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Review

Do Antioxidants Extend Longevity in Invertebrate and Vertebrate Animals?

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Abstract

In the 1950's Denham Harman proposed the Free Radical Theory of Aging whereby species lifespan and individual longevities are the consequence of free radical driven damage to biomolecules. This led to decades of research to ascertain the effect of altered antioxidant defense systems on aging and mortality in an array of species using reverse genetics and dietary manipulation. Within invertebrates, the data generally support the Free Radical Theory in that overexpression of antioxidant enzymes or dietary supplementation with antioxidant compounds increases longevity and resistance to oxidative damage. Likewise, genetic knockdown of antioxidant defenses generally shortens longevity within invertebrates. On the other hand, for endothermic vertebrates (i.e., birds and mammals) the results have been equivocal. Downregulation of antioxidant enzymes typically results in an increased oxidative burden, but without an appreciable effect on longevity, while dietary supplementation with antioxidants has little-to-no effect, at least at the concentrations used. Upregulation of antioxidant enzyme genes also fails to increase longevity in vertebrates most of the time. Interestingly, manipulating antioxidant defenses in fishes increases longevity in conjunction with reduced oxidative damage akin to what is seen in invertebrates. Since invertebrates and fishes are both exothermic this raises the possibility that the evolution of endothermy interferes with the ability of antioxidants to slow the aging process.

Keywords
Antioxidant; oxidative stress; longevity; eukaryotes

1. Introduction

Aging is an unavoidable, universal, phenomenon that impacts all organisms. Many hypotheses have been proposed to explain both cellular and molecular mechanisms of aging with the Free Radical Theory of Aging first suggested by Denham Harman being one of the most prevalent [1]. In short, it posits that aging is due to the accumulation of molecular damage downstream of free radicals and other reactive oxygen species (ROS) that are an inevitable consequence of metabolism and various exogenous sources [2] and has been cited as central to regulating human longevity [3, 4].

Ultimately, oxidative stress is the consequence of an imbalance between the production of ROS in cells and tissues and the antioxidant mechanisms meant to combat them which leads to the accumulation of oxidative damage. As the name implies, ROS are highly reactive molecules that act as oxidizing agents in reduction-oxidation (i.e., redox) reactions and can damage nucleic acids (both DNA and RNA), proteins, and lipids. If enough damage accumulates, it can lead to cell death [5].

Organisms have evolved multiple endogenous antioxidant defenses, most notably antioxidant enzymes (e.g., catalase) that are responsible for the direct conversion of ROS to less damaging or inert molecules. In addition, there are endogenous substrates that are differentially oxidized or reduced when coupled with specific enzymes (e.g., glutathione and glutathione reductase). Finally, many substances found in foodstuffs or medicinal plants can serve as exogenous antioxidants, especially polyphenols and carotenoids [6]. Nutritional antioxidants act through various mechanisms but are mainly free radical scavengers that directly neutralize free radicals, reduce peroxide concentration, or quench iron to decrease ROS production [7].

Here, we will address whether there is evidence for antioxidants acting as a buffer against the aging process. This is not a new question [8, 9] and we apologize to researchers whose work is not included, but the goal of this paper is to serve as a refresher about the uncertainty surrounding this topic, while providing an update on what has been learned during the past 10-plus years using additional models, rather than being a comprehensive review. Specifically, given the underlying assumption that mitigating the detrimental effects of ROS in intact organisms should extend longevity, the prevailing hypothesis is that an increase in antioxidant defense mechanisms coupled with reduced oxidative damage should extend longevity. The converse is that increased oxidative damage, regardless of cause, should reduce longevity. More recently, interest in the effect of various anti-aging strategies on the healthspan of an individual, defined as the time spent free from chronic illness and disabilities associated with aging, has increased but the relevance of extending healthspan, or even what it means, is still debated, and will not be addressed here [10].

1.1 Endogenous Antioxidants

Antioxidants can inhibit or delay the oxidation of a substrate when present [11] and are broadly divided into one of two classes: (1) enzymatic antioxidants and (2) non-enzymatic antioxidants.

