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Biochimica et Biophysica Acta 1362 (1997) 116–127

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Mini-review

Lipid peroxidation, antioxidant protection and aging

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Received 1 May 1997; revised 14 August 1997; accepted 19 August 1997

Abstract

The free radical hypothesis of aging proposes that deleterious actions of oxygen-derived radicals are responsible for the functional deterioration associated with aging. Because cellular membranes house the production apparatus of these radicals and because membranes suffer great damage from these radicals, modification of membrane lipids has been proposed to play a major role in the process of aging. Although the relationships between lipid peroxidation and aging have been investigated extensively, the studies have produced conflicting results. Increased lipid peroxidation and decreased antioxidant protection frequently occur, but they are not universal features of aging. Instead, age-dependent changes in these parameters appear to be species-, strain-, sex- and tissue specific. Potential correlations between lipid peroxidation and transition metal concentrations or between lipid peroxidation and declining antioxidant protection have been obscured by the contradictory nature of the findings. Future studies should focus on new approaches for the measurement in vivo lipid peroxidation and on identification of the critical targets of lipid peroxidation. © 1997 Elsevier Science B.V.

Keywords: Aging; Lipid peroxidation; Iron; Antioxidant

1. Introduction

In its original form, the free radical theory of aging proposed that aging is due to the accumulation of unrepaired damage from free radical attack on cellular components [1]. Modern thinking interprets the free radical hypothesis in terms of oxidative stress, and the reformulated theory proposes that aging is caused by a shift in the balance between the pro-oxidative and anti-oxidative processes in the direction of the pro-oxidative state. It is postulated that aging results from an increase in oxidative damage to lipids, proteins, or DNA or, alternatively, from the effect of the oxidative stress on the regulation of genes that govern developmental processes, including differentiation and aging [2–4].

One of the fundamental assumptions of the free radical theory is that the free radicals that contribute to the aging process are derived directly or indirectly from oxygen. As lipid peroxidation traditionally has been regarded as the major process that produces damage from oxygen radicals, the oxygen-based theory is viewed by some as a lipid peroxidation theory of aging. This idea is reinforced by the fact that oxidized lipid residues are major components of lipofuscin, the fluorescent pigment that accumulates with age in most tissues [5,6]. Several offshoots of the theory have been proposed to explain the link between peroxidation of membrane lipids and the functional deterioration that characterizes the process of

Abbreviations: TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde; GSH, reduced glutathione; GSSG, glutathione disulfide; ESR, electron spin resonance

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PII S0925-4439(97)00067-7

aging [7]. One of these hypotheses is that age-related peroxidation of membrane lipids brings about fluidity changes in the membranes, which in turn disrupt vital functions, such as signal transduction or the maintenance of selective permeability to ions [8–12]. Another hypothesis holds that age-associated pathologies are related to increases in lysosomal enzyme activities caused by destabilization of the lysosomal membrane [13]. Other ideas involve the production and progressive accumulation of dysfunctional DNA or protein molecules, arising from adduct formation with lipid peroxidation products [11,14–19].

Although the relationships between lipid peroxidation and the process of aging have been investigated extensively, a number of issues remain unresolved. This review article addresses the controversial findings that have been obtained regarding age-associated changes in lipid peroxidation and antioxidant status and considers the extent to which lipid peroxidation may contribute to the aging process. The chemistry of lipid peroxidation, role of transition metals, and effects of antioxidants have been reviewed elsewhere in detail [20–22]. In addition, the relationships between aging and oxidative damage to proteins or DNA are beyond the scope of this review, and the reader is directed to the excellent articles of other gerontologists for this information [3,16,23–26].

2. Age-associated changes in lipid peroxidation

2.1. Thiobarbituric acid reactive substances (TBARS)

A number of studies have examined the effect of aging on various measures of lipid peroxidation in tissues of mammalian origin. Most of these studies have measured TBARS content as a marker of endogenous lipid peroxidation, and the results have been contradictory. Typical findings are shown in Fig. 1, which contains results on TBARS from our studies with aging Fischer 344 rats [27]. The median survival age for this strain of rats is 24 to 29 months, and the ages studied represent young adulthood (4–5 months), middle age (14–15 months), and old age (26–29 months). In our study, livers were removed and quickly frozen in liquid nitrogen, homogenates were prepared with buffer solution containing EDTA, and the homogenates were immediately acidified with thiobarbituric acid (TBA) reagent. The results show that aging was associated with a 50% increase in TBARS in livers of male rats, but the effect of aging in female rats was a 50% decrease in hepatic TBARS. Curiously, the age-dependent differences in TBARS concentrations were not related to changes in antioxidant molecules, as will be discussed below.

