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# Update on the oxidative stress theory of aging: Does oxidative stress play a role in aging or healthy aging?

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## Abstract

The oxidative stress theory of aging predicts that manipulations that alter oxidative stress/damage will alter aging. The gold standard for determining whether aging is altered is lifespan, i.e., does altering oxidative stress/damage change lifespan? Mice with genetic manipulations in the antioxidant defense system designed to directly address this prediction have, with few exceptions, shown no change in lifespan. However, when these transgenic/knockout mice are tested using models that develop various types of age-related pathology, they show alterations in progression and/or severity of pathology as predicted by the oxidative stress theory; increased oxidative stress accelerates pathology and reduced oxidative stress retards pathology. These contradictory observations might mean a) oxidative stress plays a very limited, if any, role in aging but a major role in healthspan; and/or b) the role that oxidative stress plays in aging depends on environment. In environments with minimal stress, as expected under optimal husbandry, oxidative damage plays little role in aging. However, under chronic stress, including pathological phenotypes that diminish optimal health, oxidative stress/damage plays a major role in aging. Under these conditions, enhanced antioxidant defenses exert an “anti-aging” action, leading to changes in lifespan, age-related pathology, and physiological function as predicted by the oxidative stress theory of aging.

### Keywords

oxidative stress; aging; disease; lifespan; healthspan

## Introduction

Through the years, hundreds (and perhaps more) of different hypotheses have been proposed as potential reasons why organisms age [1]. Most of these ideas have been shown to be outright wrong, others have lost favor due to lack of support, but a few have remained as potential,

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fruitful avenues of biogerontological research. One of the most studied and accepted hypotheses for the molecular basis of aging has been the oxidative stress theory of aging. For more than 50 years, numerous reports have examined the links between oxidative stress, longevity, and age-related disease. This theory was first conceptualized by Denham Harman as the free radical theory of aging and suggested that oxygen free radicals (specifically hydroxyl, $OH\cdot$, and hydroperoxyl, $HO_2\cdot$) formed endogenously as byproducts from normal oxygen-utilizing metabolic processes can play an essential role in the aging process [2]. Later, Harman added a slight modification to this theory to bring special attention to role of the mitochondria in the aging process because these organelles are a major site of reactive oxygen species (ROS) production [3]. Continuing to today, numerous studies have shown that oxidative damage increases with age in many organisms and that many different forms of ROS may be the culprits of accumulated oxidative damage (reviewed in [4]). Thus, Harman's original hypothesis has been refined in such a way to address the role of all forms of ROS in regulating aging and now is generally termed the oxidative stress theory of aging [5]. The basis of this theory is that a chronic state of oxidative stress exists in all cells of aerobic organisms even under normal physiological conditions because of an imbalance between prooxidants and antioxidants [5]. This imbalance leads to the accumulation of oxidative damage to cellular macromolecules that increases during aging and contributes to the progressive decline in the function of cellular processes. Under this mechanistic framework, the regulation of oxidative stress may directly control the aging process.

In its strictest interpretation, the oxidative stress theory of aging predicts that a reduction of oxidative stress, either by reducing the prooxidant load or increasing antioxidant defenses or some combination of both, should increase lifespan. Extending this notion, the oxidative stress theory of aging would predict that a long lifespan should be correlated with a reduction of oxidative stress. Previous support for the oxidative stress theory of aging has primarily focused on finding support for the latter prediction; that is, such studies have tested whether long-lived animals show reduced oxidative damage or increased oxidative stress resistance. In most mammalian models, lifespan can be extended through either means of experimental intervention, such as calorie restriction (CR), or through genetic mutation. For more than 80 years, CR, or the reduction of caloric intake without malnutrition, has been shown consistently to extend the lifespan of multiple and various animal models, including mice and rats [6]. Studies have found generally that the long lifespan associated with CR is associated with a reduction of oxidative damage/stress; most studies testing rodents that have undergone CR have shown reductions in levels of oxidized protein, lipid, and DNA, reduced rates of mitochondrial ROS production, and increased resistance to oxidative stress when compared to rodents fed ad libitum (reviewed in [4], [7–10]). Similarly, several genetic mutations shown to extend lifespan also have tended to show a reduction in oxidative damage/stress, though the results have been less consistent than those for CR. For example, Ames dwarf mice have a mutation in the *Prop-1* gene that leads to the defective development of the anterior pituitary and subsequent alteration of the hormonal milieu [11]. Ames dwarf mice also live ~40% longer than control mice [12] and show reduced protein oxidation, lipid peroxidation, and DNA oxidation, although these results vary among the tissues [13,14]. Ames dwarf mice, as well as the phenotypically similar Snell dwarf mice, also have been shown to be resistant to oxidative stress (i.e., lethality of paraquat, a generator of superoxide), and cells cultured from these mice are resistant to oxidative stress [15–19]. Snell dwarf mice are also resistant to the effects of 3-nitropropionic acid (3-NPA), which induces mitochondrial free radical generation in tissues, though sensitive to acetaminophen, the toxicity of which is thought due in part to oxidative stress [10,20]. Other less well-studied longevity mouse mutants commonly show a correlation between long life and reduced oxidative stress/damage or increased resistance to stress, including mice lacking the signaling protein $p66^{Shc}$ ($p66^{Shc-/-}$) [21–23], mice with a 50% reduction in the receptor for IGF-I ($Igf1R^{+/-}$) [24], and mice lacking the receptor for growth hormone ($GHR^{-/-}$) [16,25,26].

Much has been discovered about the role of oxidative stress in the mammalian aging process by studying how the aging rate of a single species like the mouse can be altered by CR or genetic mutation. However, the aging process itself seems to be remarkably similar across mammalian species even though the rate of aging differs vastly, from short-lived species like mice that may live only to 4 years of age, to humans that may live more than 100 years, or even to certain whale species that may live multiple centuries [1,27]. Thus, comparisons among species having different rates of aging might be used to clarify whether oxidative stress/damage is correlated with longevity throughout the animal species and therefore may be a determinant of species lifespan. In numerous previous studies, comparisons among species that exhibit differences in lifespan of two- to ten-fold have suggested that long-lived species tend to show reduced oxidative damage [28,29], reduced mitochondrial ROS production [30–34], increased antioxidant defenses [35–38], and increased resistance to oxidative stress both *in vivo* and *in vitro* [29,39–43]. However, there has been some ambiguity in the relation between lifespan and oxidative stress/damage; some studies have found a lack of correlation of oxidation with lifespan, or even an increase in oxidative damage/stress associated with long lifespan, for various reasons [33,44–46]. As such, the question of whether reduced oxidative stress is a common correlate among all long-lived species is still a matter of debate and warrants further investigation.

While the results described above suggest that reduced oxidative stress is a common correlate among different models of extended longevity, the complexity of these models can weaken their support for the oxidative stress theory of aging. Rodents undergoing CR, the genetic mutant mice described above, and indeed different long-lived species show many alterations among many different potential lifespan-extending pathways compared to their short-lived "controls"; these include differences in hormonal profiles, DNA repair mechanisms, and protein expression patterns, and in the case of comparative studies, differences in parameters like ecological niches or body sizes. Thus, it is difficult to interpret whether long life in these models is caused solely by their response to oxidative stress, or whether this is required at all. However, several sets of experiments have been designed to more directly address the particular question of whether an alteration in the oxidative stress response can alter lifespan. These approaches commonly utilize recombinant animal models to alter the activity of different components of the antioxidant defense system, either alone or in combination, with the goal of testing what role increasing or reducing oxidative stress has on lifespan.

