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Membrane alteration as a basis of aging and the protective effects of calorie restriction

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Abstract

As has been experimentally determined, oxidative modification to biological systems can be extensive, although the identification and stochiometric relation of the reactive species that cause these alterations have not been fully elucidated. In this review, arguments are presented to support the notion that the combined effects of membrane lipid peroxidation and its by-products, reactive aldehydes are likely responsible for membrane-associated functional declines during aging. As evidence for a systemic response to overall oxidative stress, the molecular inflammation hypothesis of aging is discussed by considering that the activation of inflammatory genes act as a bridge linking normal aging to pathological processes.

Keywords: Biological membranes; Oxidative stress; Lipid peroxidation; Calorie restriction; Mitochondria; Reactive aldehydes

1. Introduction

No one would argue the importance of the cell membrane to the maintenance of cellular function and homeostasis. The cell membrane is a structural barrier that plays an essential role in protecting cellular integrity by restricting traffic between inside and outside the cell. Functionally, the membrane acts as a guard that regulates movement into the cytoplasm and as a transducer to amplify message signaling through the cytoplasm into the nucleus. The variety of intracellular metabolic requirements plus the extracelluar signals that are directed from outside the cell necessitate that the membrane be extremely responsive and dynamic in order to properly execute given functions (Vereb et al., 2003). Such complicated tasks are only possible when the membrane's chemical composition is stable and the complex infrastructure remains intact throughout the organism's lifespan (Yu, 1993).

Aging is often defined as time-dependent deleterious changes in function, accompanied by an increased inability to withstand stresses that make the organism vulnerable to disease. Biological metabolic systems are known to have evolved oxidation/reduction-coupling processes under aerobic conditions; thus, oxidative threats and innate protective mechanisms are deemed a part of life processes and traits. Because aging phenomena apparently occur in all biological systems examined so far, age-related changes in biological membrane systems should be no exception. Several investigators, including Spiteller (2002), postulate that the deterioration of membrane integrity is the underlying cause of the aging process. Earlier, Zs-Nagy (1994) proposed the essentiality of the cellular membrane in maintaining intracellular homeostasis during aging. Recent publications (Hulbert, 2003; Vereb et al., 2003) drew renewed attention to current views on membrane structure and its functional significance under normal conditions and during the aging process.

The cellular membrane's chemical composition, because of oxidation-responsive unsaturated fatty acids and redox-sensitive protein moieties, such as histidine and sulfhydryl groups as well its as membrane-associated, oxidant-producing activities, make it uniquely susceptible to various pro-oxidants including oxygen, free radicals and other

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reactive species (RS), particularly in the presence of transition metals such as Cu or Fe (Warner and Starke-Reed, 1997). The susceptibility of membrane lipids to oxidative alterations is related to two inherent properties, the chemical reactivity of the fatty acids composing the membrane bilayers and the cellular activities involved with membrane oxidation/reduction reactions. The first property is the peroxidizability of lipids caused by the unsaturation and conjugation of fatty acid double bondings. The second property relates to reactive species production, including the production of free radicals oxidants or reactive aldehydes, from the membrane sites, as a majority of sub and cellular membranes participates in such activities (Kikugawa, 1991). In a recent review, Spiteller (2001) gathered evidence on damaging oxidation by-products that indeed are generated from changes in membrane structure and not by superoxide as commonly assumed.

To appreciate the overall impact of oxidative stress on the membrane, it is essential to consider at least four related, but discrete aspects of oxidative stress: (1) the identity of the reactive species, (2) the specific targets of oxidative modification, (3) selective anti-oxidative defense systems aimed at reactive species and (4) the efficient repair and removal processes of oxidatively damaged cellular components (Yu, 1994). Because full discussions on these four aspects of oxidative stress are out of the scope of this paper, only relevant aspects pertaining to membrane modifications as potential targets of oxidative stress are considered.

2. Oxidative stress and membrane alterations

Before discussing specific topics related to oxidative membrane damage from free radicals and lipid peroxidation, a brief discussion on the major force influencing the cellular redox state of an organism would be helpful.

