PARADOXICAL ROLES OF ANTIOXIDANT ENZYMES:
BASIC MECHANISMS AND HEALTH IMPLICATIONS

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ABSTRACT: Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated from aerobic metabolism, as a result of accidental electron leakage as well as regulated enzymatic processes. Because ROS/RNS can induce oxidative injury and act in redox signaling, enzymes metabolizing them will inherently promote either health or disease, depending upon the physiological context. It is thus misleading to consider conventionally-called antioxidant enzymes to be largely, if not exclusively, health-protective. Because such notion is nonetheless common, we herein attempt to rationalize why this simplistic view should be avoided. First we give an updated summary of physiological phenotypes triggered in mouse models of overexpression or knockout of major antioxidant enzymes. Subsequently, we focus on a series of striking cases that demonstrate “paradoxical” outcomes, i.e. increased fitness upon deletion of antioxidant enzymes or disease triggered by their overexpression. We elaborate mechanisms by which these phenotypes are mediated via chemical, biological, and metabolic interactions of the antioxidant enzymes with their substrates, downstream events and cellular context. Furthermore, we propose novel treatments of antioxidant enzymes-related human diseases by deliberate targeting dual roles of the pertaining enzymes, and outlined potential of “antioxidant” nutrients and phytochemicals, via regulating the expression or function of antioxidant enzymes, in preventing, treating, or aggravating chronic diseases. We conclude that “paradoxical” roles of antioxidant enzymes in physiology, health, and disease derive from sophisticated molecular mechanisms of redox biology and metabolic homeostasis. Simply viewing antioxidant enzymes always beneficial is not only conceptually misleading but also clinically hazardous if such notions underpin medical treatment protocols based upon modulation of redox pathways.

Key Words: Antioxidant Enzyme, Knockout, Overexpression, Oxidative Stress, Redox Signaling

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Antioxidant enzymes are often discussed in scientific research and daily life as key players of metabolism that promote healthy cells, tissues and organisms. The term best relates to enzymes that lower the levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS), or counteract their downstream cellular effects of excessive oxidation. ROS and RNS are produced from aerobic biogenesis or by oxidative enzymes, and being chemically reactive they have the capacity to damage cellular components. Nature has evolved three layers of antioxidant defense in the body. Small molecular antioxidants, including uric acid, glutathione (GSH), and vitamins C and E, offer the first line of defense to scavenge ROS/RNS directly and thus prevent or delay the initiation of various oxidative stresses. Damage-removing or repairing enzymes function as the last defense to regenerate biomolecules damaged from oxidative injury. Between these two layers, antioxidant enzymes serve as an intermediate defense to detoxify ROS/RNS into less reactive species. Superoxide (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$) represent arguably the best-known and most-produced ROS, with the former scavenged by superoxide dismutases (SOD)$^1$ and the latter by catalase (CAT), glutathione peroxidases (GPX), and peroxiredoxins (PRX). Thioredoxin reductases (TrxR) are in addition required to maintain functions of thioredoxins (Trx), PRX, methionine sulfoxide reductases (Msr) and many other redox-regulatory enzymes/proteins by regenerating protein thiols (411) in parallel with glutaredoxins (Grx).

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$^1$Genes and proteins are in this article typically named according to HUGO (http://www.genenames.org) and MGI (http://www.informatics.jax.org/mgihome/nomen/gene.shtml) guidelines for human and mouse, respectively, unless other names or abbreviations are by convention more prevalent in the literature. In some cases mice have been studied with overexpression from human transgenes, which may be somewhat confusing in terms of nomenclature. We believe, however, that the references given in the Tables of this review article will serve as useful reference material for any reader interested in the exact gene constructs that are being discussed.
utilizing GSH to catalyze reduction of protein disulfide substrates (180, 395), These ROS-
metabolizing and reductive enzymes, which also play important roles in RNS homeostasis, are
widely considered to be the major antioxidant enzymes and are the focus of this review article.

During the past two decades, developments of antioxidant enzyme gene knockout and
overexpression mouse models (Table 1) have enabled us to not only verify “anticipated”
metabolic health-promoting functions of these enzymes, but also to reveal often neglected
“paradoxical” roles of antioxidant enzymes triggering metabolic disorders. It has indeed become
clear that many antioxidant enzymes are more than just protective ROS/RNS scavengers. They
regulate many redox signaling pathways and may also exhibit pro-oxidant functions or functions
independent of their redox activities.

A number of chronic diseases are associated with genetic or metabolic alterations of antioxidant
enzymes, displaying either lower or increased activities, depending upon the actual enzyme and
disease. Meanwhile, certain “antioxidant” nutrients and phytochemicals are able to regulate
antioxidant enzyme expressions or functions with health implications. Therefore it is important
to have a better understanding of the “paradoxical” functions of antioxidant enzymes in
physiology, which explain how their overexpression can promote disease or their deletion can be
health-promoting. It is our goal that this review will help to support awareness of the involved
molecular mechanisms and thus be useful in advancing a more balanced view of antioxidant
enzyme and redox biology in medicine.
II. IMPACTS OF KNOCKOUT AND OVEREXPRESSION

Superoxide- and H₂O₂-metabolizing enzymes, including SOD, catalase, GPX and PRX, are generally considered to be the primary antioxidant enzyme defense system in the body. However, the only antioxidant enzymes that have thus far been found to be essential for mouse embryonic development and thus lethal when genetically deleted are GPX4, the two genes for cytosolic and mitochondrial TrxR, Txnr1 (51, 305) and Txnr2 (123), their cognate substrate Trxs, Txn1 (438) and Txn2 (490), as well as an essential gene for synthesis of GSH (604).

Deletion of the Trsp1 (encoding selenocysteine tRNA (tRNA^[Ser]Sec) gene that is required for synthesis of all selenoproteins is also embryonically lethal (54). Genetic deletion of several other antioxidant enzymes trigger strong phenotypes even if not being embryonically lethal, while certain antioxidant enzymes seem to have an impact only under severe oxidative stress or in specific tissues.

While the SOD family represents the only enzymes able to scavenge O₂⁻, catalyzing its disproportionation into O₂ and H₂O₂, multiple classes of enzymes detoxify H₂O₂ or organic peroxides. Catalases scavenge H₂O₂ by catalyzing its disproportionation into O₂ and H₂O. Some selenoproteins and thiol peroxidase such as GPXs, and PRXs, catalyze the 2-electron reduction of peroxides to form water using reducing equivalents from GSH or Trx, respectively. The functions of GPXs and PRXs are thus intimately coupled to those of glutathione reductase (GR) and TrxR, enzymes that catalyze the reduction of oxidized GSH (GSSG) and Trx, respectively, using reducing equivalents from NADPH (185, 411). In addition, exciting progress has been made in understanding functions of Se-dependent methionine-R-sulfoxide reductase 1 (MsrB1).
that is a Trx-dependent enzyme. Much progress has also been made in the understanding of selenoprotein P (Sepp1) and \textit{Trsp} gene that are both crucial entities for Se homeostasis and functions of all selenoproteins. Because prior reviews have discussed many of the detailed phenotypes in mice with knockout or overexpression of different antioxidant enzymes (62, 69, 71, 80, 122, 337, 361, 377, 499), we will provide herein only an updated synopsis on the same subject, as a basis for the subsequent chapter focusing on their “paradoxical” roles.

\textbf{A. Superoxide Dismutase Family}

\textbf{1. SOD1}

Knockout of \textit{Sod1} does not cause embryonic lethality in mice, but results in impairment of the reproduction function of both males and females. Whereas the males produce sperm with decreased motility and fertilizing ability (207, 658), the females display a marked increase in postimplantation embryonic lethality (264) associated with elevated two-cell arrest or cell death (334). The \textit{Sod1}\textsuperscript{-/-} mice develop anemia (299) and type 1-like diabetes (684). These mice also show a reduced lifespan, a high incidence of hepatocarcinogenesis in late life, and oxidative damage-accelerated spontaneous mutations in liver and kidney (73, 167). Although mice overexpressing SOD1 are apparently normal with a reduced mutation frequency in cerebellum (357), these animals exhibit certain abnormalities found in patients with Down's syndrome (23, 24, 524, 580).
Knockout and overexpression of SOD1/Sod1 exert negative and positive impacts, respectively, on mouse susceptibility or resistance to neurodegenerative disorders, cerebral and myocardial injuries, diabetic syndrome, and tissue intoxications and dysfunctions (Table 2). However, most, if not all, of the reported “mechanisms” are associations between phenotypes and genetic manipulations, without genuine knowledge of the exact molecular mechanisms that lead to the observed phenotypes. Importantly, the association of SOD1 mutations with familial amyotrophic lateral sclerosis (ALS) does not seem to be due to effects on enzyme activity but rather an increased propensity for protein aggregation (203, 373, 474, 491). Impacts of Sod1 knockout or SOD1 overexpression in mice on neurodegenerative disorders may be related to effects on Aβ oligomerization (477), dopaminergic neurodegeneration (746), lipid peroxidation (536, 647), and protein nitration (294). In cell studies, altering the enzyme affects dopamine autoxidation and changes of GSH (242), and neuroinflammation driven by activation of nuclear factor-κB (NFκB), release of nitric oxide (NO), and proinflammatory cytokines (153).

Overexpression of SOD1 protects against various brain and neurological injuries by: 1) attenuating the mitochondria-mediated apoptosis pathway (e.g., release of cytochrome c and nuclear translocation of endonuclease G) (624, 737); 2) suppressing the induced expression of matrix metalloproteinases (467); and 3) activating Akt/glycogen synthase kinase 3β (GSK-3β) survival signaling (169, 313). Meanwhile, the protection against cerebral ischemia is conferred in part by up-regulating Akt and down-regulating p38 mitogen-activated protein kinase (MAPK), and NFκB (100). In contrast, Sod1 knockout potentiates mice to ischemic injuries by activating NFκB (100), and lung dysfunction by increasing nuclear factor of activated T-cells (NFAT) and NFATc3 activities (546). Sod1 knockout can also trigger kidney dysfunction by enhancing the
oxidative stress-induced phosphorylation and the conversion of iron responsive protein-1 (IRP1)
to the iron responsive element (IRE)-binding form, which may accelerate the reabsorption of iron
by renal tubular cells (734). Inhibition of matrix protein synthesis induced by high glucose (129)
and the NO-O$_2^-$ interaction (148) contributes to the protection of SOD1 overexpression against
diabetic nephropathy. Seemingly, several, if not all, of these SOD1-altered phenotypes are
associated with specific redox signaling effects, rather than a direct free radical scavenging.

2. SOD2

$Sod2^{-/-}$ mice, unlike $Sod1^{-/-}$ mice, develop cardiomyopathy and neonatal or perinatal lethality,
despite variations in postnatal survival time and neuronal injury (370, 392). Thus, the reported
phenotypes of $Sod2$ knockout are mostly derived from haplodeficiency or tissue-specific
inactivation of the gene. While $Sod1^{-/-}$ female mice become infertile (264), ovaries from
postnatal $Sod2^{-/-}$ mice undergo normal folliculogenesis and can produce viable offspring when
transplanted to the bursa of wild-type hosts, suggesting the enzyme dispensable for the ovarian
function (443). Interestingly, strain-dependent overexpression of SOD2 is associated with growth
retardation and decreased fertility in transgenic mice (542) (Table 3). Although the $Sod2^{+/–}$ mice
are viable and no more sensitive to hyperoxia (304), their mitochondria show decreased
respiratory capability and elevated induction of the permeability transition (697).

Likewise, knockout and overexpression of $Sod2$/SOD2 produces negative and positive impacts,
respectively, on mouse susceptibility or resistance to a number of acute or chronic disorders
(Table 3). Such opposite effects of the enzyme on neurodegenerative disorders are related to
regulating mitochondrial ROS generation and function, shifting the amyloidogenic Aβ composition (435), slowing amyloid deposition and memory deficit (164, 435), and modulating dopaminergic neurodegeneration (11, 342). Similarly, the effects on ischemic cerebral injuries are through regulations of blood-brain barrier, matrix metalloproteinases (MMPs), and inflammatory responses (425). Knockout of Sod2 aggravates cellular senescence and aging (653, 675), though overexpression of Sod2/SOD2 fails to extend life span despite preserving age-associated loss of mitochondrial function (308, 374). Overexpression of the enzyme protects against diabetes and complication through improved mitochondrial respiration and integrity and decreased iNOS and NO production (50, 226, 273, 351, 408, 597). Interestingly, old Sod2+/− Gpx1−/− mice have an elevated incidence of neoplasms (750), suggesting that knockout of multiple antioxidant enzymes can have synergistic effects on carcinogenesis.

3. SOD3

Knockout of Sod3, unlike Sod1 or Sod2, does not affect mouse development and lifespan or further worsen the shortened lifespan of Sod1−/− mice, suggesting limited overlapping roles between these enzymes (81, 593). Respective protective and detrimental outcomes from overexpression and knockout of SOD3/Sod3 are seen in brain, heart and vascular system, kidney, lung and immune system, as well as in ischemic injuries and carcinogenesis (Table 4).

This extracellular SOD isoenzyme plays a pivotal role in protecting against a number of lung disorders including oxidative injury, inflammation, and fibrosis (7, 8, 81, 189, 212, 248, 318, 492, 528, 539, 625, 672). Different from those lacking Sod1, Sod2, or catalase, the Sod3−/− mice are
susceptible to hyperoxia and induced oxidative injury (81). Protective roles of the enzyme in pulmonary fibrosis have been thoroughly reviewed (205), and its unique importance in pulmonary function is attributed to its extracellular localization, the pathological importance of extracellular matrix expression, and cytokine release elicited by extracellular ROS. Involved signaling events include modulation of transforming growth factor beta (TGF-β) and early growth response protein 1 (Egr-1) expression (672), preserving angiogenesis (528), and maintaining NO bioavailability and subsequent modulating cGMP and NFκB activity (7). The unique protection by SOD3 against lung oxidative insults offers potential of administrating the enzyme to relieve pulmonary disorders (205). Moreover, the distribution of SOD3, rather than the total SOD activity, in the extracellular space is crucial for protecting heart against the pressure overload as this insult renders the Sod3−/− mice elevated myocardial O2− production and nitrotyrosine formation, increases of ventricular collagen I & III, MMP-2 and -9, and decreases in ratio of GSH/GSSG (glutathione disulfide) (414). Knockout of Sod3 renders mice susceptible to the collagen-induced arthritis (570), while overexpression of SOD3 in mouse synovial tissue attenuates the inflammatory arthritis (736), via opposite modulations of the production of the pro-inflammatory cytokines such as IL-1β and TNFα, and MMPs. Knockout of Sod3 also impairs renal-vascular function, in part by decreasing Akt and eNOS phosphorylation and heme oxygenase 1 activity (326).

B. Catalase

Catalase is ubiquitously expressed, and is predominantly located in peroxisomes of all types of mammalian cells with the exception of erythrocytes (669) and human vascular cells (607). A
certain activity of catalase is also detected in mitochondria of rat heart (541). In humans, acatalesemia is a comparatively common genetic disease with near-total lack of catalase, which is typically considered to be asymptomatic but may be associated with increased risk of a number of diseases (224). \textit{Cat}^{-/-} mice show normal development and fertility (268), and are not more susceptible to the hyperoxia-induced lung injury than the wild-type controls. Overexpression of catalase in mitochondria prolongs the lifespan of mice and attenuates age-associated pathological changes (137, 504, 584, 654). The overall outcomes of \textit{Cat} knockout are rather limited, especially in comparison with those associated with \textit{Sod}. This may in part be due to the fact that GPX and PRX (90, 184) play major roles in removing H$_2$O$_2$ at relatively low concentrations in the cells, whereas the contribution of catalase increases when intracellular H$_2$O$_2$ is high (428).

In contrast, promotion of health by \textit{CAT}/\textit{Cat} overexpression has been shown in many tissues and conditions (\textbf{Table 5}). Overexpression of \textit{CAT} protects against cardiovascular injuries or dysfunction (424, 723, 724), which is particularly relevant due to the lack of the enzyme activity in the human vascular smooth muscle and endothelial cells (607). Aortas from apolipoprotein E knockout mice overexpressing \textit{CAT} show smaller and relatively early stages of atherosclerotic lesions compared with the control (722). Cardiac-specific overexpression of rat \textit{Cat} attenuates the paraquat-induced myocardial geometric and contractile alteration by alleviating JNK-mediated endoplasmic reticulum stress (208), prolongs lifespan, and suppresses aging-induced cardiomyocyte contractile dysfunction and protein damage (712). Also, this specific overexpression of rat \textit{Cat} rescues the anthrax lethal toxin- or lipopolysaccharide-induced cardiac contractile dysfunction by alleviating oxidative stress, autophagy, and mitochondrial injury (317,
The elevated catalase antagonizes the alcohol dehydrogenase-associated contractile depression after acute ethanol exposure in murine myocytes, partially through improving intracellular Ca\(^{2+}\) handling and ablation of alcohol dehydrogenase-amplified JNK activation and Erk de-activation (748, 749). Comparatively, the endothelium specific overexpression of CAT shows a weak protection against myocardial or vascular ischemia/reperfusion injury, despite preserving the responsiveness of the heart to adrenergic stimulation (704). Knockout of Cat accelerates diabetic renal injury through upregulation of TGF-β and collagen secretion (289), whereas overexpression of Cat protects against the pathogenesis via attenuation of angiotensinogen and Bax function and normalized expression of angiotensin converting enzyme 2 (ACE-2) (58, 603).

**C. Glutathione Peroxidase Family**

GPX enzymes utilize reducing equivalents from GSH to reduce peroxides (60, 94, 185). Eight isoforms of GPX are known, of which five are selenoproteins (GPX1-4 and GPX6). The three selenium-independent GPX enzymes rely upon thiol rather than selenol chemistry. Among the GPX enzymes, GPX1 is the most abundant and ubiquitous isoform. GPX6 is found as a selenoprotein only in humans, while the orthologous Gpx6 has a catalytic cysteine (Cys) in mice and several other species (353). Several recent reviews have summarized physiological roles of GPX enzymes in relation to other selenoproteins (61, 360, 361, 554). Our discussion herein mainly summarizes the key findings from genetic mouse models.

1. **GPX1**
Gpx1 is not essential for survival or reproduction, despite its protection against cataract and slight growth retardation (110, 141, 172, 265). Knockout of Gpx1 sensitizes mice to pro-oxidant-induced oxidative injuries, whereas overexpression of the enzyme confers extra protection against such injuries in various tissues (Table 6). The elevated susceptibility of the Gpx1−/− mice to various acute oxidative injuries, including increased lethality induced by high doses of paraquat and diquat, relates to accelerated oxidation of NAD(P)H, proteins, and lipids (108, 111, 141, 195, 196). The importance and mechanism for Gpx1 protection depends upon the intensity of stress as well as antioxidant status of the challenged animals (108, 111, 141, 195, 196, 376) but high levels of dietary vitamin E do not replace protection of Gpx1 (113). It should also be noted that Gpx1 is one of the most Se-responsive selenoproteins, whereby low dietary Se intake rapidly lowers its expression in most tissues (631), suggesting that the Se status of the control mice will affect the comparative outcome of Gpx1 removal.

Protections against disease conditions by GPX1 are illustrated by increased susceptibility of Gpx1−/− mice and resistance of Gpx1 overexpressing mice to various oxidative insults, including ischemia/reperfusion and hypoxic ischemic injury in the brain, heart, and liver (128, 195, 341, 397, 564, 595, 691, 732). Furthermore, Gpx1 protects against cardiomyopathy induced by coxsackievirus B3 through suppression of viral genome mutation (37), atherosclerosis in a pro-diabetic ApoE−/− mouse model (115, 384, 652), doxorubicin-induced and angiotensin II-mediated functional declines and cardiac hypertrophy (15, 206, 714, 733), defective blood flow and epithelial progenitor circulation in a model of ischemia-induced angiogenesis (202), diabetic
nephropathy in association with fibrosis and inflammation (115, 640, 641), and detrimental effects of cigarette smoking or influenza A infection in the lung (165, 726).

Although the exact molecular mechanisms for involvement of Gpx1 in the above-described pathogeneses are largely unknown, the existing evidences point out ROS scavenging and redox signaling as the main modes of action. The \textit{Gpx1}^−/− mouse brain shows elevated oxidative stress, caspase-3 cleavage, and 3-nitrotyrosine formation (128, 341). The decreased migration of endothelial progenitor cells in the \textit{Gpx1}^−/− mice toward vascular endothelial growth factor (VEGF) and capability of these cells in promoting the formation of vascular network are indeed related to the elevated intracellular ROS levels (202). The protection of Gpx1 against diabetic nephropathy is associated with decreases of hydroperoxides, 8-isoprostane, nitrotyrosine, 4-hydroxynonenal, and proteins implicated in fibrosis and inflammation (115, 640, 641).

2. \textbf{GPX2}

GPX2 was first found in the gastrointestinal tissues (117). There are no \textit{Gpx2} transgenic mouse lines reported (\textbf{Table 1}). Like the \textit{Gpx1}^−/− mice, \textit{Gpx2}^−/− mice appear normal unless they are stressed by oxidative challenges (678). The Gpx1 expression is up-regulated in the colon and ileum of \textit{Gpx2}^−/− mice (186), which may explain why they do not develop cancer spontaneously but develop squamous cell tumor when additional stress such as UV exposure is employed (678). Likewise, spontaneous polyps are developed in \textit{Gpx1}^−/−\textit{Gpx2}^−/− mice, probably due to elevated intestinal lipid peroxidation with onset of inflammatory bowel disease (118, 174) (\textbf{Table 7}). Notably, nuclear factor (erythroid-derived 2) (NF-E2)-related factor (NRF2), a redox-sensing
transcription factor, may counteract oxidative injuries partially through up-regulation of Gpx2, at least in lung (613). Given its high expression in the gastrointestinal tract, GPX2 likely exerts antioxidant or anti-tumorigenic functions there, in association with GPX1 and NRF2. However, a basic question still remains as whether knockout of Gpx2 itself elevates intracellular H$_2$O$_2$ levels or affects NRF2 (352).