However, some non-enzymatic antioxidants require an enzyme to catalyze specific redox reactions central to their effects [11]. The role of enzymatic antioxidants is to directly remove free radical species from both the intracellular and extracellular compartments. This involves the conversion of a highly reactive molecule such as superoxide to a less reactive species (hydrogen peroxide). The less reactive species can then be rendered inert, for example via its conversion to water. This often occurs in the presence of cofactors such as copper, zinc, manganese, or iron [12]. In some cases, antioxidant enzymes work in tandem to convert hydroperoxides into non-radical, non-reactive thermally stable products [13].

Superoxide dismutases (SODs) are critical primary antioxidants found in both prokaryotes and eukaryotes thought to protect against senescence, and the accumulation of tissue damage due to lipid peroxidation and/or protein denaturation. Primary antioxidants such as SODs are the most potent defense mechanism against free radicals and harmful inflammatory reactions [13]. Cu-Zinc SOD is found predominantly in the cytoplasm and is responsible for the dismutation of superoxide radicals to form hydrogen peroxide (H_2O_2) [14] while Mn-SOD is found in the mitochondrial matrix and is crucial to detoxifying singlet oxygen radicals generated by mitochondrial respiration [15]. Although not relevant to mammals, Fe-SOD is typically found in prokaryotes and the chloroplast of some plants and is crucial in the conversion of superoxide to oxygen and hydrogen peroxide [16, 17].

Although not as reactive as other ROS's, H_2O_2 has the potential to cause significant damage if left unchecked [18, 19]. More than likely, the antioxidant enzyme catalase (CAT) has the widest taxonomic distribution, having been described in all aerobic organisms and is responsible for catalyzing the detoxification of H_2O_2 to water and molecular oxygen. Glutathione peroxidase also reduces H_2O_2 and other peroxides and is part of a larger system involving glutathione reductase, glutathione-s-transferase and the protein glutathione. Glutathione is the most abundant antioxidant protein found in eukaryotic cells where it serves as a co-factor for glutathione peroxidase and glutathione reductase [5, 13]. Glutathione can also directly react with free radicals to prevent damage to crucial cellular components [20]. For glutathione peroxidase to be able to reduce H_2O_2 it needs glutathione reductase to maintain a pool of cysteine-thiol groups in a reduced state [21]. Meanwhile, glutathione-s-transferases are involved with the direct detoxification of xenobiotics and the reduction of lipid peroxides due to glutathione peroxidase activity [20]. Finally, the thioredoxin (Trx) system serves as an antioxidant defense mechanism in vertebrates via the maintenance of a reducing environment. This is accomplished by catalyzing electron flux from nicotinamide adenine dinucleotide phosphate ($NADP^+$) through thioredoxin reductase which reduces target proteins by utilizing conserved thiols [22]. See Figure 1 for an overview of these processes. In humans, two forms of thioredoxin have been identified which are in the cytosol (Trx1) and mitochondria (Trx2), respectively [23].

