Minireview

Oxidative stress, aging and longevity in *Drosophila melanogaster*

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Abstract Free radicals produced during normal metabolism cause damage to macromolecules. The free radical theory of aging proposes that the organism is unable to repair all of them and that, with time, unrepaired damages accumulate and put the organism at risk: in other words, free radicals provoke aging and death. This article reviews both the results of adding antioxidants to food on longevity in *Drosophila melanogaster*, as well as the studies on antioxidant enzymes (inactivation in vivo, null mutants, overexpression). It is concluded that antioxidant enzymes are probably poorly connected to the normal aging process, but they allow the organism to cope with stressful conditions. **© 2001 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.**

Key words: Free radical theory of aging; Antioxidant enzyme; Antioxidant compound; Aging; Longevity; *Drosophila melanogaster*

1. Introduction

In 1928, Pearl [1] proposed the rate of living theory stating that life span is inversely related to metabolic rate. Harman [2] accepted that aging 'seems to be a more or less direct function of the metabolic rate' and proposed a biochemical explanation of this relationship, the free radical theory of aging. This theory proposes that highly reactive derivatives of oxygen, the free radicals, are produced during normal metabolism. The organism is then unable to counteract all the damage to macromolecules because the balance between oxidants and antioxidants, such as free radicals detoxifying enzymes, is in favor of oxidants. With time, unrepaired damage is expected to accumulate and put at risk the homeostasis of the organism, i.e. to provoke aging and death.

This minireview is not intended to cover biochemical aspects of the free radical theory (see, e.g. [3]), but to summarize current evidence gathered from *Drosophila melanogaster* research, a model that is widely used in free radical research.

Some expectations can be drawn from the free radical theory. Adding antioxidants to the diet, which changes the balance between oxidants and antioxidants, should increase longevity. Increasing the activity of antioxidant enzymes is also expected to increase longevity. Obviously, decreasing this activity should bring the opposite result. Compilation of the available data will lead us to propose that, while there is no doubt that free radicals may have a negative effect, particularly in some age-related diseases (see e.g. [3]), it is uncertain that they explain the normal aging process.

According to the free radical theory, a higher metabolism leads to a higher production of free radicals, and thus to faster aging and shortened longevity. However, long-lived lines of flies have been reported to have the same metabolic rate (μl O_2/mg/h) as short-lived ones and thus a higher lifetime metabolic potential (ml O_2/mg/lifetime) [4]. In the same way, there is no correlation between the activity score at young age [5], or the mean lifetime activity score (e.g. [6]), and longevity. Negative correlations are expected if there were a tight relationship between energy expenditure and longevity. Clearly speaking, this idea of a tight relationship has been abandoned, even by supporters of the free radical theory [7], and will not be considered further.

2. Adding antioxidants to food

Various antioxidants have been added to food, either during the adult stage or both developmental and adult stages. One difficulty of this procedure is that the amount of ingested food is usually unknown, and that lethality associated with high doses may reflect feeding rejection and thus starvation [8].

Antioxidants were reported to increase longevity in some studies, but not in others (Table 1). It is unfortunate that other traits have not always been studied, because antioxidants can have deleterious effects on traits [9–11] that, as a consequence, increase longevity. For instance, thioproline was reported to increase longevity [12,13] and mating ability at old age [12], but also to decrease metabolic rate, mean weight at eclosion and development speed [9]. Compounds other than antioxidants can also increase longevity: urea, a toxic metabolic waste of flies, increases longevity of mated flies, but not of virgin ones [14]. Since urea decreases fecundity, a trade-off between reproduction and longevity could explain the increased longevity of mated flies (virgin females have a lower fecundity than mated ones). Such trade-offs could explain some positive effects of antioxidants. Therefore, caution is warranted before concluding that antioxidants increase longevity and it would be useful to discover antioxidants with

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Abbreviations: Cu/Zn SOD, cytosolic superoxide dismutase; Mn SOD, mitochondrial superoxide dismutase; CAT, catalase; GR, glutathione reductase; GPX, glutathione peroxidase; GSH, glutathione; hsp70, 70 kDa heat-shock protein

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effects on longevity only, because side effects can be a source of confusion.