3. GPX3

GPX3 is mainly synthesized in proximal convoluted tubule cells of kidney (22). While the majority of renal GPX3 is secreted into plasma, some retains at the basement membranes to account for 20% of total selenium in kidneys (429, 505). Independent of its peroxidase activity, this enzyme transfers Se from the dams to the fetus (72), while Sepp1 instead of Gpx3 provides Se to neonates via the milk (257). Knockout of Gpx3 and overexpression of GPX3 in mice produce essentially opposite impacts on ROS-related events (Table 7). The Gpx3$^{-/-}$ mice display cerebral infarctions, along with elevated oxidative stress, blood clot, the induction of P-selectin, and lowered plasma cGMP level (311) and colitis-associated carcinoma with increased inflammation in the colon (32). Overexpression of GPX3 renders mice resistant to acetaminophen (APAP) overdose (458) but leads to hyperthermia (457). Thus, some physiological effects of Gpx3/GPX3 modulation can be viewed as unexpected if the enzyme would solely have a role in extracellular H$_2$O$_2$ scavenging. It is thereby possible that it has yet unrecognized physiological functions that are not directly related to the extracellular enzymatic activity.
4. GPX4

GPX4 has three isoforms in cytosol, mitochondria, and sperm nucleus, and enzymatically exhibits substrate preference toward phospholipid hydroperoxide (667). Interacting with guanine-rich sequencing-binding factor 1, GPX4 suppresses lipid peroxidation and apoptosis during embryogenesis (664). Because the global knockout of Gpx4 renders embryonic lethality, tissue-specific and Gpx4 isoform-specific conditional knockout mice have been generated (Table 7). Collectively, increased levels of lipid peroxides by localized Gpx4 deficiency lead to: 1) endothelial cell death and thrombus formation in a vitamin E-dependent manner (707); 2) 12/15-lipoxygenase dependent apoptosis-inducing factor (AIF) translocation and neuronal apoptosis (590); 3) mitochondrial potential decline and infertility of spermatozoa (292); and 4) defective photoreceptor maturation (663). Recently it was shown that cell death by ferroptosis is triggered upon genetic removal of Gpx4 in either kidney (194) or T cells (441). Clearly, Gpx4 is important for protections against the detrimental effects of lipid peroxidation, but the enzyme also has an intriguing peroxidase-independent structural role in sperm maturation (667).

Results from isoform-specific knockout of Gpx4 indicate that: 1) mitochondrial Gpx4 protects against apoptosis during hindbrain development (52); 2) mitochondrial Gpx4 suppresses protein thiol content, and is essential for male fertility (581); and 3) nuclear Gpx4 is essential for atrium formation (52), but indispensable for sperm maturation (581). Because the mitochondrial or nuclear Gpx4−/− mice are viable, the cytosolic Gpx4 confers the embryonic lethality phenotype of the global Gpx4 knockout. Reciprocally, overexpression of GPX4/Gpx4 in the global Gpx4−/− mice, detected only in liver and heart, can rescue their embryonic lethality and attenuate the
induced mitochondrial potential declines (393, 548) (Table 7). Similarly, the mitochondrion-specific $Gpx4$ overexpression maintains mitochondrial membrane potentials and protects against ischemia/reperfusion in the heart (136).

**D. Thioredoxin Reductase (TrxR) Family**

TrxRs are a family of NADPH-dependent selenoproteins, which play important roles as key propagators of the Trx system and thus several Trx-dependent enzymes, including PRX, Msr, ribonucleotide reductase (RNR), sulfiredoxin, and more (17, 122, 412, 423, 571). Three mammalian genes encode different TrxR isoforms, in mice being $Txnrd1$ encoding cytosolic TrxR1 (215, 325, 507), $Txnrd2$ encoding mitochondrial TrxR2 (also called TR3) (325, 454, 565, 628) and $Txnrd3$ encoding thioredoxin glutathione reductase that is mainly expressed in spermatids of the testis and seems to be important for spermatogenesis (211, 623, 626, 627, 659).

All $Txnrd$ genes are transcribed in a complex manner, resulting in divergent forms of each isoenzyme that differ from each other mainly in their N-terminal domains (88, 95, 139, 211, 442, 455, 507, 572, 573, 622, 629), potentially reflecting many levels of regulation. The phenotypes of mouse knockout models targeting the $Txnrd1$ and $Txnrd2$ genes are summarized in Table 8. No knockout models targeting $Txnrd3$ have yet been reported and overexpression of TrxR isoenzymes is difficult to obtain, due to their intricate expression patterns.

1. **TrxR1**
The full Txnrd1⁻/⁻ knockout mice display early embryonic lethality, with one study reporting lethality between embryonic days 8.5 and 10.5 mainly due to decreased cellular proliferation (305), and the other study embryonic death before day 8.5 with a lack of formation of mesoderm (51). Differences in genetic targeting between these studies, one removing the last exon of the gene (305) and the other removing the first exon (51), may possibly help to explain the different phenotypes. Notably, the knockout in mice of the Trx1 gene encoding Trx1 (see below) that is the presumed main substrate of TrxR1, gives even earlier embryonic death than upon TrxR1 removal (438). This suggests that functions of TrxR1 and Trx1 are not always directly linked in a physiological setting, which may be due to the fact that the GSH system can also keep Trx1 reduced through Grx activities (162). It is, however, clear that TrxR1 is an essential enzyme for embryonic development in mice.

Heart-specific Txnrd1⁻/⁻ mice are normal (305), as are mice with neuron-specific deletion of the enzyme (617). Interestingly, however, expression of the enzyme in glial cells is essential for normal development of the central nervous system (617). When deleted in either hepatocytes, mouse embryonic fibroblasts or B-cell lymphoma cells, the Nrf2-driven and mainly GSH-dependent enzyme systems are typically strongly upregulated (302, 430, 520, 535, 634). In fact, it was found that the Nrf2 induction can be so strong upon TrxR1 deletion or inhibition that cells become even more resistant to certain events of oxidative challenge, than those having normal expression of TrxR1 (63, 405, 634). These apparently paradoxical impacts on mouse susceptibility to stress upon TrxR1 removal will be further elaborated in the following chapters.

2. **TrxR2**
Similarly to TrxR1, the mainly mitochondrial isoenzyme TrxR2 is essential for embryonic development. Interestingly, however, Txnrd2 knockout yields early embryonic death in a more tissue specific manner, presenting liver apoptosis, impaired hematopoiesis and insufficient heart development (123). Knockout of mitochondrial Trx2 that is presumed to be the main substrate of TrxR2, however, displays a more severe phenotype with massive widespread apoptosis and open anterior neural tube (490). This illustrates that the functions of TrxR2 are not always directly linked to those of Trx2, which is similar to the situation with Trx1 and TrxR1 (see above).

There was a lack of overt phenotype when TrxR2 was conditionally knocked out in the nervous system (617) or in B- and T-cells (209), while its conditional knockout in heart produced obvious detrimental effects (123, 280). Recently, it was also shown that TrxR2 knockout in tumor cells prevented tumor growth because of a lack of hypoxia-inducing factor (HIF) function and JNK activation (254). These observations suggest that although most cells and tissues are dependent upon mitochondrial function, the physiological effects of genetic deletion of the mitochondrial TrxR2 enzyme are more specific than what would be explained by a generally impaired mitochondrial function in the whole organism.

E. Additional Mouse Models for Knockouts of Selenoproteins

Most, if not all, of the 24-25 selenoproteins in the mammalian proteomes (353) presumably have redox activity. Readers are referred to other recent reviews for a full survey of these proteins (84, 124, 250, 322, 360). However, in the context of this article it is worth considering MsrB1, a
Trx1-dependent selenoenzyme, and Sepp1, as their physiological antioxidant roles have been
studied using several genetic mouse models. Knockout of MsrB1 renders mice prone to lipid
peroxidation and protein oxidation in tissues as well as defective actin polymerization in
macrophages upon lipopolysaccharide challenge (190, 371) (Table 9). Neuronal protection by
Sepp1, a predominant extracellular selenoprotein that delivers selenium from liver to other
tissues and has peroxidase activity (576, 639), may be attributed to its selenium transport
function, because deletion of its C-terminal region being rich in selenocysteine residues (amino
acids 240-361) was sufficient to produce severe neurodegeneration in mice (258, 533, 583).
Liver-specific expression of SEPP1 in Sepp1−/− mice enhances their brain selenium content and
rescues the neurological defects (559), further supporting the important role of this selenoprotein
in the selenium transport.

The redox activity of all selenoenzymes depends on the function of selenocysteine (Sec), which
is cotranslationally incorporated at re-defined specific UGA codons in a process that requires
tRNA^[Ser]Sec, the transcriptional product of the Trsp gene. Because Trsp−/− mice are embryonically
lethal (54), various conditional knockouts and variants of Trsp have been made to study roles and
regulations of selenoproteins in specific tissues, resulting in several interesting phenotypes
(Table 9). Intriguingly, knockout of Trsp in endothelial cells causes embryonic lethality and in
muscle and liver induces postnatal death (76, 609). Global or conditional Trsp−/− mice expressing
wild-type or mutant Trsp transgene have also been generated (78, 591). These Trsp-altered
mouse models help understand tissue-specific functions of selenium, and allow for recapitulation
of mechanisms behind the classical selenium-deficiency syndrome, Kashin-Beck disease (161).
A recent review (361) offers detailed discussion on the pleiotropic effects of Trsp targeting that
are likely to be derived from the combined effects of modulation of multiple selenoproteins at once.

**F. Thioredoxin Family**

Trxs are small thiol-disulfide oxidoreductases with a Cys-Gly-Pro-Cys active site and are present in all living cells. The reduced forms with a dithiol motif in the active site catalyze disulfide reduction reactions, generating oxidized forms of Trx with a disulfide in the active site, which is again reduced by NADPH via TrxRs (276, 396). The isoforms of Trx have a broad range of functions in mammalian cells (18), including to serve as electron donors for Prxs that are controllers of the intracellular redox state together with GSH (274), and being major protein S-denitrosylases (91). The structure of Trxs comprises a central core of β-strands surrounded by α-helices that defines the Trx-fold, now known to be present in a large number of proteins denoted the Trx superfamily of proteins. This includes Grx (395), glutathione S-transferases, GPXs, PRXs and proteins of the protein disulfide isomerases (PDI) family, which are all built from Trx domains (21). In the context of this review the main results of genetic mouse experiments for analyses of Trx1, Trx2, Grx1 and Grx2 are discussed as follows.

1. **Trx1**

Trx1 (encoded by *Txn* in mice) is ubiquitously expressed in the cytosol/nucleus, and has a large number of functions in cellular redox control and antioxidant defense (18). One of those functions is to provide reducing power to RNR that is essential for DNA synthesis. Because the
global knockout of *Txn* in mice induces early embryonic lethality (437), shortly after
implantation with differentiation and morphogenesis defects, studies in adult mice were instead
enabled using a dominant-negative mutant line in which the active site Cys-32 and Cys-35
residues were altered to Ser (dnTrx-Tg) (140). These functionally Trx1-deficient mice display
decreased Trx activity in the lung and are sensitive to ambient air at room temperature. These
mice experience genotoxic stress, as evidenced by decreased activities of aconitase and NADH
dehydrogenase, lower mitochondrial energy production, but increased levels of p53 and
Gadd45α expression. These dnTrx-Tg mice are also manifested with increased levels of pro-
inflammatory cytokines (140), which are aggravated by exposure to hyperoxia. In contrast,
overexpression of enzymatically active Trx1 in the lung (140) helps maintain redox balance and
mitochondrial function with decreased inflammation. Mice overexpressing *TXN* have increased
resistance to a range of oxidative stress insults (643). In addition, Trx1 has been shown to protect
against joint destruction in a murine model of arthritis (657). Overexpression of the protein
furthermore seems to promote fetal growth by reducing oxidative stress in the placenta (665),
prevent diabetic embryopathy (314), and extend mainly the earlier part of the life span in mice
with a prolonged youth phenotype (527).

Trx1 is secreted from cells under inflammation and oxidative stress and is detectable in plasma
(482). Of particular interest is that the extracellular Trx1 is taken up by cells and has been
proposed as an effective antioxidant therapy (439, 483, 688). Its presumed antioxidant and anti-
apoptotic properties are tightly coupled with the reduced form of Trx1 binding to thioredoxin
interacting protein 1 (TXNIP) (475, 735) or apoptosis signaling kinase (ASK1) (290, 310).
Extracellular Trx1 is however also found in plasma as a truncated form called Trx80, resulting
from α-secretase cleavage (213) and known to act as an inflammatory mediator (Th1) via effects on the immune system and monocytes (522). Both Trx1 and Trx80 seem to have a positive effects protecting from Alzheimer’s disease in the brain (213). Because Trx80 lacks redox activity together with TrxR1 (523) and since extracellular forms of these proteins are likely to remain oxidized, it is possible and even likely that some of their physiological roles are unrelated to redox activities.

2. Trx2

Trx2, with a mitochondrial leader sequence, is targeted to the mitochondria, where it plays a crucial role in controlling of ROS by acting as a reductant of Prx3 in concert with the GSH system and Grx2 (240, 744). Knockout of the Trx2 gene (490) induces embryonic lethality with massively increased apoptosis and exencephaly with open anterior neural tube. Cardiac specific deletion of Trx2 (283) produces spontaneous dilated cardiomyopathy at one month of age, with increased heart size, reduced ventricular wall thickness, and progressive decline in left ventricular contractile function result in mortality due to heart failure at young age. In cardiomyocyte-specific Trx2−/− mice, mitochondrial function and ATP production are declined and ASK1-dependent apoptosis accelerated. Interestingly, humans with dilated cardiomyopathy have lowered Trx2 protein levels in heart tissue, suggesting that these mice could be a good model of the human disease (283).

3. Grx1
Grx1 catalyzes GSH-disulfide oxidoreduction reactions (275), de-glutathionylation of S-gluthionylated proteins (277) and reduction of Trx1 by GSH when TrxR is inactivated (162). Surprisingly, knockout of Grx1 (267) results in only a mild phenotype without major effects on ischemia reperfusion injuries. However, knockout of the gene offers protection against inflammation or defective revascularization in diabetes (4, 270), which will be further elaborated in the following chapter.

4. Grx2

Grx2 is encoded by a gene resulting in splice variants including Grx2a located in mitochondria and Grx2c in the cytosol/nucleus. Knockout of Grx2 (710) induces early onset of age-dependent cataract in mice. Grx2 is also required to control mitochondrial function since knockout affects cardiac muscle (426, 427), giving rise to larger hearts and high blood pressure.

G. Peroxiredoxin Family

The PRX enzymes are a family of abundantly present 20-30 kDa peroxidases (185, 562, 706). These homodimeric proteins fall into three varieties distinguished by their reaction mechanisms and the number of cysteine residues required for catalysis: typical 2-Cys (in mammals PRX1-4), atypical 2-Cys (mammalian PRX5), and 1-Cys (mammalian PRX6) (561, 594, 706). Both types of 2-Cys PRX utilize the reducing power of NADPH via the Trx/TrxR system to reduce their active site disulfides, formed upon catalysis with peroxide reduction, back to active dithiols. On the other hand, 1-Cys PRX, mainly utilize GSH as the reducing agent (706). Furthermore, the various PRX isoforms exhibit different subcellular localizations (271, 706). As the PRX enzymes
are highly abundant, accounting for as much as 1% of soluble cellular protein (673, 706) and are 
excessively reactive with H$_2$O$_2$, they are likely to be critical for both oxidative stress protection 
as well as redox signaling (562, 616, 698, 699).

1. **Effects of Prx knockout**

Mice lacking Prx1–4 and 6 are viable, but exhibit increased ROS levels and sensitivity to 
oxidative insults (300, 375, 389, 461, 484, 683). In general, both Prx1$^{-/-}$ and Prx2$^{-/-}$ mice appear 
healthy and are fertile, but have hemolytic anemia and increased atherosclerotic plaques (339, 
518), suggesting that Prx1 and Prx2 protect red blood cells from oxidative stress (375, 484).

Indeed, they exhibit splenomegaly, Heinz bodies in their blood, and morphologically abnormal 
red blood cells, which are high in ROS (375, 484). Prx3$^{-/-}$ mice are healthy in appearance and 
could grow to maturity, but exhibit elevated intracellular ROS, including in lung tissue (389).

Intratracheal inoculation of lipopolysaccharide to the Prx3$^{-/-}$ mice results in pronounced lung 
inflammation (389). Likewise, Prx6$^{-/-}$ mice also appear normal, but are very sensitive to 
oxidative insults (461, 683).

2. **Effects of Prx overexpression**

Overexpression of the PRX enzyme genes generally confers protection against different forms of 
oxidative stress. For instance, overexpression of Prx3 in heart mitochondria of mice suppressed 
cardiac failure after myocardial infarction (440). In addition, the Prx3 overexpressing mice have 
lower mitochondrial H$_2$O$_2$ concentrations and are protected against hyperglycemia and glucose
intolerance (102). Overexpression of Prx2 inhibits the ischemic damage of neurons (56).

Overexpression of PRX4 in the pancreas of mice suppresses the TRAIL-mediated apoptosis, protects pancreatic islet β-cells against injury caused by single high-dose streptozotocin (STZ)-induced insulitis, and attenuates inflammation (155). When Prx6 is overexpressed, development of cataract in mouse and rat lenses are significantly delayed (355). These transgenic mice exhibit extra resistance to the lung injury induced by hyperoxia (687). However, global Prx6 overexpression does not protect against diet-induced atherosclerosis despite lowering levels of H$_2$O$_2$ (531).
III. PARADOXICAL OUTCOMES

Although knockout of several antioxidant enzymes is detrimental and their overproduction beneficial to health, the opposite impacts have also been increasingly observed. This chapter describes a series of such apparently “paradoxical” cases that reveal metabolic benefits of deleting major antioxidant enzymes, or harmful effects of overexpressing them.

A. SOD Family

1. Elevated resistance to APAP toxicity by knockout of Sod1

APAP, also known as acetaminophen or paracetamol, is the active component of Tylenol and many other over-the-counter analgesics. A life-threatening hepatotoxicity of APAP overdose depends upon the liver enzyme CYP2E1 (cytochrome P450 2E1) that catalyzes biotransformation of APAP to a highly reactive intermediate, N-acetyl-p-benzoquinoneimine (NAPQI), which in turn can cause depletion of hepatic GSH and excessive liver necrosis (306).

Interestingly, genetic deletion of several antioxidant enzymes yields increased APAP resistance in mice, which has been reported for Gstp1 (255), TrxR1 (see below) and Sod1.

While an intraperitoneal injection of 600 mg APAP/kg results in 75% mortality in wild-type mice within 20 h, all such treated Sod1−/− mice survive for the entire 70 h duration of study (379).

Moreover, the Sod1−/− mice survived nearly three times as long as, and showed much less hepatic injuries, than wild-type mice following both higher (1,200 mg/kg) and lower (300 mg/kg) doses of APAP injection, respectively. As shown in FIGURE 1, this astonishing resistance to APAP
intoxication is associated with at least four separate mechanisms. Firstly, these mice have a 50% reduction in activity of the NAPQI-producing enzyme CYP2E1 (cytochrome P450 2E1) in liver. The down-regulated CYP2E1 activity thus helps attenuate NAPQI formation and the resultant GSH depletion and protein adduct formation. Indeed, hepatocytes isolated from Sod1\(^{-/-}\) Gpx1\(^{-/-}\) mice display a lower susceptibility to APAP-induced cell death, but higher susceptibility to NAPQI toxicity as compared with cells from wild-type mice (754). Secondly, hepatic protein nitration plays a crucial role in mediating APAP-induced hepatotoxicity (343). Knockout of Sod1 nearly completely blocks APAP-induced hepatic protein nitration (379). This is intriguing as the enzyme knockout or depletion presumably elicits elevated O\(_2^{-}\) production and thus subsequent peroxynitrite formation for protein nitration, provided that NO is available. Strikingly, SOD1 was previously shown to catalyze peroxynitrite-mediated nitrotyrosine formation in vitro (298). Later, the enzyme was demonstrated to be required for the protein nitration mediated by APAP or LPS in murine liver (758). Thirdly, compensatory inductions of other protective antioxidant enzymes (379, 756) and, fourthly a blunted cell death signaling (757) also attribute to the APAP resistance of the Sod1\(^{-/-}\) mice. Seemingly, the above-described Sod1 deficiency-derived protection against the APAP overdose is cytosolic-specific. The mitochondrial Sod2\(^{+/+}\) mice are actually more prone to the APAP-induced liver toxicity than their wild-type controls, potentially through prolonged JNK activation, exaggerated mitochondrial dysfunction with nuclear DNA fragmentation and necrosis (200, 545). It remains unclear whether Sod2\(^{+/+}\) mice are altered with expression of CYP2E1 and metabolism of APAP. However, SOD2 may serve a more important role than SOD1 as mitochondrion is a main target of the APAP toxicity. As the protein level of hepatic CYP2E1 in the Sod1\(^{-/-}\) is not altered (379), the activity loss probably results from an oxidative modification. However, another group failed to detect similar decreases in the baseline
activity of CYP2E1 in Sod1\textsuperscript{-/-} mice, despite conflicting effects of ethanol on the enzyme activity between their own studies (133, 329).

