when it was noted that GPX1 levels were increased in these patients (80). It was shown that the imatinib blocked Bcr-Abl signaling and in turn decreased mTOR signaling, which increased GPX1 and 4 activity. Direct inhibition of mTOR with rapamycin resulted in increased GPX1 activity highlighting a mechanism of direct control of GPX1 and 4 function by mTOR. In the heart it has been shown that mTOR inhibition during ischemic events is cardio-protective (91). Thus, it is possible that the upregulation of GPX1 and/or 4 function may be a major cellular requirement for cardiac protection during ischemia.

Se supplementation has been shown to protect against cardiac damage resulting from diabetes. Using a streptozotocin (STZ)-induced diabetes model in rats, Se supplementation was shown to protect the ultrastructure of the heart against diabetes-induced alterations and restore altered mechanical and electrical activities (92). Over-expression of GPX1 in transgenic animals has been shown to disrupt regulation of insulin resistance and obesity in mice (93). However, the cardiac-specific effects of GPX1 over-expression were not evaluated in this study. In a study that compared two different models of diabetes (KKAy mice as a model of obese insulin-resistant diabetes, and STZ-induced diabetic mice as a model of insulin-deficient diabetes) (94), distinct responses of GPX1 expression were observed. In conditions of insulin-resistance, both superoxide dismutase 1 (SOD1) and GPX-1 mRNA levels were increased in kidney, but only GPX1 mRNA was increased in the heart. In contrast, mRNA levels for these antioxidant enzymes were unaltered during STZ-induced insulin-deficient diabetes. This is consistent with results from a study showing unaltered GSH/GSSG ratios in STZ-treated mice (95). Thus, Se may reduce cardiac reactive oxygen species during diabetes through the actions of GPX-1, which detoxifies H₂O₂, but other selenoproteins may also contribute to the protective effects observed with Se supplementation.

The strongest evidence of dietary Se affecting cardiovascular disease in humans involves Keshan disease, a cardiomyopathy occurring in a region of China with poor Se soil (96, 97), where low body Se is associated with congestive heart failure. Se deficiency was considered a major cause since Keshan disease was first reported in 1935. Oral Se supplementation was found to completely prevent Keshan disease, but the seasonal and annual incidence of the disease suggested an infectious co-factor was involved. It was later shown that coxsackie virus B3 (CVB3) was a co-factor in the pathogenesis of Keshan disease (98–100). Subsequently, several insightful experiments conducted in mice provided evidence that Se deficiency enhances oxidative stress, which increases mutations and virulence of this virus

(101). Most compellingly, a non-myocarditic strain of CVB3 inoculated into Se deficient mice acquired genetic mutations found in more virulent strains of CVB3 and produced cardiomyopathy. When the virulent strain was transferred into Se adequate hosts, these mice also developed cardiomyopathy. Similar changes from avirulent to virulent strains and similar cardiomyopathies were observed when inoculating GPX1-null mice (102), highlighting the role of high H₂O₂ levels in mediating this disease and the importance of GPX1 in preventing it. This was supported by recent analyses of myocardial samples from Keshan disease patients that showed lower levels of GPX1 in these tissues compared to healthy controls that correlated with higher levels of a marker of oxidative stress, 8-hydroxy-2-deoxy guanosine (103).

In addition to GPX1, GPX3 (plasma GPX) also protects the heart from oxidative stress due to its ability to serve as a scavenger of ROS in extracellular spaces. GPX3 also resides in the vasculature and thereby is an important enzyme for protecting against stroke, highlighted in a study showing a role for GPX3 in regulating the bioavailability of NO produced from platelets and vascular cells (104), presumably through indirect reduction of peroxides. This was followed up with a study showing that decreased GPX3 activity led to platelet hyper-reactivity and an increased risk of thrombosis (105). The clinical significance of this was supported with a study showing that impaired metabolism of ROS as a result of reduced GPX3 activity resulted in insufficient NO levels that affected normal platelet inhibitory mechanisms and predisposed study subjects to arterial thrombosis (106). In vitro hypoxia is a strong transcriptional regulator of GPX3 expression (107). This finding led to clinical studies demonstrating associations between polymorphisms in the GPX3 promoter and the risk of ischemic stroke (108, 109). However, others were unable to confirm these findings (110).