Moreover, the positive effects of antioxidants on longevity are often considered as arguments in favor of the free radical theory. Yet, epithalamin, which has no effect on longevity, has been reported to decrease lipid peroxidation and increase SOD and catalase (CAT) activities [15]. In other words, increased longevity and decreased free radical attacks are not necessarily associated.

In 1988, Massie [13] concluded that several antioxidants fail to increase longevity and that the search for efficient antioxidants 'would be facilitated if more investigators would publish their negative results'. It may be added that observing an increased longevity is not sufficient to conclude that there is an improved balance between oxidants and antioxidants. Measuring other traits, particularly those possibly involved in trade-offs with longevity, is needed. Clearly enough, the experiment then becomes rather difficult and better procedures to test the free radical theory may be required.

3. Modifying the activity of antioxidant enzymes

D. melanogaster flies possess cytosolic Cu/Zn and mitochondrial Mn mitochondrial SOD, CAT and glutathione (GSH) reductase (GR), but they have no GSH peroxidase (GPX) [16]. SOD converts O_2^- to H_2O_2, while CAT and GPX convert H_2O_2 to H_2O. GR converts the oxidized form of GSH to the reduced form. In accordance with the free radical theory, increasing or decreasing the activity of these enzymes should increase or decrease longevity, respectively. This activity can be decreased either by chemical inhibition in vivo or by creating mutant flies with a lowered enzyme activity (Table 2). The activity can also be increased using transgenesis (Table 3).

Table 1
Effect on median or mean longevity of male flies with antioxidants added in food

Antioxidant	Percent change	Ref.
Thiazolidine	−2.0	[13]
Vitamin E	**13.7**	[39]
Dinitrophenol	**12.3**	[39]
Nordihydroguaiaretic acid	12.0	[39]
Thiocentrophenoxine	**14.7**	[10]
Propyl gallate	**34.2**	[11]
N-Acetylcysteine	−1.9	[13]
N-Acetylcysteine	**26.6**	[43]
GSH and ascorbic acid	0.0	[13]
Luminol	−2.1	[13]
Plastanox	−3.7	[13]
Pyrogallol	−12.1	[13]
L-Pyroglutamic acid	3.8	[13]
D-Sorbitol	1.9	[13]
Thiodiproponic acid	−11.5	[13]
Thioproline	**16.0**	[12]
Thioproline	**30.4**	[39]
Thioproline	**6.1**	[13]
Xanthine	1.8	[40]
Ascorbic acid	1.7	[41]
Vitamin A palmitate	**17.0**	[42]
Ascorbyl palmitate	−3.2	[13]
Melatonin	0.0	[15]
Epithalamin	0.0	[15]

The compound may be given during both the developmental (from egg to pupae) and the adult stages or during the adult stage only. Significant positive effects are shown in bold characters. When different doses have been used, the best result is given. Note that the same antioxidant may appear several times in the table, with contrasting results.

3.1. Inactivation of enzymes in vivo and in mutants

It seems that no attempt has been made to decrease in vivo the SOD activity in *D. melanogaster*. In *Musca domestica*, the SOD inactivation by diethyldithiocarbamate added in drinking water was paradoxically reported to increase mean longevity by around 40%, with no effect on maximum longevity, i.e. the life span of the longest lived flies. As expected, SOD activity decreased (ca −30%). The CAT activity and the concentration of inorganic peroxides did not vary and the concentration of GSH increased [17]. The authors concluded that the 20% reduction in metabolic rate and the increased GSH concentration explained the increased longevity. However, the results also indicate that SOD is not crucial in normal adult life, since a decrease of its activity is associated with increased life span.

The CAT activity has been suppressed in *D. melanogaster* using the herbicide 3-amino-1,2,4-triazole. A 95% reduction of CAT had no effect on longevity in flies in normal conditions, but strongly decreased survival when flies were given H_2O_2 [18]. CAT seems to be rather useless in normal adult life and to be called for only when facing a strong oxidative threat.

Concerning mutants with decreased enzymatic activity, a sterile cytosolic superoxide dismutase (Cu/Zn SOD) null mutant (no detectable SOD activity) has been reported to live shorter (−80%) and to be less resistant to paraquat (a generator of O_2^-) than controls [19]. A partial Cu/Zn SOD null mutant (3.5% of the SOD activity of the control line) had a similar longevity to controls but a higher sensitivity to paraquat [20]. Finally, a Cu/ZnSOD deficient mutant (60% of the SOD activity of the control line) was not hypersensitive to paraquat, but its longevity was not recorded [20]. However, crosses between this mutant and a wild-type stock or the 3.5% SOD null mutant produced flies with normal longevities. These studies seem to indicate that adult life is impaired when SOD activity is absent but that only a small SOD activity is sufficient to live in normal conditions. Furthermore, about 50% of the normal SOD activity is sufficient to resist a strong oxidative stress.