2. Protection against irradiation-induced neuronal damages by knockout of Sod

Knockout of Sod1 or Sod2 decreases a baseline of neurogenesis, but ameliorates radiation-induced decline of neurogenesis (183, 286) (FIGURE 2). Following irradiation, Sod2\textsuperscript{+/--} mice preserve normal hippocampal-dependent cognitive functions and normal differentiation pattern for newborn neurons and astroglia, which otherwise are damaged in irradiated wild-type mice. However, irradiation leads to a disproportional reduction in newborn neurons of the Sod2\textsuperscript{+/--} mice following behavioral training, suggesting that Sod2 haploinsufficiency renders newborn neurons susceptible to metabolic stress (126). In contrast, irradiation of Sod3\textsuperscript{-/-} mice enhances hippocampus-dependent cognition and decreases hippocampal nitrotyrosine formation (540). These results suggest that chronically-elevated \( \mathrm{O}_2^- \) anion levels and/or the lower production of \( \mathrm{H}_2\mathrm{O}_2 \) resulting from Sod3 knockout, may be protective against irradiation-induced damages in neurogenesis and cognition. In line with this result, overexpression of SOD3 impairs long-term learning and potentiation in hippocampal area CA1, further suggesting that \( \mathrm{O}_2^- \), rather than being considered exclusively neurotoxic, may also be a signaling molecule necessary for normal neuronal function (644). The underlying molecular mechanisms and signaling pathways for these phenotypes await further investigation. Likewise, knockout of Sod1 enhances recovery after closed head injury-induced brain trauma in mice, which is associated with attenuated activation of NFκB and subsequent decreased death-promoting signals due to down-regulated \( \mathrm{H}_2\mathrm{O}_2 \) production (41).
3. Neurological disorders associated with overexpression of SOD1/Sod1

SOD1 expression is associated with two types of neurological diseases: Down's syndrome (with elevated SOD activity) and ALS (associated with SOD1 mutations) (569, 612). Indeed, SOD1-overexpressing mice manifest certain abnormalities that resemble physiological effects seen in Down's syndrome, including withdrawal and destruction of some terminal axons and development of multiple small terminals (23, 24), a defect in platelet's dense granule responsible for the uptake and storage of blood serotonin (580), thymus and bone marrow abnormalities (524), and an impairment of hippocampal long-term potentiation (201). Meanwhile, SOD1 overexpression causes mitochondrial vacuolization, axonal degeneration, and premature motor neuron death, and accelerates motor neuron degeneration in mice expressing an ALS-inducing SOD1 mutant (303). The SOD1 overexpression also impairs muscle function and leads to typical signs of muscular dystrophy in mice (525, 550). In fact, transgenic mice overexpressing SOD1 display aberrant protein expression profiles in neurons and mitochondria of hippocampus (605, 606), indicating that elevated SOD1 activity in Down's Syndrome is not just a side-effect or a compensation in response to the increased oxidative stress, but may be part of the cause for the pathophysiology.

Overexpression of SOD1 impairs peripheral nerve regeneration and increases development of neuropathic pain after sciatic nerve injury with a disturbed inflammatory reaction at the injury site (350), exacerbates abnormalities in hematopoiesis and radiosensitivity in a mouse model of ataxia-telangiectasia (529), and promotes aging as indexed by mitochondrial DNA deletion in the acoustic nerve of transgenic mice (120). Neurons from the SOD1 overexpressing mice exhibit
higher susceptibility to kainic acid-mediated excitotoxicity, associated with a chronic pro-
oxidant state as manifested by decreased cellular GSH and altered Ca homeostasis (30). All these
negative impacts, along with known biochemical and neurological mechanisms, of SOD1
overexpression on various neurological disorders are summarized in FIGURE 3.

In contrast, other studies have shown either negligible effects of *Sod1* overexpression on
toxicities induced by neurotoxins including kainite, glutamate and N-methyl-D-aspartate
(NMDA) (347, 729) or even protections against similar insults in vivo (260, 586) and in vitro (53,
92). These seemingly contradictory findings may be confounded in part with differences in
extents of Sod1 overexpression, acute vs. chronic experimental settings, the timing of
observation, and the cellular capacity of H$_2$O$_2$ catabolism at the testing condition. For example,
when treated with a O$_2^·$ donor, overexpression of *SOD1* increases neuronal vulnerability due to
increased H$_2$O$_2$ accumulation, while overexpression of the gene in astrocytes that exhibit a
greater H$_2$O$_2$ catabolism capacity than do neurons actually leads to an increased resistance to O$_2^·$
toxicity (104). Therefore, the “paradoxical” function of SOD1/Sod1 overexpression in the central
nervous system may largely rely on: 1) whether the generated extra H$_2$O$_2$ results in a burden
beyond affordable cellular clearing capacity; 2) whether the induced burst of O$_2^·$ is more
detrimental to cell survival than the converted extra amount of H$_2$O$_2$; and 3) whether effects of
the enzyme expression are unrelated to its enzymatic activity.

4. Impaired immune functions and detrimental effects by overexpression of *SOD1/Sod1*
Overexpression of SOD1 in intraperitoneal macrophages decreases their microbicidal and fungicidal activity, along with increased intracellular production and release of H$_2$O$_2$, decreased extracellular release of O$_2^-$, and inhibited NO production following endotoxin stimulation (456). It was intriguing why enzymatically derived NO production became decreased when O$_2^-$ anion levels were diminished. The authors noted that nitrocompound metabolism in macrophages was affected by the overproduction of SOD1, but did not give mechanistic explanations. Possibly the reduced activities of NFκB and Erk1/2 in the SOD1 overexpressing macrophages, which are upstream regulators of iNOS, lead to downregulation of iNOS expression and thus lower NO production. However, this hypothesis remains to be experimentally confirmed. Transgenic mice overexpressing SOD1 show no increased resistance to TNFα-induced endotoxic shock (144), but a higher sensitivity to malaria infection as reflected by an earlier onset and increased rate of mortality (220), and activation-induced DNA fragmentation in their splenic T cells (513).

Doubling the expression of SOD1 does not extend, but instead causes a slight reduction of lifespan in mice (284). Likely due to elevated chronic oxidative stress, Sod1 overexpression leads to an increased heart rate variability (646) and accelerates the loss of cone function (668). Contrary to its protection against most of ischemic injuries, overexpression of SOD1 in the in-utero ischemia/reperfusion in pregnancy led to brain damages in both adult and fetal mice (383). Sod1 $^{-/-}$ mice exposed to chronic ethanol consumption exhibit decreased alcohol dehydrogenase activity and little induced CYP2E1 activity, which suppresses ethanol metabolism and precludes the resultant steatosis (133), while these mice are more susceptible to the acute ethanol-induced liver injury (329). This apparently contrasting impact of Sod1 knockout on injuries associated
with either acute or chronic ethanol intake underscore the stress-type and/or temporal-dependence of the function and (patho)physiological relevance of this enzyme.

5. Diverse effects of SOD2/Sod2 overexpression on alcohol intoxication and cancer cell survival

While overexpression of Sod2 protects against liver mitochondrial DNA depletion and respiratory complex dysfunction after alcohol binge exposure via inhibition of the formation of peroxynitrite (433), the overexpression aggravates prolonged (7 weeks) alcohol intake-induced hepatic toxicity (368, 433) (FIGURE 4). The prolonged ethanol intake selectively triggered hepatic iron elevation, lipid peroxidation, respiratory complex I protein carbonyls and dysfunction, mitochondrial DNA lesion and depletion in Sod2 overexpressing mice. Because administration of an iron chelator (defereroxamine) prevents all these adverse effects, hepatic iron accumulation is likely the crucial factor for the metabolic disorder (368). It has been suggested that alcohol administration decreases the expression of hepcidin, leading to abnormally active duodenal ferroportin and increased intestinal absorption of iron, which gradually increases hepatic iron accumulation (246). Although it remains unclear why in the referenced study (368) the iron overload was only found in Sod2 overexpressing mice, elevated Sod2 activity was linked to hepatic iron accumulation through modulation of iron homeostasis proteins in alcoholic patients (481, 633).

A proposed mechanism for aggravated hepatotoxicity by Sod2 overexpression may be as follows: the hepatic iron overload could lead to a decreased mitochondrial manganese uptake and
increased mis-incorporation of iron in the active site, forming Fe-substituted Sod2. The Fe-Sod2 is stable and lacks superoxide dismutase activity, but gains hydroxyl radical generating activity in the presence of hydroxyl radicals derived from H$_2$O$_2$, which in turn is generated by the manganese-Sod. Consequently, increased production of hydroxyl radicals could lead to the above-mentioned lipid peroxidation and other oxidative injuries (368). Apparently, increased hepatic iron and H$_2$O$_2$ might also generate hydroxyl radicals through Fenton reactions. The anticipated diminished O$_2^-$ anion levels due to Sod2 overexpression might furthermore remove its beneficial roles in limiting propagation of lipid peroxidation and blunt alcohol-induced increases of iNOS and subsequent up-regulation of peroxisome proliferator activated receptor gamma coactivator 1 (PGC-1), which otherwise promotes mitochondrial DNA replication (368). Another contributing factor could be the decrease in the mitochondrial transcription factor A (Tfam) in Sod2 overexpressing mice following alcohol administration. However, further studies are required to clarify the different cause-effect relationships with regards to the observed phenotypes. It should be noted that in rats, overexpression of Sod2 in liver prevents steatosis, inflammation, necrosis, and apoptosis following prolonged alcohol (4 weeks) administration (694). Thus, there may also be different impact between species of Sod2 overexpression on ethanol metabolism and intoxication.

Recently, differential roles SOD2 have been proposed between early and late stages of carcinogenesis. At the early stage, a lower SOD2 level may facilitate transformed phenotypes by potentiating mitochondrial defects, whereas at the later stage a higher SOD2 level protects cell from mitochondrial injury and contributes to tumor growth and metastasis (149). The roles of SOD2 become further complicated when cancer cells are challenged with increased oxidative
stress. Overexpression of SOD2 in HeLa cervical cancer cells promotes their growth when
growth factors are withdrawal, suggesting that SOD2 may promote tumor-cell survival in vivo at
conditions unfavorable to cell growth by counteracting the intracellular oxidative processes that
can additively impair cell growth and viability (514). Moreover, overexpression of SOD2
promotes survival of cancer cells treated with radiation, cytokines or drugs (263, 387, 432, 469,
632), likely through activation of NFκB and AP-1 signaling by the SOD2-mediated conversion
of H$_2$O$_2$. Therefore, overexpression of SOD2 may promote cancer due to increased cancer cell
resistance to the cytotoxicity of therapeutic treatments.

B. Catalase

1. Diabetic developments induced by catalase overexpression

The β cell-specific overexpression of rat Cat in non-diabetic background mice shows no
detrimental effects on islet function (717) and protects against the diabetogenic effect of STZ (99,
717). However, this type of overexpression provides no protection against cytokine-mediated
toxicity in isolated islets, despite a suppression of ROS formation (99, 717). Interestingly,
overexpression of CAT in mitochondria, compared with that in cytoplasm, confers stronger
protections against the cytokine-induced cytotoxicity in insulin-producing cells (230, 407),
indicating an important role of mitochondrial ROS in the cytokine toxicity of autoimmune
diabetes.
Strikingly, β cell-specific overexpression of Cat in nonobese diabetic mice accelerates spontaneous diabetes onset in males and cyclophosphamid-induced diabetes in both males and females, and sensitizes isolated islets to cytokine injuries. FIGURE 5 depicts several described divergent effects of catalase overexpression on susceptibilities to diabetes, but none of these effects are fully understood mechanistically. There was a down-regulation of Akt/Foxo1/Pdx1 survival pathway in islets associated with the cyclophosphamid-induced autoimmune type 1 diabetes (390). It was suggested that insulin/IGF-1 mediated phosphorylation of Akt might be down-regulated by PTP-1B (a tyrosine phosphatase) that is inhibited by ROS (H$_2$O$_2$) and catalase overexpression prevented the ROS inhibition of PTP-1B (390, 574). Although there are no direct experimental data to support these notions, maintaining adequate intracellular H$_2$O$_2$ may be needed for activating protective responses of β cells in autoimmune type 1 diabetes.

In contrast, Cat overexpression consistently protects against diabetic nephropathy (58, 603) or insulin resistance-induced cardiac contractile dysfunctions (160). Overexpression of Cat also attenuates high glucose-induced reduction of endothelial cell tight-junction proteins and the subsequent brain blood barrier (BBB) dysfunction in diabetes (402). These data suggest differential roles of catalase in pancreas and other organs in diabetic vs. physiological conditions.

2. Cell type-dependent inhibition of proliferation by catalase overexpression

Elevating catalase activity, similar to that of Sod, may alter the sensitivity of cancer cells to chemotherapy (216, 416). Overexpressing CAT in the cytosolic and especially in the mitochondrial compartments of HepG2 cells potentiates TNF-α-induced apoptosis by promoting
activation of caspases-3 and -8 (26). In contrast, overexpression of Cat in a murine lymphoid cell line enhances resistance to dexamethasone-induced apoptosis and exhibits increased net tumor growth in nude mice, which is associated with a delay of mitochondrial cytochrome c release and altered glucose and energy metabolism (648, 649). Overexpression of CAT inhibits proliferation of endothelial cells (740) and vascular smooth muscle cells (66, 602) by suppressing Erk1/2 and p38 MAPK signaling (602) and promoting a Cox2-dependent apoptosis (66). This highlights the need for a physiological level of endogenous H$_2$O$_2$ for survival and proliferation of vascular cells. Interestingly, the proliferation rate is elevated in vascular smooth muscle cells of Sod1$^{+/-}$ and Sod2$^{+/-}$ mice, along with higher activity of divergent mitogenic signaling pathways. The heterozygosity of Sod1 leads to preferential activation of Erk1/2 and p38 MAPK, while that of Sod2 causes activation of JAK/STAT pathway in smooth muscle cells (422). This opposite outcome is intriguing, because overexpression of Cat presumably diminishes intracellular H$_2$O$_2$ whose formation would be supposed to be lower due to the Sod haplodeficiency. Nevertheless, these diverse effects underscore the physiological importance to tightly regulate intracellular H$_2$O$_2$ levels for control of vascular cell proliferation. Furthermore, specific overexpression of CAT in myeloid lineage cells impairs perfusion recovery associated with fewer neovascularization and blunted inflammatory response following a femoral artery ligation, suggesting that H$_2$O$_2$ derived from myeloid cells such as macrophages plays a key role in promoting neovascularization in response to ischemia and in the development of ischemia-induced inflammation (269). Notably, decreases of H$_2$O$_2$ levels upon overexpression of catalase were verified by direct assays in vascular smooth muscle cells and myeloid cells in the above-mentioned studies, but only by indirect methods in endothelial cells and lymphoid cells.
C. GPX Family

1. Improved insulin sensitivity and decreased insulin synthesis upon knockouts of Gpx1 and Sod1

While knockouts of Gpx1 and Sod1 impair islet function, pancreas integrity, and body glucose homeostasis, these mice present improved insulin sensitivity in liver and muscle (680, 684). This improvement is mainly associated with an increased phosphorylation of muscle Akt at Thr\(^{308}\) and Ser\(^{473}\) after injection of insulin (684) (FIGURE 6). Presumably, this “unanticipated” benefit is attributed to elevated intracellular ROS that inhibit protein phosphatase activities and thereby attenuate dephosphorylation of Akt (33, 680). Moreover, an increased IR\(\beta\) protein in the liver of the Sod1\(^{-/-}\), but not in the Gpx1\(^{-/-}\), mice may also contribute to the improvement (684).

Meanwhile, Gpx1\(^{-/-}\) mice are resistant to the high fat diet-induced insulin resistance and show favorable responses including decreased-expression of gluconeogenic genes (G6pc, Pck1 and Fp1), increased glucose uptake by white gastric and diaphragm skeletal muscles through membrane docking of glucose transporter 4 upon AS160 phosphorylation on Thr\(^{642}\), and enhanced insulin-induced oxidation of phosphatase and tensin homolog (Pten) and PI3K/Akt signaling (406) in their embryonic fibroblast cells.

Comparatively, the Sod1 knockout exerts stronger impacts on insulin synthesis and secretion, glucose and lipid metabolism, and islet integrity than that of Gpx1 (684). Simultaneous ablation of both enzymes does not result in additive or severer metabolic outcomes. The Sod1\(^{-/-}\) mice show more apparent pancreatitis than the Gpx1\(^{-/-}\) mice that are more susceptible to the cerulein-
induced amylase increase. Although hypoinsulinemia and decreased pancreatic β cell mass are caused by knockouts of both of Gpx1 and Sod1 via down-regulation of the key transcription factor Pdx1 in pancreatic islets, the former seems to decrease only Pdx1 protein whereas the latter exerts suppressions at three levels of the Pdx1 regulation: epigenetic, mRNA, and protein (684) (FIGURE 7). Likewise, knockout of Sod1, but not Gpx1, up-regulates protein phosphatase 2b/sterol responsive element binding protein (SREBP)-mediated lipogenesis and down-regulates the AMPK-mediated gluconeogenesis (680). Apparently, there are several overlapping as well as distinctive mechanisms for Sod1 and Gpx1 in regulation of glucose homeostasis and lipid metabolism (378). It should also be noted that reductive stress may be as destructive as oxidative stress in the etiology of diabetes and obesity (753).

2. Potentiation of the peroxynitrite-induced toxicity by GPX1/Gpx1

Peroxynitrite represents a major RNS formed from reaction of O$_2^-$ with NO, which occurs at a diffusion-limited rate (39). Although peroxynitrite induces nitration in a variety of biomolecules, a major activity indicator is the nitrosylation of protein tyrosine residues (39). Peroxynitrite-mediated protein nitration is indeed involved in the pathogenesis of many human diseases (297, 530). Impacts and mechanisms of the influence of GPX1 activity on peroxynitrite-induced oxidative damage have been studied in different systems (198, 610). FIGURE 8 summarizes “paradoxical” roles and mechanisms of bovine GPX1, Gpx1 knockout, and GPX1 overexpression in coping with the PN-mediated protein nitration and toxicity in these systems.

Using a cell-free system, Sies et al found that GPX1 can serve as a peroxynitrite reductase (610). However, that function of GPX1 could not be verified by Fu et al. (198) using primary
hepatocytes isolated from *Gpx1*−/− mice. In stark contrast, *Gpx1*−/− hepatocytes are instead extremely resistant to peroxynitrite-induced DNA fragmentation, cytochrome c release and caspase-3 activation, GSH depletion, protein nitration, and cell death (198). Interestingly, treating hepatocytes with S-nitroso-N-acetyl-penicillamine (SNAP; a NO donor) in addition to diquat (O$_2$/H$_2$O$_2$ donor) produces synergistic cytotoxicity, and protein nitration induced by these two pro-oxidants together is attenuated in *Gpx1*−/− cells (197). While knockout of *Gpx1* in mice exerts partial protection on the APAP- or LPS-induced hepatic toxicity and protein nitration (343, 755, 756), overexpressing *GPX1* sensitizes mice to the APAP-induced hepatotoxicity and lethality (458). The metabolism of APAP in *GPX1* overexpressing mice leads to a substantial decrease in the replenishment of GSH in liver and blood compared with the controls. In contrast, overexpressing *GPX3* and *Sod1* in the same study renders mice resistant to the APAP toxicity. These observations again underscore the complexity or unpredictability of seemingly similar antioxidant enzymes in coping with a given oxidative insult.

### 3. Protection against kainic acid-induced lethality and seizure by *Gpx1* knockout

Kainic acid is an analog of glutamate that is widely used to induce limbic seizures and model the disease of epilepsy in rodents (40, 217). Administration of the compound activates NMDA receptors in hippocampus and other vulnerable brain regions (31, 43). As an event following NMDA activation (364, 365), there is increased oxidative stress including formations of O$_2^-$, NO, and peroxynitrite in the central nervous system after the kainic acid injection (217, 452, 568). Thus, antioxidants such as ascorbate, GSH and EUK-134 (a synthetic SOD and catalase mimic) can decrease the neurotoxic effects of kainic acid and its seizure-associated neuropathology (421, 575). Strikingly, *Gpx1*−/− mice are much more resistant to kainic acid-induced seizure (frequency
and interval), neuronal injury, and lethality compared with wild-type controls (309). This increased resistance involves inactivation of the NMDA receptor via thiol oxidation of its NMDA receptor-1 subunit, possibly due to elevated H$_2$O$_2$ levels in the brain of Gpx1$^{−/−}$ mice, and subsequent attenuation or block of the kainic acid-induced oxidative injuries (309) (FIGURE 9).

As described above, neurons from SOD1 overexpressing mice exhibit elevated susceptibility to the kainic acid-mediated excitotoxicity (30). Therefore, certain levels of ROS or chronic oxidation in the brain are needed for a functional NMDA receptor, with long-term use of antioxidants possibly thereby leading to detrimental rather than protective effects.

4. Type 2 diabetes-like phenotypes induced by Gpx1 overexpression

Global overexpression of Gpx1 in non-obese or non-diabetic mice results in hyperinsulinemia, hyperglycemia, hyperlipidemia, insulin resistance, β cell hypertrophy, and obesity at 6 months of age (445). Diet restriction can prevent all these phenotypes except for hyperinsulinemia and hyper-secretion of insulin after glucose-stimulation (686). Thus, these two phenotypes represent primary effects of Gpx1 over-production and seem to be mediated by up-regulation of a key transcription factor (Pdx1) for β cell differentiation and insulin synthesis and secretion, as well as down-regulation of the insulin secretion inhibitor mitochondrial uncoupling protein 2 (Ucp2).

The insulin resistance in these Gpx1 overexpressing mice may be attributed to less oxidative inhibition of protein tyrosine phosphatases due to diminished intracellular ROS (H$_2$O$_2$) levels upon higher Gpx1 activity, leading to accelerated dephosphorylation of IRβ and Akt after insulin stimulation (445, 686). Meanwhile, Gpx1 overexpression also affects transcripts, proteins, and functions of other pro-insulin genes, lipogenesis rate-limiting enzyme genes, and key glycolysis (GK) and gluconeogenesis (PEPCK) enzymes in islets, liver, and muscle (526, 721). FIGURE
10 highlights the major pathways and modes of action in relation to insulin production and insulin responses, illustrating how Gpx1 overexpression can induce type 2 diabetes-like phenotypes. Dietary Se deficiency precludes Gpx1 overproduction in these mice and partially rescues their metabolic syndromes by modulating or reversing these molecular and biochemical changes (721). Similarly, dietary Se levels have indeed been shown to affect glucose metabolism and insulin sensitivity (362). However, β cell-specific overexpression of GPXI in db/db mice with mutated leptin receptor rescues β-cell dysfunction with reversed signs of diabetes at 20 weeks of age (229, 244). It should be noted that islets have relatively low baseline Gpx1 activity but display one of the highest overproductions of Gpx1 activity among all tissues in the global Gpx1 overexpressing mice. Collectively, it seems clear that GPX1/Gpx1 overproduction is beneficial at diabetic or obese pathophysiological conditions, but becomes deleterious if triggered in healthy mice with normal metabolic status.