The presence of GPX3 as one of two major plasma selenoproteins (Sel P is the other), suggests a role for this selenoprotein in modulating NO concentration or other aspects of the vascular environment. Whether GPX3 affects susceptibility to stroke or other cardiovascular disorders may require more mechanistic studies. GPX3 has also been implicated in the regulation of cardiopathology that accompanies diabetes. For example, GPX3 mRNA levels increase in hearts of STZ-treated mice compared to untreated controls (111). Furthermore, insulin treatment completely abolished the increase in GPX3 mRNA in STZ-treated mice, suggesting GPX3 mRNA levels were dependent on insulin and serum glucose levels. GPx-3 has also been suggested to play a role in preventing plasma LDL oxidation, vascular inflammation, and atherogenesis (112). Surprisingly, we found that GPX3 and not GPX1 was upregulated during myocardial hypertrophy induced with either thyroid hormone or isoproterenol treatment (113). This may suggest that the ROS are required by the myocytes for hypertrophic response to these different stimuli but the extracellular matrix requires protection conferred by increased levels of GPX3.

As mentioned above, GPX4 is an antioxidant enzyme with cytosolic, nuclear, and mitochondrial isoforms that play an essential roles in protecting cellular lipids from oxidative damage. Lipid peroxidation has been shown to result from increased ROS in several different models of myocardial hypertrophy, but limited data are available regarding a protective role for GPX4 during cardiac stress. One study has demonstrated over-

expression of GPX4 in the mitochondria, but not the cytosol, conferred protection against simulated ischemia-reperfusion in neonatal rat cardiac myocytes (NCM) (114). Work in our laboratory has shown that GPX4 mRNA and protein is upregulated in myocardial hypertrophy (113), and this selenoenzyme may protect the cellular lipids from oxidative damage during the hypertrophic response. In a cell culture model GPX4 was implicated in cell adhesion molecule expression, which plays a critical role in the development of atherosclerosis (115). In particular, GPX4 overexpression in rabbit aortic smooth muscle cells lowered IL-1 induced VCAM-1 expression either by lowering stimulatory hydroperoxides or by using hydroperoxides for protein modification. Given the high levels of expression of this enzyme, the protective role of GPX4 in cardiovascular stress warrants more attention.

While most studies have focused on the beneficial effects of increased GPX activity in cellular function, there certainly is a case to be made for possible detrimental effects of overexpressing GPX proteins that may interfere with the non-toxic role of ROS. For example, changes in ROS can regulate tyrosine phosphorylation/dephosphorylation of crucial signaling molecules and mouse liver and muscle tissues overexpressing GPX1 exhibited perturbations in ROS regulated protein phosphorylation (93). In particular, increased GPX1 protein accelerated protein tyrosine phosphatase-1b activity due to low H₂O₂ levels, which led to decreased phosphorylation of the insulin receptor and Akt proteins. On the other end of the spectrum, GPX1-deficient cells show higher levels of H₂O₂ with increased phosphorylation (116). In this manner, consideration must be given to how extremely high levels of GPX activity in cardiac tissues may disrupt cellular processes regulated by non-toxic levels of ROS.