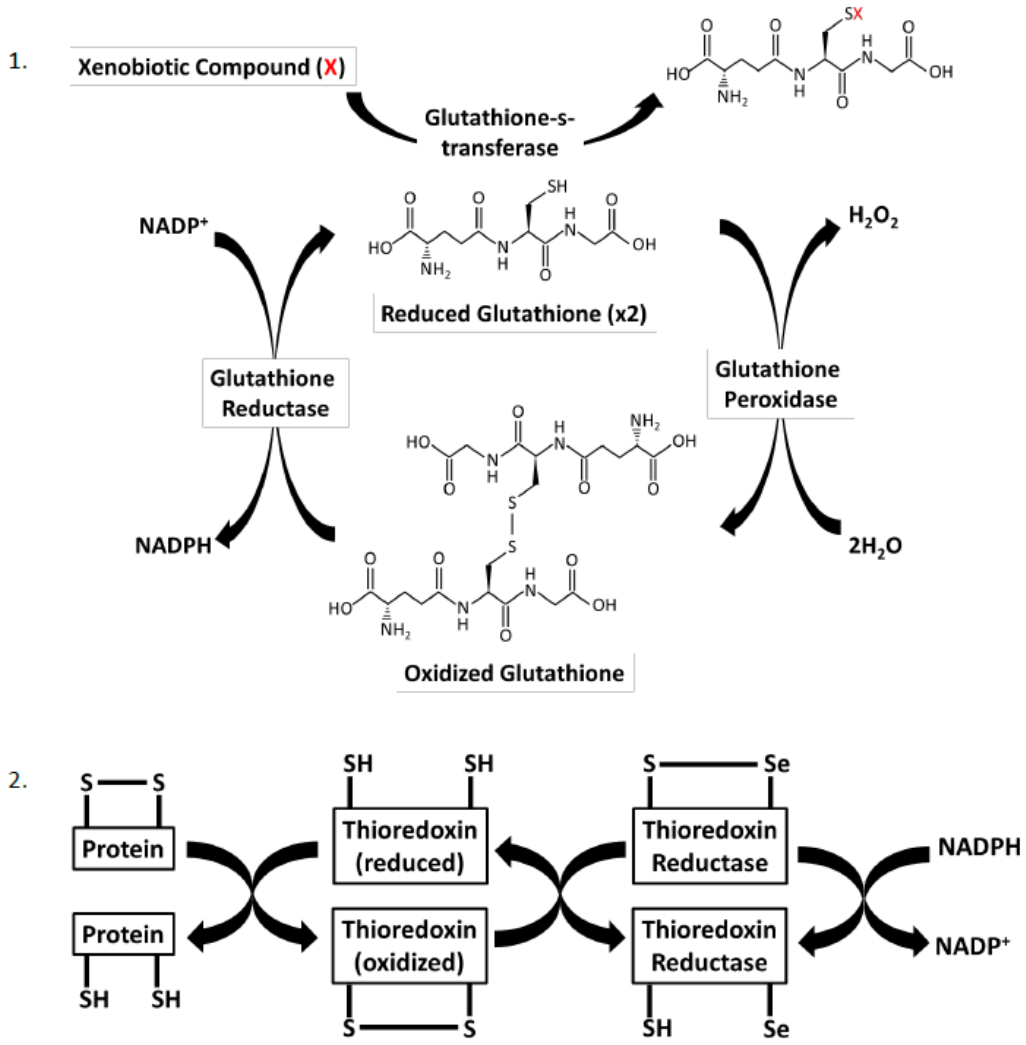

Figure 1 The glutathione and thioredoxin systems. 1) Glutathione serves as a substrate for glutathione reductase, glutathione peroxidase and glutathione-s-transferase. Glutathione peroxidase activity reduces hydrogen peroxide to water to mitigate against its damaging effect; glutathione reductase catalyzes the reduction of oxidized glutathione to regenerate the supply of reduced glutathione using nicotinamide adenine dinucleotide phosphate (NADPH) as a reducing factor; glutathione-s-transferase uses reduced glutathione for detoxification via conjugation to xenobiotic compounds. 2) In the thioredoxin system, thioredoxin reductase is used to deliver reducing equivalents from NADPH to thioredoxin with reduced thioredoxin being used catalyze the reduction of disulfides within oxidized proteins.

1.2 Exogenous Antioxidants

Non-enzymatic antioxidants act by quenching free radicals directly or indirectly via the promotion of other processes necessary for radical scavenging [13] to prevent reactive species from being formed or to remove them before harmful damage can occur [24]. Non-enzymatic antioxidants are comprised mostly of nutrients such as vitamin C (ascorbic acid), vitamin E (alpha-tocopherol), N-acetyl cysteine and carotenoids [12, 25].