5. Intriguing roles of GPX enzymes in carcinogenesis

A number of studies have revealed cancer type-, stage-, and tissue-dependent impacts of GPX enzymes on carcinogenesis (FIGURE 11). Global GPXI overexpression sensitizes mice to skin tumor formation induced by 7,12-dimethylbenz(a)anthracene/12-O-tetradecanoylphorbol-13-acetate (DMBA/TPA) (413), whereas adenoviral delivery of GPXI to pancreatic tumor xenografts actually suppresses the tumor growth in nude mice (403). While mechanisms for differential roles of GPX1 between skin and pancreatic tumors remains elusive, a deficiency of Gpx1 in cancer-free naked mole rats (323) suggests the enzyme to be dispensable for cancer prevention at least in this particular species. As discussed in Chapter II, chronic colitis (174) and inflammation-driven intestinal cancer (118) are observed in Gpx1+/−/Gpx2+/− mice (118, 174), but
not in \textit{Gpx2}$^{-/-}$ mice unless additional stress is employed (678). This implies dose-dependent or overlapping roles of the two Gpx enzymes in this regard.

Roles of GPX2 in carcinogenesis vary with cellular metabolic contexts (61, 473). In healthy (normal or precancerous) cells, the enzyme helps maintain self-renewing of the gastrointestinal epithelium, suppress inflammatory processes, and thereby inhibit carcinogenesis (61). Loss of \textit{Gpx2} induces apoptosis, mitosis, and elevated \textit{Gpx1} expression in the intestine of mice (62, 186).

In cancerous cells, however, the anti-apoptosis function of the enzyme may promote their growth and migration. After being treated with intestinal carcinogens azoxymethane/dextran sulfate, \textit{Gpx2}$^{-/-}$ mice fed a selenium-inadequate diet (0.08 mg Se/kg) showed increased tumor numbers but decreased sizes, compared with the wild-type controls (352). The elevated tumor numbers may reflect Gpx2-derived protection against carcinogenesis at the early stage of tumor formation, whereas the declined tumor sizes imply promotion of Gpx2 on tumor growth during the latter stages of tumorigenesis. In addition, up-regulation of GPX2 in colorectal cancer (59, 187) and activation by cancer-associated NRF2 and Wnt pathways (28, 336) further suggest that the enzyme may indeed be involved in the pathophysiological processes.

Several studies have collectively demonstrated both positive and negative impact of GPX3 on carcinogenesis (62). While knockout of \textit{Gpx3} sensitizes mice to chemical-induced, colitis-associated carcinoma (32), knockdown of the gene by shRNA in leukemia stem cells decreased their competitiveness and self-renewal capability (256). Apparently, most of the findings on the roles of GPX enzymes in carcinogenesis await full elucidation of the molecular and cell biological mechanisms.
D. Paradoxical effects of TrxR1 targeting by genetic modulation or drug treatment

Although TrxR1 is an essential enzyme for mouse embryogenesis, the enzyme can be conditionally deleted in a wide range of differentiated tissues without apparent phenotype (Table 8, see above) and fully inhibited for at least a week in mice by gold compound treatment without overt toxicity (615). Strikingly, genetic deletion or full inhibition of TrxR1 can instead protect cells and tissues from oxidative challenges. As in the case of global knockout of Sod1, liver-specific Txnrd1−/− mice become highly resistant to APAP-induced hepatotoxicity (302, 520). It was also found that the TrxR1 enzyme, together with GSH, is a prime target for inhibition by NAPQI, which should help explain why APAP-derived NADPQI becomes more toxic than what is seen upon mere GSH depletion using inhibition of GSH synthesis (302, 520). The protective effects of TrxR1 deletion against APAP challenge are likely to be explained by compensatory up-regulation of many Nrf2 targets in mice with hepatocytes lacking Txnrd1 with more robust GSH biosynthesis, glutathionylation, and glucuronidation systems following APAP overdose (302, 520). Indeed, “priming” of tissues for oxidative injuries by prior inhibition of TrxR1 has also been shown in lung tissue, where inhibition of the enzyme leads to better resistance to hyperoxia (63, 405), in most or all of these cases presumed to involve activation of the cell protective Nrf2 pathway (89).

Similar paradoxical roles of TrxR1 exist in carcinogenesis. Although liver-specific Txnrd1−/− mice were reported to display a much greater tumor incidence (90 vs 16%) compared with wild-type mice after diethylnitrosamine induction (79), the Trx/TrxR1 system has also been found to
promote tumor growth (19, 251). Tumors arising in mice after injection of \textit{Txnrd1}-knockdown Lewis lung carcinoma (LLC1) cells are of much smaller in size than those from mice injected with the control, malignant cells; and most importantly, these knockdown LLC1 cells lose their targeting construct or show attenuated metastasis (251, 730). The mechanisms by which the enzyme can either be cancer preventive or promoting cancer progression are not fully understood, but are likely to relate to different stages of carcinogenesis and perhaps also differ between cancer types. It is known that over-expression of TrxR1 in cancer cells correlates with tumorigenic properties and down-regulation of the enzyme inhibits growth of human hepatocarcinoma cells (204). As mentioned above, knockdown of TrxR1 in lung carcinoma cells reverses their tumorigenicity and invasive potential in a xenograft model (730). Therefore, TrxR1 enzymes have been suggested as potential targets for development of anticancer drugs (410, 485, 666). As loss of \textit{Txnrd1} renders tumors highly susceptible to pharmacologic GSH deprivation, a concomitant inhibition of both GSH and TxrR systems was recently proposed to be a strategy to kill tumor cells (245, 430). In this context it should be noted that drug-targeted inhibition may not only inhibit TrxR1, but can also convert the enzyme to a pro-oxidant NADPH oxidase upon selective modification of its Sec residue (13).

We conclude that TrxR1 can exert “paradoxical” effects in three separate forms of the enzyme, i.e. no matter whether it is overexpressed, knocked down or targeted by low molecular weight inhibiting compounds, either beneficial or detrimental physiological effects can be triggered depending upon cellular context. This is summarized in \textbf{FIGURE 12} and its diverse effects should be considered in studies aimed at understanding the physiological roles of this enzyme.
E. Hazard of Trx overexpression and benefit of Grx knockout

Increased Trx1 potentiates cadmium toxicity (218), whereas ablation of Grx1 renders mice resistant to the LPS-induced inflammation and macrophage activation associated with enhanced S-glutathionylation (4). The latter also enhances resolution of airway hyper-responsiveness and mucus metaplasia in allergic mice (270). Because the gene knockout also attenuates inflammation and expression of proinflammatory mediators in the lung, S-glutathionylation of specific target proteins may be beneficial to attenuate airway hyperresponsiveness like in asthma. Thus, inhibitors of Grx1 may be of interest clinically. Plasma Grx1 concentration is increased in patients with diabetes (163). This may be linked to defective revascularization in diabetes, since Grx1 overexpressing mice have elevated soluble vascular endothelial growth factor receptor 1 and attenuated post-ischemia limb revascularization (478).
IV. MECHANISMS AND METABOLIC RELEVANCE

The apparently paradoxical outcomes in several cases of antioxidant enzyme overexpression or genetic deletion studies clearly challenge the “prevailing” view that these enzymes are only beneficial, or that ROS/RNS are solely toxic byproducts of aerobic metabolism. It is clear that controlled production of ROS/RNS is important in signaling and that under certain conditions, antioxidant enzymes exhibit pro-oxidant activities. In all aspects of redox biology, spatial-, tissue- and temporal-specific dependences are crucial, which will also have an impact upon the physiological functions of antioxidant enzymes (307, 465, 656).

The exact mechanisms for “paradoxical” outcomes of antioxidant enzyme knockout or overexpression should undoubtedly derive from the interplay of three factors: 1) the properties and roles of their ROS/RNS substrates and products; 2) the activities and functions of the antioxidant enzymes and 3) the metabolic contexts in which these entities interact. Accordingly, we will here discuss a series of chemical, molecular, biochemical, and physiological mechanisms that need to be considered and that may help to explain the observed paradoxical roles of antioxidant enzymes. Contributions of reductant substrates such as GSH to the paradox are discussed in the context of antioxidant enzyme catalysis.

A. Multi-faced Chemical Reactivity and Metabolic Roles of ROS/RNS

1. Dose-dependent impacts of ROS/RNS
Whereas excessive levels of ROS and RNS trigger oxidative stress, appropriate levels of ROS/RNS are required for redox signaling. Apparently, antioxidant enzymes are needed to suppress excessive production of ROS and RNS. Under certain conditions, however, insufficient ROS/RNS or elevated cellular reductants can be detrimental, or, conversely, elevated ROS/RNS may be beneficial. This explains in part dose-dependent effects of ROS/RNS or roles of their metabolizing enzymes. Transgenic mice with 2- to 3-fold increased Sod2 activity in major organs are phenotypically normal and fertile (542), while a higher overexpression of the enzyme to 2.5- to 8.7-fold activity above normal decreases body size and female fertility, and causes male infertility. Transgenic lines overexpressing 60- or 100-fold catalase activity are more resistant to doxorubicin-induced cardiac injury, but further overexpression to 200-fold or higher fails to provide protection (319). While the precise molecular explanations to these observations are unknown, they likely involve effects of site-specific localization, reactivity, steady-state levels of H$_2$O$_2$, as well as differential induction of compensatory pathways, as discussed below.

2. Detrimental effects of insufficient peroxides on redox signaling

Of the primary ROS, H$_2$O$_2$ is perhaps the most important for signaling (560), with both O$_2^-$ and hydroxyl radicals having limited half-life and reactivity profiles unsuitable for diffusible signals (135, 192, 193). H$_2$O$_2$ is an ideal signaling agent because of its relatively long lifetime and selectivity for targeting of particular protein microenvironments (135, 181, 192, 193, 700). It can oxidize thiol groups of specific Cys residues to disulfides (S-S), sulfenic (S-OH), sulfinic (SO$_2$H), and sulfonic (SO$_3$H) acids (404). Over-oxidation to sulfonic acid is not implicated in redox signaling, but contributes to oxidative stress due to its irreversibility. Peroxide sensing proteins...
that utilize uniquely reactive Cys residues may include transcriptional factors (20, 487), kinases (651), phosphatases (451), ion channels (521), ubiquitin and small ubiquitin-related modifier (SUMO)-conjugating enzymes, ligases and adapter proteins (55, 157, 450, 743), as well as various metabolic enzymes (466). Most likely of all proteins to react with H$_2$O$_2$ are however peroxidases, such as GPXs or PRXs, which in turn may propagate the oxidative signal to specific downstream targets in cells (698, 699).

Many signal transduction pathways are hard-wired to redox signaling networks, due to the large number of kinases and phosphatases having reactive Cys residues that affect their activities (70, 192, 193, 673). Deliberately-produced peroxides can oxidize catalytic Cys residues in various protein tyrosine phosphatases (PTPs), thereby inactivating them (34, 146, 577, 650, 670). This in turn serves to enhance activation of related signaling pathways by preventing the PTPs-catalyzed de-phosphorylation of specific phosphorylated tyrosine residues. Apparently, this type of inhibition can be removed by a hyperactivity of peroxide-scavenging enzymes. As in the case of Gpx1-overexpressing mice that develop type 2 diabetes-like phenotypes (445, 686), the over-produced Gpx1 diminishes intracellular ROS production, reverts the inhibition of PTPs, accelerates dephosphorylation of IR and Akt after insulin stimulation, and thereby leads to insulin resistance. In contrast, knockout of Gpx1 causes accumulation of intracellular peroxide, which, via the same pathways, improves insulin sensitivity and renders the mice more resistant to high-diet induced insulin resistance (406, 684). However, the specific dose, temporal dynamics, and targeting protein phosphatases for the action of H$_2$O$_2$ in redox signaling remain largely unclear.
3. Mixed effects of peroxynitrite in cell signaling

Peroxynitrite-mediated signaling pathways are not as firmly established as those involving H$_2$O$_2$. Traditional “antioxidant” ROS-scavenging enzymes like SOD1 and GPXs have been implicated in peroxynitrite metabolism and are supposed to affect the related signaling pathways. Peroxynitrite can upregulate Src tyrosine kinases, the Akt pathway, and various mitogen-activated kinases (512). Because many mitogen-activated kinases like p38 and c-Jun are implicated in pro-apoptotic pathways, peroxynitrite is considered to be a pro-death signaling molecule. In addition, peroxynitrite has also been implicated in hypoxic signaling. Under hypoxia, cytochrome c oxidase exhibits nitrite reductase activity, reducing nitrite to nitric oxide instead of oxygen to water (85, 86). This nitric oxide then reacts with O$_2^-$ to form peroxynitrite, which can oxidize yet to be determined protein targets that signal adaptation to hypoxia (158, 534). As discussed in greater detail below, SOD1 can either increase or decrease peroxynitrite via its ability to control O$_2^-$ fluxes. Thus, paradoxical outcomes of Sod1 knockout or SOD1 overexpression may be in part derived from the unpredictable consequences of peroxynitrite modulation (298, 512, 758). The same may also be true for the case of GPX1 (197-199) but the precise roles and mechanisms of SOD1 and GPX1 in regulating peroxynitrite-mediated signaling are unclear.

B. Paradox-related Properties of Antioxidant Enzymes

1. Pro-oxidant catalysis
Despite their well-known ROS/RNS scavenging capacity, some antioxidant enzymes may also promote oxidative/nitrosative stress. One example is the conversion of TrxR1 to a prooxidant enzyme upon targeting of its Sec residue by inhibitors, as discussed above. Another illustrative example relates to the peroxidase activity exhibited by SOD1 (398, 399, 551, 745). The peroxidase cycle of SOD1 involves peroxide reducing the Cu(II) center to form O$_2^-$ radical and Cu(I), followed by another molecule of peroxide re-oxidizing Cu(I) to form Cu(II) and hydroxyl radical. These reactions can occur under severe peroxide stress, with the resulting hydroxyl radicals subsequently being able to irreversibly oxidize metal coordinating His residues and thereby inactivate SOD1 (728). In the presence of carbonate, hydroxyl radicals can also oxidize carbonate to form carbonate radicals, which can in turn oxidize a variety of other substrates, including azide, urate, and nitrite (448, 745). However, it remains unclear to which extent this chemistry happens in vivo, but how much this contributes to the SOD1 toxicity.

In some circumstances, SOD1 can also promote aberrant protein nitration, either by enhancing peroxynitrite production or by directly activating it for tyrosine nitration. Beckman and colleagues demonstrated that human SOD1 mutants that are zinc deficient, either due to mutations associated with ALS or by other interventions that limit zinc to the protein, are better at catalyzing the reduction of dioxygen to O$_2^-$, thus providing a pool of O$_2^-$ that can react with nitric oxide to form peroxynitrite (173, 655). This peroxynitrite can then go on to nitrosylate and irreversibly damage various biomolecules, serving as another mechanistic basis for a toxic gain of function associated with various ALS-associated the mutants of SOD1.
Beckman and Koppenol have also proposed that intact human SOD1 can activate peroxynitrite to nitrosylate protein tyrosine residues (38, 39). The mechanism would involve Cu(II)-catalyzed heterolytic cleavage of peroxynitrite into the nitronium cation and CuO, with the former being a potent nitrosylating agent. Indeed, Lei and co-workers demonstrated that there is a diminished or blocked protein nitration in Sod1−/− mice treated with APAP (379). They proposed that the block of hepatic protein nitration in those mice might partially explain their resistance to the APAP overdose. Adding functional holo-SOD1, but not apo-SOD1, to liver homogenates of the Sod1−/− mice mixed with a bolus of peroxynitrite indeed resulted in increased protein nitration (758).

Likewise, GPX1 bears pro-oxidant potential. Several groups have demonstrated that this enzyme can aggravate nitrosative stress in mouse models (199, 343, 376, 377, 379, 458). This effect opposes the role of GPX1 in preventing nitrosative stress by catalyzing reduction of peroxynitrite into nitrite using reducing equivalents from GSH (610). Although the precise mechanisms remain poorly understood (191, 197), attenuated protein nitration should help explain the increased resistance of Gpx1−/− hepatocytes to peroxynitrate toxicity and lack of potentiation or even protection conferred by Gpx1 knockout against APAP hepatotoxicity (199, 343, 376, 377, 379, 458).

2. “Unwanted” modulation of reducing equivalents

Excessive enzymatic removal of ROS/RNS may lead to other detrimental downstream effects. For example, consumption of GSH as a reductant substrate deplete cells of GSH and thus outweigh the benefits of GSH-dependent ROS scavenging enzymes (199, 379, 458). Although
un-catalyzed reduction of peroxide by GSH is slow (700), GPX1 is very efficient at catalyzing this reaction (235). However, GSH can directly scavenge other more reactive species, like hydroxyl radicals, HOCl, peroxynitrite, and carbonate radicals (235). It can also regenerate antioxidants vitamins C and E. This may partially explains why overproduction of GPX1/Gpx1 can result in greater sensitivity to the destructive reactivity of APAP metabolites (199, 379, 458).

Meanwhile, elevating GSH may also be detrimental via mechanisms that involve S-glutathionylation and inactivation of various key proteins. For instance, GAPDH (462), eNOS (96, 431), certain tyrosine phosphatases (1), MAPK phosphatase 1 (331), mitochondrial thymidine kinase 2 (630), and protein disulfide isomerase (715) have all been reported to be inactivated by glutathionylation. This may either protect such enzymes from further damage, but can inhibits their function. While the precise pathways and mechanisms are yet unclear, NRF2 is emerging as a major regulator of oxidative and reductive extreme conditions in metabolism (57, 321). Upon a rise of ROS levels above normal, NRF2 helps to up-regulate GSH synthase and GSSG reductase, G6PD of the pentose phosphate pathway, as well as antioxidant enzymes like TrxR1, SOD and catalases. While initially activated to protect against oxidative stress, hyperactivity of NRF2 can however result in a shift towards reductive stress, due to over-abundance of anti-oxidant factors and GSH that can lead to cardiomyopathy and hypertrophy (543). It is possible that the detrimental effects of reductive stress can be associated with elevated S-glutathionylation (227) and/or inappropriate suppression of critical ROS-dependent signaling pathways. The effects of antioxidant enzyme overexpression in this context remain to be better characterized.
3. Stress source and intensity-dependent roles

As ROS scavengers, both Gpx1 and Sod1 protect mice against the lethality and toxicity caused by ROS-generating diquat and paraquat (111, 113, 168, 199). However, the opposite is true when mice are treated with the RNS-generating APAP and kainic acids (309, 379). Indeed, the ultimate metabolic outcome from overexpression or knockout of a particular antioxidant enzyme should be decided by how the enzyme will alter the relative production and fate of ROS and RNS in a given context. Good examples are the impacts of SOD overproduction on cardiovascular diseases or myocardial ischemic injuries. First, elevated SOD may help to preserve NO bioavailability, by preventing its reaction with O$_2^-$ to form peroxynitrite, and thus allow NO to serve as a vessel relaxation factor to protect the cardiac function and survival (83, 493). On the other hand, the hyperactivity of SOD may promote formation of H$_2$O$_2$ which then triggers downstream signaling responses that may inhibit vascular pathogenesis (747). However, the role of vascular H$_2$O$_2$ can also depend upon the location, as exemplified with H$_2$O$_2$ derived from overproduced SOD3 anchored to endothelial cells, which promotes VEGFR2 signaling and then potentially aggravates angiogenesis-dependent vascular diseases (508).

Knockouts of Txnrd1, Gpx1 and Sod1 produce different phenotypes of glucose and lipid metabolism in mice (302, 680, 684). While the knockout of Sod1 elevates endogenously-derived intracellular O$_2^-$, the mice display similar impairments in islet physiology, but distinct signaling mechanism compared with the Gpx1$^+$ mice with elevated intracellular peroxides. As shown in **FIGURE 7**, Sod1 knockout down-regulates Pdx1 at three levels: epigenetic, gene, and protein, whereas the Gpx1 knockout affects only the Pdx1 protein. Interestingly, both knockouts suppress
GSIS by elevating Ucp2 expression with decreased ATP production and affecting the mitochondrial potential in islets (684). Interestingly, only the GPX mimic ebselen, but not the SOD mimic copper diisopropylsalicylate (CuDIPS), rescues impaired GSIS in islets of all test genotypes including \( \text{Gpx}^{1/-} \) and \( \text{Sod}^{1/-} \) (684). The effects of ebselen seemed to be mediated via peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1\( \alpha \)) while, in contrast, CuDIPS improves insulin secretion only in \( \text{Sod}^{1/-} \) islets with suppressed gene expression of the PGC-1\( \alpha \) pathway (685). These results demonstrate that Gpx1 and Sod1, via their respective ability to modulate different species of ROS, can differentially affect redox-sensitive pathways in regulating GSIS. However, the “sensor” in the target signal molecules or regulators that distinguish and react with the local changes of \( \text{O}_2^- \) and peroxide needs unveiling.

Even for the same oxidative insult, the necessity and mechanism of a given antioxidant enzyme will vary with stress intensity and antioxidant status. When mice are injected with a high dose of paraquat (50 mg/kg body weight) or diquat (24 mg/kg), Gpx1 becomes absolutely essential to promote mouse survival by protecting against the depletion of NADPH and redox collapse (113, 195). In contrast, \( \text{Gpx}^{1/-} \) mice tolerate low doses of paraquat (12.5 mg/kg) and diquat (6 mg/kg) well if they are fed adequate selenium to saturate the expression of other selenoproteins (111, 196). Still, minute amounts of Gpx1 activity, raised by injection of selenium in selenium-deficient mice, becomes protective against a moderate dose of paraquat-induced hepatic necrosis and apoptosis (112). Comparatively, knockout of \( \text{Sod}^{1} \) in the mice enhances sensitivity to oxidative injury induced by a similar dose of paraquat (10 mg/kg) (264), implicating that generation of \( \text{O}_2^- \) contributes more to the paraquat-induced oxidative toxicity.
4. Compensatory inductions

“Hidden helpers” or compensatory responses induced by altering the expression of antioxidant enzymes may also help to explain the mechanisms of the observed phenotypes. The protection conferred by the Sod1 knockout against the APAP toxicity is associated with a 50% activity reduction in a key APAP-biotransforming enzyme, CYP2E1 (379), which catalyzes the formation of toxic metabolites of APAP. The Sod1 knockout also results in a 40% reduction of GPX1 activity (684), which may also add to the resistance of the Sod1−/− mice to the drug overdose (756).