Mutants with no or low CAT activity (0 or 14% of the wild-type activity) were reported to live as long as controls [21], but a severely decreased longevity has also been observed in the same 0% mutant [22]. Mutants with a 50% CAT activity were not more sensitive to H_2O_2 than controls, while those with a 5% activity were reported to suffer from H_2O_2 exposure. However, this mutant had still a significant resistance when compared to a 0% mutant [23].

Studies of acatalasemic mutants show that longevity is not impaired when CAT activity is absent and that only a small activity is needed to resist a strong oxidative threat. In other words, CAT seems to be less essential than SOD, because normal life seems possible without CAT.

3.2. Overexpression of enzymes

Overexpression of CAT has been observed to have no or slightly negative effects on longevity and to increase resistance to H_2O_2 [24,25], while resistance to hyperoxia or paraquat was not increased [24]. It seems thus that CAT overexpression has no effect in normal living conditions, but has a role in

Table 2
Median or mean longevity and resistance to oxidative stress of flies with a reduced or abolished antioxidant enzyme activity

Enzyme	Activity	Longevity	Stress resistance	Ref.
CAT	5% (chemical)	normal	decreased (H_2O_2)	[18]
Cu/Zn SOD	0% (mutant)	−80%	decreased (Pq)	[19]
Cu/Zn SOD	3.5% (mutant)	normal	decreased (Pq)	[20]
Cu/Zn SOD	60% (mutant)	not determined	normal (Pq)	[20]
CAT	0% (mutant)	normal	not determined	[21]
CAT	14% (mutant)	normal	not determined	[21]
CAT	0% (mutant)	−76%	not determined	[22]
CAT	0% (mutant	not determined	decreased (H_2O_2)	[23]
CAT	5% (mutant)	not determined	decreased (H_2O_2)	[23]
CAT	50% (mutant)	not determined	normal (H_2O_2)	[23]

For the 'Activity' column, 100% is the enzymatic activity of the appropriate control line. Longevity experiments used males, but the sex is unknown for [22]. Stress resistance reports the resistance of males or females to paraquat (Pq) or H_2O_2.

stress resistance. This conclusion is similar to that drawn from CAT inactivation studies.

Overexpression of Cu/Zn SOD did not increase longevity or resistance to paraquat [20], but slightly increased longevity and resistance to ionizing radiation and a negative effect on resistance to paraquat have also been reported [26]. By contrast, flies overexpressing bovine Cu/Zn SOD lived slightly longer than controls, were more resistant to paraquat, hyperoxia and heat shock, but not to H_2O_2 [27,28]. Overexpression of Cu/Zn SOD has also been reported to strongly increase longevity (48%), in a dose-dependent relationship between overexpression and life span extension [25]. Finally overexpression limited to motorneurons of human SOD was observed to increase longevity by up to 40% [29]. Overexpressing SOD thus provides contrasting results, because longevity increases or not, depending on the experiments. SOD overexpression either increases, decreases or does not change stress resistance, depending on the stress examined.

Overexpression of SOD or CAT does not increase life span in some studies. However, the tandem overexpression of CAT and SOD could give a different picture, because the substrate of CAT is the product of the SOD action. In one study, overexpression of both CAT and SOD increased longevity in eight lines, decreased it in one line, and had no effect in six lines [30]. Other authors reported that simultaneous overexpression of CAT and SOD did not further increase the longevity than overexpression of SOD alone and was slightly detrimental in some lines, probably because SOD overexpression was reduced in the presence of CAT inserts [25]. Furthermore, resistance to H_2O_2 was not clearly increased when compared to lines overexpressing CAT only [25].

A 5% lower longevity and a similar resistance to hyperoxia compared to controls have been reported in flies overexpressing mitochondrial superoxide dismutase (Mn SOD), as well as a 3% increased resistance to heat [31]. However, Tower has indicated [32] that a submitted article reports that Mn SOD overexpression increases longevity.