Results from studies of age-associated changes in tissue levels of TBARS are summarized in Table 1. Sex-dependent differences in the effects of aging are evident for liver and brain of Wistar rats as well as for liver of Fischer 344 rats [11,27–32]. In addition, age-associated changes that are seen in some organs are not evident in others. The data for male Wistar rats, for example, indicate that TBARS may increase with age in liver and brain, but not in heart or lung [28,30,31]. Although fewer studies have used mice, these studies also have produced conflicting results [33–36]. For example, hepatic TBARS concentrations were elevated in old female C57BL mice but were unaffected by aging in males [33,34]. These effects of age and gender differ from those reported for rat liver, where elevated TBARS are observed in old males, but not in old females; likewise, the effect of aging on TBARS in mouse kidney contrasts with that reported for rat kidney (i.e., unchanged in mice vs. decreased in rats) (Table 1). Altogether, the findings suggest that age-associated changes are species-, strain-, sex-, and tissue-specific, and that increased lipid peroxidation is not an inevitable consequence of aging in any organ.

There are many uncertainties regarding conclusions based on data obtained using the thiobarbituric acid (TBA) assay. Many investigators report their results as malondialdehyde (MDA), although the assay is not specific for MDA. In most cases, investigators do not publish information about sample preparation or specific details about procedures for the assay. These details are important to know, because artifacts can arise from the exposure of tissues to oxygen. For example, Massie et al. reported that storing samples prior to analysis yielded TBA values that were higher and more erratic than those obtained with fresh tissue homogenates [33]. Furthermore, the production of TBA reactants during the period of reaction is affected by metal ions, antioxidants and chelating agents, as well as the type and concentration of acid added with the TBA [37]. These problems may have contributed to the inconsistencies and contradictions

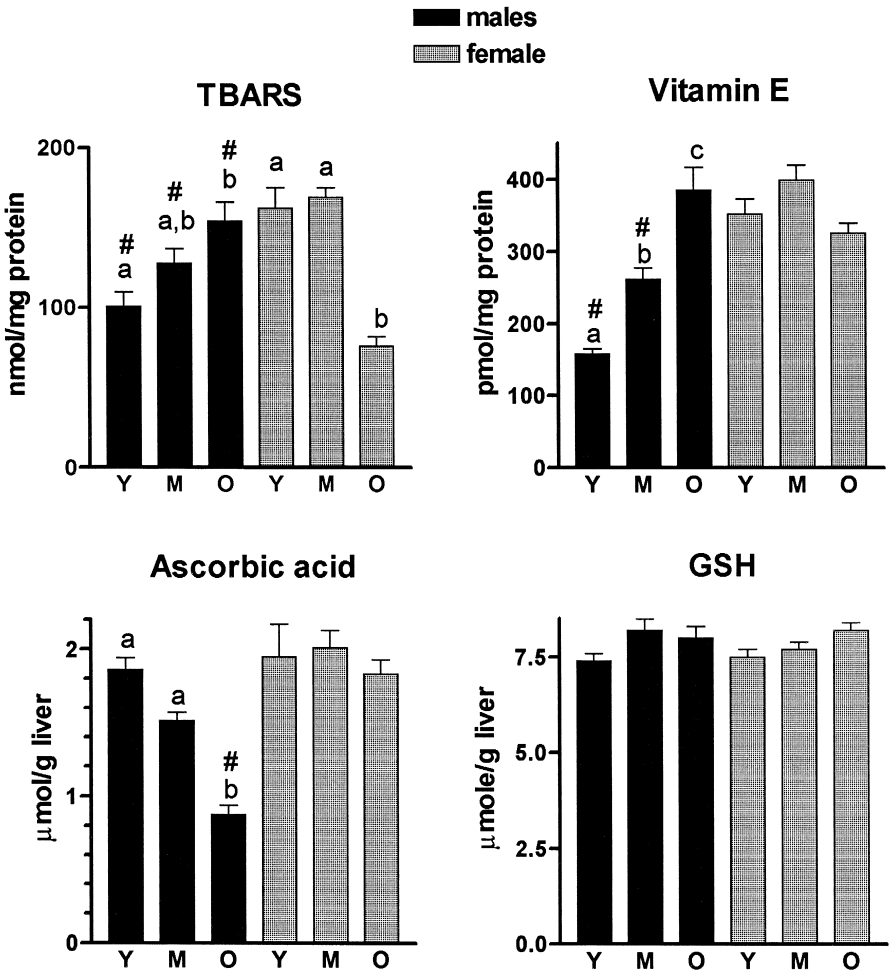

Fig. 1. Effect of age and sex on contents of TBARS and antioxidant molecules in rat liver. TBARS (MDA equivalents) and antioxidant concentrations were measured in whole-cell homogenates prepared from livers of Fischer 344 rats, as described previously [27]. Values are means of 6–7 rats aged 4–5 months (Y), 14–15 months (M), or 26–29 months (O). Different letters (a,b,c) denote significant differences between means of age groups within a given sex ($P < 0.05$, analysis of variance followed by Tukey's test). The absence of letters indicates there are no age-associated differences. Significant differences between sexes ($P < 0.05$) are designated by a # symbol over the mean for male rats.

in the findings from aging studies. However, if the medium used for tissue preparation contains a chelating agent (e.g., EDTA) and an antioxidant (e.g., butylated hydroxytoluene), and if conditions for the assay are kept constant, then comparisons among age groups within the same study should be valid, even though absolute values for TBARS cannot be compared from one study to the next.