As elaborated above, NRF2 is a redox-sensitive transcription factor that controls protective responses to oxidative stress (419, 463). The protein is normally sequestered in the cytosol by Keap1 and marked for proteasomal degradation. Under oxidative stress, Keap1 becomes oxidized and NRF2 can then translocate to the nucleus where it initiates transcription of selected antioxidant enzyme genes (419, 496). In the Sod1−/− mice, a greater fraction of Nrf2 is localized in the nucleus and up-regulates gene expression of many antioxidant proteins including glutathione S-transferases, sulfiredoxins, TrxR1, GSH synthases and other reductases (239). The up-regulation of these enzymes provides an increased antioxidant capacity in Sod1−/− mice against the APAP-derived oxidative stress. Likewise, the liver specific knockout of Txnrd1 enhances mouse resistance to the APAP toxicity by up-regulation of the Nrf2-target genes and proteins, with more robust GSH biosynthesis, glutathionylation, and glucuronidation systems (302, 520). The increased resistance to acute lung injury induced by endotoxin in acatalasemic mice (759) results from the H₂O₂-mediated down-regulation of cytokine expression in macrophages via
inhibition of NFκB activation. With these compensatory mechanisms revealing intricate pathways of physiological coordination to cope with redox imbalances, caution should be given to evaluate functions of antioxidant enzymes as absolute or isolated entities, as they will always be context-dependent.

5. Overlapping and coordinated functions

Catalase and GPX1 are two major antioxidant enzymes that are both responsible for removal of H$_2$O$_2$ although via distinct mechanisms (94, 184). Double-knockout mice deficient in both of these enzymes were generated to reveal insights into the overlap between these potentially redundant H$_2$O$_2$-detoxification systems (333). Interestingly, hepatic lipid peroxidation is not elevated in mice deficient in Gpx1 alone compared with that of wild-type mice (113), yet it is increased in mice lacking both Gpx1 and catalase (333).

In other cases, intrinsic expression of multiple isoforms of the same antioxidant enzymes in cells makes interpretations of oxidant-mediated diseases difficult. GPX1 is ubiquitous in all types of cells and GPX2 is in epithelium of the gastrointestinal tract (60, 117, 177). $Gpx1^{-/-}$ and $Gpx2^{-/-}$ mice are grossly normal. However, $Gpx1^{-/-}Gpx2^{-/-}$ mice develop spontaneous ileocolitis and intestinal cancer (118, 174, 175). This occurrence of cancer is also associated with an increased rate of mutation in the intestine (372). Collectively, these results suggest that the two enzymes cooperatively attenuate intestinal flora-induced inflammation by removing H$_2$O$_2$ and alkyl hydroperoxides, thereby suppressing the vicious cycles of the inflammatory response and oxidant-mediated mutations and cancer.
Over-production of either SOD2 or Cat, each having its distinct target of ROS, in pancreatic β cells of mice significantly delays but does not prevent the onset of diabetes induced by STZ compared with wild-type mice (99). However, the STZ-triggered increase in blood glucose is more effectively attenuated by overexpression of both enzymes in mice, suggesting that both O$_2^-$ and H$_2$O$_2$ contribute to the dysfunction and death of pancreatic β cells caused by STZ. In contrast, double knockouts of Sod1 and Gpx1 did not produce more aggravated effects than the single knockout of Sod1 on mouse susceptibility to pro-oxidant toxicity, loss of islet beta cell mass and insulin synthesis, and dysregulation of glucose metabolism (376, 684, 758). Thus, overlapping or coordination between antioxidant enzymes is not a universal feature.

Double knockouts of antioxidant enzymes can also yield unexpected novel insights into mammalian redox control. This was recently exemplified when Txnrd1 was conditionally deleted from hepatocytes in mice lacking a functional Gsr gene, thus leading to livers lacking both of the two major cytosolic NADPH dependent oxidoreductases TrxR1 and GR, presumed to be required for essentially all NADPH dependent reductive activities in the cytosol. These mice were, surprisingly, found to be both viable and fertile. The reductive power was instead supplied solely by dietary methionine that became converted to GSH, which was likely used in single-turnover reactions and thus these livers avoid the reliance on NADPH (171).

6. Non-redox functions
SOD1 has been shown to play roles in copper and zinc metabolism (132, 690, 709). In Baker’s yeast, overexpression or deletion of SOD1 affects the cell resistance or sensitivity to copper and zinc toxicity or deprivation, respectively (132, 709). More recent work suggests that SOD1 is also important for communicating the cellular stress response to low zinc (278, 709). Most interestingly, a group have demonstrated (656) a role for SOD1 in cell signaling independent of its role in $\text{O}_2^-$ disproportionation. They found that under oxidative stress, SOD1 translocated to the nucleus where it acted as a transcriptional activator of genes involved in oxidative resistance and repairing. Indeed, yeast cells expressing an allele of SOD1 that cannot get into the nucleus are more sensitive to oxidative stress. SOD1 represents ~1% of total cellular protein (~10-50 $\mu$M), and less than 1% of total SOD1 enzyme may be needed to protect against various oxidative insults (127, 557). Thus, the recently-discovered novel functions of SOD1, besides $\text{O}_2^-$ scavenging, help explain its high cellular abundance and perhaps its paradoxical roles.

Another antioxidant enzyme that exhibits non-redox functions is PRX1 (307). The enzyme forms oligomeric species that exhibit chaperonin activity upon oxidation of certain Cys to sulfinic acid. Such a mechanism enables the PRX family enzymes, which are better at scavenging low concentrations of peroxide, to be converted to chaperones to ensure proper protein folding under severe oxidative stress and high peroxide fluxes (409, 560, 705). Undoubtedly, the discovery of SOD1 as a novel transcriptional factor and PRX1 as a chaperone offers a new direction to elucidate the underlying mechanism for paradoxical roles of antioxidant enzymes. Additional non-redox functions of antioxidant enzymes include the role of Gpx4 as a structural protein in sperm mentioned above, or the cytokine-like properties of extracellular Trx1 or Trx80. It is possible, perhaps even likely, that additional non-redox related functions of classically
considered antioxidant enzymes will be discovered, which should help in interpreting the phenotypes seen upon their genetic deletion or overexpression.

C. Metabolic Context and Reaction Environment Affecting Roles of Antioxidant Enzymes

1. Physiological vs. pathophysiological conditions

Antioxidant enzymes may exert different impact in physiological compared to pathological processes. While overexpressing \( Gpx1 \) induces type 2 diabetes-like phenotypes in mice without diabetic or obese-prone genetic background (445), the β cell-specific overexpression of \( GPX1 \) in \( ob/ob \) mice actually, in stark contrast, reverses hyperglycemia and improves β-cell volume and granulation (244). Similarly, knockout of \( Gpx1 \) impairs insulin synthesis and secretion in mice fed the normal diet (684), but enhances mouse resistance to a high-fat diet-induced insulin resistance (406). Therefore, roles of antioxidant enzymes under “normal” and “diseased” conditions should not be extrapolated or inferred from each other.

2. Temporal dependence of physiological effects

Short-term benefits of antioxidant enzyme alterations may lead to long-term harms, and \textit{vice versa}. Indeed, overexpression of \( Gpx1 \) alone or in combination with \( SOD1 \) and \( SOD3 \) protects mouse islets from oxidative injury and improves islet graft function (480). However, the long-term over-production of \( Gpx1 \) results in hyperinsulinemia, insulin resistance, and obesity (684). In contrast, knockout of \( Sod1 \) offers extra resistance to APAP overdose and insulin sensitivity in
the young adult mice (379, 684), but leads to hepatocarcinogenesis in later life (167). When the hippocampal long-term potentiation (LTP), one of the major cellular mechanisms for learning and memory ability, is impaired in young (2-months old) SOD1 overexpressing mice (201), the aged (2-years old) transgenic mice actually exhibit an enlarged LTP (316) and consequently a better performance in spatial memory (315) compared with the wild-type mice. These differences implied a strong age-dependence for the effects of the Sod1 deficiency and/or Sod1-derived peroxide based on the brain function.

3. Subcellular location-dependence effects

Sub-cellular localizations of particular antioxidant enzymes have profound effects on their roles, which need to be considered in interpretations of the mechanistic results. Overexpressing extracellular GPX3 protects mice from the APAP toxicity, while the overexpression of intracellular GPX1 sensitizes mice to the toxicity (458). Likewise, the β-cell specific overexpression of cytoplasmic Cat and the metallothionein gene, but not the mitochondrial Sod2, accelerates the cyclophosphamide-induced and spontaneously-developed diabetes in the non-obese diabetic male mice (390). Overexpression of CAT in mitochondria, but not in the peroxisomes or nuclei, extends the median and maximal lifespan of the mice by 20% (584). This indicates that the interactions between ROS/RNS and their metabolizing enzymes should not be extrapolated from different subcellular compartments.

Likewise, peroxide-derived from the yeast SOD1 protein that is proximal to a membrane bound casein kinase is required to regulate energy metabolism in response to oxygen and glucose
availability (557). The yeast SOD1 protein that is not targeted to the cytosol, or other SOD isoforms that are targeted to the cytosol like mitochondrial SOD2 or Candida Albicans SOD3 are unable to regulate casein kinase signaling. Similarly, a small fraction of cytosolic PRX1 and PRX2 is associated with lipid rafts proximal to NADPH oxidase enzymes. Only the lipid raft associated PRX1, but not cytosolic PRX1, is found to be phosphorylated at Tyr194 by a protein tyrosine kinase (PTK) of the Src family when cells are stimulated by growth factors (705).

Phosphorylation of PRX1 near membranes has the effect of inactivating the enzyme, which promotes peroxide-mediated signals to propagate.

4. Cell-compartmentalization and tissue heterogeneity of transgenes

Different types of cell may not respond the same toward similar changes of antioxidant enzymes. While the cardiac-specific overexpression of CAT/Cat generates many benefits for prolonging lifespan and protecting against cardiac injuries (208, 317, 660, 712, 748, 749), the same specific overexpression in the endothelium shows little protection against myocardial or vascular ischemia/reperfusion injury (704). In either tissue-specific overexpression of a given antioxidant enzyme, such as catalase in cardiomyocytes and pancreatic β cells (319, 717), or ubiquitous overexpression of an antioxidant enzyme in mice, the intended overexpression may be very heterogeneous in different types of cells within an organ. Likewise, extents of a global overexpression of a transgene in mouse tissues may be restricted to certain organs, but not as widely spread as the corresponding endogenous mouse genes or the genes whose promoters are used in the transgene constructs (such as the human β-actin promoter) (266, 494, 509, 716, 727).
The heterogeneity of transgene expression cannot be appropriately assessed when homogenate of the entire organ is used for expression study. To circumvent this problem, large genomic fragments containing the genes of interest have been used to overexpress SOD1 and CAT (103). However, whether the specificity of transgene expression can also be applied to each individual type of cells within each organ is still an open question.

Heterogeneity of transgene expression is also shown even in mice carrying an identical transgene. For example, the same 14.5-kb genomic fragment containing the entire human SOD1 gene has been used independently by several laboratories to generate transgenic mice (87, 170, 231, 681). Although the SOD1 overexpression protects heart against an in vitro model of ischemia/reperfusion in two independent lines of transgenic mice, the cell specificity of overexpression in these mice is quite different. The gene is overexpressed in both endothelial cells and cardiomyocytes in one line of transgenic mice (681), but exclusively in coronary vascular cells including endothelial cells and smooth muscle cells but not cardiomyocytes in another line (106). Therefore, immune-histochemical studies are needed to identify the types of cells expressing the transgene in the target organs, and more than one line of transgenic mice carrying the same transgene should be employed in physiological studies to ensure reproducibility of the experiments. The latter approach is even more critical when homozygous transgenic mice are used in the experiments, because the transgene occasionally disrupts the expression of a normal mouse gene at the site of integration by the mechanism referred to as “insertional mutagenesis,” leading to a phenotype that is unrelated to the expression of the transgene (708). As a given antioxidant enzyme may not be sufficiently overexpressed in
targeted cells within an organ that are vulnerable to a particular oxidant-mediated injury, a
negative result does not rule out the enzyme function in defense against the injury in those cells.

Furthermore, the tissue heterogeneity of the transgene expression may also affect human
implications of findings from a particular animal model. Noteworthy, SOD3 in human aorta
accounts for approximately 50% of the total SOD activity, whereas the enzyme in rat aorta
represents only 5% of the total SOD activity due to a key amino acid difference that affects
tissue binding in vessels (178, 619). As a result, the rat essentially lacks vascular SOD3 and,
consequently, the observed protection of SOD3 against vascular diseases in rat models may be
easily over-interpreted.

5. Genetic background of mouse models

Most transgenic and knockout mice are initially generated in a mixed genetic background (272,
515) and it will take 10 to 12 generations of backcrossing to become congenic. Because this
crossing may take several years, most of the phenotypic studies, at least initially, are performed
on mice in a mixed genetic background. Such studies should be interpreted with caution, since
the genetic background of the mice may contribute to the observed phenotypes. For example,
strain C57BL/6J (B6) mice are more susceptible to hyperoxia-induced lung injury than C3H/HeJ
(C3) mice (287). Further studies using linkage analysis have shown that the B6 mice carry a
nucleotide substitution in the promoter region of the Nrf2 gene (116). This Nrf2 polymorphism
co-segregates with the susceptible phenotype of B6 mice to hyperoxia. Therefore, when SOD2
transgenic mice in a B6 X C3 mixed genetic background are used for study of hyperoxia-induced
lung injury, tolerance to exposure is determined by both the origin of the \textit{Nrf2} allele and expression of the \textit{SOD2} transgene (266). Therefore, control experiments should be conducted for functional studies in mice with the identical genetic background, preferably littermates of the experimental mice.

6. \textbf{Heterozygous mouse models and human relevance}

To date, most of the phenotypic studies have been performed using homozygous knockout mice (if viable) in comparison with wild-type mice. However, studies using heterozygous mice with a partial deficiency may be more relevant to human diseases, since humans being fully devoid of a protein or enzyme are very rare. Although relatively limited studies have documented the phenotypes of such mice that express approximately a half of the normal amount of enzyme, some results are very intriguing. For example, under normal physiological conditions, the time to development of malignant tumors in \textit{Prx1} \textit{+/-} mice is between those of \textit{Prx1} \textit{-/-} and wild-type mice. In addition, hemolytic anemia was first observed in the \textit{Prx1} \textit{+/-} mice at 12 months of age compared with 9 months of the null mice, whereas wild-type mice are free of this disease (484). Therefore, a partial deficiency in \textit{Prx1} results in phenotypes being intermediate between complete deficiency and normal in mice. On the contrary, while \textit{Sod1} \textit{+/-} females show a declined fertility (489), the fertility of the \textit{Sod1} \textit{+/-} females are normal (264, 443). In response to trauma-induced dysfunction of mitochondrial respiration in brain, \textit{Cat} \textit{+/-} mice are as vulnerable as \textit{Cat} \textit{-/-} mice (268). In contrast, the phenotype of \textit{Sod1} \textit{+/-} mice resembles that of wild-type mice in response to acute paraquat toxicity (10 mg/kg body weight) (264). Therefore, the effect of a partial deficiency in antioxidant enzyme on untreated mice and oxidant-mediated disease models
varies from gene to gene. While future research on the physiological role of antioxidant enzymes should consider more partial knockdown or knockout models, current findings from the homozygous knockout mouse models need to be verified in human studies.

In summary, we have postulated a series of mechanisms in this chapter that should underpin the “paradoxical” outcomes of antioxidant enzyme deletion or overexpression. **FIGURE 13** highlights the central concept that effects of antioxidant enzyme modulation arise from a complex interplay between the activities of the antioxidant enzymes with their ROS/RNS substrates (and reductants such as GSH), as well as the importance of the environmental context in which they operate. It is our hope that this figure, along with our deliberations, prompt readers to recognize that the mechanisms by which nature masterfully orchestrates these seemingly paradoxical events are evolved to maintain redox homeostasis, and are critical towards understanding both health and disease.
A. Antioxidant Enzymes in relation to Human Diseases

Catalase-deficient patients, classified as acatalasemic or hypocatalasemic, are found in many countries (495). These patients can have different alterations of the catalase gene including substitution (692, 693), deletion (262), and insertion (222, 225). Being apparently healthy, patients with acatalasemia may display increased risks of a progressive oral gangrene (166, 637, 638) and type 2 diabetes mellitus, especially in females (223). Still, the rather common occurrence of this autosomal recessive disease and its mild symptomatology suggests that catalase has mainly redundant activities with regards to human H$_2$O$_2$ removal pathways.

Two well-known neurodegenerative diseases: familial ALS and Down’s syndrome, exemplify the significant health implications of antioxidant enzymes in a “paradoxical” manner. While dominantly-inherited mutations of SOD1 gene account for 20% of the familial ALS cases (569), the pathophysiology seems to be due to a gain of mutant protein toxicity independent of the normal enzymatic activity of SOD1. Several lines of transgenic mice expressing Sod1 mutants have indeed displayed pathological characteristics reminiscent of those seen in ALS (67). The Down’s syndrome patients usually display a 50% increase in SOD activity (14, 131, 612).

Although transgenic mouse models have been developed for this disease (23, 24, 524, 580), the underlying mechanisms of SOD1 toxicity in Down’s syndrome are not understood (143, 366). In addition, mutations of SOD2 in humans are associated with idiopathic cardiomyopathy, sporadic motor neuron defect and cancer (261). However, Sod2$^{-/-}$ mice generated by targeted disruption
only partially recapitulate these human symptoms; rather, these mice display metabolic
phenotypes including fatty liver and cardiomyopathy (388). Indeed, polymorphisms of SOD
enzymes, catalase and GPX1 have been implicated in association with a number of human
metabolic disorders such as diabetes and cardiovascular diseases, as well as cancers [reviewed in
(130, 253)]

Altered nutritional selenium intake has long been implied in several diseases that are believed to
be explained mainly by aberrant selenoprotein functions (554). The first examples of genetically
and molecularly defined diseases of insufficient selenoprotein synthesis were found to relate to
mutations in the selenium-binding protein-2 involved in translational insertion of Sec into
selenoprotein and leading to complex diseases with hypothyroidism as a main symptom (25, 150,
582). These patients are however only partially deficient in selenoproteins and considering that
deletion of Trsp and some of the selenoproteins in mice is embryonically lethal (see above) it is
unlikely that patients would survive with a total lack of selenoprotein synthesis, but additional
polymorphisms and other aberrations in specific selenoproteins are likely to be discovered in
relation to disease.

Among the genetic variants of GPX enzymes, GPX1Pro198Leu polymorphism is the most
studied case. In a small randomized trial with 37 morbidly obese women (BMI > 45), this variant
precluded the protection against DNA breaks by daily supplementation of one Brazil nut daily
(290 µg Se/day) for 8 weeks (121). Furthermore, the same variation is associated with decreased
selenium status in Alzheimer’s patients (75), lowered GPX activity and increased breast cancer
risk in Danish women (553), predisposition to colorectal adenomas or carcinomas based on the
Norwegian cohort NORCCAP (241), and increased prevalence of cardiovascular disease on the cohort of 184 Japanese with type-2 diabetes (238). These associations appear to be specific, as no such relationship was found between the same variant and the risk of basal cell carcinoma in the cohort of 317 Danish (677). Another GPX1 variant (C198T) lowering the enzyme activity was identified in the South Indian population, which resulted in increased incidences of type 2 diabetes (C/T, 1.4-fold and T/T, 1.8-fold) (547).

Several single nucleotide polymorphisms on GPX2 are found to affect Barrett’s esophagus and esophageal adenocarcinoma (479). Polymorphisms of GPX3 are known to suppress the expression of this gene and serve as a risk factor for thrombosis in cerebral veins (676). In the 3’-untranslated region of GPX4, the T/C variation at position 718 is linked to cancer susceptibility, with the T variant being associated with a lower risk for developing colorectal cancer (44).

Likewise, polymorphisms in transcription factor binding sites of the PRX6 promoter are associated with less favorable overall survival in breast cancer patients (589).

B. Novel Treatments of Antioxidant Enzyme-related Diseases

The impact of antioxidant enzymes in disease may possibly offer novel treatment options for redox-related diseases, provided that the molecular mechanisms are known and can be specifically targeted. RNA interference (RNAi) technologies may thus possibly be developed for treatment of ALS originated from single nucleotide polymorphisms in SOD1 (472, 569). Mice expressing the missense mutant SOD1G93A-targeting shRNA were created to prove the principle of therapeutic potential (154, 544, 552, 566, 713).
Commonly used drugs for treating cardiovascular diseases, such as β-adrenoceptor blocker carvedilol, ACEs, and statins (2, 738), bear SOD-like activities that suppress O$_2^\cdot$. A GPX mimic may be used to improve GSIS impaired by the GPX1 deficiency (685). Overexpressing one or several antioxidant enzyme genes proves effective to prolong the survival of islet graft against the anticipated host oxidative attack (480). When large doses of chemotherapeutic agents or radiation induce severe oxidative stress, treating the patients with antioxidant enzyme mimics may help restore their redox homeostasis (359).

Meanwhile, there have been many studies aiming for virally mediated approaches to increase expression of antioxidant enzymes for protective effects in models of hypertension, restenosis, myocardial infarction, stroke, and other diseases [see (711) for a review]. For example, a gene delivery of antioxidant enzymes such as GPX1 and SOD1 was shown to attenuate oxidative stress in the brain of rodent models of HIV-associated neurocognitive disorders, Parkinson's disease, and diabetic complications (5, 6, 453, 564). Similarly, gene delivery for expression of SOD3 protects against the monocrotaline-induced hypertension in the lung of rats (312).

However, the safety and efficacy of gene therapy are still a concern, and therapeutic potentials of viral delivery of antioxidant enzyme genes to specific tissues remains an open question.

In contrast, inhibiting a given antioxidant enzyme or specifically silencing its gene expression may help treat disorders related to a gain of enzymatic function. As stated above, there is a great potential of using RNAi to specifically suppress the toxic mutant of Sod1 gene associated with ALS (154, 544, 552, 566, 713). In addition, microRNA (miRNA), regulators of mRNA stability...
and translation, has been recently proposed as biomarkers for a variety of diseases (449).