Initial studies utilizing this approach were generally performed in invertebrate models for several reasons, including the relative ease of performing genetic manipulation and, perhaps more important, the short lifespan of these models. However, the results of lifespan studies from these invertebrate models have been at times confusing; in some models, the alteration of antioxidant function has a significant effect on lifespan, but for others, the interpretation of whether lifespan has been altered is more complex. For example, the chronological and/or clonal lifespan of yeast (*Saccharomyces cerevisiae*) seems to be diminished by ablation of the activity of various antioxidants including superoxide dismutases and methionine sulfoxide reductases [47–50]. Conversely, overexpression of these antioxidants seems to extend the lifespan of this model organism [48,50,51]. However, in the roundworm *Caenorhabditis elegans*, the absence of superoxide dismutase genes has shown contradictory results; *C. elegans* without superoxide dismutase show no effect on lifespan, or a surprising extension of lifespan, even while enhancing the sensitivity of worms to oxidative stress and increasing the accumulation of oxidized proteins [52–55]. Treatment of *C. elegans* with superoxide dismutase mimetics has been reported to extend their lifespan [56], but these experiments have yet to be successfully repeated in other laboratories [57,58]. In the fruit fly *Drosophila melanogaster*, the absence of CuZn superoxide dismutase diminishes lifespan by ~80% and increases sensitivity to oxidative stress, whereas flies with one-half the normal amount of this enzyme have a normal lifespan [59,60]. Flies lacking Mn superoxide dismutase do not develop to

adulthood, but those with only 50% reduction of this enzyme are slightly shorter-lived than control [61,62]. In *Drosophila*, overexpression of these enzymes, and other components of the antioxidant defense system such as catalase, thioredoxin, and methionine sulfoxide reductase, has either extended lifespan or had no effect depending on the particular technique utilized for generating the transgenic animals [63–71]. The technicalities of these particular experiments, and potential interpretations, are beyond the scope of this review, but have been discussed elsewhere in detail [4,72,73].

The work in invertebrate models has been, for the most part, extremely useful in attempting to address the role of oxidative stress in the aging process. For many reasons, however, it is not clear how much of this information may pertain to vertebrate models. For example, the evolutionary distance between invertebrates such as *S. cerevisiae*, *C. elegans*, and *Drosophila* and vertebrate models like the mouse, rat, and even humans is great and it is unclear to what extent mechanisms of aging (or of any particular physiological function) may be shared among these species. In addition, the aging process itself is complex and, in mammals, is a risk factor for the development of many different age-related diseases, some of which have no clear orthologues in invertebrate species; e.g., cancer, cardiovascular disease, and diabetes. Oxidative stress has been shown to be important in the development and progression of these and other diseases [74]. While the aging process directly affects the development of these diseases, it is likely that there also may be complex interplays between disease and aging that would be lost in invertebrate models. Thus, although there are valid reasons to study mechanisms of aging in invertebrate models, we may be able to develop a deeper understanding of the aging process in humans, including the associated development of age-related disease, by utilizing vertebrate models like the mouse and rat. To this end, we will devote the remainder of this review to current research directly testing the oxidative stress theory of aging in mice and whether the data support the oxidative stress theory of aging.

## Do alterations in the expression of antioxidant genes in laboratory mice regulate lifespan?

As argued above, the oxidative stress theory of aging, under its strictest interpretation, predicts that a reduction of oxidative stress, either by reducing the prooxidant load or increasing antioxidant defenses, or in some combination of both, should increase lifespan. Conversely, this theory also would predict that increased oxidative stress should result in a reduction in lifespan. While the first prediction may be relatively straightforward (i.e., reduced oxidation increases lifespan) and likely offers the greatest insight into mechanisms regulating aging, the second prediction can be more complex to interpret because conditions that lead to the reduction of lifespan may have little in common with the actual aging process [75]. However, the testing of models with increased oxidative damage is still important as these results may be taken as support against the oxidative stress theory of aging if these models prove to have no lifespan reduction compared to control.

In mammals, direct testing of the oxidative stress theory of aging has been undertaken primarily by utilizing mouse mutations generated to either lack or reduce particular antioxidants (“knockout”) or to overexpress particular antioxidants to some level greater than found in wild type animals (“transgenic”). While studies using invertebrate models have shown that altering antioxidant defense systems have yielded variable results on lifespan (discussed above), studies in mice have generally shown a relatively uniform pattern. Unfortunately, this pattern has not, so far, generated much support for the oxidative stress theory of aging being a likely molecular mechanism regulating longevity.

## Knockout models

**Superoxide dismutases—**CuZn superoxide dismutase (CuZnSOD; Sod1) is the major cytoplasmic superoxide scavenger and is also located in the mitochondrial intermembrane space [76,77]. Although mice lacking Sod1 (*Sod1*$^{-/-}$) are viable and grow relatively normally to adulthood, these mice exhibit signs of extremely elevated oxidative stress relative to wild type mice. Plasma $F_2$-isoprostanes, a measure of tissue-wide lipid peroxidation, are elevated two- to three-fold [78], and oxidative damage to DNA, protein carbonyls, and lipid peroxidation in tissues are all considerably higher than in control animals [79]. Interestingly, the average and maximum lifespan of *Sod1*$^{-/-}$ mice is approximately 30% shorter relative to that of control mice both in the inbred C57BL/6 genetic strain [79] and in a model of mixed genetic background [80]. While initially this might seem to be solid support for the oxidative stress theory of aging, interpretation of the lifespan of this genetic mutant must be approached with some caution. While some age-related pathologies are accelerated in these mice, they also exhibit some pathologies that are uncommon to mice; in particular, the major cause of death of *Sod1*$^{-/-}$ mice is hepatocellular carcinoma, which is not commonly found in C57BL/6 mice [79]. Thus, it might be argued [75] that these mice may not exhibit "accelerated aging" due to the lack of Sod1, but rather that the lack of Sod1 renders these mice particularly prone to non-aging related diseases or pathologies caused by oxidative stress. In mice with a 50% reduction in Sod1 (*Sod1*$^{+/-}$), no reduction in lifespan has been reported, though oxidative damage in these animals has not been investigated thoroughly [72,79].

Complementary to CuZnSOD, Mn superoxide dismutase (MnSOD; Sod2) is the main scavenger of superoxide in the mitochondrial matrix [76]. Unlike Sod1 knockout mice, mice with a complete knockout of Sod2 are not viable beyond the neonatal stage of development [81,82]. However, just prior to death these mice do show extreme increases in levels of oxidative stress [83] and are sensitive to hyperoxia [84] and ischemia/reperfusion [85]. Mice heterozygous for ablation of Sod2 (*Sod2*$^{+/-}$; i.e., a 50% reduction in Sod2) also have elevated markers of oxidative damage but are completely viable to adulthood. *Sod2*$^{+/-}$ mice show increased protein carbonylation (though no increase in oxidation of the amino acid, methionine), oxidation of both nuclear and mitochondrial DNA, reduced mitochondrial function in several tissues [86–90], and increased sensitivity to oxidative stress [88,89,91]. Surprisingly, the lifespan of *Sod2*$^{+/-}$ mice is no different from control mice, although the incidence of cancer with age is elevated relative to wild type mice [89]. One interpretation of these findings then would be that Sod2 is important for life (as complete ablation of Sod2 causes neonatal death), but that a reduction of Sod2, which increases oxidative damage, does not alter the fundamental aging process. However, these results also suggest that susceptibility to particular diseases (such as cancer) might be dependent on oxidative stress/damage.

Mice lacking the third primary superoxide dismutase, extracellular superoxide dismutase (ECSOD; Sod3), also have been generated and examined in terms of lifespan. ECSOD is the major SOD of extracellular fluids and interstitium, with the lung being the tissue having greatest expression [92]. Mice lacking ECSOD (*Sod3*$^{-/-}$) are viable, show modest increases in lipid peroxidation [80], and are sensitive to oxidative stress in the form of hyperoxia [93] and ischemia/reperfusion of multiple tissues [94,95]. These mice are generally healthy and show no reduction in lifespan relative to control animals [80], again suggesting that oxidative stress (from the lack of Sod3 in this model) alone does not regulate mammalian aging. However, there has been little characterization of the oxidative damage status of these mice.