2.2. Other indices of endogenous lipid peroxidation

Other investigators have studied the influence of aging on lipid peroxidation using measures of endogenous lipid peroxidation other than the TBARS assay. Expiration of ethane and pentane is considered to be one of the best indices of lipid peroxidation in vivo, and three studies have measured exhaled alkanes in aging male rats [38–40]. Although the exhalation of pentane increased markedly as a consequence of aging, ethane exhalation increased only marginally or not at all. Although the results for pentane suggest that aging is accompanied by an increase in the rate of lipid peroxidation; other factors also contribute to the rate of alkane exhalation. Metabolism of alkanes occurs in vivo and is more rapid for pentane than for ethane [41]. Moreover, the rate-limiting step in the

Table 1
Tissue content of TBARS in aging rats and mice

Animal			Organ					
Species	Strain	Sex	Liver	Brain	Heart	Kidney	Lung	Ref.
Rat	F344	M	↑ [a]	–	–	–	–	[27]
Rat	F344	F	↓	–	–	–	–	[27]
Rat	F344	F	↔	↔	–	–	↔	[32]
Rat	F344	M	↔	–	–	–	–	[14]
Rat	Wistar	M	↑	↑	↔	–	–	[28]
Rat	Wistar	F	↓	↓	↔	↓	–	[29]
Rat	Wistar	M	↔	↔	–	–	↔	[30,31]
Mouse	C57BL	M	↔	–	–	↔	–	[33]
Mouse	C57BL	F	↑	–	–	–	–	[34]
Mouse	$C3B10RF_1$	F	↔	–	–	–	–	[35]
Mouse	$B6D2F_1$	NS	–	↔	–	–	–	[36]

[a]Arrows indicate increased, decreased or unchanged TBARS content in organs of old (24–32 months) vs. mature (4–9 months) animals. NS = Not specified.

metabolism is oxidation by cytochrome P450 [41], and it is well recognized that cytochrome P450-dependent biotransformation decreases with age in male rats [42–44]. Thus, the increase with age in pentane exhalation could be a result of decreased pentane clearance, rather than increased lipid peroxidation.

Other markers of endogenous lipid peroxidation that have been studied as a function of aging include tissue concentrations of lipid hydroperoxides or phosphatidylcholine hydroperoxides and lipid, protein or DNA adducts of lipid peroxidation products (i.e., adducts of MDA or 4-hydroxy-2-nonenal) [17,19,24,45–48]. The findings indicate that tissue contents of hydroperoxides and aldehyde-adducts either increase with age or remain unchanged, depending on the organ examined. For example, Miyazawa et al. [47] found that phosphatidylcholine hydroperoxide concentrations increased in brain and liver but not lung of aging rats, and Draper et al. [19] found that deoxyguanosine-MDA adducts increased with age in DNA from liver and kidney but not in DNA from testes. Using a totally different approach, Zhan and coworkers compared the electron spin resonance (ESR, or electron paramagnetic resonance) spectra produced by tissue plugs from liver and sex glands of aging rats [39]. Tissue samples were frozen in liquid nitrogen and cut into small rods, and the frozen rods were placed directly into the lumen of an ESR machine. ESR spectra were obtained that were consistent with a lipid peroxyl radical and a semiquinone radical; the signals were significantly reduced in organs of old rats compared with those of young-adult and middle-aged rats. Thus, inconsistent findings regarding age-associated changes also have been obtained with indices of lipid peroxidation other than the measurement of TBARS.

Conflicting results from different studies may be explained in part by species-, strain-, sex- and tissue-specificity in the effects of aging, as mentioned above. In addition, the discrepancies may relate, to an important extent, to the use of undefined chow diets that contain a variety of products, which may vary in amount from batch to batch and from manufacturer to manufacture and which often include fish products with unknown amounts of oxidized lipids. Furthermore, most of the studies have not considered gross and histopathological abnormalities that are common in aged animals, nor have they been conducted using pathogen-free animals. Thus, factors that contribute to the state of oxidative stress in vivo also may have confounded the results of studies on aging and lipid peroxidation.