Although numerous miRNAs targeting antioxidant enzymes have been identified in cultured cells (243, 682), little is known about the reciprocal interactions between antioxidant enzymes and miRNA expression during pathogenesis.

Many types of drug-resistant cancer cells express high levels of antioxidant enzymes such as SOD, GPX, and PRX (64, 517). Pre-treating these cells with specific antioxidant enzyme antagonists or genetically silencing the target gene shall improve the anti-cancer drug efficacy (741). Similarly, pre-conditioning the antioxidant enzyme status may help minimize toxicities of commonly used drugs. Theoretically, hepatotoxicity of APAP may be attenuated if the patients are treated with TrxR1, SOD1 or GPX1 inhibitors, perhaps along with some GPX3 mimic, before administration. This notion is based on the fact that knockout of Txnrd1, Sod1 or Gpx1 renders mice resistant to the drug-induced protein nitration and toxicity (see above), but overexpression of GPX3 protects against APAP hepatotoxicity (458).

Targeting of antioxidant enzymes may possibly also be applied to treat chronic diseases such as type 2 diabetes. Insulin resistance is the hallmark of the disease, and is inversely related to the oxidative inhibition of protein phosphatases in GSIS. When Gpx1 overexpression diminishes of intracellular H₂O₂ and lifts the oxidative inhibition of protein phosphatases, causing insulin resistance (445), knockout of Gpx1 and Sod1 alone or together improved insulin sensitivity via the opposite mechanism (684). Therefore, the injected insulin could be more effective in lowering blood glucose, if GPX1 and SOD1 are temporarily down-regulated prior to insulin
administration. Clearly, such clinical protocols based upon findings from mouse experiments need to be studied and duly verified in human studies.

C. Antioxidant Nutrients

1. Perception and mixed outcomes of intervention trials

Antioxidant nutrients in foods often refer to vitamins C and E, carotenoids (particularly β-carotene), and certain trace elements such as selenium and zinc. Antioxidants are widely used to preserve food and beverages and to promote value-added product sales because of their perceived health benefits (182, 233). Indeed, many people believe that “antioxidant is good, more antioxidant is better” (234). However, more than 100 nutritional intervention trials conducted during the past 20 years (45-47) have shown disappointing outcomes of administering high or pharmacological doses of dietary antioxidant nutrients (46, 221, 233, 235, 537). In contrast, supra-nutrition of selenium and elevated serum selenium concentrations are associated with increased risk of type 2 diabetes (9, 119, 134, 363, 620). Although a re-analysis of the data from the large Se and Vitamin E Cancer Prevention (SELECT) trial (400) found the risk for increased prevalence of diabetes to be attributed to vitamin E supplementation (340), this controversial finding underscores the potential risk of over-dosing antioxidant nutrients. It also points out the need for a thorough understanding of selenium biology before large nutritional selenium trials are initiated or when their results are to be interpreted (249, 555).

2. Mode of actions by antioxidant nutrients
Antioxidant nutrients may contribute to overall antioxidant defence and interact with antioxidant enzymes in several ways. First, some of these nutrients like vitamins C and E directly scavenge ROS and RNS. Secondly, some of them serve as co-factors of antioxidant enzymes. Examples include selenium in the form of Sec in GPX, copper, zinc, and manganese in SOD, and iron in catalase. Dietary selenium deficiency is related to several diseases as is selenium toxicity (554). While iron and zinc deficiencies are quite common, deficiencies of manganese and copper are rare in humans. However, supplementing these nutrients to adequate subjects does not likely elevate their pertaining antioxidant enzyme activities because the activities are supposed to be saturated by those nutrients at the requirement levels. That fact may partially explain the lack of positive effects of long-term supplementation of antioxidant nutrients in adequate subjects.

Thirdly, antioxidant nutrients regulate antioxidant enzyme gene expression and protein production. For example, dietary vitamin E seems to down-regulate certain selenoprotein gene expressions (282) and up-regulate SOD activity (506). However, optimal intakes of antioxidant nutrients for the balance between body antioxidant enzymes and ROS/RNS still remain elusive.

3. Effects of phytochemicals on antioxidant enzymes

Plant foods contain a diverse range of secondary metabolites of bioactive molecules (phytochemicals) (380). Although these low molecular weight compounds do not seem to decrease systemic oxidative damage, polyphenols, carotenoids and tocopherols may reach high concentrations in the gastrointestinal tract and exert effects there (236, 237). Moreover, some of these phytochemicals (e.g., polyphenols) can exert pro-oxidant effects.
As discussed above, NRF2 plays a key role in maintenance of cellular redox homeostasis under oxidative stress (49, 328, 464). Phytochemicals such as EGCG, curcumin and isothiocyanates may induce oxidative or covalent modification of thiols in cysteine residues of NRF2, resulting in dissociation of NRF2 from Keap1 and its translocation to the nucleus where NRF2 can regulate gene expression of more than 200 antioxidant and phase II detoxifying enzymes (74, 252, 645). Thus, using naturally-occurring phytochemicals to up-regulate NRF2 may be a strategy for preventing or treating chronic diseases due to insufficient NRF2 activities (49, 156, 214). However, many of these compounds also inhibit TrxR1 (89) and the resulting long-term impact on disease must be better understood before guidelines on prevention through supplementation with phytochemicals should be given.

NRF2 can exert different roles in effects of various phytochemicals on cancer prevention and development (29, 35, 145, 420, 460, 464, 488, 596). Many phytochemicals characterized as NRF2 inducers (751) can be either chemopreventive or oncogenic (618). This has promoted scientists to search for NRF2 inhibitors. For example, brusatol isolated from the seeds of *Brucea sumatrana*, may inhibit NRF2 and enhance the efficacy of chemotherapeutic drugs in a mouse xenograft model (558). A coffee alkaloid trigonelline inhibits NRF2 and renders pancreatic cancer cells susceptible to anti-cancer drug-induced apoptosis (16). Meanwhile, NRF2 can act as a protooncogene (596), suggested that protective effects of its activities might exist only in normal non-cancerous cells and tissues. Overexpression of *Nrf2* indeed causes chemoresistance (742), whereas NRF2 inhibitors can overcome it (488). The complex nature between interactions
of phytochemicals with NRF2 will require a genuinely-personalized use of such compounds for cancer prevention or treatment (49, 210).
VII. CLOSING REMARKS

Strict control of ROS and RNS at physiological levels is essential to avoid disease, neither too much nor too little being good. Recently, James Watson hypothesized that several chronic diseases such as diabetes, dementias, cardiovascular disease and certain types of cancers may all be linked to a failure to generate sufficient ROS \(^{(689)}\). Another, complementary, theory is the Triage theory proposed by Bruce Ames underscoring that distortions in trace element usage by age underpins several diseases, which thereby also includes effects on several antioxidant enzymes \(^{(10, 444)}\).

In this review, we have attempted to provide comprehensive analyses of the paradoxical functions of SOD, catalase, GPX, TrxR, Trx, Grx, and PRX enzymes, along with other selenoproteins and selenoprotein synthesis-related \(Trsp\), in metabolism, health, and disease.

While paradoxes associated with these enzymes signify an alternative requirement for the body to maintain metabolic balance, harmony, and homeostasis, our understanding of the underlying mechanisms is far from clear. We do not know how antioxidant enzymes respond to demands for a tight control of their substrates or products in specific tissues or whole body. We know very little of novel functions of antioxidant enzymes independent of redox modulation. Their pro-oxidant catalytic potential and mechanism are not fully recognized or understood. Likewise, little is revealed regarding feedback mechanism of individual antioxidant enzymes and global coordination of different enzyme families in coping with various ROS/RNS-initiated events.
Because most of our discussions are based on animal experiments, many of the findings need to be verified in humans. It is clear that a number of human diseases are associated with genetic defects or polymorphism in specific antioxidant enzymes. However, specific, sensitive, and reliable indicators of *in vivo* redox status are yet explored to identify the optimal range of the antioxidant enzyme activities and ROS/RNS tone required by individuals according to personal genetic makeup, life style, and living environment. While mechanisms outlined in this review for the paradoxical roles of antioxidant enzymes may lead to alternative therapy strategies, the challenge will be to identify surfeits and deficits among the complex array of given diseases to design the most effective treatment. Antioxidant nutrients and phytochemicals can affect production of ROS/RNS, functions of antioxidant enzymes, and the balance between the two. It remains to be found out when and how these supplements are beneficial, wasteful, or even detrimental. In conclusion, antioxidant enzymes and their ROS/RNS substrates represent a pair of natural complements. Basic mechanisms and clinical implications for their interdependence and counterbalance in physiology and health warrant intensive research.
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FIGURE LEGENDS

FIGURE 1. Protective mechanisms conferred by the Sod1 knockout against APAP toxicity. The associated mechanisms include: 1) inhibition of protein nitration; 2) up-regulation of GR and TrxR activities; 3) down-regulation of microsomal P450 enzyme CYP2E1 activity, decreasing NAPQI production and the subsequent GSH depletion; and 4) inhibition of cell death signaling. Collectively, the Sod1−/− mice, as shown in the bottom graph, display a 100% survival rate over 70 h, while 75% of the wild-type mice die within 20 h after an intraperitoneal injection of 600 mg APAP/kg of body weight (from reference 379). APAP, acetaminophen; Bcl-XL, B-cell lymphoma-extra large; CYP2E1, cytochrome P450 2E1; GR, glutathione reductase; GSH, glutathione; IκBε, inhibitor of NFκB, epsilon; JNK, c-jun N-terminal kinase; NAPQI, N-acetyl-p-benzoquinoneimine; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; p21, cyclin-dependent kinase interacting protein 1; PARP, poly(ADP-ribose) polymerase; and TrxR, thioredoxin reductase.

FIGURE 2. Protections conferred by knockouts and haploid insufficiencies of Sod1, Sod2, and Sod3 against neural and cognitive damages induced by irradiation and brain trauma. Whereas mechanisms for the protections against the irradiation-induced damages await further investigation, the enhanced recovery from the brain trauma in the Sod1−/− mice is associated with attenuated H2O2 production and the subsequent NFκB activation. NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells.
FIGURE 3. Induction or potentiation of various neurological disorders by SOD1 overexpression. While mice overexpressing SOD1 develop signs of Down’s syndrome and muscular dystrophy, the overexpression promotes or exacerbates pathogeneses of other listed disorders including amyotrophic lateral sclerosis that is induced by the overexpression of mutant SOD1. Respective biochemical and neurological mechanisms for the impacts of SOD1 overexpression, along with their change directions (up and down arrows), are schematically shown for each of the disorders. GSH, glutathione.

FIGURE 4. Aggravation of prolonged alcohol intake-induced hepatic toxicity by Sod2 overexpression. The main proposed mechanism is that Sod2 overexpression leads to accumulation of hepatic iron that partially replaces manganese in the active site of Sod2 to form Fe-Sod2. Consequently, Fe-Sod2 catalyzes production of hydroxyl radicals from H$_2$O$_2$ that cause lipid peroxidation and mitochondrial damage. Likely, the increased hepatic iron and H$_2$O$_2$ may also enhance production of hydroxyl radicals through Fenton reactions. In fact, the exacerbated effect of Sod2 overexpression can be prevented by iron chelators. Meanwhile, the anticipated diminished levels of superoxide anion may remove its beneficial role in limiting the propagation of lipid peroxidation and blunt the ethanol induction of iNOS and subsequent up-regulation of PGC-1, leading to mtDNA depletion. Fe-Sod2, iron-substituted superoxide dismutase 2; iNOS, inducible nitric oxide synthase; mtDNA, mitochondrial DNA; NO, nitric oxide; and PGC-1, peroxisome proliferator activated receptor gamma coactivator 1.

FIGURE 5. Divergent effects of catalase overexpression on susceptibility to diabetes. The β-cell specific overexpression of catalase in non-obese diabetic mice leads to early onset of
spontaneous diabetes in male mice, with accelerated occurrence of diabetes by inhibition of the Akt-Foxo1-Pdx1 survival pathway in islets following cyclophosphamide administration. In contrast, such overexpression prevents diabetogenic effects of streptozotocin in non-diabetic mice. In insulin-producing cells, finally, overexpression of catalase in mitochondria renders strong resistances to cytokine-induced cytotoxicity, whereas its overexpression in cytoplasm leads to weak protection. Akt, protein kinase B; Foxo1, forkhead box O1; and Pdx1, pancreatic and duodenal homeobox 1.

**FIGURE 6.** Comparative mechanisms for improved insulin sensitivity in Sod1<sup>-/-</sup> and Gpx1<sup>-/-</sup> mice. Knockout of Sod1 elevates hepatic IRβ protein and muscle Akt phosphorylation after insulin stimulation, whereas knockout of Gpx1 induces only the latter. Meanwhile, embryonic fibroblast cells from Gpx1<sup>-/-</sup> mice are manifested with enhanced Pten oxidation and PI3K/Akt activation after insulin addition. These changes are presumably (dashed arrows) upstream of Akt phosphorylation and result in improved insulin sensitivity. However, such impact of Sod1 deletion has not been tested yet (question mark). In addition, Gpx1<sup>-/-</sup> mice fed a high fat diet display, following insulin challenge, enhanced glucose update through membrane docking of glucose transporter 4 upon AS160 phosphorylation on Thr<sup>642</sup>. Akt, protein kinase B; AS160, the 160 kDa substrate of Akt; IRβ, β subunit of insulin receptor; PI3K, phosphatidylinositol-3-kinase; and Pten, phosphatase and tensin homolog.

**FIGURE 7.** Distinctive mechanisms between knockouts of Sod1 and Gpx1 in lowering pancreatic islet β cell mass and plasma insulin concentration via down-regulation of the key transcription factor Pdx1. While knockout of Gpx1 decreases only the Pdx1 protein in islets,
knockout of Sod1 exerts suppression at three levels of Pdx1 regulation: epigenetic, mRNA, and protein. The down regulation of Pdx1 mRNA and protein upon Sod1 knockout coincides with decreased mRNA and protein levels of Foxa2, a transactivator of Pdx1, as well as attenuated binding of Foxa2, H3 acetylation, and H3K4 trimethylation in the proximal region of the Pdx1 promoter. Foxa2, forkhead box A2; H3, histone-3; K4, lysine-4; ORF, open reading frame; and Pdx1, pancreatic and duodenal homeobox 1.

FIGURE 8. Paradoxical roles of bovine GPX1, Gpx1 knockout, and GPX1 overexpression in coping with PN-mediated protein nitration and toxicity in cell-free system, primary hepatocytes, and mice. Different insult-mediated responses with net impacts of enzyme expression, and reported or proposed mechanisms are summarized in this figure. APAP, acetaminophen; DQ, diquat; GSH, glutathione; GST, glutathione S-transferase; PN, peroxynitrite; and SNAP, S-nitroso-N-acetyl-penicillamine.

FIGURE 9. Mechanisms of protection conferred by Gpx1 knockout against kainic acid-induced neurotoxicity. Gpx1 deficiency may elevate H2O2 production that can oxidize thiols in the NMDA receptor-1 subunit, which deactivates the NMDA receptor and subsequently attenuates or blocks kainic acid-induced oxidative stress and injuries. This oxidative stress can also be protected by antioxidants. EUK-134, a synthetic SOD and catalase mimic; GSH, glutathione; and NMDA, N-methyl-D-aspartate.

FIGURE 10. Molecular and biochemical mechanisms for the type 2 diabetes-like phenotypes induced by Gpx1 overexpression in mice. The diminished H2O2 accumulation in pancreatic islets may enhance β cell mass and insulin synthesis and secretion via modulation of key signaling
genes and proteins at epigenetic, mRNA, and(or) protein levels. These effects lead to
hyperinsulinemia and hyper-secretion of insulin. Meanwhile, Gpx1 overexpression also impairs
insulin responsiveness in liver and muscle and disturbs lipogenesis, glycolysis, and
gluconeogenesis in those tissues. The reported modes of action for those impacts include
modulation of key gene expression, protein function, and enzyme activities. The outcomes from
these effects in insulin-responsive tissues are reflected by insulin resistance, hyperglycemia,
hyperlipidemia, and obesity. The overall phenotypes from GPx1 overexpression in either insulin
producing or insulin responsive tissues, resemble type 2 diabetes. Representative key factors for
each of the main pathways or phenotypes are listed in brackets. Acc1, acetyl-coenzyme A
carboxylase 1; Beta2, neurogenic differentiation 1; Cat, catalase; Cfos, fbj murine osteosarcoma
viral oncogene homolog; Fasn, fatty acid synthase; Foxa2, forkhead box a2; Ins1, Insulin 1; IRβ,
the β-subunit of insulin receptor; Kir6.2, the KCNJ11 subunit of ATP-sensitive K⁺ channel; p53,
transformation related protein 53; Pdx1, pancreatic and duodenal homeobox 1; Pparγ,
peroxisome proliferator-activated receptor γ; Pregluc, Preproglucagon; Sur1, sulfonylurea
receptor; Ucp2, uncoupling protein 2; Akt, protein kinase B; GK, glucokinase; PEPCK,
phosphoenolpyruvate carboxykinase; and Δψ, mitochondrial membrane potential.

FIGURE 11. Intriguing roles of GPX isoenzymes in carcinogenesis. Overexpression of GPX1 in
mice promotes DMBA/TPA-induced skin cancer, whereas adenoviral delivery of GPX1 to
pancreatic tumor xenografts slows tumor growth in nude mice. This contrast illustrates tissue- or
stage-specific roles of GPX1 in carcinogenesis. As depicted in the middle yellow box, Nrf2 and
β-catenin that are associated with cancer, were shown to up-regulate GPX2 expression in
cultured human cells. Knockout of Gpx2 either stimulates or inhibits AOM-DSS-induced
intestinal tumorigenesis at early or late stages, respectively. The relative stage-specific effects
are indicated on the pink-colored triangle box, exemplifying the temporal dependence of the GPX enzyme in carcinogenesis. In addition, knockout of $Gpx3$ in mice promotes AOM/DSS-induced colitis-associated carcinoma, but knockdown of the enzyme by shRNA inhibits leukemia stem cell renewal. This comparison illustrates the cancer type- and/or model-specific role of the GPX enzyme in carcinogenesis. AOM/DSS, azoxymethane/dextran sodium sulfate; DMBA/TPA, 7,12-dimethylbenz[a]anthracene/12-O-tetradecanoylphorbol-13-acetate; Nrf2, NF-E2-related factor 2; and shRNA, small hairpin RNA.

**FIGURE 12.** Paradoxical effects of TrxR1 overexpression, genetic loss or drug inhibition. Three separate states of TrxR1 can have either beneficial (top) or detrimental (bottom) effects in mammals, as summarized in this figure. Native TrxR1 promotes cell viability through diverse functions of the Trx system, including support of Prxs, Msrs, and RNR as well as modulation of redox signaling pathways. However, cancer cells may also rely on TrxR1 activity to proliferate and the enzyme can thus promote cancer progress as well as metastases. Genetic targeting of TrxR1 is embryonically lethal, while conditional knockout in differentiated tissues such as hepatocytes result in Nrf2 activation and an increased resistance to oxidative challenges. When targeted by inhibitors, the TrxR1 enzyme can also gain an NADPH oxidase activity in addition to loss of its native Trx1 reducing capacity, which further activates Nrf2 but can also trigger cancer cell death, toxic side effects in normal tissues and increased dependence upon GSH for survival. APAP, acetaminophen; Msrs, methionine sulfoxide reductases; NAPQI, $N$-acetyl-$p$-benzoquinoneimine; Nrf2, NF-E2-related factor 2; Prxs, peroxiredoxins; RNR, ribonucleotide reductase; Trx, thioredoxin; and TrxR1, thioredoxin reductase-1.
FIGURE 13. Scheme of mechanisms of paradoxical outcomes upon modulation of antioxidant enzyme status. In general, either detrimental or beneficial impacts of antioxidant enzyme overexpression or knockout arise from complex interplays among redox active enzymes, their substrates, and the enzymatic reaction environment. Different ROS/RNS species and antioxidant enzymes discussed in this review are illustrated, along with their representative features (in black text) of chemistry, free radical biology, and metabolism that may all trigger paradoxical outcomes. Specifically, the dose, reactivity, and localization of ROS/RNS substrates can lead to differential impacts on oxidative stress and redox signaling pathways. Impacts and mechanisms of reductant substrates (e.g., GSH) in the “paradox” are shown in the context of antioxidant enzyme catalysis. The antioxidant enzymes can themselves contribute to the paradoxical outcomes by acting as pro-oxidants, either by catalyzing production of certain ROS/RNS or over-consuming reducing equivalents, depleting ROS/RNS required for signaling, acting on non-canonical substrates, exhibiting non-redox functions, inducing compensatory responses, or having overlapping functions with other enzymes. The environmental context, i.e. physiological (non-stress) or pathophysiological (metabolic stress, oxidative injury, nutrient deficiency, or drug toxicity) state, the experimental model, as well as spatial or time constraints, will determine the final phenotype. Thus, apparent paradoxes in antioxidant enzyme overexpression and knockout studies should be viewed in a well-defined physiological context as a combined interactions of all of these factors. Cat, catalase; Gpx, glutathione peroxidase; Grx, glutaredoxin; MsR, methionine sulfoxide reductase; Prx, peroxiredoxin; ROS, reactive oxygen species; RNS, reactive nitrogen species; Sepp1, selenoprotein P; Trsp, selenocysteine tRNA gene; Sod, superoxide dismutase; Trx, thioredoxin; and TrxR, thioredoxin reductase.
Table 1 Commonly-used mouse models for antioxidant enzyme overexpressing and knockout