**Glutathione peroxidases—**Glutathione peroxidases (Gpx) play an important role in the detoxification of peroxides to protect cells in nearly all mammalian tissues. Glutathione peroxidase 1 (Gpx1), the most abundant isoform of the mammalian Gpxs, is ubiquitously expressed, and is responsible for much of the detoxification of $H_2O_2$ within the cytoplasm

[96]. Despite the importance of Gpx1 in the cellular antioxidant defense system, mice lacking Gpx1 (*Gpx1*$^{-/-}$) are viable and have a relatively normal phenotype [97]. *Gpx1*$^{-/-}$ mice show elevated levels of lipid peroxidation markers in the vascular system [98], but not in plasma [99]. *Gpx1*$^{-/-}$ mice, and cells derived from these mice, are also exquisitely sensitive to several forms of oxidative stress including $H_2O_2$, paraquat, diquat, 3-NPA, malonate, and MPTP [91,100–104]; however, *Gpx1*$^{-/-}$ mice show no increased susceptibility to hyperoxia [97]. Despite the importance of Gpx1 to peroxide detoxification, and to oxidative stress resistance, *Gpx1*$^{-/-}$ mice show no reduction in lifespan relative to control mice [72,105] nor do they show any difference from control animals in cause of death, i.e., *Gpx1*$^{-/-}$ mice die from the same diseases at the same rate as control mice [105].

Glutathione peroxidase 4 (Gpx4) differs from Gpx1 in its specificity for detoxification of lipid peroxides, including phospholipid hydroperoxides and hydroperoxides of cholesterol esters [106,107]. Gpx4 is ubiquitiously expressed at low levels (compared to Gpx1), yet unlike Gpx1, Gpx4 is required for embryonic development as mice lacking Gpx4 do not develop beyond stage E7.5 [108,109]. However, mice with a 50% reduction in Gpx4 (*Gpx4*$^{+/-}$) are viable to adulthood, and show reduced expression of this protein in all tissues [109]. Cell lines derived from *Gpx4*$^{+/-}$ mice are sensitive to multiple forms of oxidative stress [109,110] as are the mice themselves [109]. In addition, cell lines from *Gpx4*$^{+/-}$ mice show elevated levels of oxidized lipids and oxidized DNA [109], and the mice themselves have elevated levels of the lipid oxidation marker $F_4$-neuroprostane in the brain (though no difference in DNA oxidation in the liver nor in $F_2$-isoprostanes in the plasma) [111]. Surprisingly (based on the prediction of the oxidative stress theory of aging), *Gpx4*$^{+/-}$ mice show a slight, statistically significant extension of median lifespan (~7%) accompanied by a slight delay in the incidence of cancer [111]. However, these mice show no differences from control mice in median or maximum lifespan, suggesting that the lack of Gpx4 is not detrimental to aging and in fact may be beneficial in the case of certain diseases (i.e., delayed cancer onset).

**Methionine sulfoxide reductases—**Methionine sulfoxide reductase A (MsrA) is not an antioxidant per se, but functions in the antioxidant defense system by reducing methionine sulfoxide (Met-O) residues to unoxidized methionine (Met), thereby protecting proteins specifically from oxidative stress [90,112]. It also has been proposed that MsrA, in addition to repairing oxidized residues, may act to regulate overall antioxidant activity by reducing the highly oxidation prone methionine residues, thereby protecting other macromolecules from oxidation and, in effect, acting as a “free radical sink” [113]. Mice lacking MsrA (*MsrA*$^{-/-}$) are viable and grow normally to adulthood [114]. Relative to control mice, *MsrA*$^{-/-}$ mice show increased levels of protein oxidation both in protein carbonyls in kidney (though not in brain, lung, or liver) and of Met-O in the plasma [114,115]. *MsrA*$^{-/-}$ mice are also sensitive to oxidative stress induced either by hyperoxia [114] or by injection with paraquat [116], and cells from *MsrA*$^{-/-}$ mice are sensitive to several different oxidizing agents including paraquat, $H_2O_2$, tert-butyl hydroperoxide, and hypochlorite [116]. These data support the hypothesis that MsrA may act as a more general “sink” for free radicals and protect cells from oxidative stress. Initially, it was reported that *MsrA*$^{-/-}$ mice were short-lived with a lifespan ~40% shorter than control mice [114]. However, our group repeated the lifespan study with *MsrA*$^{-/-}$ mice and found no difference in lifespan between mice lacking MsrA and control [116]. Several possibilities might exist for the discrepancies between the two studies (i.e., small numbers of animals in the original study, potential maternal effects), but differences in animal husbandry appear to be the major reason for the discrepancy in lifespan. In the original study by Moskovitz, the mean lifespan of control animals was relatively short (approximately 1/3 shorter than observed in our study), which might suggest that the animals were under some form of chronic stress or disease, again questioning whether the short lifespan in that original study really represented “accelerated aging” [75]. The disparate findings in these two studies highlight the importance of replicating lifespan studies under optimal husbandry conditions. Based on our

lifespan data, it appears that the lack of MsrA, like the other antioxidants described above, seems to have little effect on mouse lifespan, suggesting further that the oxidative stress theory of aging may have little to do with regulating mammalian aging under optimal husbandry conditions. Interestingly, mice lacking an isoform of another methionine sulfoxide reductase, MsrB1, are also viable, show increased levels of oxidative damage, and have a lifespan that does not differ from control mice through >20 months of age [117].

**Thioredoxin 2—**Thioredoxins (Txn) catalyze disulfide bond reductions in multiple substrate proteins and can perform as antioxidants by detoxifying peroxides through peroxiredoxins and by reducing protein disulfides and methionine sulfoxides, either directly or through the actions of other oxidoreductases [118]. Thioredoxin 2 (Trx2) is the mitochondrial form of thioredoxin, and complete ablation of this enzyme is detrimental to development; Trx2 null mouse embryos do not progress beyond stage E8.5 due to massive cellular apoptosis [119]. However, *Trx2*$^{+/-}$ mice are viable and show ~50% reduction of Trx2 expression in all tissues studied [120]. These mice show evidence of heightened oxidative stress, elevated mitochondrial $H_2O_2$ release, and increased levels of oxidized lipids, proteins, and nucleic acids [120]. In addition, these mice are sensitive to oxidative stress in the form of paraquat [120]. The difference in lifespan between *Trx2*$^{+/-}$ mice and control mice is not statistically significant, though the mean and maximum lifespan of *Trx2*$^{+/-}$ mice is slightly lower than that of control mice (7% and 16% respectively) [72]. The lack of a significant difference might be due to a relatively small sample size, but a cautious interpretation is that a reduction of Trx2 does not diminish mouse lifespan.

**Knockouts of multiple antioxidants—**The antioxidant defense system developed in higher eukaryotes is complex and compromised of many different antioxidants and oxidation repair systems. Because of the many backup and redundant antioxidant systems, it might be argued that alterations in just a single constituent may have little effect on lifespan [4,89]. Our laboratory group has approached this potential issue by generating mice with reductions in multiple antioxidants through targeted breeding. Mice generated to lack Sod1 and either completely lack a second antioxidant (Gpx1; *Sod1*$^{-/-}$/*Gpx1*$^{-/-}$) or have a 50% reduction in a second antioxidant (Sod2; *Sod1*$^{-/-}$/*Sod2*$^{+/-}$, Gpx4; *Sod1*$^{-/-}$/*Gpx4*$^{+/-}$) show no further reduction in lifespan beyond that of *Sod1*$^{-/-}$ mice alone [72]. Data from other laboratories show that mice lacking both Sod1 and Sod3 (*Sod1*$^{-/-}$/*Sod3*$^{-/-}$) are also no shorter lived than the *Sod1*$^{-/-}$ mice alone, even though levels of the oxidative damage marker, isoprostane 8-iso-prostaglandin $F_{2a}$ were further elevated in the double knockout mice [80]. Our group also generated mice with a 50% reduction in Sod2 and either a complete loss of Gpx1 (*Sod2*$^{+/-}$/*Gpx1*$^{-/-}$) or 50% reduction in Gpx4 (*Sod2*$^{+/-}$/*Gpx4*$^{+/-}$); both sets of mice show no alteration of lifespan from control mice [72,105]. In *Sod2*$^{+/-}$/*Gpx1*$^{-/-}$ mice, DNA oxidation and protein carbonyl levels are significantly increased relative to control mice [105], and these mice (and cells from these mice) are even more sensitive to oxidative stress than are control, *Sod2*$^{+/-}$ or *Gpx1*$^{-/-}$ mice [91]. Somewhat surprisingly based their normal lifespan, cancer burden is increased in *Sod2*$^{+/-}$/*Gpx1*$^{-/-}$ mice [105]. Lastly, mice lacking Gpx1 and with a 50% reduction in Gpx4 (*Gpx1*$^{-/-}$/*Gpx4*$^{+/-}$) show no difference from control mice in mean, median, or maximum lifespan [72]. Together, these data suggest that even the loss of these antioxidants, even in combination, does not negatively affect murine lifespan except in the case of Sod1 (with the caveat regarding lifespan vs. pathology discussed above).