Vitamin C is a water-soluble antioxidant found in both the intracellular and extracellular compartments where it directly scavenges oxygen free radicals in aqueous environments and defends against the oxidation of cholesterols [26]. Vitamin C also assists with converting vitamin E from its oxidized to its reduced form [18]. Foodstuffs with an abundance of vitamin C include fruits, vegetables, beef, cereals, poultry, and fish [13]. Meanwhile, Vitamin E is a lipid soluble antioxidant present in the cell membrane and is a component of lipoproteins where it serves to inhibit lipid peroxidation. Vitamin E is abundant in vegetable oils, mangoes, almonds and other nuts, and broccoli [27]. The lipid soluble precursor for vitamin A synthesis, β-carotene, can function synergistically with vitamin E to protect against lipid peroxidation [13]. N-acetyl cysteine (NAC) is used for glutathione synthesis, but since it contains a thiol group, NAC can donate electrons for the detoxification of ROS and the protection of sulfhydryl-containing proteins from oxidative damage [28]. Typically, NAC levels are maintained via the consumption of cysteine, which is found in high protein foods [29]. Carotenoids are pigments that play a key role in protecting plants against photo-oxidative processes and are efficient antioxidants that scavenge singlet molecular oxygen and peroxyl radicals [30]. In humans, carotenoids are part of the antioxidant defense system and work synergistically with other antioxidants [30].

The tenets of the Free Radical Theory of aging implicate ROS, and the induction of oxidative stress, as a causal agent in the aging process. Hence, interventions to reduce oxidative stress should slow aging and increase species' lifespan. Although they are often used interchangeably, lifespan and longevity are not equivalent. Specifically, there are differences in lifespans among species, while differences in longevity occur among individuals of the same species. This is important because a species' lifespan is the consequence of evolution and its effect on a suite of parameters that contribute to the unique physiology of an individual species. Differences in longevity among individuals of the same species, meanwhile, are the consequence of an individual's unique allelic composition and how it shapes its response to exogenous inputs.

1.3 Impact of Enzymatic and Non-enzymatic Antioxidants on Vertebrate Longevity

Among vertebrates, there is little evidence to suggest that antioxidant defenses are correlated with differences in lifespan or longevity. For example, in a study of antioxidant enzyme activity in heart, brain and liver of 14 mammalian and avian species, only Mn-SOD and catalase demonstrated a significant relationship with lifespan, while there was no relationship for Cu-Zinc SOD, glutathione peroxidase, or glutathione reductase activity [31]. No appreciable difference in Mn-SOD, catalase, and glutathione peroxidase activity in tissues from mice relative to naked mole rats, an extremely long-lived rodent model of aging, has also been reported [32]. Data from dermal fibroblasts collected from several mammalian species gave parallel results to whole organ lysates [33], while a negative relationship between species lifespan and antioxidant activity of lung has been reported [34]. In birds, there is some evidence for increased antioxidant capacity and long-life among species [35], but maybe only for specific antioxidant defenses [36]. Within fishes there is a positive relationship between antioxidant activity in in some, but not all, organs [37].

At the individual species level, within mice overexpression of Cu-Zinc SOD had no effect on longevity [38]. This finding has been repeatedly demonstrated in other rodent models [38, 39]. In the case of thioredoxin and thioredoxin reductase, a negative relationship between their activity and species lifespan was observed in both mammals and birds [40, 41]. Genetic manipulation of

Trx1 and Trx2 in C57BL/6 mice did increase their resistance to oxidative stress and reduced the degree of oxidative damage, but there was a statistically insignificant effect on longevity [23, 42]. Interestingly, while genetic knockout of antioxidant enzyme genes can reduce longevity in vertebrate models [43-48] there are many notable exceptions even in the presence of increased oxidative damage. This has been shown most robustly in genetically modified mice [39, 42, 49-51], but more recent studies using short-lived fish have also shown no correlation between the degree of oxidative stress and mortality [52]. An important caveat for these studies is that the genetic manipulations used typically only result in a single change in the "dose" of the gene being tested. That is, the dosage is altered to a specific degree because of the manipulation that was used. For example, expression may be increased or decreased by 50%, but the degree to which it changes varies from one study to the next. Typically, a range of differences in the degree of "dosing" within the same population is not generated and so it is possible that the ideal "dose" has been missed. The expression system used may also influence the outcome [53].