2.3. Magnitude and significance of age-associated differences

A major unanswered question is the biological importance of the magnitude of the reported age-related changes in endogenous lipid peroxidation. For the most part, age-associated increases in various indices of lipid peroxidation are in the range of 1.5 to 2-fold. Can this level of lipid peroxidation cause enough membrane damage to produce detriments in function? Toxicological studies in isolated hepatocytes and isolated perfused liver indicate that extremely high levels of intracellular reactive oxygen are needed to cause acute cell injury, amounts that are several orders of magnitude greater than can be generated by intracellular processes under pathophysiological conditions [49]. However, the amount of reactive oxygen needed to produce significant damage in a chronic exposure situation in vivo is unknown. Thus, there is a need to assess the consequences of a two-fold increase in lipid peroxidation over the lifetime of an animal. Such an assessment would require the establishment of appropriate physiological parameters for measurement, i.e., the

identification of valid indices of pathological injury and/or biological markers of aging.

2.4. Lipid peroxidation potential

The findings considered thus are far from studies aimed at investigating the effects of aging on endogenous, or in vivo, lipid peroxidation. Also, there have been investigations that employed in vitro lipid peroxidation systems and membranes isolated from animals of different ages to determine the effect of aging on lipid per-oxidation potential [50–52]. The general conclusions from recent studies are that lipid peroxidation potential is mainly a reflection of the degree of unsaturation of the fatty acids present in the membranes and that fatty acid unsaturation is a function of both diet and animal age [53,54].

3. Age-associated changes in transition metal concentrations

Transition metals promote lipid peroxidation in two ways: (1) by catalyzing the formation of oxygen free radical species capable of initiating lipid peroxidation and (2) by catalyzing the decomposition of preformed lipid peroxides to propagate lipid peroxidation. The idea that elevated metal content of tissues might be responsible for age-associated increases in lipid peroxidation is not new. In fact, this possibility was put forth more than a decade ago by Massie and coworkers, who reported that total iron concentrations in organs from male C57BL mice were elevated in old age [33]. Since then, evidence has accumulated demonstrating age-associated increases in transition metals, especially iron and copper, in tissues of animals, including humans [55–61]. It should be pointed out, however, that elevated metal content is not a universal finding; like the changes in lipid peroxidation and antioxidant protection, age-associated changes in metal content are tissue- and sex-specific. As an example of the tissue specificity, Sherman et al. reported that copper levels in male rats increased with age in liver, kidney and spleen but not in lung and heart [59]. Similarly, Persigehl et al. found that trace element concentrations in human organs depended on age and environmental influences but showed no clear correlation to either parameter [56].

Nevertheless, several lines of evidence suggest that iron ought to be involved in the lipid peroxidation that occurs with aging [62]. Age pigments contain large amounts of iron, and there are reasons to believe that transition metal catalysis may be required for pigment formation. For example, enhanced iron levels in the medium of cultured rat cardiac myocytes markedly increased lipofuscin accumulation, while deferoxamine had the opposite effect [63]. The administration of iron to house flies also increased lipofuscin accumulation and, at the same time, shortened their life spans by one half [64]. Furthermore, dietary tea, which inhibits the absorption of iron, prolonged the life span of fruit flies [65]. In light of the latter findings, it would seem important to determine if the life span of rodents could be extended by lowering their tissue concentrations of iron by chronic administration of an iron chelating agent, for example.

There is some evidence also, based on serum ferritin studies, that iron stores increase with age in humans as well as animals [66–71]. However, serum ferritin concentrations may not be an accurate reflection of body iron stores, as conditions such as inflammation, liver disease and increased red cell turnover elevate serum ferritin concentrations to a disproportionate degree relative to body stores of iron. Measurements of actual iron concentrations in human organs have not demonstrated age-related increases in all tissues. Notably, four studies measured hepatic iron concentrations and found no age-dependent elevation [55,56,70,71], although a trend towards increased iron stores was observed in women following menopause [70,71]. Interestingly, Halgren and Sourander found that nonheme iron increased with advancing age in almost every region of the human brain, but iron concentrations in the brain were independent of the amount of iron in the liver [55]. These findings suggest that hepatic iron stores also are a poor reflection of the iron content of other body tissues. Additional information about the influence of aging on iron stores in human organs is clearly needed.

Most of the iron in vertebrate tissues is tightly bound to proteins, and is not available for the promotion of lipid peroxidation. Our studies in hepatocytes of male rats indicated that approximately 5% of the total nonheme iron in rat liver is present in the form

Table 2
Low-molecular-weight-chelatable iron and total nonheme iron in hepatocytes of mature and old rats

Age (months)	LMWC iron (nmol Fe^{2+}/mg protein)	Total nonheme iron (nmol Fe/mg protein)
6	0.11 ± 0.02	2.74 ± 0.14
27	0.44 ± 0.05 [a]	7.53 ± 0.30 [a]

Low-molecular-weight-chelatable (LMWC) iron and total nonheme iron were measured in freshly isolated hepatocytes of mature and old male Fischer 344 rats, using ferene as the chromogen [102]. Values are means ± SE for six hepatocyte preparations.
[a] Significantly different from mature rats ($P < 0.05$).

of low-molecular-weight chelatable iron (Table 2). Interestingly, we found that concentrations of low-molecular-weight chelatable iron and of total nonheme iron were 3 to 4 times higher in hepatocytes of old rats than in those of mature rats, providing a possible cause for an age-related increase in lipid peroxidation. On the other hand, total nonheme iron concentrations were still higher in livers of old female rats (unpublished data), yet TBARS concentrations were lower in old females than in old males. The latter findings are consistent with a previous study with mice in which elevations in total iron concentrations were not accompanied by increases in TBARS [33]. Thus, the importance of age-associated elevations of iron stores to the lipid peroxidation that is associated with aging remains controversial and warrants further attention.