Finally, overexpression of GR did not increase longevity in normoxia but increased survival under hyperoxia conditions [33]. Recently, however, it has been reported that GR does not exist in *D. melanogaster* [34].

As an attempt to measure the rate of aging, and not only longevity, authors have observed negative geotaxis, i.e. the capacity to climb up the vertical side of a vial. No effect of overexpression of Cu/Zn SOD [25], bovine Cu/Zn SOD [28], Mn SOD [31], or of a putative GR [33] has been observed. By contrast, flies overexpressing both CAT and SOD climbed better than controls when getting old [30].

Table 3
Median or mean longevity and resistance to oxidative stress of transgenic flies with increased antioxidant enzyme activity

Enzyme	Activity (%)	Longevity	Stress resistance	Ref.
CAT	180	normal	normal (Pq and hyperoxia) or increased (H_2O_2)	[24]
CAT	150–250	normal or slightly decreased	increased (H_2O_2)	[25]
Cu/Zn SOD	130–170	normal	normal (Pq)	[20]
Cu/Zn SOD	150	slightly increased	increased (radiation) or decreased (Pq)	[26]
Cu/Zn SOD	132	slightly increased	increased (Pq, hyperoxia, heat) or normal (H_2O_2)	[27,28]
Cu/Zn SOD	100–150	increased	not determined	[25]
Cu/Zn SOD	in motorneurons only	increased	not determined	[29]
Cu/Zn SOD and CAT	126–132 (CAT) 143–173 (SOD) (results of three lines with increased longevity)	increased (eight lines), decreased (one line), normal (six lines)	not determined	[30]
Cu/Zn SOD and CAT	150 (CAT) 140 (SOD)	same as SOD lines	increased (H_2O_2), but similar to overexpressing CAT lines	[25]
Mn SOD	105–216	slightly decreased	normal (hyperoxia) or slightly increased (heat)	[31]
GR?	100–200	normal	increased (hyperoxia)	[33]

For the 'Activity' column, 100% is the enzymatic activity of the appropriate control line. Longevity experiments used males, except for CAT overexpression [25] that used females. Stress resistance reports the resistance of males or females to various stresses, such as paraquat (Pq) or H_2O_2. Note that, according to a recent study [34], GR does not exist in *D. melanogaster*, which is the reason for the quotation mark after GR in the first column.

To summarize, SOD overexpression clearly increased (40–50%) longevity in two studies [25,29] and the overexpression of both CAT and SOD was also efficient (up to 33%, in 8 lines out of 15) [30]. Nevertheless, no effect of overexpression of CAT [24,25] or SOD [20], as well as slight positive effects of SOD overexpression [26,27], were also reported. A delay in aging, as assessed by the study of behavior, was observed in one study only. Finally, overexpression increased stress resistance in most of the studies.

4. Conclusions

Most of the studies manipulating the level of antioxidant enzymes indicate that a percentage of their activity is required to live a long life, provided no strong oxidative stress is encountered. In other words, the normal enzyme activity level is sufficient to counteract the normal production of free radicals in everyday life. Furthermore, overexpression does not seem to delay behavioral aging, except in one study. It seems, therefore, that antioxidant enzymes have a small role during normal aging and are not a strong determinant of individual longevity, even if they are widely used in stressful conditions. If stressed, the organism takes the advantage of antioxidant enzymes and the supplement of activity provided by overexpression may be useful.

Antioxidant enzymes could thus be considered mainly as stress enzymes, as it is the case with heat-shock proteins. 70 kDa heat-shock protein (hsp70) is produced in response to a strong stress (e.g. [35]) but, contrary to the claim that flies overexpressing hsp70 live longer (e.g. [36]), it does not seem to be the case [37]. It could thus be hypothesized that different shields are available when facing a stress; among them are antioxidant enzymes and heat-shock proteins. These shields may, however, be considered as the airbags of a car: they will be used during a car crash, but they are of no use for everyday driving.

For the time being, we may accept that 'there is no key evidence at present that establishes a direct causal link between oxidative stress and the rate of aging' [38]. However, SOD overexpression strongly increased life span in two studies [25,29]. Forthcoming experiments will probably determine whether these results can be generalized or are better explained, for instance, by difficulties linked to the transgenesis technique [31,32].

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