Taken as a whole, the data from the knockout mice described above suggest that a reduction in one or more of the key antioxidants 1) generally increases the accumulation of several forms of oxidative damage; 2) generally renders these mice, or cells from these mice, sensitive to various forms of oxidative stress; and 3) generally has little effect on lifespan or the aging process, except potentially in the case of Sod1. Thus, the data from a large number of antioxidant knockout mouse models do not generally support the oxidative stress theory of

aging; i.e., reducing antioxidant defenses, which increases oxidative stress, does not negatively affect lifespan.

## Transgenic models

As mentioned above, the prediction that reduced oxidative stress should extend lifespan should be relatively straightforward. A number of transgenic mice that overexpress different antioxidants have been generated to test this hypothesis.

**Superoxide dismutases—**Lifespan studies have been performed on two separate groups of mice overexpressing CuZnSOD (Sod1 TG). The initial study generated mice that ubiquitously overexpressed human Sod1 approximately two- to five-fold depending on the tissue tested [121]; these mice were generated in the CD1 background strain. Epstein's group reported that mice either hemi- or homozygous for Sod1 TG show no difference in lifespan from control mice [121]. Our own group generated a similar Sod1 TG mutant in the C57BL/6 background strain; these mice also ubiquitously express human Sod1 at levels two to five times that of the expression of the exogenous mouse Sod1 [122]. Cell lines derived from embryonic Sod1 TG mice are resistant to agents like paraquat, which generate superoxide but not to peroxides, demonstrating an increase in superoxide-specific activity [123,124]. Sod1 TG mice also tend to be more resistant to oxidative stress induced by paraquat, and show reduced levels of isoprostane accumulation following treatment with diquat [72]. However, the Sod1 TG mice generated by our group, like the initial report on Sod1 TG mice, also showed no extension of lifespan [72,125]. These findings suggest that protection from cytosolic superoxide by overexpression of Sod1 does not alter lifespan despite reducing oxidative stress. It must be mentioned here that overexpression, generally extremely high overexpression, of some antioxidant genes (such as Sod1 or Sod2) can actually be detrimental in some physiological aspects [126,127]; therefore, it might be argued that even two- to five-fold overexpression, as utilized in the above studies, may not be ideal for lifespan extension.

At least two laboratory groups have generated transgenic mice overexpressing Sod2 (Sod2 TG). Epstein's group generated Sod2 TG mice using a genomic fragment of the endogenous mouse Sod2 gene; these Sod2 TG mice show an approximately two-fold overexpression of Sod2 in all tissues examined [127]. Our group has shown that these mice have reduced accumulation of protein and lipid oxidation with age, and that they have an increased resistance to oxidative stress (paraquat) as do cells derived from these animals [128,129]. However, we also found that Sod2 TG mice live no longer than control and that they show no major alterations in cause of death [128]. Ho et al. generated a second strain of Sod2 TG mice using a transgene with the Sod2 cDNA fused to β-actin promoter [130]. These mice express Sod2 at two- to four-fold higher levels than exogenous Sod2 and show reduced mitochondrial superoxide production in the brain and protection from hyperoxia [130]. These Sod2 TG mice were found to show a 4% increase in mean and 18% increase in maximum lifespan [131]; however, no formal statistical analysis of lifespan was presented in this report, which brings into question whether a statistically significant difference in lifespan actually exists [132–134]. The potential difference in lifespan might also be attributed to different genetic background strains used in the two studies (C57BL/6 [128] vs. B6C3 [130]). Certainly these differences warrant further exploration, but neither study as published provides strong evidence that protection against superoxide in the mitochondrial compartment by Sod2 can positively affect lifespan.

**Catalase—**Catalase is a ubiquitously expressed antioxidant enzyme primarily located in the peroxisomes; this enzyme catalyzes the decomposition of hydrogen peroxide to oxygen and water [96]. Two groups have generated mice that overexpress catalase to assess the effect of this enzyme on lifespan. Rabinovitch's group generated three lines of mice that overexpress

human catalase in peroxisomes, in the nucleus, or in mitochondria [135]. The lines overexpressing catalase in the peroxisomes (PCAT) or nucleus (NCAT) showed slight increases (~10%) in median lifespan that did not reach statistical significance, and showed no change in maximum lifespan. However, the line overexpressing catalase in the mitochondria (MCAT) showed a significant extension of median and maximum lifespan (~20%). MCAT mice show catalase activity levels 50-fold higher than control mice in the mitochondrial fractions isolated from cardiac tissue. MCAT mice also show reduction in the age-related increase in oxidation of nucleic acids, reduced $H_2O_2$ production from heart mitochondria, and reduced $H_2O_2$-induced aconitase inactivation [135]. Our group also generated catalase transgenic mice (Cat TG) overexpressing the human catalase gene two- to four-fold higher in its normal cellular location, the peroxisome. Cat TG-derived embryonic fibroblasts are resistant to hydrogen peroxide toxicity, though not superoxide toxicity, demonstrating an increase in catalase-specific activity [123]. Cat TG mice show reduced DNA oxidation in all the tissues studied compared to control mice [72]; however, we found that Cat TG mice show no alteration of lifespan compared to control mice [72,125]. It is unclear why MCAT mice may be long-lived, while Cat TG mice show no difference in lifespan, but it must be mentioned that the normal cellular location of catalase is within the peroxisomes. It may be that protection from mitochondria-originated oxidative stress may be more important for lifespan than the cytosolic oxidative stress protection provided by peroxisomal catalase. The MCAT data do support the oxidative stress theory of aging, but perhaps more strongly support the hypothesis that altering the reduction of $H_2O_2$ in the mitochondria might affect multiple signaling pathways, or metabolic processes, and that these changes may be most responsible for the increase in lifespan.

**Glutathione peroxidase 4—**Our group also generated mice that overexpress human Gpx4 in all tissues at levels three- to five-fold greater than endogenous Gpx4 expression (Gpx4 TG). These mice show reduced lipid peroxidation and reduced liver cell apoptosis following diquat injection relative to that of control animals [136]. Further, embryonic fibroblast cell lines from Gpx4 TG mice are resistant to several forms of oxidative stress as are neuronal cell lines derived these animals [136,137]. However, Gpx4 TG mice are not long-lived relative to control mice, suggesting again that Gpx4 has little effect on aging rate in mice [72].