Roles for thioredoxin reductases in cardiac redox regulation

Thioredoxins are small proteins that regulate intracellular redox involved in a variety of cellular functions including DNA metabolism and repair, transcription, intracellular signaling, and cell-cell communication (117, 118). Thioredoxins are used by several cellular enzymes as cofactors in dithiol-disulfide exchange reactions and this is a major mechanism by which a reduced environment is maintained within cells, particularly serving to maintain reduced cysteine groups (119). The activities of thioredoxins are dependent upon Txnrd enzymes, which use NADPH/H⁺ as a reducing agent to regenerate reduced thioredoxins, which are in turn used to reduced oxidized cysteine residues in cellular proteins (Fig. 2). There are three Txnrd enzymes, all of which are selenoproteins. Txnrd1, 2, and 3 are localized to the cytosol/nucleus, mitochondria, and testis, respectively (120–122). ROS like H₂O₂ are considered cellular regulators mainly by their reversible oxidation of sulfur on cysteine residues that leads to the formation of disulfide bonds. Txnrds regulate levels of reduced thioredoxin that control the formation or elimination of disulfide bonds through oxidative or reducing reactions, respectively. This may alter the structure of a signaling protein and thereby regulate its activity (14).

Roles for thioredoxins in cardiovascular disease have been previously reviewed (123–125), and several studies have shown that these redox-regulating molecules control a variety of cardiac functions. For example, mice with heart-specific expression of human thioredoxin-1

exhibit reduced levels of hypertrophy and oxidative stress in response to pressure overload (126). Moreover, expression of dominant-negative thioredoxin-1 in the heart increases oxidative stress and induces cardiac hypertrophy under basal conditions. While studies involving thioredoxins implicate the TR enzymes due to the roles of these selenoproteins in regenerating reduced thioredoxins, it must be kept in mind that Txnrds enzymatically reduce other substrates including lipoic acids, NK-lysin, ascorbate, and ubiquinone (127–129), any of which may also modify cardiac function.

Shifting the redox balance toward an oxidative state activates hypertrophic and apoptotic signaling in cardiac myocytes in response to a variety of stimuli (130–134). The cytoplasmic/nuclear thioredoxin-1/Txnr1 system is important for reversing oxidation of free thiols on cysteine residues. There is emerging evidence that the thioredoxin-1/Txnr1 system plays a key role in regulating cell-signaling events during myocardial remodeling (123, 125). A specific example is the S-thiolation of Ras, the small G protein that plays a crucial role in regulating hypertrophic growth in cardiac myocytes in response to stimuli such as α -adrenergic receptor (α -AR) agonists and mechanical strain (135). Alpha-AR-stimulated hypertrophic signaling in adult rat cardiac myocytes is partly mediated via a thioredoxin-1-sensitive oxidative modification of thiols on Ras (136). In this sense, Se levels may regulate myocardial remodeling through the selenoprotein, Txnr1, and its role together with thioredoxin-1 in reversible reduction of signaling molecules. In fact, we found that cytoplasmic/nuclear Txnr1 was induced in the heart during myocardial hypertrophy, while mitochondrial Txnr2 was constitutively expressed at high levels (113). This may reflect a more flexible need for reduced thioredoxin in the cytoplasm and nucleus depending on metabolic needs of the myocytes, whereas the mitochondria may require a more consistent supply of reduced thioredoxin.

The methionine sulfoxide reductase B1 selenoenzyme and oxidative stress in the heart

As described above, ROS are important regulators of cell signaling and cytoskeleton dynamics through the oxidation of amino acid side chains. Traditionally, ROS have been considered as cellular regulators by their reversible oxidation of sulfur on cysteine residues that leads to the formation of disulfide bonds. In this manner, the formation or elimination of disulfide bonds through oxidative or reducing reactions, respectively, may alter the structure of a signaling protein and thereby regulate its activity. However, the oxidation/reduction of sulfur on methionine (Met) residues within proteins has emerged as an important mechanism of redox regulation of the activity of certain signaling molecules. ROS can oxidize Met to a mixture of two diastereomers, Met-S-sulfoxide (Met-S-SO) and Met-R-sulfoxide (Met-R-SO), that are stereoselectively reduced back to Met by methionine sulfoxide reductases A (MsrA) and B (MsrB1, 2 and 3), respectively (137). MsrB1 is the only selenoprotein in this family of enzymes while MsrA, MsrB2, and MsrB3 contain cysteine in place of selenocysteine in their catalytic centers (17).