<table>
<thead>
<tr>
<th>Enzyme/Protein</th>
<th>Overexpression</th>
<th>Knockout</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nature of the transgene</td>
<td>Altered site</td>
</tr>
<tr>
<td><strong>Cu,Zn-superoxide dismutase (SOD1)</strong></td>
<td>The entire human SOD1 gene contained in a 14.5-kb genomic fragment</td>
<td>Brain, liver, heart, and lung</td>
</tr>
<tr>
<td></td>
<td>The entire human SOD1 gene contained in a 64-kb genomic fragment</td>
<td>Brain, heart, kidney, liver, lung, skeletal muscle, and spleen</td>
</tr>
<tr>
<td><strong>Mn-superoxide dismutase (SOD2)</strong></td>
<td>A human SOD2 expression construct driven by 3.7 kb of the promoter and 5' flanking sequences of the human surfactant protein C gene</td>
<td>Lung</td>
</tr>
<tr>
<td></td>
<td>A human SOD2 expression construct controlled by 3 kb of 5' flanking sequence plus 5' untranslated region and intron 1 of the human β-actin gene.</td>
<td>Brain, eye, heart, lung, skeletal muscle, spleen, and tongue</td>
</tr>
<tr>
<td></td>
<td>The entire mouse Sod2 gene contained in a 14-kb genomic fragment</td>
<td>Brain, heart, kidney, liver, and lung</td>
</tr>
<tr>
<td></td>
<td>A human SOD2 expression construct driven by 570 bp of 5' flanking sequence and promoter of the rat insulin 1 gene</td>
<td>Pancreatic β-cells</td>
</tr>
<tr>
<td></td>
<td>A human SOD2 expression vector controlled by a 2-kb promoter and 10-kb enhancer of the mouse Tie2 (a vascular endothelial-specific receptor tyrosine kinase) gene</td>
<td>Endothelial cells</td>
</tr>
<tr>
<td></td>
<td>A human SOD2 expression construct controlled by a 5.5-kb mouse genomic fragment containing the last intron of the β-myosin heavy chain (MHC) gene to exon 3 of the α-MHC gene</td>
<td>Heart</td>
</tr>
<tr>
<td><strong>Extracellular superoxide dismutase (SOD3)</strong></td>
<td>A human SOD3 expression construct controlled by 3 kb of 5' flanking sequence plus 5' untranslated region and intron 1 of the human β-actin gene.</td>
<td>Brain, heart, and skeletal muscle</td>
</tr>
<tr>
<td></td>
<td>A human SOD3 expression construct driven by 3.7 kb of the promoter and 5' flanking sequences of the human surfactant protein C gene</td>
<td>Lung</td>
</tr>
<tr>
<td><strong>Catalase</strong></td>
<td>A rat CAT expression construct</td>
<td>Heart</td>
</tr>
<tr>
<td>Gene Name</td>
<td>Description</td>
<td>Tissue(s)</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>------------------------------------------------------------------------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td><strong>(CAT)</strong></td>
<td>downstream of a 5.5-kb mouse genomic fragment containing the last intron of the β-myosin heavy chain (MHC) gene to exon 3 of the α-MHC gene</td>
<td>Liver and gut</td>
</tr>
<tr>
<td>A human CAT expression construct controlled by a 2.8-kb mouse α-fetoprotein enhancer element I fused to 1.8 kb of the human β-globin promoter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A rat CAT expression construct driven by 570 bp of 5' flanking sequence and promoter of the rat insulin I gene</td>
<td>Pancreatic β-cells</td>
<td>(717)</td>
</tr>
<tr>
<td>Three human CAT expression constructs (peroxisome-, nucleus-, and mitochondria-targeted) driven by the cytomegalovirus enhancer element and a chicken β-actin promoter</td>
<td>Brain, heart, kidney, skeletal muscle, and spleen</td>
<td></td>
</tr>
<tr>
<td>The entire human CAT gene contained in a 80-kb genomic fragment</td>
<td>Brain, heart, kidney, liver, lung, skeletal muscle, and spleen</td>
<td></td>
</tr>
<tr>
<td><strong>Glutathione peroxidase 1 (GPX1)</strong></td>
<td>A human GPX1 expression construct controlled by the promoter, exon 1, and intron 1 of the mouse hydroxymethylglutaryl-coenzyme A reductase gene</td>
<td>Brain, heart, kidney, and liver</td>
</tr>
<tr>
<td>A human GPX1 expression construct controlled by rat insulin II promoter</td>
<td>Pancreas</td>
<td></td>
</tr>
<tr>
<td>The entire mouse Gpx1 gene contained in a 5.3-kb genomic fragment</td>
<td>Brain, eye, heart, lung, skeletal muscle, spleen, pancreas, and tongue</td>
<td></td>
</tr>
<tr>
<td><strong>Gastrointestinal glutathione peroxidase (GPX2)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Glutathione peroxidase 3 (GPX3)</strong></td>
<td>A human GPX3 expression construct controlled by the promoter, exon 1, and intron 1 of the mouse hydroxymethylglutaryl-coenzyme A reductase gene</td>
<td>Kidney, brain, and lung</td>
</tr>
<tr>
<td><strong>Phospholipid hydroperoxide glutathione</strong></td>
<td>A rat mitochondria-targeted Gpx4 expression driven by the human cytomegalovirus</td>
<td>Mitochondria of the heart</td>
</tr>
<tr>
<td>Peroxiredoxin I (PRX1)</td>
<td>Prx1</td>
<td>Global</td>
</tr>
<tr>
<td>------------------------</td>
<td>------</td>
<td>--------</td>
</tr>
<tr>
<td>Peroxiredoxin II (PRX2)</td>
<td>Prx2</td>
<td>Global</td>
</tr>
<tr>
<td>Peroxiredoxin III (PRX3)</td>
<td>Prx3</td>
<td>Global</td>
</tr>
<tr>
<td>Peroxiredoxin IV (PRX4)</td>
<td>Prx4</td>
<td>Global</td>
</tr>
<tr>
<td>Peroxiredoxin VI (PRX6)</td>
<td>Prx6</td>
<td>Global</td>
</tr>
<tr>
<td>Thioredoxin 1 (TXN1 or TRX1)</td>
<td>Txn1/Trx1</td>
<td>Global</td>
</tr>
<tr>
<td>Thioredoxin 2 (TXN2 or TRX2)</td>
<td>Txn2/Trx2</td>
<td>Global</td>
</tr>
<tr>
<td>Thioredoxin reductase 1 (TrxR1)</td>
<td>Txnrd1</td>
<td>Global, neurons, liver, heart</td>
</tr>
<tr>
<td>Thioredoxin reductase 2 (TrxR2)</td>
<td>Txnrd2</td>
<td>Global, neurons, heart</td>
</tr>
<tr>
<td>Selenoprotein P (SEPP1)</td>
<td>Sepp1</td>
<td>Global</td>
</tr>
<tr>
<td>Glutaredoxin 1 (Grx1 or Glrx1)</td>
<td>Glrx1</td>
<td>Global</td>
</tr>
<tr>
<td>Glutaredoxin 2 (Grx2 or Glrx2)</td>
<td>Grx2</td>
<td>Global</td>
</tr>
<tr>
<td>Gene</td>
<td>Description</td>
<td>Tissue/Expression</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Selenocysteine tRNA (Trsp)</td>
<td>A 1.93-kb genomic DNA containing the entire mouse <em>Trsp</em> gene with mutations of T→C at position 9 and A→G at position 37.</td>
<td>Brain, kidney, liver, and testes.</td>
</tr>
<tr>
<td>Methionine sulfoxide reductase A (MSRA)</td>
<td>Three mouse MsrA expression constructs (wild-type, mitochondria-targeted, and cytosolic) controlled by the cytomegalovirus enhancer and chicken β-actin promoter.</td>
<td>Liver, skin fibroblasts.</td>
</tr>
<tr>
<td>Methionine sulfoxide reductase B (MSRB)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Gene expression and tissue distribution for *Trsp* and *MsrA* and *MsrB1* genes.
### Table 2. Physiological impacts or pathological responses of superoxide dismutase-1 overexpression and knockout in mice

<table>
<thead>
<tr>
<th>Organ/Condition</th>
<th>Phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Overexpression</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain and neurological system</td>
<td>Ameliorate brain injuries induced by cold or subarachnoid hemorrhage (in rats) via suppressing MMP-2 and MMP-9 or activation of Akt/GSK-3β.</td>
<td>(93, 169, 313, 467)</td>
</tr>
<tr>
<td></td>
<td>Protect vulnerable motor neurons after spinal cord injury via attenuating the mitochondrial apoptosis pathway (in rats)</td>
<td>(624, 737)</td>
</tr>
<tr>
<td></td>
<td>Attenuate kainic acid-induced neurotoxicity in hippocampus and striatum</td>
<td>(260, 586)</td>
</tr>
<tr>
<td></td>
<td>Alleviate phenotypes of Parkinson’s disease by elevating dopamine and suppressing lipid peroxidation, protein nitration</td>
<td>(294, 536, 647)</td>
</tr>
<tr>
<td>Vascular system</td>
<td>Protect against post-angioplasty response and neointimal formation (adenovirus-mediated gene overexpression in rabbit tissue)</td>
<td>(358)</td>
</tr>
<tr>
<td>Lung</td>
<td>Alleviate pulmonary oxygen toxicity and prolong survival</td>
<td>(695)</td>
</tr>
<tr>
<td>Ischemic injury</td>
<td>Protect against cerebral ischemic injury (in mice/rats)</td>
<td>(100, 345, 476)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Protect against diabetogenesis and diabetic nephropathy by suppressing glomerular nitrotyrosine formation and matrix protein synthesis</td>
<td>(129, 148, 354)</td>
</tr>
<tr>
<td>Cancer</td>
<td>Reduce mutation frequency in cerebellum</td>
<td>(357)</td>
</tr>
<tr>
<td><strong>Knockout</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain and neurological system</td>
<td>Undergo marked hypertrophy and altered responses to acetylcholine in cerebral arterioles</td>
<td>(36, 152)</td>
</tr>
<tr>
<td></td>
<td>Vulnerable to axonal injury such as axotomy and ischemic insults, and altered calcium homeostasis in spinal motor neurons</td>
<td>(556, 611)</td>
</tr>
<tr>
<td></td>
<td>Increase susceptibility to MPTP-induced phenotypes of Parkinson’s disease</td>
<td>(746)</td>
</tr>
<tr>
<td></td>
<td>Drive phenotypes of Alzheimer’s disease such as Aβ oligomerization and memory loss</td>
<td>(477)</td>
</tr>
<tr>
<td></td>
<td>Display a modified distribution of fiber types and fiber loss, muscle atrophy and weakness</td>
<td>(348, 349, 367)</td>
</tr>
<tr>
<td>Vascular system</td>
<td>Lead to dysfunctions in endothelial-dependent vasodilation and myogenic tone, and accelerated vascular aging in endothelial progenitor cells</td>
<td>(125, 151, 152, 228, 674)</td>
</tr>
<tr>
<td>Lung</td>
<td>Increase NFAT activity and NFATc3 nuclear localization resulted from elevated superoxide/H$_2$O$_2$ ratio, induce spontaneous pulmonary hypertension in pulmonary arteries</td>
<td>(546)</td>
</tr>
<tr>
<td>Liver</td>
<td>Alter hepatic gluconeogenesis, glycolysis, and lipogenesis, and induce lipid accumulation by impaired lipoprotein secretion</td>
<td>(662, 680)</td>
</tr>
<tr>
<td>Ischemic injury</td>
<td>Enhance sensitivity to acute paraquat and alcohol-induced liver toxicity</td>
<td>(264, 329)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Impair neovascularization induced by hindlimb ischemia</td>
<td>(228)</td>
</tr>
<tr>
<td></td>
<td>Aggravate ischemia/reperfusion-induced myocardial, hippocampal and renal injuries (in mice/rats)</td>
<td>(100, 719, 731)</td>
</tr>
<tr>
<td></td>
<td>Accelerate diabetic renal injury</td>
<td>(147)</td>
</tr>
<tr>
<td></td>
<td>Impair islet function, pancreas integrity, and body glucose homeostasis by elevating islet superoxide, upregulating p53 phosphorylation and downregulating Foxa2/Pdx1 pathway</td>
<td>(684)</td>
</tr>
<tr>
<td></td>
<td>Increase susceptibility to ocular disorders such as cataract and progressive retinal cell loss</td>
<td>(247, 295, 500-503)</td>
</tr>
<tr>
<td>Immune response</td>
<td>Increase susceptibility to the experimental autoimmune encephalomyelitis</td>
<td>(436)</td>
</tr>
<tr>
<td></td>
<td>Cause anemia and autoantibody production by elevating oxidative stress in erythrocytes</td>
<td>(299)</td>
</tr>
<tr>
<td>Kidney</td>
<td>Exhibit an increase in phosphorylation of iron regulatory protein 1 (IRP1) in kidney, leading to increased binding to iron-responsive elements (IREs)</td>
<td>(734)</td>
</tr>
<tr>
<td>--------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td></td>
<td>Susceptible to hydronephrosis- and salt-induced hypertension and histopathological changes</td>
<td>(82)</td>
</tr>
<tr>
<td>Cancer</td>
<td>Show reduced lifespan and increased carcinogenesis in late life with oxidative damage-accelerated spontaneous mutations in liver and kidney</td>
<td>(73, 167)</td>
</tr>
<tr>
<td>Others</td>
<td>Lead to embryonic two-cell arrest or cell death, and impaired sperm motility and fertilizing ability</td>
<td>(207, 334, 658)</td>
</tr>
<tr>
<td></td>
<td>Induce age-related dysfunction of the lacrimal gland, potentiate hearing loss, cochlear pathology, bone stiffness/strength, skin morphology and wound healing</td>
<td>(301, 346, 446, 614)</td>
</tr>
</tbody>
</table>
Table 3. Physiological impacts and pathological responses of superoxide dismutase-2 overexpression and knockout in mice

<table>
<thead>
<tr>
<th>Organ/Condition</th>
<th>Phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Overexpression</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain and neurological system</td>
<td>Attenuate MPTP-induced phenotypes of Parkinson’s disease</td>
<td>(342)</td>
</tr>
<tr>
<td></td>
<td>Attenuate phenotypes of Alzheimer’s disease by reducing hippocampal oxidative stress, modulating Aβ deposition and composition, and slowing memory deficit</td>
<td>(164, 435)</td>
</tr>
<tr>
<td>Lung</td>
<td>Prevent hypoxia-mediated decrease in Na,K-ATPase and alveolar fluid reabsorption (adenovirus-mediated gene overexpression in rats)</td>
<td>(401)</td>
</tr>
<tr>
<td>Liver</td>
<td>Protect against liver mitochondrial DNA depletion and respiratory complex dysfunction after an alcohol binge</td>
<td>(433)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Ameliorate high-fat diet-induced insulin resistance in rat skeletal muscle (electroporation delivery of expression vector to rat muscle)</td>
<td>(50)</td>
</tr>
<tr>
<td></td>
<td>Prevent retinal VEGF expression and retinopathy in diabetic mice</td>
<td>(226, 351)</td>
</tr>
<tr>
<td></td>
<td>Normalize contractility in diabetic cardiomyocytes with improved mitochondrial respiration</td>
<td>(597)</td>
</tr>
<tr>
<td>Ischemic injury</td>
<td>Reduce ischemia/reperfusion-induced vascular endothelial cell death and protects against blood-brain barrier damage</td>
<td>(425)</td>
</tr>
<tr>
<td></td>
<td>Reduces neuronal vulnerability to forebrain ischemia (injection of astrocyte-specific expression vector to rat brain)</td>
<td>(718)</td>
</tr>
<tr>
<td></td>
<td>Protect against myocardial ischemia/reperfusion-induced injury</td>
<td>(107)</td>
</tr>
<tr>
<td>Aging</td>
<td>Preserve age-associated loss of mitochondrial DNA mass and function of ATP generation</td>
<td>(308, 374)</td>
</tr>
<tr>
<td><strong>Knockout</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain and neurological system</td>
<td>Show selective cerebral vascular dysfunction and accelerated disorganization of distal nerve axons following nerve injury</td>
<td>(179, 459)</td>
</tr>
<tr>
<td></td>
<td>Exacerbate phenotypes of Alzheimer disease, Parkinson’s disease and ALS</td>
<td>(11, 12, 385)</td>
</tr>
<tr>
<td></td>
<td>Exhibit neurodegenerative phenotypes including frequent, spontaneous motor seizures</td>
<td>(188, 394)</td>
</tr>
<tr>
<td>Liver</td>
<td>Exaggerate APAP-induced liver toxicity, mitochondrial dysfunction and DNA fragmentation</td>
<td>(200, 545)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Result in severe central nervous system degeneration and subsequent gait deformities, seizures, and perinatal lethality in type 2 diabetes</td>
<td>(497)</td>
</tr>
<tr>
<td>Heart</td>
<td>Induce cardiac mitochondrial dysfunction, severe lipid peroxidation and spontaneous apoptosis in myocardium, and maladaptive cardiac hypertrophy</td>
<td>(563, 621, 671)</td>
</tr>
<tr>
<td>Vascular system</td>
<td>Lead to increased vascular oxidative stress with aging and endothelial dysfunction in large and mesenteric arteries</td>
<td>(65, 138, 498, 720)</td>
</tr>
<tr>
<td></td>
<td>Up-regulate transferrin receptor and down-regulate mitochondrial biogenesis and metabolism in erythroid cells</td>
<td>(434)</td>
</tr>
<tr>
<td>Kidney</td>
<td>Develop hypertension, mild renal damage and interstitial inflammation in aged mice</td>
<td>(516, 567)</td>
</tr>
<tr>
<td>Cancer</td>
<td>Elevate incidence of neoplasms in aging Sod2+/Gpx1−/− mice</td>
<td>(750)</td>
</tr>
<tr>
<td>Ischemic injury</td>
<td>Increased susceptibility to cerebral ischemia/reperfusion with activation of MMPs, inflammation, blood-brain barrier breakdown and high brain hemorrhage rates</td>
<td>(425)</td>
</tr>
<tr>
<td>Aging</td>
<td>Lead to reduced lifespan and premature onset of aging-related phenotypes</td>
<td>(653, 675)</td>
</tr>
<tr>
<td>Others</td>
<td>Reduce contractile muscle function and aerobic exercise capacity during aging</td>
<td>(335, 417, 418)</td>
</tr>
<tr>
<td></td>
<td>Ocular pathology including progressive retinal thinning</td>
<td>(578)</td>
</tr>
<tr>
<td></td>
<td>A significant decrease in the respiratory capability and an increased rate of induction of the permeability transition in mitochondria</td>
<td>(697)</td>
</tr>
<tr>
<td>Organ/Condition</td>
<td>Phenotype</td>
<td>Reference</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>---------------------------------------------------------------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td><strong>Overexpression</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Brain and neurological system</strong></td>
<td>Protect against brain injury induced by subarachnoid hemorrhage, hyperoxia or cold</td>
<td>(447, 510, 739)</td>
</tr>
<tr>
<td></td>
<td>Improve behavioral outcome from closed head injury</td>
<td>(532)</td>
</tr>
<tr>
<td></td>
<td>Protect against aging-induced memory and cognitive impairments</td>
<td>(381, 382)</td>
</tr>
<tr>
<td>Vascular system</td>
<td>Reduce cuff-induced arterial neointimal formation (adenovirus-mediated gene expression in rat tissue)</td>
<td>(511)</td>
</tr>
<tr>
<td><strong>Lung</strong></td>
<td>Attenuate pulmonary oxygen toxicity by increasing cGMP activity and reducing of NFκB activation (aerosolized delivery of expression plasmid to neonatal rabbits) or by attenuating neutrophil inflammatory responses</td>
<td>(7, 189)</td>
</tr>
<tr>
<td></td>
<td>Preserve pulmonary angiogenesis by retaining VEGF, VEGFR1, VEGFR2 and PECAM-1</td>
<td>(528)</td>
</tr>
<tr>
<td></td>
<td>Inhibit the development of hypoxia- or fibrosis-induced pulmonary hypertension and vascular remodeling, and ameliorate established pulmonary hypertension</td>
<td>(8, 492, 672)</td>
</tr>
<tr>
<td></td>
<td>Attenuate radiation-, endotoxin (adenovirus-mediated expression)-, influenza- or air pollutant-induced lung injury</td>
<td>(212, 248, 318, 539, 625)</td>
</tr>
<tr>
<td>Immune response</td>
<td>Attenuate inflammatory arthritis by suppressing the production of proinflammatory cytokines and MMPs</td>
<td>(736)</td>
</tr>
<tr>
<td><strong>Ischemic injury</strong></td>
<td>Increased resistance to heart or cerebral ischemia/reperfusion injuries</td>
<td>(97, 98, 493, 598, 600)</td>
</tr>
<tr>
<td></td>
<td>Improve recovery from surgical hind-limb ischemia (adenovirus-mediated gene expression)</td>
<td>(579)</td>
</tr>
<tr>
<td><strong>Cancer</strong></td>
<td>Inhibit chemical-induced skin carcinogenesis</td>
<td>(332)</td>
</tr>
<tr>
<td><strong>Heart</strong></td>
<td>Exacerbate pressure overload-induced left ventricular hypertrophy and dysfunction</td>
<td>(414)</td>
</tr>
<tr>
<td><strong>Lung</strong></td>
<td>Increased susceptibility to hyperoxia</td>
<td>(81)</td>
</tr>
<tr>
<td><strong>Kidney</strong></td>
<td>Exhibit renal histopathological abnormalities, hypertension, endothelial dysfunction, and reduced eNOS and Akt activity</td>
<td>(326)</td>
</tr>
<tr>
<td>Immune response</td>
<td>Increased susceptibility to the collagen-induced arthritis</td>
<td>(570)</td>
</tr>
<tr>
<td><strong>Ischemic injury</strong></td>
<td>Worsen the outcome from cerebral or skeletal muscle ischemia/reperfusion</td>
<td>(519, 599)</td>
</tr>
<tr>
<td>Organ/Condition</td>
<td>Phenotype</td>
<td>Reference</td>
</tr>
<tr>
<td>-----------------</td>
<td>---------------------------------------------------------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Heart Overexpression</td>
<td>Preserve the responsiveness of the heart to adrenergic stimulation</td>
<td>(704)</td>
</tr>
<tr>
<td></td>
<td>Attenuate cardiac contractile dysfunction induced by paraquat, anthrax, LPS, acute ethanol, or hypoxia-reoxygenation, through alleviating events such as JNK-mediated ER stress</td>
<td>(105, 208, 317, 660, 748, 749)</td>
</tr>
<tr>
<td></td>
<td>Prevent progressive myocardial remodeling, including myocyte hypertrophy, apoptosis, and interstitial fibrosis due to overexpression of Gαq</td>
<td>(538)</td>
</tr>
<tr>
<td></td>
<td>Protect against acute and chronic doxorubicin-induced cardiotoxicity</td>
<td>(319, 320)</td>
</tr>
<tr>
<td>Vascular system</td>
<td>Prevent pathological mechanical changes underlying abdominal aortic aneurysm formation</td>
<td>(424)</td>
</tr>
<tr>
<td></td>
<td>Reduce pressure response to norepinephrine or angiotensin II by eliminating H$_2$O$_2$ in arterial wall</td>
<td>(723)</td>
</tr>
<tr>
<td></td>
<td>Inhibit toxin-accelerated atherosclerosis in the hypercholesterolemic ApoE/ mice</td>
<td>(722, 724)</td>
</tr>
<tr>
<td>Kidney</td>
<td>Inhibit the development of hypertension and renal injury in the angiotensinogen transgenic mice</td>
<td>(219)</td>
</tr>
<tr>
<td>Ischemic injury</td>
<td>Render the heart resistant to myocardial ischemia/reperfusion injury</td>
<td>(386)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Increase resistance to STZ-induced β-cell injury and diabetic effect including an attenuation of renal angiotensinogen and proapoptotic gene expression</td>
<td>(58, 99, 717)</td>
</tr>
<tr>
<td></td>
<td>Cardiac overexpression rescues insulin resistance-induced cardiac contractile dysfunction</td>
<td>(160)</td>
</tr>
<tr>
<td></td>
<td>Prevent hypertension and progression of nephropathy-induced cardiac contractile dysfunction and normalizing ACE-2 expression in type 1 diabetes</td>
<td>(603)</td>
</tr>
<tr>
<td>Aging</td>
<td>Mitochondrial overexpression prolongs lifespan with delayed age-associated pathologies including cardiac aging and cataract, decreases malignant nonhematopoietic tumor burden in old mice and enhances hippocampus-dependent memory with a reduction of anxiety</td>
<td>(137, 504, 584, 654)</td>
</tr>
<tr>
<td></td>
<td>Cardiac overexpression prolongs lifespan and attenuates aging-induced cardiomyocyte contractile dysfunction and protein carbonyl formation</td>
<td>(712)</td>
</tr>
<tr>
<td>Knockout</td>
<td>Show a decreased efficiency in brain mitochondrial respiration following cortical oxidative injury</td>
<td>(268)</td>
</tr>
<tr>
<td>Brain</td>
<td>Render remnant kidneys increased susceptibility to oxidant tissue injury and progressive renal fibrosis after nephrectomy</td>
<td>(344)</td>
</tr>
<tr>
<td>Kidney</td>
<td>Accelerate STZ-induced diabetic renal injury with increased expression of glomerular TGF-β and collagen α</td>
<td>(289)</td>
</tr>
<tr>
<td>Organ/Condition</td>
<td>Phenotype</td>
<td>Reference</td>
</tr>
<tr>
<td>-----------------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td><strong>Overexpression</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain and neurological system</td>
<td>Protect against 6-OHDA-induced neurotoxicity, trauma-induced mitochondrial dysfunction, cerebral/ischemia reperfusion and hypoxic ischemic injury</td>
<td>(42, 564, 595, 691, 716)</td>
</tr>
<tr>
<td>Heart</td>
<td>Resistant to ischemia/reperfusion injury and doxorubicin-induced cardiomyopathy and mitochondrial dysfunction</td>
<td>(714, 733)</td>
</tr>
<tr>
<td>Liver</td>
<td>Protect against paraquat-induced hepatotoxicity Enhanced susceptibility to acetaminophen toxicity</td>
<td>(111) (458)</td>
</tr>
<tr>
<td>Diabetes and metabolic disorders</td>
<td>Contribute to hyperinsulinemia in association with the transcription factor PDX1 and mitochondrial uncoupling protein 2 β-cell-specific overexpression of human GPX1 rescues β-cell dysfunction and reverses diabetes in 20-week-old db/db obesity mice Gpx1 overexpression mice are obese</td>
<td>(686) (229, 244) (445)</td>
</tr>
<tr>
<td><strong>Knockout</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain and neurological system</td>
<td>Exacerbate neuronal toxicity induced by Aβ, malonate, 3-nitropropionic acid, and 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine Resistant to kainic acid-induced seizure and neurodegeneration</td>
<td>(128, 341) (309)</td>
</tr>
<tr>
<td>Heart</td>
<td>Susceptible to ischemia/reperfusion injury in male mice Mutate the benign coxsackievirus B3 and induce myocarditis Susceptible to doxorubicin- and angiotensin II-induced aortic and cardiac dysfunction and oxidative stress Accelerated progression of atherosclerosis under a diabetic ApoE−/− background on a high fat diet (21% fat, 0.15% cholesterol)</td>
<td>(397, 732) (37) (15, 206) (384, 652)</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aging</td>
<td>No apparent phenotype except for cataract</td>
<td>(142, 265)</td>
</tr>
<tr>
<td>Carcinogenesis</td>
<td>Gpx1−/−Gpx2−/− mice have spontaneous polyps formation and inflammation-induced tumor formation in the gastrointestinal tract</td>
<td>(174)</td>
</tr>
</tbody>
</table>
Table 7. Physiological impacts and pathological responses of glutathione peroxidases 2-4 overexpression and knockout in mice