**Thioredoxin 1—**Thioredoxin 1 (Trx1) is the cytosolic form of thioredoxin; mice generated to overexpress human Trx1 (Trx1 TG) three- to six-fold (depending on the tissue) are resistant to UV-induced oxidative stress and to oxidative stress induced by ischemia/reperfusion [138, 139]. In addition, these mice are relatively long-lived; Trx1 TG mice show greater mean (35%) and maximum (22%) lifespan relative to control mice [138]. However, in the original longevity study by Yodoi's group, the mean lifespan of both the transgenic (~22 months) and control mice (~15 months) was relatively short compared to that which is typically found for laboratory stocks of the same genetic background (C57BL/6). These mice were bred in a specific-pathogen free environment, and no data were provided on causes of death, making it difficult to explain why control mice in this experiment are so much shorter lived than in other laboratories [72, 89,111,116]. Without understanding the cause of the reduced lifespan for control mice, it is difficult to interpret these data as strong support for the oxidative stress theory of aging.

**Transgenics expressing multiple antioxidants—**As mentioned in the section on knockout animals, it might be somewhat naïve to think that alteration of a single antioxidant might alter to any significant degree something as a complex as the aging process. To test what effect overexpression of multiple antioxidants has on lifespan, several different double transgenic animals (i.e., mice that overexpress two different antioxidants) have been generated. Mice overexpressing catalase specifically in the peroxisome (PCAT) and also Sod1 were reported to show a significant extension of median lifespan compared to both control (18.5%)

and PCAT (7%) mice; however, there was no effect on maximum lifespan in these mice [135]. Our group found no lifespan extension in mice that overexpress both catalase and Sod1 (Cat TG/Sod1 TG) [72,125], although cells from these mice are resistant to cell death caused by either superoxide or $H_2O_2$ [123,140]. We also found no alteration of lifespan in mice that overexpress both Sod1 and Sod2 (Sod1 TG/Sod2 TG), though cells from these mice are more resistant to superoxide toxicity than cells of either Sod1 TG mice or Sod2 TG mice [72].

The results from the transgenic mice studies suggest that overexpression of antioxidants in murine models can 1) diminish the accumulation of several forms of oxidative damage; 2) render the mice, and cells derived from the mice, resistant to various forms of oxidative stress; but 3) with few exceptions, have little effect on the lifespan of these mice. Together with the data from knockout mice, these findings suggest that alteration of oxidative defense systems, alteration of oxidative damage accumulation, and alteration of oxidative stress resistance have little, if any, effect on the regulation of mice lifespan when under optimal husbandry conditions in the laboratory environment.

## Do alterations in the expression of antioxidant genes in laboratory mice regulate age-related physiological and functional decline?

For years, longevity or lifespan has been the parameter by which researchers have determined whether a treatment or gene mutation has a significant effect on aging. However, aging itself is a constant, progressive process wherein healthy, young adult organisms become frail, old organisms with a greater susceptibility to illness, injury, and death [141]. This process affects multiple organs, tissues, and cell types and involves nearly every biochemical and physiological function of the body that has been measured [1]. In mammalian models of extended lifespan, generally, the onset and progression of most age-related diseases is delayed or reduced and the age-related decline in physiological functions is retarded. For example, most physiological, biochemical and pathological changes with age are delayed or reduced in CR rodents compared to ad libitum fed controls (reviewed in [6]). Age-related development of insulin resistance [142], declines in immune function [143,144], and alterations in neural and behavioral parameters [145,146] are all reduced by CR, and the onset and incidence of age-associated diseases including neoplasia and nephropathies are delayed [147–149], among many other examples. Additionally, the long-lived Ames/Snell dwarf mutations show reduced occurrence or severity of age-related pathologies such as collagen aging [150], osteoarthritis [151], the development of cataracts [152], glomerular nephrosis [152], age-related cognitive decline [153,154], and the slowed progression of multiple forms of spontaneous neoplasia [152,155]. Such measures of healthy aging, i.e., periods of good health, free of disease and pathology within a lifespan may be useful in determining the effects of “anti-aging” treatments on particular tissues or organ systems. Thus, there is a growing emphasis to monitor healthspan in addition to lifespan in determining the effect of a manipulation on aging.

The strongest interpretation of the oxidative stress theory of aging predicts that longevity is influenced by oxidative damage. However, oxidative stress is known to be critically important to many pathologies, including the development of many age-related diseases like various forms of neoplasia, cardiovascular disease in its many forms, and insulin resistance and diabetes [74]. The data in the previous section showed little support for oxidative stress in the regulation of aging as measured by mean or maximum lifespan; but, as will be discussed below, there are some clear trends for alteration of aging in the antioxidant knockout/transgenic models when changes in biomarkers of health, or possible disease, or physiological function are examined. As such, there may be dissociation between aging (measured by longevity) and healthy aging (as measured by healthspan) in these models.

As discussed above, very few of the antioxidant mouse models show any decrease in lifespan compared to control animals, and those that do (i.e., *Sod1*$^{-/-}$, *MsrA*$^{-/-}$ mice in one study) may develop serious diseases, calling into question whether the reduction in lifespan is related to the aging process. However, many of these models have been reported to show accelerated development of some age-related disease or accelerated decline of physiological function. For example, *Sod1*$^{-/-}$ mice show an acceleration of age-related hearing loss [156,157] and higher incidence of both macular degeneration [158] and cataract formation [159]. In addition, these mice show rapid declines in fertility [160], increased vascular hypertrophy and decreased endothelial relaxation [161], and acceleration of age-related skeletal muscle atrophy and motorneuron deficiencies [78,162]. Surprisingly, *Sod2*$^{+/-}$ mice seem to show no significant difference from control mice in several biomarkers for advancing age including skin collagen aging, cataract formation, and immune cell proliferation; however, these mice do tend to have increased neoplasia burden [89]. In addition, *Sod2*$^{+/-}$ mice seem to develop insulin resistance with age relative to control mice [163]. Mice lacking Gpx1 develop early cataract formation [164,165], impaired endothelium-dependent vasodilator function [98] and a reduced ability to undergo cellular proliferation and differentiation [102,166]. These mice also show reduced liver mitochondria function relative to control mice [167]. Unlike the findings with *Gpx1*$^{-/-}$ mice, no difference was found between *Gpx4*$^{+/-}$ mice and control in the age-related incidence of cataracts [111]. While adult *MsrA*$^{-/-}$ mice generally appear quite normal, some groups have described neuromuscular ataxia with age [114], accelerated age-related hippocampal degeneration [168], and accelerated cardiac dysfunction [169,170]. Within our group, however, we have found no gross abnormalities, particularly with behavior, in these mice even in old age [116]. Of note, little has been reported on the overall health (and decline of health with age) of mice that overexpress specific antioxidants. It has been reported that MCAT mice show a significant delay in the age-related development of cataracts [135], diminished age-related hearing loss [171] and a delay in the progression of cardiac aging and reduced cardiac lesions [172,173] and tend to show reduced malignant non-hematopoietic tumor burden and reduced systemic inflammation [173].

Overall, the current data (summarized in Table 1) suggest that age-related diseases and declines in physiological function are altered when the expression of antioxidant genes are manipulated. Again, although it may be difficult to ascribe negative physiological effects in knockout mice solely to increases in oxidative stress, the data from MCAT mice suggest that overexpression of an antioxidant, and the concurrent protection against oxidative stress, can be preventative for some physiological declines associated with aging. It is important to remember that these differences in physiological functions have been measured in the same mice that show no, or little, difference in lifespan from controls. These tantalizing results certainly warrant further exploration, particularly into the design of accurate measures of murine health and further clarification of the health status of all antioxidant models. Overall though, these data may support the idea that healthspan can be regulated by oxidative stress independently of lifespan; this may be particularly evident in the mouse models under optimal husbandry conditions where lifespan may be greatest due to the minimization of negative extrinsic factors. As such, these findings might suggest that the reduction of oxidative stress/damage in mammals might then be key to increasing the healthy period of life, a time free of disease and pathology.

## Do alterations in the expression of antioxidant genes in laboratory mice regulate susceptibility to age-related disease models?