MsrB1 was identified in our laboratory as one selenoprotein that was highly upregulated in hearts during cardiac stress (113). In particular, triiodothyronine- or isoproterenol-treatment

of mice for seven days induced both H₂O₂ and caspase-3 treatment in hearts, but antioxidant systems mitigated oxidative damage to cells during treatments and no cell death was observed. Analyses of mRNA and protein expression for selenoproteins revealed that MsrB1 was consistently and dramatically increased in oxidatively stressed hearts. A closer examination of the Msr family of enzymes in terms of protein and activity revealed that MsrB1 was the only induced Msr during cardiac stress, suggesting a particularly important protective or regulatory role for this selenoenzyme during hypertrophy induced with either triiodothyronine or isoproterenol. In this sense, ROS damage to proteins causing Met to Met-R-SO conversion may induce increased expression of MsrB1 that acts to reverse oxidative damage to proteins and minimize damage to the cardiomyocytes as myocardial hypertrophy ensues. The notion of the MsrB1 playing an inducible role in stressed hearts compared to MsrA is consistent with results from ischemia-reperfusion studies in rat hearts (138).

An alternative explanation for the induced expression and activity of MsrB1 during cardiac stress with triiodothyronine and isoproterenol may involve the dynamic reorganization of actin. It was recently shown in macrophages that MsrB1 plays a unique role together with the pro-oxidant enzymes, Mical1 and 2, in regulating actin assembly and disassembly (139). Two Met residues in actin are stereo-specifically converted to Met-R-SO by Mical1 and Mical2 and reduced back to Met by the selenoprotein MsrB1, supporting actin disassembly and assembly, respectively. Thus, during the myocardial hypertrophy and remodeling that arises from the treatment of mice with triiodothyronine or isoproterenol, actin remodeling via reversible Met-R-SO may rely on induced expression of MsrB1 to stabilize assembly similar as that observed in macrophages.

This special role of MsrB1 and Met-R-SO in regulating actin dynamics does not mean that MsrA plays no role in redox regulated signaling in the heart. In fact, a number of studies have demonstrated that MsrA is crucial for reducing two sulfoxidated Met residues on calcium/calmodulin-dependent protein kinase II (CamKII) that regulates its activity in a calcium-independent manner (140–142). Conversion of Met to Met-S-SO on CamKII regulates cardiotoxic effects of aldosterone, β -adrenergic antagonists, and angiotensin II (143, 144). In contrast to the regulation of actin assembly, the redox regulation of CamKII appears to involve MsrA instead of MsrB1. In fact, an abundance of data from the laboratory of Dr. Mark Anderson have shown that MsrA overexpression is sufficient to protect the heart from a variety of diseases involving oxidative stress including atrial fibrillation, heart failure, arrhythmias, and others (141, 145). This supports the notion that stereoselective oxidation of Met residues in individual cellular proteins to either Met-S-SO or Met-R-SO determines which Msr is involved in reversing the residue back to Met and thereby regulating the actions of that individual cellular factor (Figure 3). In addition to actin and CamKII, it is highly likely that other cellular protein factors will be identified that are regulated by Met sulfoxidation and this will provide important insight into the role of MsrB1 as well as MsrA in the heart under different types of stress.

Honorable mention: other important selenoproteins

While roles for GPXs, TRs, and MsrB1 in the heart have been established, other selenoproteins have been studied in relation to cardiac stress with findings worthy of mention. Polymorphisms in the gene encoding the ER membrane selenoprotein, SelS, have been suggested to play a potential role in the development of subclinical cardiovascular disease in the context of type-2 diabetes (146). Also, studies in a Finnish cohort suggested that variation in the SelS locus may have an effect on cardiovascular disease morbidity, especially in females (147). SelK is also an ER membrane protein that was shown by our laboratory to be required for expression of the scavenger receptor, CD36, in macrophages and thereby promote foam cell formation and atherogenesis in mouse aortas (34). This represents one case in which deficiency of an individual selenoprotein actually protects against cardiovascular disease. SelK has also been implicated with SelS in regulating ER stress induced by misfolded proteins (31), and ER stress is an important factor regulating the apoptosis/survival decisions of cardiomyocytes during a variety of stresses. In fact, several oxidoreductase selenoproteins like SelM, SelN, SelT, and Sep15 reside in the ER and may regulate the response to ER stress in the heart.