<table>
<thead>
<tr>
<th>Organ/Condition</th>
<th>Phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Over expression</td>
<td>Heart</td>
<td>Mitochondrial Gpx4 (rat) attenuates ischemia/reperfusion cardiac injury</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>Human GPX4 protects against diquat-induced apoptosis and oxidative stress</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased expression of human GPX3 in the plasma by 50% renders the mice resistant to APAP-induced hepatotoxicity and a thermosensitive phenotype</td>
</tr>
<tr>
<td>Knockout</td>
<td>Brain and neurological system</td>
<td><em>Gpx3</em>−/−: display cerebral infarctions</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Gpx4</em>−/−: accumulate oxidized lipids and senile plagues</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mitochondrial <em>Gpx4</em>−/−: apoptosis-induced cerebral degeneration in the hindbrain</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Gpx4</em>−/− (neuron-specific): neurodegeneration, corrected by α-tocopherol</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Gpx4</em>−/− (endothelium-specific): vitamin E-dependent suppression of angiogenesis in aortic explants</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nuclear <em>Gpx4</em>−/−: retardation in atrium formation</td>
</tr>
<tr>
<td></td>
<td>Carcinogenesis</td>
<td><em>Gpx2</em>−/−: severe inflammation and colon carcinoma induced by AOM/DSS; prone to UV-induced squamous cell tumor</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Gpx3</em>−/−: prone to colitis-associated carcinoma and increased inflammation in the colon</td>
</tr>
<tr>
<td></td>
<td>Aging</td>
<td><em>Gpx4</em>−/−: a slight lifespan extension (1029 vs 963 days)</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td><em>Gpx4</em>−/− (photoreceptor-specific): degeneration and apoptotic death of photoreceptor cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Gpx4</em>−/− (spermatocytes-specific): infertility; reduced forward mobility and mitochondrial membrane potential in the spermatozoa</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Mitochondrial Gpx4</em>−/−: infertility; impaired sperm quality and severe structural abnormalities in the midpiece of spermatozoa</td>
</tr>
<tr>
<td>Gene/Isoenzyme/Deletion</td>
<td>Organ (Genetic model)</td>
<td>Phenotype</td>
</tr>
<tr>
<td>------------------------</td>
<td>-----------------------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Txnrd1 (TrxR1, TR1)</strong></td>
<td>Ubiquitous deletion</td>
<td>Early embryonic lethality</td>
</tr>
<tr>
<td></td>
<td>Heart (MLC2a-driven knockout)</td>
<td>No apparent phenotype and no effect on infarct size after cardiac ischemic/reperfusion injury</td>
</tr>
<tr>
<td></td>
<td>Nervous system (Nestin-driven knockout)</td>
<td>Smaller mice with ataxia and tremor, cerebellar hypoplasia, ectopically located and abnormal Purkinje cells, disorganized Bergmann glial network.</td>
</tr>
<tr>
<td></td>
<td>Neurons (Ta1-driven knockout)</td>
<td>No apparent phenotype</td>
</tr>
<tr>
<td></td>
<td>Liver (Alb-driven knockout)</td>
<td>Strong upregulation of Nrf2-targeted genes, no apparent growth defect in non-treated liver but metabolic switch with accumulation of glycogen or lipids and significantly increased resistance to acetaminophen challenge</td>
</tr>
<tr>
<td></td>
<td>B-cell lymphoma (mb-1-driven knockout in λ-myc lymphoma model)</td>
<td>No effect on tumor growth except induction of an absolute requirement of the tumors on GSH upon Txnrd1 knockout</td>
</tr>
<tr>
<td></td>
<td>Hepatocellular carcinoma (induced by diethylnitrosamine in Alb-driven knockout)</td>
<td>Strongly increased propensity for hepatocarcinogenesis</td>
</tr>
<tr>
<td><strong>Txnrd2 (TrxR2, TR2)</strong></td>
<td>Ubiquitous</td>
<td>Early embryonic lethality with impaired heart development, anemia, lack of hematopoiesis and liver apoptosis</td>
</tr>
<tr>
<td></td>
<td>Nervous system (Nestin-driven knockout)</td>
<td>No apparent phenotype</td>
</tr>
<tr>
<td></td>
<td>Heart (MLC2a-driven knockout)</td>
<td>Congestive heart failure with signs of dilated cardiomyopathy, death within hours after birth</td>
</tr>
<tr>
<td></td>
<td>Heart (tamoxifen-induced α-myosin heavy chain-driven knockout)</td>
<td>Impaired cardiac function at rest and more severe injuries after cardiac ischemia/reperfusion, with NAC treatment normalizing the observed phenotypes</td>
</tr>
<tr>
<td></td>
<td>B- and T-cells (CD4- and CD19-driven knockouts)</td>
<td>No apparent phenotype</td>
</tr>
</tbody>
</table>
Table 9. Physiological impacts and pathological responses of overexpression, knockout, and transgene of other selenium-dependent proteins and Trsp in mice

<table>
<thead>
<tr>
<th>Models</th>
<th>Phenotypes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trsp transgene</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lacking the STAF-binding site</td>
<td>Tissue-specific decrease in selenoprotein expression (brain, muscle &gt; lung, spleen &gt; liver, kidney; no change in heart and testes)</td>
<td>(77)</td>
</tr>
<tr>
<td></td>
<td>Tissue- and selenoprotein-specific changes in selenoprotein expression (+GPX1, †TrxR1, liver &gt; testes); pyogranulomatous inflammation in various tissues</td>
<td>(470, 471)</td>
</tr>
<tr>
<td></td>
<td>mTOR-dependent increase of muscle growth after exercise</td>
<td>(279)</td>
</tr>
<tr>
<td></td>
<td>Severe neurological defects and mortality in these mice on a Se-deficient or high Se (2.25 ppm) diet</td>
<td>(324)</td>
</tr>
<tr>
<td></td>
<td>Increased susceptibility to azoxymethane-, diethylnitrosamine-, and C3(1)-induced carcinogenesis in intestines, liver, and prostate, respectively; no changes on TGFα-induced hepatocarcinogenesis</td>
<td>(159, 296, 324, 470)</td>
</tr>
<tr>
<td></td>
<td>Increased X-ray-induced micronuclei formation in the erythrocytes</td>
<td>(27)</td>
</tr>
<tr>
<td></td>
<td>Defective immune responses after the lungs were targeted with viral infection of influenza</td>
<td>(601)</td>
</tr>
<tr>
<td>Mutation at position 37 (A → G)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hematopoietic cells (Mxl-driven)</td>
<td>(327)</td>
</tr>
<tr>
<td></td>
<td>Prone to hemolytic anemia and defective oxidative homeostasis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Macrophage (LysM-driven)</td>
<td>(635)</td>
</tr>
<tr>
<td></td>
<td>Increased oxidative stress, induction of Nrf2 expression, defective immune response and expression of fibrosis-associated genes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T cells (Lck-driven)</td>
<td>(608)</td>
</tr>
<tr>
<td></td>
<td>Defective T cell maturation and antibody responses upon T cell receptor stimulation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neuron (Tal1-driven)</td>
<td>(588, 701, 702)</td>
</tr>
<tr>
<td></td>
<td>Defects in interneuron development and cerebellar hypoplasia, increased striatal neuronal loss with movement disorder, and seizure due to spontaneous epileptiform activity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Endothelial cells (Tie2E2-driven)</td>
<td>(609)</td>
</tr>
<tr>
<td></td>
<td>Embryonic lethal. 14.5 days embryos are smaller with underdeveloped vascular system, limbs, tail and head</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Osteochondroprogenitor (Col2a1-driven)</td>
<td>(161)</td>
</tr>
<tr>
<td></td>
<td>Growth retardation and delayed skeletal ossification reminiscent of Kashin-Beck disease</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Skin (K14-driven)</td>
<td>(592)</td>
</tr>
<tr>
<td></td>
<td>Small body size, alopecia, flaky and fragile skin, and early regression of hair follicles</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heart and skeletal muscle (MCK-driven)</td>
<td>(609)</td>
</tr>
<tr>
<td></td>
<td>Die 12 days after birth with acute myocardial failure</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mammary gland (MMTV- or Wap-driven)</td>
<td>(288)</td>
</tr>
<tr>
<td></td>
<td>Increased mammary carcinogenesis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Liver (Alb-driven)</td>
<td>(76, 587, 591)</td>
</tr>
<tr>
<td></td>
<td>Premature death at 1-3 months of age, no changes in brain Se levels, and increased apolipoprotein E and cholesterol levels in plasma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kidney (NPHS2-driven)</td>
<td>(48)</td>
</tr>
<tr>
<td></td>
<td>No effect on streptozotocin-induced diabetes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prostate epithelium (ARR2PB-driven)</td>
<td>(415)</td>
</tr>
<tr>
<td></td>
<td>Early onset of intraepithelial neoplasia</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Trsp conditional knockout</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mutant G37 transgene under global Trsp&lt;sup&gt;+&lt;/sup&gt;</td>
<td>(78)</td>
</tr>
<tr>
<td></td>
<td>Reduced fertility in males and litter size in females</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A34 or G37 transgene in liver-specific Trsp&lt;sup&gt;−&lt;/sup&gt;</td>
<td>(591)</td>
</tr>
<tr>
<td></td>
<td>Reversal of the elevated levels of apolipoprotein E and cholesterol in the plasma</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Sepp1</strong>&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>(259, 533, 583)</td>
</tr>
<tr>
<td></td>
<td>Neuronal degeneration, loss of 55% Se in the brain, degenerated and dystrophic axons in the cervical spinal cords and the brainstem</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Sepp1</strong>&lt;sup&gt;2240-361&lt;/sup&gt;</td>
<td>(258)</td>
</tr>
<tr>
<td></td>
<td>Decreased Se levels in the brain</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>MsrB1</strong>&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>(190, 371)</td>
</tr>
<tr>
<td></td>
<td>Oxidation of protein, lipid, and GSH in liver and kidney; actin fragmentation</td>
<td></td>
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</tbody>
</table>
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control oxidative phosphorylation in cardiac muscle by mediating deglutathionylation reactions.


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APAP Overdose

- Peroxynitrite
  - *Sod1*<sup>−/−</sup>
- Protein Nitration
  - *Sod1*<sup>−/−</sup>

CYP2E1

NAPQI

- GSH Depletion
  - *Sod1*<sup>−/−</sup>

Cell Death Signaling

- JNK↑
- Bcl-X<sub>L</sub>
- p21↓
- IkBε↓

GSH Depletion

- Caspase3
- PARP
- NFκB

Survival, %

Hours

- Wild-type
- *Sod1*<sup>−/−</sup>

600mg/kg APAP

F1
Cognitive Damage
Astrogliosis & Neuronal Death
NFκB
Sod1−/
Sod2+/−
[Sod2+/−]
[Sod3−/−]
Neurogenesis Damage
Differentiation Pattern Change
Cognitive Damage
Hippocampal Nitration
Astrogliosis & Neuronal Death
[Sod1−/−] [H₂O₂] NFκB
Brain Trauma
SOD1 Overexpression

Chronic Oxidative Stress ($H_2O_2 \uparrow$)

- Induce/lead to
- Promote/exacerbate

Kainic Acid Neurotoxicity
Sciatic Nerve Injury
Acoustic Nerve Aging
Down’s Syndrome
Muscular Dystrophy
Amyotrophic Lateral Sclerosis

Inflammation
Mitochondrial Damage
Axonal Degeneration

Ca$^{2+}$ Homeostasis
DNA Breakage
Neurotransmitter Uptake
Lipid Peroxidation
OH$^\bullet$

GSH
SOD1 Overexpression

F3
Prolonged Ethanol Intake

Liver of Sod2 Overexpressing Mice

Fe

Fe-Sod2 + H₂O₂

•OH

Fe-Sod2

O₂⁻

iNOS

NO

PGC-1

Lipid Peroxidation

mtDNA Lesion & Depletion

Respiratory Complex I Protein Carbonyls

↑ Increase
↓ Decrease
↓ Blunted induction
Cyclophosphamide

Nonobese Diabetic Mice

Streptozotocin

Non-Diabetic Mice

Cytokines

Insulin-Producing Cells

Male Spontaneous Diabetes

β-Cell Specific Cat Overexpression

Akt-Foxo1-Pdx1 survival pathway

Autoimmune Type 1 Diabetes

Diabetes

Cytotoxicity

CAT Overexpression in Cytoplasm

CAT Overexpression in Mitochondria

Weak
Liver IRβ↑

Embryonic Fibroblast
Pten Oxidation↑
P13K/Akt↑

Sod1−/−

Muscle pAkt^Thr308↑
pAkt^Ser473↑

Insulin Sensitivity↑

Muscle pAS160^Thr642↑

Gpx1−/−

High Fat Diet

Muscle pAkt^Thr308↓
pAkt^Ser473↓
Sod1\(^{-/-}\)

Promoter

Foxa2
mRNA → Protein

H\(_3\)K\(_4\) Trimethylation

Pdx1

ORF

DNA

Gpx1\(^{-/-}\)

β Cell Mass
Plasma Insulin
<table>
<thead>
<tr>
<th>Condition</th>
<th>Insult</th>
<th>Impact</th>
<th>Mechanism</th>
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<tbody>
<tr>
<td>Cell-Free System</td>
<td>Bovine GPX1 Protein Nitration</td>
<td>Protection</td>
<td>As PN Reductase</td>
</tr>
<tr>
<td>Primary Hepatocytes</td>
<td>PN DNA Fragmentation Caspase-3 Activation</td>
<td>Protection</td>
<td>GSH Sparing</td>
</tr>
<tr>
<td></td>
<td>Gpx1-/- Protein Nitration</td>
<td>Protection</td>
<td>Sod2 Induction?</td>
</tr>
<tr>
<td></td>
<td>SNAP +DQ Protein Nitration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mice</td>
<td>APAP Death</td>
<td>Partial Protection</td>
<td>GST Elevation?</td>
</tr>
<tr>
<td></td>
<td>GPX1 Overexpression Death</td>
<td>Potentiation</td>
<td>GSH Depletion</td>
</tr>
<tr>
<td></td>
<td>APAP Death</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Kainic Acid

NMDA Receptor Activation

Oxidative Stress (O₂⁺, NO, ONOO⁻)

Seizure
Neuronal Injury
Lethality

Ascorbate
GSH
EUK-134

Thiol Oxidation
(NMDA Receptor-1 Subunit)

[\text{H}_2\text{O}_2]\uparrow

Gpx₁⁻⁻
Gpx1 Overexpression

Type 2 Diabetes-Like Phenotypes

Mode of Actions
- Epigenetics
- Gene Expression
- Protein Modulation
- Enzyme Activity

Insulin Responsiveness
- Insulin Signaling
  (p-IRβ, p-Akt Thr308/Ser473)
- Lipogenesis
  (Hepatic Acc1, Fasn, Pparγ, p53)
- Glycolysis
  (Hepatic Gk1 and GK activity)
- Gluconeogenesis
  (Muscle PEPCK activity)

Insulin Production
- β Cell Mass
  (Beta2, Foxa2, Cfos, Pdx1, p53)
- Insulin Synthesis
  (Pdx1, Ins1)
- Insulin Secretion
  (Ucp2, Pregluc, Sur1, Kir6.2, Cat, ΔΨ)

Type 2 Diabetes-Like Phenotypes

[\text{H}_2\text{O}_2]
DMBA/TPA-Induced Skin Tumor

Pancreatic Tumor

Nrf2 and β-catenin → GPX2↑

Intestinal Carcinogenesis

Gpx2−/−

GPX1 Overexpression

AOM/DSS-Induced Colitis-Associated Carcinoma

Leukemia Stem Cell Self-Renewal

Gpx3 Targeting

Mice

Knockout

Cells

shRNA

Inhibition

Promotion
Inhibitors of TrxR1

Gene Targeting

Native TrxR1

Inhibitors of TrxR1

Drug Targeting of TrxR1

Genetic Loss of TrxR1

Beneficial Effects

• Activation of Nrf2, with increased resistance to oxidative stress
• Hampered carcinogenesis

• Promotes cell viability and proliferation through Trx system, support of Prxs, Msrs and RNR
• Redox regulation of normal cell function

• Activation of Nrf2 pathways
• Production of pro-oxidant NADPH oxidase activities, resulting in increased anticancer efficacy

Detrimental Effects

• Embryonically lethal
• Results in absolute dependence upon GSH

• Support of cancer cell growth and formation of metastases

• Increased dependence upon GSH
• Risk of NADPH oxidase-like toxicity in normal tissues?

Hepatocytes
• APAP (NAPQI)
• Cisplatin

Tumor Cells

Embryos

F12
Pro-oxidant Catalysis
Reducant Consumption
ROS/RNS Depletion
Substrate Specificity
Compensation
Overlapping Function
Non-redox Function

Antioxidant Enzymes

Substrates (ROS/RNS)

Paradoxical Outcomes

Detrimental

Beneficial

F13