It is clear that we currently have a limited knowledge base on how, and whether, oxidative stress can control the general decline in normal physiological functions with age. One major problem with this approach is that inbred strains of laboratory mice generally succumb to very particular, often strain-specific set of diseases and pathologies. The data from these strains of mice then can be limited, and differences in health parameters between antioxidant mutant and

control mice may be skewed towards particular disease states. Another way to approach this question is to test how oxidative stress can modulate the progression of various age-related diseases using mouse models of specific diseases. Several different models have been utilized in attempt to mimic some forms of human-like diseases in rodents, or to widen the spectrum of diseases from which common laboratory mice die. One of the first steps in addressing these questions might then be to look at the effect of particular antioxidants (using the mouse models described above) within the parameters of these disease models. In such an experimental design, it may be possible to determine the effect of an antioxidant on general health, or healthspan, while under the challenge of a chronic disease state.

For example, the leading cause of death for people living in the United States is cardiovascular disease, and the mortality risk of this disease increases dramatically with age, i.e., the old are much more likely to both develop and die from cardiovascular disease than are the young. However, cardiac decline is difficult to measure in most mouse strains [174], and atherosclerosis is generally not a normal phenotype of mice without utilizing an experimental intervention [175]. By using models of cardiovascular disease, the effect of particular antioxidants on health and lifespan in such conditions can be easily tested. For example, mice lacking apolipoprotein E (*ApoE*$^{-/-}$) have decreased serum apolipoprotein E and exhibit lipid abnormalities and atherosclerosis even on a low-cholesterol diet. Mice lacking ApoE and also deficient in Sod2 (*ApoE*$^{-/-}$/*Sod2*$^{+/-}$) show an exacerbation of endothelial dysfunction relative to *ApoE*$^{-/-}$ mice, suggesting mitochondria-derived ROS may be important for the progression of this aspect of the disease [176]. However, the lack of Sod3 is shown to have little effect on the pathology of *ApoE*$^{-/-}$ mice, suggesting that extracellular SOD may have little effect on certain aspects of atherosclerosis [177]. Mice lacking Gpx1 (*ApoE*$^{-/-}$/*Gpx1*$^{-/-}$) are also prone to atherosclerosis in the ApoE model [178], but not in a model of atherosclerosis that is dependent on a high fat/high cholesterol diet [179]. Overexpression of Sod1 (*ApoE*$^{-/-}$/Sod1 TG) seems to have little effect in the ApoE model, but overexpression of catalase, or of catalase and Sod1 (*ApoE*$^{-/-}$/Cat TG; *ApoE*$^{-/-}$/Cat TG/Sod1 TG) seems to be protective [180]. Overexpression of Gpx4 (*ApoE*$^{-/-}$/Gpx4 TG) also appears to reduce the number and size of atherosclerotic lesions in ApoE mice, as well as reduce vascular oxidative stress [181]. Other models of cardiovascular disease also have been shown to be responsive to alterations of oxidative stress. For example, lack of Sod3 exacerbates hypertension in a kidney clip model [182] and heart damage in an aortic constriction model of cardiac hypertrophy [183]; and vessels from *Sod2*$^{+/-}$ mice and *Gpx1*$^{-/-}$ mice are sensitive to low density lipoprotein (LDL) oxidation whereas those from Sod1 TG and Cat TG mice are resistant [184]. Additionally, *Gpx1*$^{-/-}$ mice develop significantly worsened myocarditis than control animals in a viral infection model [185]. Overall, these data make the overwhelming case that genetic alterations to antioxidant genes resulting in alterations to oxidative stress/damage play an important role in many aspects of cardiovascular disease. Further, there is a clear differentiation in the health of antioxidant knockout and transgenic mice when challenged in these models. Importantly, these studies were generally performed using the same antioxidant mouse models that have no significant alteration of lifespan under optimal husbandry conditions. These results suggest that while oxidative stress may not limit lifespan under optimum laboratory conditions, it may be a critical determinant of the development of one of the most common age-related mortality risks in human and thus, be a determinant of lifespan under conditions that promote cardiac decline.

Another disease of growing significance to humans is type 2 diabetes mellitus, the most common form of diabetes. In the United States alone, more than 20 million people are currently diagnosed with diabetes, with almost 60 million more diagnosed with pre-diabetes conditions. The prevalence of type 2 diabetes is age-dependent, as more than 20% of people older than 60 years of age have diabetes compared to only about 8% of the population as a whole. Complications from diabetes include cardiovascular disease, blindness, nerve damage, and

kidney damage and nephropathy; thus, the increasing incidence of diabetes in the population is a significant health concern [186]. Again, most of the commonly used laboratory mouse strains do not exhibit type 2 diabetes without utilizing a dietary or genetic intervention, but several models have been developed to test different aspects of diabetes and diabetic complications [187]. In many different models, it is clear that oxidative stress plays a key role in the development of both type 2 diabetes and many of the complications associated with the disease. For example, *Sod1*$^{-/-}$ mice show increased retinal damage in two different diabetic models [188], increased diabetes-induced cataract formation [189], and increased diabetes-induced nephropathy [190]. Conversely, Sod1 TG mice show protection against diabetes-induced retinopathy [188,191]. Mice overexpressing catalase specifically in the kidney are protected from the renal disease associated with diabetes [192]. Similarly, overexpression of Sod2 specifically in the heart protects against diabetes-induced left ventricular hypertrophy [193]. Increasingly, diets high in fat content are being used as an experimental paradigm for westernized human diets, obesity, and the development of type 2 diabetes. MCAT mice are protected from the development of insulin resistance caused by high fat diet feeding [194], as are Sod2 TG mice [163], suggesting the great importance in the role of mitochondria-derived ROS in the development of obesity-related insulin resistance. Surprisingly, *Gpx1*$^{-/-}$ mice seem to be resistant to many of the effects of high fat feeding [195]; however, this phenotype seems to be attributed to increased ROS-dependent signaling rather than oxidative damage. Overall, these data are consistent with the growing number of reports showing that oxidative stress may be a major regulator of the development of insulin resistance, diabetes, and complications from diabetes that are seen with age [196–198]. Again, most of these experiments were performed using lines of mutant mice that show little or no alteration of lifespan from control; yet, relatively clear differences in health between antioxidant knockout and transgenic mice appear when in the context of these diabetes models. Thus, health, and perhaps longevity, may be regulated by oxidative stress when under metabolic challenge.

Aging also has long been recognized as a major risk factor for many neurodegenerative diseases, including one of the most common forms of age-associated neural decline, Alzheimer's disease (AD). This is a progressive and fatal disease affecting more than 5 million people in the United States and is the seventh leading cause of death in this country. Again, although laboratory mice do not develop the common pathologies associated with AD, several genetic mouse models of AD have been developed. One of the most common is a model that overexpresses the mutant form of amyloid precursor protein (APP TG) that, in humans, leads to the Swedish variant of AD [199]. The reduction of Sod2 in APP TG mice (APP TG/*Sod2*$^{+/-}$) accelerates the behavior and learning deficits common to APP TG mice and also accelerates AD-like pathology including amyloid deposition and neurodegeneration [200–202]. The reduction of Gpx4 mice (APP TG/*Gpx4*$^{+/-}$) significantly increases the amyloid plaque burden and increases measurable levels of amyloid-β (Aβ) deposition compared to APP TG mice [203]. Conversely, Sod2 overexpression in APP TG mice (APP TG/Sod2 TG) reduces the amyloid plaque burden and neurodegeneration and rescues the memory and learning deficits found in APP TG mice [204,205]. Thus, modulation of antioxidant expression can affect the progression of AD in this model; further investigation into the health of these animals beyond brain-specific effects might be useful in determining whether overall health might be altered as well. For example, overexpression of Sod1 causes a significant increase in the lifespan of APP TG mice (APP TG/Sod1 TG) [206]. This lifespan extension occurs without reduction in AD pathology, so it remains to be determined whether this extension is merely a consequence of protection against some side effect of AD (such as neuronal degeneration) or a more general effect, but it is intriguing that lifespan under a chronic disease state might be modulated by superoxide dismutase. Oxidative stress may also play a critical role in the development of other neurological disorders such as Parkinson's disease (PD) and Amyotrophic Lateral Sclerosis (ALS). PD is a degenerative disorder of the central nervous system characterized by tremor, stiffness, and slowing of movement caused by loss of