Dietary supplementation of antioxidants has also failed to demonstrate a convincing positive effect on species longevity. For example, NAC supplementation in mice did significantly increase longevity in male mice, but there was no effect in females [54]. However, the increase in male longevity may actually be the consequence of reduced food intake (i.e., dietary restriction) rather than a direct effect of NAC itself. Meanwhile, Vitamin E supplementation has given mixed effects in that several studies have failed to show an increase in longevity in mice [55-60], while others reported a significant increase in longevity even in the absence of a demonstrable effect on oxidative damage [61]. Vitamin C supplementation has also been reported to increase longevity in mice, but similar to NAC supplementation this effect may be secondary to reduced food intake [62]. Mice fed a mixture of antioxidants showed an increase in longevity if the supplementation began early in life, but the appreciable variation in the median longevity among the controls (i.e., in the absence of antioxidant supplementation) makes the significance of this finding uncertain [63] while other studies demonstrating a diet-induced increase in antioxidant gene expression did not increase longevity in mice [48, 64]. Finally, a mitochondrial targeted antioxidant supplement found that housing condition was an important modulator of the effect on longevity [65].

The lack of a consistent and/or convincing effect of dietary antioxidant supplementation on longevity extends to species other than mice. For example, Selman et al. [60] fed a diet containing either Vitamin C or Vitamin E to a species of vole and found that longevity was significantly *decreased*. Vitamin C supplementation in guinea pigs also showed no beneficial effect [66] and human trials have largely failed to show a beneficial effect of dietary antioxidants as well, including vitamin C, vitamin E, vitamin A, and β-carotene. And similar to voles, the risk of mortality was actually increased when antioxidant supplements were provided to humans in some cases [25, 67]. Taken together, these inconsistent results have led to the speculation that there may be a beneficial effect of dietary antioxidants that is a consequence of something other than their antioxidant properties, such as altered tumor growth [59]. Not surprisingly, species identity has been implicated as well, with a krill oil supplemented diet shortening the longevity of mice while increasing the longevity of an annual fish (*Nothobranchius guentheri*) that has become an increasingly popular model for vertebrate aging research [68]. Indeed, longevity in *N. guentheri* and another fish species (medaka, *Oryzias latipes*) is increased in conjunction with a reduction in markers of oxidative stress when fed dietary supplements [69, 70]. As with gene dosage, it is possible the proper dose of antioxidant supplementation has not been achieved for a given species and/or the feeding duration

itself was insufficient (or too long) [68]. The findings for antioxidant manipulation in vertebrates is summarized in Table 1.

Table 1 Effect of antioxidants on vertebrate longevity.

Endogenous Antioxidant	Organism	Effect on Longevity	Source	Reference
Mn-Sod	Deer, Cow, Bat, Mice, Pig, Rabbit, Finch, Squirrel, Quail, Sheep, Guinea pig, Human	Positive	Intracellular antioxidant activity in heart, liver, brain	Page et. al 2010
	Human, Dog, Horse, Monkey, Naked Mole Rat, Fish	None	Antioxidant activity in skin fibroblasts	Brown and Stuart, 2007 Trenzado *et al.* 2013
	Deer, Cow, Bat, Mice, Pig, Rabbit, Finch, Squirrel, Quail, Sheep, Guinea pig, Human, Dog, Horse, Monkey, Naked Mole Rat	Negative	Antioxidant activity in lung tissues	Campo *et al.*, 1994
Cu-Zinc SOD	Deer, Cow, Bat, Mice, Pig, Rabbit, Finch, Squirrel, Quail, Sheep, Guinea pig, Human	None	Intracellular antioxidant activity in heart, liver, brain	Page *et al.*, 2010
Catalase	Deer, Cow, Bat, Mice, Pig, Rabbit, Finch, Squirrel, Quail, Sheep, Guinea pig, Human	Positive	Intracellular antioxidant activity in heart, liver, brain	Page *et al.*, 2010
	Deer, Cow, Bat, Mice, Pig, Rabbit, Finch, Squirrel, Quail, Sheep, Guinea pig, Human, Dog, Horse, Monkey, Naked Mole Rat	Negative	Antioxidant activity in lung	Campo *et al.*, 1994
Glutathione peroxidase	Deer, Cow, Bat, Mice, Pig, Rabbit, Finch, Squirrel, Quail,	None	Intracellular antioxidant activity in heart, liver, brain	Page *et al.*, 2010