Thyroid hormone metabolism is important for both heart development and health of the mature heart. Dysregulation of thyroid hormone levels by the DIO1-3 selenoenzymes is crucial for preventing hyperthyroidism, which produces a well recognized spectrum of cardiovascular perturbations including increased heart rate and contractility, elevated cardiac output, and eventual cardiac hypertrophy (148, 149). DIO2 is the main DIO selenoenzyme expressed in the heart and its activity is important for proper cardiac function. For example, elevated DIO2 activity in the heart enhances cardiac contractility and ameliorates deterioration of cardiac function caused by pressure overload (150).

Conclusions

The relationship between dietary Se and cardiovascular disease is complex. Most of the available data suggest that inadequate Se intake increases susceptibility to a number of disorders involving cardiac stress as well a vascular disease. However, the potential benefits of Se supplementation in preventing human cardiovascular disorders remains unproven. The underlying rationale is that increased Se supplies the individual with higher levels of antioxidant selenoproteins in most tissues, including the heart and vasculature. The selenoproteins best characterized in cardiovascular disease are GPX1, 3, and 4, as well as Txnrd1 and 2. This is a very short list and much work is needed to uncover roles for other selenoproteins. More definitive evidence of protective roles for dietary Se and specific selenoproteins will require better in vivo models. This understanding will lead to a new perspective on cardiac pathology and the foundation for potential therapeutic efforts to modulate selenoproteins and selenium metabolism in the heart.

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Physiological location	Catalyzed reaction	Significance in cardiovascular system
Cytoplasm of most nucleated cells, including cardiomyocytes, fibroblasts, endothelial cells, and others.		Detoxifies H ₂ O ₂ , thereby reducing oxidative damage to cellular lipids, proteins, and nucleic acids. Important for protection from oxidative stress during ischemia-reperfusion and other disorders.
Extracellular spaces of cardiac tissue and vasculature		Secreted into extracellular spaces and plasma; detoxifies peroxides and thereby protects extracellular matrix in heart as well as platelets and vascular cells in circulation.
Cytoplasm, nucleus, and mitochondria of most nucleated cells, including cardiomyocytes, fibroblasts, endothelial cells, and others.		Detoxifies lipid hydroperoxides in the plasma membrane and organelle membranes under basal conditions and conditions of oxidative stress like ischemia-reperfusion, hypertrophy, etc.

Figure 1.
The properties of GPX enzymes relevant to cardiovascular stress.

Figure 2.

The mechanism of redox regulation by Txnrd enzymes. Electrons are taken from nicotinamide adenine dinucleotide phosphate (NADPH) via Txnrd and are transferred to the active site of thioredoxin, which in turn maintains reduced forms of cellular proteins. Flavin adenine dinucleotide (FAD) is a redox cofactor bound to Txnrd and the active center with selenocysteine and cysteine residues contain Se and S in their side-chains, respectively. In addition to thioredoxin, other macromolecules and small molecules may be reduced by Txnrd enzymes.

Physiological location	Catalyzed reaction	Significance in cardiovascular system
Expressed in the cytoplasm of cells; induced in the heart under stress.		Reduces Met-R-SO in proteins such as polymerized actin, which may be important during myocardial hypertrophy.
Expressed in the cytoplasm, nucleus and mitochondria; constitutively expressed in heart.		Reduces Met-S-SO in proteins such as CAMKII, which is important in a variety of cardiac stresses.

Figure 3.
The role of methionine sulfoxide reductase selenoenzyme MsrB1 (SelR) and the cysteine-containing enzyme MsrA.