dopamine. Sod1 TG mice are protected from the physiological (including loss of dopamine and neuronal death) and functional (locomotor) changes associated with two different PD models including MPTP injection and paraquat+maneb injection [207,208]. Overexpression of Gpx1 (Gpx1 TG) also protects mice in the paraquat+maneb PD model [207]. Thus, protection against oxidative stress in the form of superoxide (Sod1) or peroxides (Gpx1) minimizes the effects of PD in these models. ALS is a progressive, fatal neurodegenerative disease caused by the degeneration of motor neurons. One of the common mouse models of this disease is generated by overexpression of a mutant Sod1 that leads to protein aggregation and toxicity [126,209]. Reduction of Sod2 (*Sod2*$^{+/-}$) can increase the progression of ALS, as measured by neuromuscular function, and reduce the lifespan of some particular ALS mouse models, but not all [210,211]. Together, these findings show that alterations of the expression of antioxidant genes can greatly influence the progression of neurological disorders like AD, PD, and ALS. Further, these findings support the hypothesis that even though oxidative stress may have little influence on lifespan under optimum husbandry conditions, it is important for the maintenance of health under certain neurological disorders including age-related disorders common to humans.

Inflammatory processes, particularly those mediating chronic inflammation, have been implicated as being a primary cause of multiple chronic and age-related diseases and may be a critical determinant of the aging process itself [212]. The underlying biology connecting inflammation with various disease processes is unclear, but it may be that oxidative stress is key. Understanding how particular antioxidant manipulations can regulate inflammatory responses then may be an important piece to our understanding of the aging process. Sod1 TG mice seem to be resistant to certain kinds of inflammation; these mice are resistant to allergen challenge [213] and also show resistance to kanamycin-induced hearing loss [214]. Overexpression of Sod3 specifically in the lungs protects from lipopolysaccharide (LPS) - induced lung inflammation [215]. Mice overexpressing Trx1 (Trx1 TG, though on a different background than those in lifespan studies) are protected against the induced pathology in an allergen-induced asthma model [216]. Trx1 TG mice also show protection against the development of emphysema caused by chronic exposure to cigarette smoke [217], protection in a model of pancreatitis [218], and protection against joint destruction in an arthritis model [219]. The role of inflammation in aging still needs further clarification, but these results suggest that alterations in oxidative stress by changing the levels of antioxidant enzymes could be critical for maintenance of health under chronic inflammatory states.

Overall, these findings (summarized in Table 2) clearly support the idea that the progression and severity of many age-related diseases can be exacerbated or blunted by altering oxidative stress through modulation of the expression of antioxidant genes. It is important to reiterate that many of these diseases studied were performed using the same antioxidant mouse models that show no difference in lifespan under optimal husbandry conditions. Thus, under conditions designed to minimize the effect of particular diseases or pathologies on longevity, oxidative stress does little to affect lifespan, but health, both under optimal conditions and disease models, can be strongly affected by antioxidant status. Further, it under certain disease models, it appears that lifespan can be altered by oxidative stress; for example, the lifespan of AD mice is lengthened by overexpression of Sod1 [206] and the lifespan of ALS mice can be shortened by reduction of Sod2 [210,211]. So clearly, there is a divergence between the mechanisms that control lifespan under optimal laboratory conditions and those that control health, disease, and perhaps lifespan under challenging conditions. Future questions might then revolve around attempting to delineate what role oxidative stress may play under both states.

## Conclusions: Does alteration of antioxidant function in laboratory mice regulate aging or healthy aging?

The gold standard in determining whether a particular mutation or treatment affects aging has been, and likely always will be, whether or not the lifespan of an organism is affected. It is argued that the measurement of lifespan should be conducted in an optimal environment to eliminate or minimize deaths from non-aging causes, e.g., infectious disease, inflammation, and stress [133,134]. Under these conditions, the differences in lifespan observed with a particular manipulation can generally be interpreted as actually affecting the aging process, and not simply protecting against early mortality due to non-aging causes. There is little direct evidence to support the oxidative stress theory of aging under these conditions as there are few differences in lifespan of different mouse models with genetic alterations in the antioxidant defense system using such husbandry conditions. However, when the expression of antioxidant genes are studied in the context of age-related disease models, alterations in the antioxidant defense system dramatically impact disease progression or severity as predicted by the oxidative stress theory of aging.

How can these apparently contradictory observations on the effect of manipulating antioxidant genes on lifespan and age-related pathology be reconciled? One potential explanation is that oxidative stress does not play a role in aging but does play a role in the progression of age-related pathologies, i.e., oxidative stress/damage plays a role in healthspan or healthy aging. If this explanation is correct, it would be the first demonstration that a manipulation can alter healthspan but not have an impact on lifespan in mice. This explanation would also suggest that investigators should consider putting more emphasis on parameters such as age-related changes in health, function, and physiology in studies on aging rather than relying solely on lifespan.

A second potential explanation for these contradictory findings is that the role oxidative stress in aging depends on the environment. In an environment with minimal stress, such as that of long-lived mice maintained under optimal husbandry, oxidative damage plays little if any role, i.e., aging arises from other factors or mechanisms. However, when an organism is exposed to chronic stress over its lifespan from sources such as sub-optimal husbandry or from pathological phenotypes that may diminish optimal health, oxidative stress/damage plays a major role in aging. Under these conditions, an enhanced antioxidant defense system exerts an “anti-aging” action, leading to changes in lifespan, age-related pathology, and physiological function as predicted by the oxidative stress theory of aging. This explanation would account the discrepancy reported in the lifespan of *MsrA*$^{-/-}$ mice [114,116]. Initially, Moskovitz et al. [114] reported that *MsrA*$^{-/-}$ mice have a shorter lifespan than wild type mice; however, we observed no difference between the lifespans of *MsrA*$^{-/-}$ and wild type mice [116]. As noted above, the lifespan of wild type mice in the study by Moskovitz et al. [114] was very short compared to that of our study, e.g., a mean survival of 680 days vs. 925 days. We propose that the decrease in the lifespan of the *MsrA*$^{-/-}$ mice observed by Moskovitz et al. [114] arose because the mice were maintained under sub-optimal conditions, i.e., they were under increased stress. Thus, the reduction in lifespan disappeared when the *MsrA*$^{-/-}$ mice were maintained under optimal conditions used in our study [116]. This explanation also is supported by the study of Orr et al. [65] showing that the effect of overexpression of Sod1 on lifespan varied in different strains of *Drosophila*. The increase in lifespan with Sod1 overexpression correlated with the lifespan of the particular strain of fly tested; short-lived flies, which could be under greater stress, showed the greatest increase in lifespan, while the longest-lived flies showed little or no increase in lifespan.

In summary, the current data showing the effect of alterations in the antioxidant defense system on the lifespan of mice (as well as various invertebrate models) seriously call into question the

classical interpretation of the oxidative stress theory of aging, i.e., that oxidative damage plays a major role in the mechanism of the aging process. Based on the current data, if oxidative stress/damage plays a role in aging, it is much more limited than previously thought. However, oxidative stress/damage may play a role in healthspan, i.e., the period of life when the animal is free of age-related pathologies, or may play a stronger role under conditions where an animal is exposed to a chronic stress over its lifespan and where oxidative stress/damage may accelerate several features of the aging process. Perhaps by examining both the health and lifespan of antioxidant mouse models under optimal, healthy conditions as well as under chronic stress or disease states, we might be able to understand the larger role that oxidative stress plays in healthy aging (Figure 1).