Oxidative stress is commonly referred to as a condition under which oxidative modification, i.e., damage is inflicted by reactive species (Sies, 1997). In biochemical and physiological terms, oxidative stress denotes a shift in the oxidation/reduction balance in favor of oxidation, which can impose an undue challenge to the biological system just like an acidic condition, challenges the body's pH buffering system.

It is worthy pointing out briefly some major differences between the original free radical theory of aging and the oxidative stress hypothesis of aging (Yu and Yang, 1996). In the original proposal of the free radical theory of aging, only superoxide and hydrogen peroxide were listed as reactive oxygen species that are linked to the metabolic rate of the organism. However, it is now clear that a host of other chemical entities, including non-radicals such as singlet oxygen (Klotz et al., 2000) and reactive aldehydes (Uchida, 2003) are shown to cause oxidative stress in entire biological systems, including the membrane structure. At present, there is no firm evidence to indicate that the free radical production is necessarily connected with metabolic rate and/or increases with age (Yu, 1996; Merry, 2002). One additional difference between the two hypotheses is that the original free radical hypothesis proposed that disease processes leads to aging, which is hard to accept based on what is known about aging today (Yu and Yang, 1996).

About the connection between metabolic rate and aging – an idea that has been in the aging literature since Rubner's proposal on the metabolic theory of aging in 1908 – Hulbert et al. (2004) recently examined this connection and found no straight correlation between the two. Thus, it seems clear that neither the rate of aging nor an organism's longevity are dependant on its metabolic rate or coupled with mitochondrial free radical production, as Rasmussen et al. (2003) also concluded. In fact, a recent paper by Speakman et al. (2004) showed that mice with higher metabolic rates lived longer. Thus, the experimental evidence supporting a common belief that mitochondrial aging is a biological aging clock (which is derived from the free radical theory of aging) is further weakened in wake of these new revelations.

Among most the likely targets of oxidative stress is the lipid–protein structured biological membrane complex for several reasons. For instance, most cellular activities-associated with the membrane involve reactive species production; lipids are exquisitely susceptible to oxidation and proteins contain various redox-sensitive moieties. As early as the 1970s, researchers interested in membrane biochemistry started to document evidence on age-related changes in various cellular organelles (Hegner, 1980). Schroeder (1984) proposed the membrane asymmetry hypothesis of aging based on the possibility of the re-arrangement and re-orientation of different phospholipid subclasses across the bilayer structure for the maintenance of membrane integrity during aging. Zs-Nagy (1994) proposed a membrane hypothesis of aging based on his long-held view that changes in the intracellular osmotic property and ionic permeability are keys to age-related cellular changes. More recently, Else and Hulbert (2003) developed the membrane pacemaker hypothesis of aging based on findings of the life-extending action of calorie restriction's ability to modify the fatty acid composition of the membrane.

Evidence shows that oxidatively modified proteins from reactive oxygen- or nitrogen-derived species also are found in aged tissues (Stadtman, 1988; Warner and Starke-Reed, 1997). However, evidence from the literature also shows that many oxidatively modified proteins may be the result of by-products such as reactive aldehydes caused by the lipid peroxidation process. Kikugawa (1991) delineates protein damage caused by oxidized lipids and cross-linked proteins derived from lipid hydroperoxides. Lucas and Szweda (1998) report adduct formation by the reactive aldehyde, 4-hydroxy-2-nonenal (HNE) in membrane proteins. Although no damage was found to membrane proteins specifically, the degree of membrane fatty acid unsaturation mediated oxidatively damaged proteins and mitochondrial DNA in liver and brain as reported recently (Pamplona et al., 2004).

3. Oxidative stress and mitochondria

Biological sources of free radicals and reactive species are widely spread throughout the cell, but quantitatively mitochondria are considered the major site responsible for intracellular superoxide production. Loschen et al. (1974) first reported evidence for the mitochondrial generation of superoxide radicals and showed that superoxide is the precursor of mitochondrial hydrogen peroxides. Subsequent studies identified two loci of superoxide generation, both localized in the inner membrane of the mitochondrial respiratory chain. One of the suggested sites is the ubiquinone-cytochrome *b* region, probably occurring through the autoxidation of ubisemiquinone. The superoxide production rate is expected to be higher in state-4 respiration with low ADP when respiratory chains are in a reduced condition. This rate is low in state-3 or in the presence of uncouplers when oxygen consumption is high and the components of the respiratory chain are oxidized. Most recently, Nisoli et al. (2004) published an in-depth review of basic bioenergetics and other related topics to mitochondrial function.