Glutathione peroxidase	Sheep, Guinea pig, Human Deer, Cow, Bat, Mice, Pig, Rabbit, Finch, Squirrel, Quail, Sheep, Guinea pig, Human, Dog, Horse, Monkey, Naked Mole Rat	Negative	Antioxidant activity in lung	Campo *et al.*, 1994
Glutathione Reductase	Deer, Cow, Bat, Mice, Pig, Rabbit, Finch, Squirrel, Quail, Sheep, Guinea pig, Human	None	Intracellular antioxidant activity in heart, liver, brain	Page *et al.*, 2010
	Deer, Cow, Bat, Mice, Pig, Rabbit, Finch, Squirrel, Quail, Sheep, Guinea pig, Human, Dog, Horse, Monkey, Naked Mole Rat	Negative	Antioxidant activity in lung	Campo *et al.*, 1994
Thioredoxin Reductase	Mouse, Syrian hamster, Norway rat, Mongolian gerbil, Squirrel, bat, guinea pig, sheep, deer, dog, pig, cow, quail, zebra finch	Negative	Liver, heart, brain	Pamplona and Costantini, 2011; Salway *et al.*, 2010
Thioredoxin 1/2	Mouse	Positive*	Overexpression	Roman *et al.*, 2020; Perez *et al.*, 2011
Exogenous Antioxidant				
N-Acetyl cysteine (NAC)	Mouse (Male) Mouse (Female)	Positive** Negative	Dietary Supplementation	Flurkey *et al.*, 2010 Lipman *et al.*, 1998
Vitamin E	Mouse Mouse Vole Human	None Positive Negative None***	Dietary Supplementation	Navarro *et al.*, 2005 Selman *et al.*, 2013 Shields *et al.*, 2021 Bjelakovic *et al.*, 2012,2013
Vitamin C	Mice Guinea Pigs Human	Negative None None***	Dietary Supplementation	Massie *et al.*, 1984 Davies *et al.*, 1977 Shields *et al.*, 2021

β-carotene	Human	None***	Dietary Supplementation	Bjelakovic *et al.*, 2012,2013 Shields *et al.*, 2021 Bjelakovic *et al.*, 2012,2013
Krill Oil	*Nothobranchius guentheri*	Positive	Dietary Supplementation	Leng *et al.*, 2022
B-Glucan	*N. guentheri, Oryzias latipes*	Positive	Dietary Supplementation	Song *et al.*, 2020 Sanchez *et al.*, 2018

*Although the results show positive effects on longevity the data collect was statistically insignificant

**Positive effect on longevity could be due to reduced food intake (i.e., dietary restriction)

***In most scenarios the effects of the antioxidants had no impact on longevity, but in some cases increased mortality risk

1.4 Impact of Enzymatic and Non-enzymatic Antioxidants on Invertebrate Longevity

Due to the relative ease of use and rapid turnaround time, genetically modified invertebrate models were the most heavily used early in the investigation of antioxidant defense genes and lifespan regulation. Overall, these works generated a strong body of evidence suggesting enhanced antioxidant defenses have a significant positive effect on invertebrate lifespan and/or longevity with yeast (*Saccharomyces* spp.), fruit flies (*Drosophila melanogaster*) and the nematode worm *Caenorhabditis elegans* being the best studied. This contrasts with the mixed results obtained from vertebrate models reviewed above.