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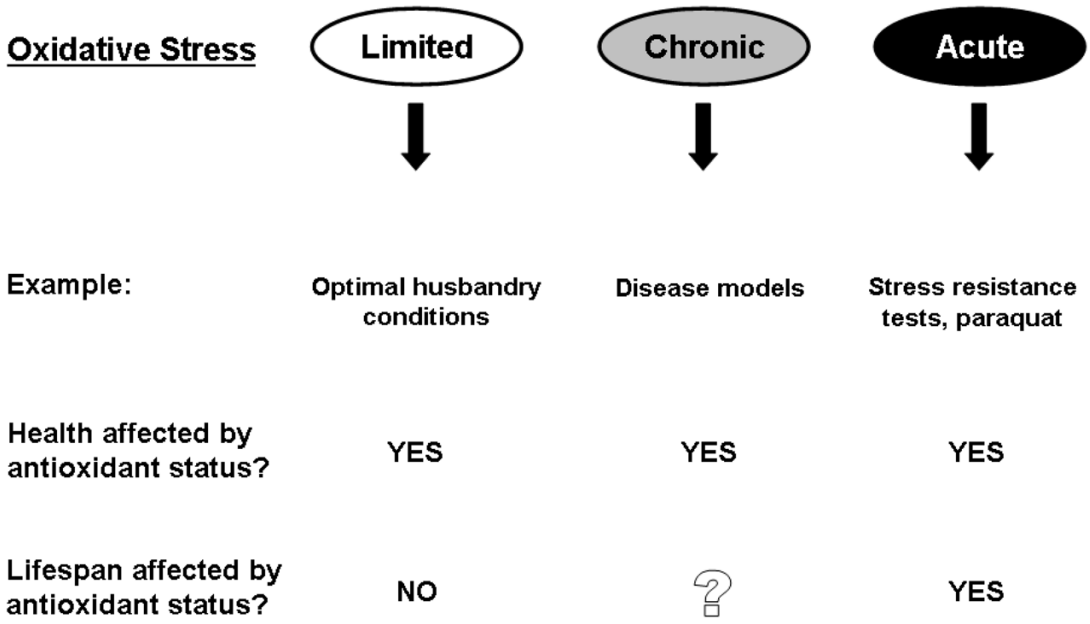


**Figure 1.**

By clarifying health and lifespan under different environmental conditions, we may be able to clarify the role of oxidative stress in regulating lifespan. Lifespan is relatively unaffected by oxidative stress under optimal husbandry conditions (limited oxidative stress), but healthy aging as measured by healthspan, can be altered by oxidative stress. Clearly, under conditions of acute stress, such as during oxidative stress resistance tests (i.e., paraquat), health and lifespan are limited by antioxidants. Recent evidence has shown that under certain disease conditions, like cardiovascular disease, diabetes, or neurodegeneration, chronic oxidative stress can modulate the progression of disease; it remains to be seen what effect this has on lifespan under these condition.

## Table 1

Effect of genetic manipulation of antioxidant defenses on age-related disease during normal aging

| Gene | Description of animal model | Effect | Refs |
|---|---|---|---|
| Cu/Zn-Superoxide Dismutase | *Sod1*$^{-/-}$ | Hearing loss, increase in macular degeneration and cataract, decreased fertility, and skeletal muscle atrophy. | [78,156–162] |
| Mn Superoxide Dismutase | *Sod2*$^{+/-}$ | Increased tumor burden, insulin resistance. | [88,163] |
| Glutathione peroxidase 1 | *Gpx1*$^{-/-}$ | Cataract formation, impaired circulatory vasodilation. | [98,164,165] |
| Methionine Sulfoxide reductase A | *MsrA*$^{-/-}$ | Accelerated age-related hippocampal degeneration, accelerated cardiac dysfunction | [114,168–170] |
| Catalase | MCAT | Delay in cataract formation, delay in cardiac dysfunction, reduced hearing loss. | [171–173] |

## Table 2

Effect of genetic manipulation of antioxidant defenses on age-related disease in challenged animal models.

| Disorder | Gene altered | Description of animal model | Effect | Refs |
|---|---|---|---|---|
| **Neurodegenerative diseases** | | | | |
| | Cu/Zn-Superoxide Dismutase | CD-1 mice overexpressing human SOD1. | Protection against neuronal cell death after transient focal ischemia. | [220] |
| | | APP TG mice overexpressing human SOD1 (APP TG/Sod1 TG) | Increased lifespan of APP TG mice | [206] |
| | | Sod1 TG in PD models (MPTP, PQ+maneb) | Reduced neural death and loss of dopamine, improved locomotion | [207,208] |
| | Mn-Superoxide Dismutase | | | |
| | | APP TG mice heterozygous for Sod2 (APP TG/*Sod2*$^{+/-}$) | Increased Aβ plaques, neurodegeneration | [200–202] |
| | | APP TG mice overexpressing Sod2 (APP TG/Sod2 TG) | Reduced Aβ plaques, neurodegeneration | [204,205] |
| | | ALS model (SOD1$^{G93A1Gur}$) heterozygous for Sod2 (*Sod2*$^{+/-}$). | Increased disease progression (ALS animal model) | [210,211] |
| | Glutathione peroxidase 1 | Gpx1 TG in PD model (PQ +maneb) | Reduced neural death and loss of dopamine, improved locomotion | [207] |
| | Glutathione peroxidase 4 | APP heterozygous for Gpx4 (*Gpx4*$^{+/-}$). | Increased amyloid plaques | [203] |
| **Cardiovascular-related diseases** | | | | |
| | Catalase | (*Apo E*$^{-/-}$/Cat TG) | Retardation of atherosclerosis | [180] |
| | Cu/Zn –Superoxide dismutase/Catalase | (*ApoE*$^{-/-}$/Sod1 TG/Cat TG) | Retardation of atherosclerosis | [180] |
| | Glutathione peroxidase 4 | (*ApoE*$^{-/-}$*/Gpx4 Tg)* | Retardation of atherosclerosis | [181] |
| | Mn-Superoxide Dismutase | (*ApoE*$^{-/-}$/*Sod2*$^{+/-}$) | Increased endothelial dysfunction | [176] |
| | Glutathione peroxidase 1 | (*ApoE*$^{-/-}$/*Gpx1*$^{-/-}$) | Increased progression of atherosclerosis | [178] |
| | | *Gpx1*$^{-/-}$ with viral-induced myocarditis model | Increased myocarditis after viral infection. | [185] |
| | Extra cellular Cu/Zn- Superoxide dismutase | *Sod3*$^{-/-}$ with kidney clip model | Increased hypertension | [182] |
| **Diabetes** | | | | |
| | Cu/Zn-Superoxide Dismutase | *Sod1*$^{-/-}$ with streptozotocin-induced diabetes mellitus | Increased cataract formation | [188,189] |
| | | | Increased nephropathy | [190] |
| | | Sod1 TG with streptozotocin-induced diabetes mellitus | Protection from nephropathy | [188,191] |
| | Mn-Superoxide Dismutase | Sod2 TG under high fat diet feeding | Protection against insulin resistance | [163] |
| | | Type 1 diabetic mouse overexpressing Sod2 in the heart | Protection against diabetic cardiomyopathy | [193] |
| | Catalase | MCAT under high fat diet feeding | Protection against insulin resistance | [194] |

| Disorder | Gene altered | Description of animal model | Effect | Refs |
|---|---|---|---|---|
| | | Type 2 diabetic mouse overexpressing Cat in the kidney | Protection from nephropathy | [192] |
| | Glutathione peroxidase 1 | *Gpx1*$^{-/-}$ under high fat diet feeding | Protection against insulin resistance | [195] |
| **Inflammation** | | | | |
| | Cu/Zn-Superoxide Dismutase | Sod1 Tg | Resistance to allergen challenge and kanamycin-induced hearing loss. | [213,214] |
| | Extra celular Cu/Zn- Superoxide dismutase | Sod3 Tg | Protection against LPS-induced inflammation in lung. | [215] |
| | Thioredoxin 1 | Trx1 Tg | Protection against allergen-induced asthma model, cigarette smoke- induced emphysema, and joint destruction in arthritis model. | [216–219] |