4. Age-associated changes in antioxidant protection

4.1. Antioxidant enzyme activities

The influence of aging on antioxidant defenses has been investigated extensively. Many studies have shown that one or more of the antioxidant enzymes or antioxidant molecules decrease as a consequence of aging. This has led to the belief that aging is associated with a decrease in antioxidant status and that age-dependent increases in lipid peroxidation are a consequence of diminished antioxidant protection. A close examination of the published data, however, indicate that age-related changes in antioxidant defenses are quite varied.

The influence of aging on hepatic antioxidant enzyme activities of male and female Fischer 344 rats are shown in Fig. 2 [27]. Catalase, glutathione (GSH) peroxidase, Cu, Zn-superoxide dismutase, and glutathione disulfide (GSSG) reductase activities were measured in post-mitochondrial supernatants prepared from livers of rats whose ages ranged from young adulthood to old age. Age-associated increases in activity were as prevalent as decreases; and the age-associated changes that were observed differed between sexes. For example, catalase activity decreased with age in males and increased with age in females.

Fig. 3 shows data from a parallel study, also in Fischer rats, that demonstrate the effect of aging on the mitochondrial activities of hepatic antioxidant enzymes [72]. The changes found for mitochondrial enzymes differ from those found for cytosolic enzymes. Significant differences between young-adult and old rats were demonstrated for GSH peroxidase, Mn-superoxide dismutase and GSSG reductase activities. These differences included age-related increases as well as decreases and were different for males than for females. We concluded that age-associated increases in antioxidant enzyme activities counterbalanced the decreases, so that overall antioxidant protection was not significantly affected as a consequence of aging [72].

Our findings on antioxidant enzyme activities in livers of aging Fischer rats are not unusual. Age-associated changes in antioxidant protection appear to depend on species, strain, sex and tissue studied. This concept is illustrated by the data in Table 3, which contains a selection of the reported findings on age-dependent changes in GSH peroxidase activity. The variation in results for GSH peroxidase activity in rat heart, i.e., decreased, increased or unchanged in old age [73–76], is a good example of the inconsistency in findings. Results for other antioxidant enzymes are equally variable [27,29–32,72–83].

We recently extended our studies of age-dependent changes in antioxidant enzyme activities to include several oxidoreductases that regulate the thiol-disulfide redox state. We found no age-dependent differences in thiotransferase, protein disulfide isomerase or thioredoxin reductase activity in livers of male and female Fischer 344 rats [84]. There was also no change in the concentration of protein sulfhydryl