For example, overexpression of superoxide dismutases increases chronological (but not replicative) longevity in yeast [71], as well as *D. melanogaster* and *C. elegans* [72-75], although the increase in longevity did not coincide with a decrease in oxidative damage in *C. elegans*. In fact, protein oxidation was increased in this model leading the authors to speculate that overexpression of superoxide dismutase resulted in changes in longevity-promoting transcriptional activity, rather than an antioxidant effect *per se* [76, 77]. Increased SOD gene activity has also been reported to be necessary for longevity-assurance in a long-lived mutant strain of *C. elegans* [78]. Interestingly, SOD mutants with reduced enzyme activity display a marked increase in oxidative damage but have a normal longevity [79] that is partially dependent on the cellular location that a specific isoform is normally expressed, for example in the mitochondria versus the cytosol [79-81].

On the other hand, Orr and Sohal [82] reported increased longevity in fruit flies overexpressing SOD that was associated with reduced protein oxidation and that the loss of SOD activity significantly reduced longevity. Another study also reported a positive effect on longevity when SOD is overexpressed, but it was not statistically significant [83]. Interestingly, there is no beneficial effect of catalase overexpression on longevity in *D. melanogaster* despite an increased resistance to hydrogen peroxide induced toxicity [72, 84], while RNAi-mediated reduction in catalase activity shortens the longevity of both sand flies (*Lutzomyia longipalpis*) and *D. melanogaster* [85, 86]. This is true for RNAi-mediated downregulation of SOD in *D. melanogaster* as well [86], but longevity is unaffected in an SOD hypomorph [87].

Other enzymatic antioxidants that extend longevity in invertebrates when overexpressed include thioredoxin in both *D. melanogaster* and *C. elegans* [78, 88, 89]. In budding yeast, increased peroxiredoxin activity increases replicative lifespan via scavenging peroxide and due to its chaperone activity [90]. Tissue-specific overexpression peroxiredoxin in the neurons of *D. melanogaster* also increased longevity when expressed in the cytoplasm of the endoplasmic reticulum alone [91, 92]. Meanwhile, reduced glutathione peroxidase activity in *C. elegans* shortens longevity [93] but reduced peroxiredoxin activity has no effect [94].

As with vertebrates, there has been a long-standing interest in screening dietary supplements for their anti-aging properties using invertebrate models [95]. Indeed, dietary supplementation using well-known antioxidants (e.g., Vitamins C and E) increases longevity under varying conditions, and in varied genetic backgrounds, in fruit flies [96] while Vitamin C supplementation increases longevity in yeast and *C. elegans* [97, 98]. As is often the case, too much Vitamin C is toxic, however [96]. This parabolic relationship between dose and longevity is also observed with N-acetylcysteine (NAC) supplementation. For example, *C. elegans* and *Drosophila* spp. given a low dose of NAC have significantly increased longevity, while a high dose is detrimental [99, 100]. In the case of Vitamin E supplementation, it is more complicated. In budding yeast, Vitamin E reduces replicative lifespan [101], but increases longevity in *C. elegans* despite no effect on the degree of superoxide damage [102, 103]. In *D. melanogaster,* the specific form of Vitamin E used was important, as well as the dose at which it was administered [55, 104]. Carotenoid supplementation has not been well-studied, but it does prolong longevity in the mealworm beetle (*Tenebrio molito*), but most likely due to its effects on immune function and not oxidative stress [105]. In both *D. melanogaster* and *C. elegans*, carotenoid supplementation does increase longevity [106-108].

In addition to these more traditional antioxidants, multiple studies have demonstrated a beneficial effect (i.e., increased survival) of various supplements that act either directly as antioxidants or that induce antioxidant activity in invertebrate models. Many of these compounds are derived from plants, such as hawthorn [109] and canary seed [110] or from fungi, such as shitake mushroom [111], but also include synthetic drugs [112]. While most of these studies have been conducted in *C. elegans,* similar findings have been seen with plant extracts in *D. melanogaster* [113-115], yeast [116, 117] and rotifers [118]. The findings for antioxidant manipulation in invertebrates are summarized in Table 2.