Table 1**Summary of Selenoprotein Functions**

Selenoprotein	Abbreviation(s)	Function and Significance
Cytosolic glutathione peroxidase	GPX1	Reduces cellular H ₂ O ₂ . GPX1 knockout is more susceptible to oxidative challenge. Overexpression of GPX1 increases risk of diabetes.
Gastrointestinal glutathione peroxidase	GPX2	Reduces peroxide in gut. GPX1/GPX2 double knockout mice develop intestinal cancer, one allele of GPX2 added back confers protection.
Plasma glutathione peroxidase	GPX3	Reduces peroxide in blood. Important for cardiovascular protection, perhaps through modulation of Nitrous Oxide levels.
Phospholipid hydroperoxide glutathione peroxidase	GPX4	Reduces phospholipid peroxide. Genetic deletion is embryonic lethal; GPX4 acts as crucial antioxidant, and sensor of oxidative stress and pro-apoptotic signals.
Olfactory glutathione peroxidase	GPX6	Importance unknown.
Thioredoxin reductase Type I	TrxR1, Trxrd1, TR1	Localized to cytoplasm and nucleus. Genetic deletion is embryonic lethal. Regenerates reduced thioredoxin; controls glucose-derived H ₂ O ₂ .
Thioredoxin reductase Type II	TrxR2, Trxrd2, TR3	Localized to mitochondria. Genetic deletion in mice is embryonic lethal, but TXNRD2-deficiency in humans leads to glucocorticoid deficiency.
Thioredoxin reductase Type III	TRxR3, Trxrd3, TR2, TGR	Testes-specific expression.
Deiodinase Type I	D1, DIO1	Important for systemic active thyroid hormone levels.
Deiodinase Type II	D2, DIO2	Important for local active thyroid hormone levels.
Deiodinase Type III	D3, DIO3	Inactivates thyroid hormone.
Selenoprotein H	Sel H	Nuclear localization, involved in transcription. Essential for viability and antioxidant defense in <i>Drosophila</i> .
Selenoprotein I	Sel I, hEPT1	Possibly involved in phospholipid biosynthesis.
Selenoprotein K	Sel K	Transmembrane protein localized to ER and involved in calcium flux in immune cells. Also associated with ER associated degradation.
Selenoprotein M	Sel M	Thioredoxin-like ER-resident protein that may be involved in regulation of body weight and energy metabolism.
Selenoprotein 15	Sep 15	Thiol-disulfide oxidoreductase involved in protein folding in the ER. Possibly a pro-cancer selenoprotein and knockout mice are protected from chemically induced colon carcinogenesis.
Selenoprotein N	Sel N, SEPN1, SepN	Potential role in early muscle formation; mutations lead to multiminicore disease and other myopathies. Knockout affects muscle function and also impairs lung function independent of respiratory muscles.
Selenoprotein O	Sel O	Contains a Cys-X-X-Sec motif suggestive of redox function, but importance remains unknown. Localized to mitochondria across mouse tissues.
Selenoprotein P	Sel P	Selenium transport to brain and testes - Sel P knockout leads to neurological problems and male sterility. Sel P also functions as intracellular antioxidant in phagocytes.
Selenoprotein R	Sel R, MsrB1	Functions as a methionine sulfoxide reductase and Sel R knockouts show mild damage to oxidative insult and impaired actin dynamics.
Selenoprotein S	Sel S, SEPS1, SELENOS, VIMP	Transmembrane protein found in ER. Complexes with many proteins and may be involved in ER associated degradation.
Selenoprotein T	Sel T	ER protein involved in calcium mobilization.

Selenoprotein	Abbreviation(s)	Function and Significance
Selenoprotein V	Sel V	Testes-specific expression.
Selenoprotein W	Sel W, SEPW1	Putative antioxidant role, perhaps important in muscle growth.
Selenophosphate synthetase	SPS2	Involved in synthesis of all selenoproteins, including itself.

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