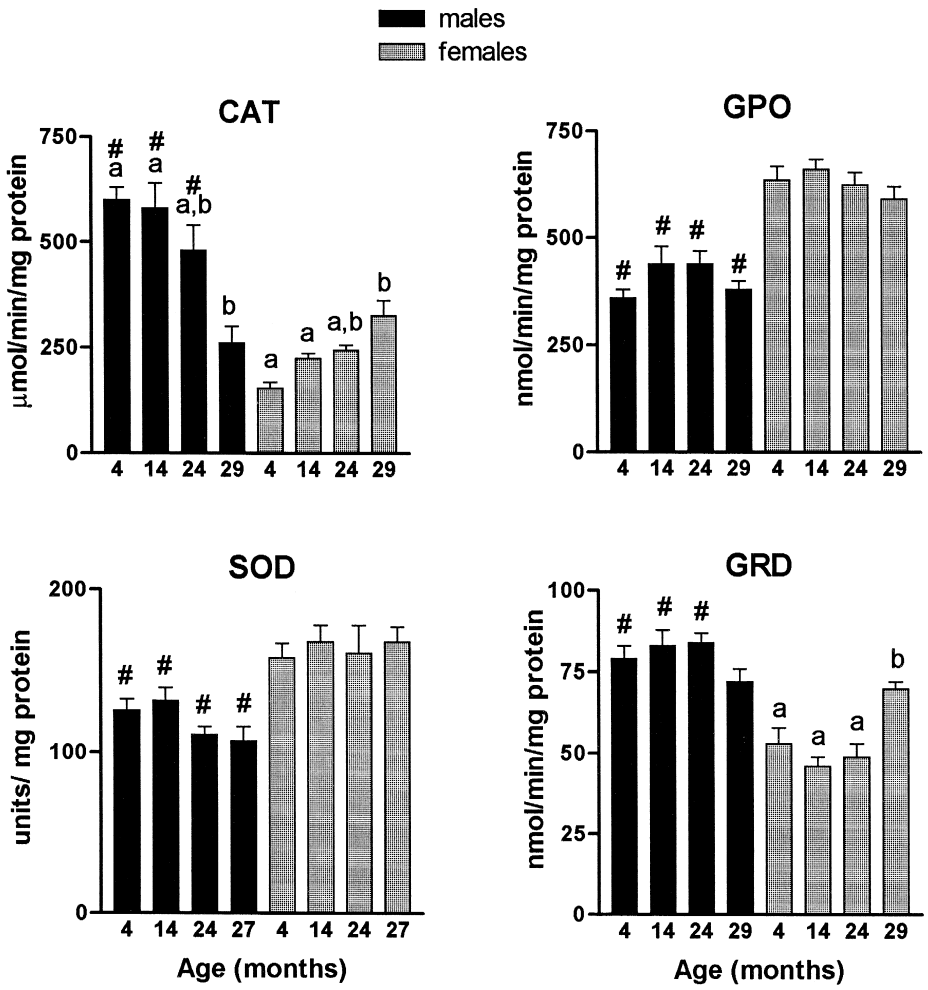

Fig. 2. Effect of age and sex on antioxidant enzyme activities in rat liver cytosol. Catalase (CAT), GSH peroxidase (GPO), Cu, Zn-superoxide dismutase (SOD) and GSSG reductase (GRD) activities were measured in post-mitochondrial supernatants of livers from male and female Fischer 344 rats [27]. Values are means ± SE for six rats. Different letters denote significant differences between means of age groups within a given sex ($P < 0.05$, analysis of variance followed by the Scheffe test). The absence of letters indicates there are no age-associated differences. Significant differences between sexes ($P < 0.05$) are designated by a # symbol over the mean for male rats (Adapted from Rikans et al. [27]).

groups. Our previous studies had shown that liver GSH concentrations are unaffected by aging, and that GSSG reductase activity is either unchanged (in males) or increased (in females) as a consequence of aging [27,85,86]. These findings demonstrate that the capacity to maintain a reduced intracellular state can be well preserved in old age.

4.2. Antioxidant molecules

Results from studies of the influence of aging on the tissue contents of antioxidant molecules also have led to contradictory conclusions regarding age-related differences in antioxidant status and their contribution to the increased lipid peroxidation observed in some tissues of old animals. The effect of aging on tissue GSH content is particularly controversial, with some investigators reporting substantial decreases in old age and others reporting an absence of change [83]. Recent results for human subjects illustrate the inconsistencies that typically are found. GSH concentrations in gastric mucosa decreased as a function of age but not liver cirrhosis, whereas GSH levels in plasma decreased with cirrhosis but not with aging [87]. Another problem is that the effects of aging on tissue levels of lipid peroxidation products do not

correlate well with changes in antioxidant molecules, as illustrated by results from our studies in Fischer rats (Fig. 1). We found that age-dependent differences in hepatic TBARS concentrations could not be explained on the basis of the major biological antioxidants, namely, vitamin E, GSH, and ascorbic acid. One might point to the decrease in ascorbate to account for the increase in TBARS in livers of old male rats, but, in the light of the 2.5-fold increase in vitamin E content, it would be difficult to argue that antioxidant protection was diminished significantly in these livers. Moreover, the TBARS content of female rat liver decreased with age, yet concentrations of biological antioxidants remained the same. An alternative explanation for our data is that the age-associated changes in biological antioxidants could have been inconsequential because of an excess of antioxidant protection in rat liver (see below). In addition, the increase in TBARS in male livers may have been caused by an age-associated decrease in MDA metabolism [88,89] rather than an increase in lipid peroxidation.