Table 2 Effect of antioxidants on invertebrate longevity.

Endogenous Antioxidant	Organism	Effect on Longevity	Source	Reference
Mn-Sod	Yeast			Fabrizio *et al.*, 2003
	Worms	Positive	Overexpression	Melov *et al.*, 2000
	Fruit Flies			Curtis *et al.*, 2007
Cu-Zinc Sod	Yeast	Positive		Harris *et al.*, 2005
	Worms	Positive	Overexpression	Doonan *et al.*, 2008
	Flies	Positive		Sun and Tower, 1999
Catalase	Yeast	Positive	Overexpression	Fabrizio *et al.*, 2003
	Fruit Flies	Positive	RNAi-mediated reduction	Sun and Tower, 1999
	Sand Flies	Positive	Overexpression	

Thioredoxin (Trx1)	Worms Fruit Flies	Positive Positive	Overexpression	Diaz-Albiter *et al.,* 2011 Miranda-Vizuete *et al.*, 2006 Oberacker *et al.*, 2018
Thioredoxin (TrxT)	Fruit Flies	Positive	Overexpression	Umeda-Kameyama *et al.*, 2007
Peroxiredoxin	Yeast Fruit Flies Worms	Positive Positive* None	Increased activity Overexpression in ER and cytoplasm Decreased activity	Roger *et al.,* 2020 Lee *et al.,* 2009 Ranjan *et al.,* 2013
Glutathione Peroxidase	Worms	Negative	Decreased activity	Sakamoto *et al.,* 2014
Exogenous Antioxidant				
N-Acetyl cysteine (NAC)	Worms Fruit Flies	Positive* Positive*	Dietary Supplementation	Oh *et al.*, 2015 Brack *et al.*, 1997
Vitamin E	Yeast Worms Fruit Flies	Negative Positive Mixed Results	Dietary Supplementation	Lam *et al.*, 2010 Harrington and Harley, 1998 Driver and Georgeou, 2003
Vitamin C	Yeast Worms	Positive Positive	Dietary Supplementation	Owsiak *et al.*, 2010 Shibamura *et al.*, 2009
Carotenoids	Fruit Flies *Tribolium* Fruit Flies Worms	Positive* Positive Positive Positive	Dietary Supplementation	Bahadorani *et al.*, 2008 Dinhaut *et al.*, 2017 Yazaki *et al.*, 2011 Lashmanova *et al.,* 2015

*Parabolic effect of dosage where high levels lead to a decrease in longevity

2. Conclusions

Within invertebrate models, genetic manipulation of endogenous antioxidant defenses has the expected outcome on longevity, be it increased or decreased dependent on the situation, while supplementation with various exogenous antioxidant compounds has a beneficial effect in most cases. Not unexpectedly, there are exceptions to these findings due to a variety of mitigating factors, such as species, strain, specific manipulation (including dosing), *et cetera,* but the consensus seems to be that oxidative stress and longevity are strongly correlated within invertebrates. Within vertebrates, however, the conclusions are muddled. A little over 10 years ago, the Free Radical Theory of Aging was called into question based largely on the lack of a tangible effect of genetic

models of impaired oxidative stress resistance on longevity within mice, as well as inconclusive findings using dietary supplementation with various antioxidant compounds [8, 9]. Since that time, additional studies in various vertebrate models, using different experimental approaches, has done little to change this notion, perhaps except for increased oxidative stress consistently reducing longevity in fishes. However, fishes are exotherms, just as invertebrates are, but mice (i.e., mammals) are endotherms. So too are birds, which also fail to show a consistent effect of oxidative stress and/or antioxidant capacity on the aging process [119]. Taken together, this suggests that the relevance of oxidative stress as a potential modulator of the aging process in humans is minimal, although it certainly is involved in numerous pathophysiological processes.

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Author Contributions

SB and EB wrote and edited the manuscript with support from JMH. JMH conceived the framework and direction of the manuscript.

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Competing Interests

The authors have declared that no competing interests exist.

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