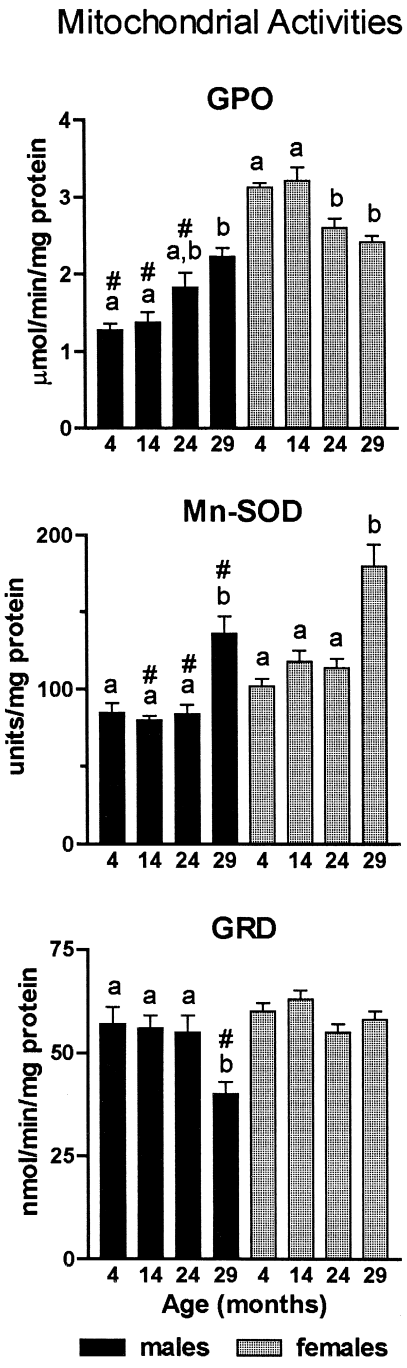

Fig. 3. Effect of age and sex on antioxidant enzyme activities in rat liver mitochondria. GSH peroxidase (GPO), Mn-superoxide dismutase (SOD), and GSSG reductase (GRD) activities were measured in liver mitochondria of male and female Fischer 344 rats [72]. Different letters (a,b) denote significant differences between means of age groups within a given sex ($P < 0.05$, analysis of variance followed by Tukey's test). The absence of letters indicates there are no age-associated differences. Significant differences between sexes ($P < 0.05$) are designated by a # symbol over the mean for male rats (Adapted from Rikans et al. [72]).

4.3. Magnitude and significance of age-related changes in antioxidant status

The biological significance of the reported changes in antioxidant molecules and antioxidant enzyme activities can be questioned. The data in Fig. 1 are fairly typical, in that age-associated decreases in activity are in the order of 25 to 30%. Some of the contradictions between findings from different groups of investigators have occurred because decreases that are not statistically significant in one study are slightly more substantial in another study of same model; the former are reported as maintenance of protection, while the latter are extolled as evidence of a significant decline in antioxidant defense. Moreover, there may be an excess of antioxidant protection in many tissues [3]. For example, studies in isolated hepatocytes indicated that catalase activity could be inhibited more than 70% without affecting sensitivity to diquat-induced cell killing [89]. Likewise, studies

Table 3
GSH peroxidase activity in organs of aging rats and mice

Animal			Organ						
Species	Strain	Sex	Liver	Brain	Heart	Kidney	Muscle	Lung	Ref.
Rat	F344	M	↔ [a]	–	–	–	–	–	[27]
Rat	F344	M	↓	↔	↓	↓	–	–	[76]
Rat	F344	F	↓	Cerebrum ↔	–	–	–	↔	[32]
Rat	F344	F	↔	–	–	–	–	–	[27]
Rat	Wistar	M	↔	↔	–	–	–	↔	[30,31]
Rat	Wistar	M	↓	↓	↓	↓	↓	↓	[39]
Rat	Wistar	M	–	–	–	–	–	↑	[77]
Rat	Wistar	M	↑	–	–	–	–	–	[78]
Rat	Wistar	M	↓	–	↔	–	–	–	[75]
Rat	Wistar	M	–	Caudate putamen ↑ Par.temp.cortex [b] ↑ Substantia nigra ↔ Thalamus ↓	–	–	–	–	[79]
Rat	Wistar	F	↓	↔	↔	↓	–	–	[29]
Rat	Wistar	F	–	–	–	–	–	↓	[77]
Rat	Wistar-Furth	M	↔	–	–	–	↑	–	[80]
Rat	Sprague-Dawley	M	–	–	↑	–	↑	–	[74]
Mouse	C57BL	M	↓	–	↔	↓	–	–	[81]
Mouse	C57BL	M	–	↑	↔	↓	–	–	[82]
Mouse	C57BL	F	↓	–	–	–	–	–	[35]

[a] Arrows indicate unchanged, decreased or increased activity in organs from old (23–30 months) vs. mature (3–10 months) animals.
[b] Parieto-temporal cortex.

with *Drosophila melanogaster* mutants demonstrated that only 50% of the normal Cu, Zn-superoxide dismutase activity and 5–14% of the normal catalase activity were sufficient to withstand experimentally induced oxidative stress and to achieve a normal life span [90,91]. Thus, it is difficult to know if decreases of 25 to 30% in antioxidant enzyme activities jeopardize the antioxidant status of the cell or have any relevance to the aging process.

5. Causality of lipid peroxidation in the aging process

One of the problems faced by gerontologists is that of separating events that cause aging from those that are caused by aging processes. The evidence regarding lipid peroxidation is a clear example of this dilemma. In support of a causal role for lipid peroxidation is the substantial body of evidence that shows a positive correlation between concentrations of lipid peroxidation products and animal age. Furthermore, caloric restriction, the only treatment known to retard the process of aging and extend the life span of animals, inhibits the age-associated increase in lipid peroxidation and prevents the decline in antioxidant protection [10,34,39,45,53,76,83,92]. The obvious caveat is that age-associated changes in a number of factors are reversed by caloric restriction and it remains to be shown that any of these factors is a direct cause of aging.

Conversely, the elevations in products of lipid peroxidation that have been observed in aging tissues may be caused by age-related alterations in processes other than lipid peroxidation. In this regard, there is good evidence that aging is associated with a decrease in the metabolism of certain products of lipid peroxidation. For example, age-associated diminutions have been reported for metabolism of MDA and 4-hydroxy-2-nonenal by liver fractions and isolated hepatocytes [48,88,89]. Although an effect of aging on the biotransformation of ethane and pentane has not been reported, it seems likely that metabolism of these hydrocarbons, may also decrease in old age, especially in male rats where several cytochrome P450-dependent biotransformation reactions are

markedly diminished as a consequence of aging [42–44].

6. Conclusions

Although there are numerous reports of age-associated elevations in lipid peroxidation, reports of decreased or unchanged lipid peroxidation have also been frequent. The same applies to age-associated decreases in antioxidant defenses. The effects of aging appear to be species-, strain-, gender- and tissue specific, yet there is no particular pattern that explains why lipid peroxidation is increased with age in some cases (tissues, strains, etc.) and not in others. It is clear that additional correlative data will not provide new insight into the role of lipid peroxidation in the aging process.

Quantitative considerations are frequently overlooked aspects of free radical injury in vivo, and this aspect of oxygen-induced damage needs to be addressed in the context of aging. At present there is very little information about the consequences of chronic exposure to pathophysiological levels of oxidative stress in vivo. It is possible that the magnitude of the changes that occur in some tissues as a consequence of aging may not be detrimental to normal function.

Evidence to prove or disprove a lipid peroxidation theory of aging awaits the development of new approaches to test the hypothesis. One approach would be to develop ways of manipulating rates of lipid peroxidation in vivo by magnitudes that are physiologically relevant and then determine whether or not these manipulations affect the life spans of mammals. This would also require the development of improved methods for the measurement of lipid peroxidation in vivo. Past investigations have relied mostly on the measurement of lipid peroxidation products; the problem with that approach is that age-dependent effects on the processes responsible for the removal or repair of these products have not been considered. Measurement of ethane and pentane exhalation is the best method available at present for the estimation of lipid peroxidation in vivo. Yet several factors besides the rate of lipid peroxidation contribute to the rate of alkane exhalation, including dietary macronutrients, oxygen concentration at the site of production, and alkane biotransformation; and compensation for age-dependent changes in these influences is rarely made. New advances are also needed in the identification of the critical target(s) of lipid peroxidation. The formulation of testable hypotheses regarding the causality of lipid peroxidation depends on the development of specific ideas regarding sites and consequence of the peroxidative damage to membrane lipids.

Many theories have been proposed regarding the basic nature of aging, but none has been validated as an explanation for intrinsic biological aging. Most gerontologists take the view that theories of aging are not mutually exclusive and that aging is a multicausal phenomenon. It seems likely, therefore, that lipid peroxidation could be one of several factors that contribute to the process of aging, but the contradictory nature of the findings to date suggest that lipid peroxidation is not a major cause of aging.

7. Future perspectives

The free radical hypothesis of aging is very attractive because it provides an explanation for biological aging that can be investigated at the molecular level. The basic tenet of the hypothesis is that age-related loss of function is due to the progressive and irreversible accrual of molecular oxidative damage to essential macromolecules [1,26,93]. The fact that reactive oxygen species are produced continuously and at a high rate as by-products of aerobic metabolism suggests that the mitochondrion is the critical site of the oxidative damage, and data are accumulating to suggest that molecules other than membrane phospholipids are the critical targets [12,24,82,94]. Some evidence indicates that the accrual of oxidatively damaged DNA may be a major cause of the physiological changes associated with the aging process [24,95–97]. For example, steady-state levels of 8-oxoguanine, a product of hydroxyl radical- and singlet oxygen-mediated DNA damage, increase with age in a variety of tissues and could be responsible for age-related increases in somatic mutations or mitochondrial DNA deletions [95,97]. There is also evidence to suggest that oxidation of proteins may be a major cause of aging [14–16,82,94,98,99]. For example, studies with ad libitum fed and caloric restricted mice demonstrated that protein oxidative

damage is associated with aging, and that dietary restriction, which prolongs life span, lessens this damage [82]. Another way that oxidative stress may contribute to cellular aging is by affecting gene expression [3,4,100,101]. In this respect, aging-induced up-regulation of the nuclear binding activities of NF-κB, a transcription factor that responds to oxidative stress, was recently demonstrated in mouse cardiac muscle [101]. The free radical hypothesis of aging provides a framework for many interesting avenues of investigation and it is clear that additional research is needed to complete our understanding of the role of oxidative stress in the aging process.

Acknowledgements

The authors' studies were supported by Grant No. AG04984 from the National Institute on Aging.

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