The amount of free radicals generated by mitochondria has been estimated to range from 1% to as much as 4% of the oxygen respired by the organism, depending on the physical activity. Since approximately 80% of superoxides produced become hydrogen peroxide through the actions of mitochondrial Mn/Zn-dependent superoxide dismutase, mitochondria are expected to be the most affected by oxidative stress (Sastre et al., 1996; Hagen et al., 1997). Shigenaga and Ames (1994) have produced an excellent review on age-related alternations of mitochondrial structure and function.

Among the several well-known reactive species, lipid-derived aldehydes, 4-hydroxy-2-nonenal (HNE) and 4-hydroxy-2-hexenal (HHE) are most studied for their cytotoxic action in eliciting oxidative stress. HNE is a major aldehyde produced during the peroxidation of n-6 polyunsaturated fatty acids (PUFA), such as linoleic and arachidonic acids. It is estimated that the endogenous concentration of HNE could reach as much as 5 mM (Uchida, 2003). Many of the potentially harmful actions of HNE include the inhibition of mitochondrial ATP translocase and the modification of GSH and SH levels. The activation of mitochondrial permeability transition (MPT) by reactive aldehydes from membrane lipid peroxidation is well-demonstrated (Kristal and Yu, 1998). The opening of the mega channel by these reactive species allows free passage of solutes under 1500 Da, thereby destroying the proton gradient and preventing oxidative phosphorylation.

Lucas and Szweda (1998) reported HNE's modification of mitochondrial proteins during the reperfusion of hearts that were isolated from young and old rats. These investigators found the formation of two proteins adducts in the older, but not the younger heart, indicating the senescent heart to be more vulnerable to lipid peroxidation. HHE, a peroxidative product of n-3 PUFA, has been shown to affect age-dependent MPT with an exquisite sensitivity (Kristal et al., 1996; Kristal and Yu, 1998). Two interesting papers on the cellular and molecular action of HHE were published recently by Chung's laboratory: Je et al. (2004) showed the up-regulation of mitogen-activated protein kinase (MAPK) by HHE through NF-κB activation in endothelial cells and Lee et al. (2004) reported the induction of endothelial apoptosis by HHE. Another important feature of reactive aldehydes is the inactivation redox-sensitive proteins such as histidine, cysteine and lysine moieties (Uchida, 2003).

The reason reactive aldehydes should receive a close attention is because of their unique properties contrasted with free radicals. For instance, compared to oxygen- or nitrogen-derived oxidants, reactive aldehydes have a much longer half-life (i.e., minutes instead of microseconds to nanoseconds for most free radicals). Further, the non-charged structure of aldehydes allows them to migrate with relative ease through hydrophobic membranes and hydrophilic cytosolic media, thereby extending the migration distance far from the production site. Based on these features alone, these aldehydes can be more destructive than free radicals and may have far-reaching damaging effects on target sites within or outside membranes.

In addition to mitochondria, the biological sources of oxidative stress are multiple and are widely distributed throughout the cell. Examples of the cellular activities and metabolic processes contributing to the production of reactive species are the microsomal electron transport system, various immune cells, prostaglandin synthesis, many membrane-associated oxidases and the interconvertable xanthine oxidase/dehydrogenase system, all of which can produce substantial amounts of reactive oxidants as part of normal functions under physiological conditions. Although oxidative damage to biological systems is commonly accepted, in most cases, the precise identity of the responsible reactive oxidant and quantitative measurements has yet to be established. A case in point is a recent publication by Hoffmann et al. (2004), who failed to obtain direct evidence on the destruction of nuclear DNA by reactive oxygen species generated from the mitochondrial respiratory chain in HeLa cells.

4. Lipid peroxidation during aging

Lipid peroxidation is an oxidative process in which unstable fatty acids, due to their conjugated double bond structure, undergo oxidative modification initiated by reactive species, including O_2, singlet oxygen and free radicals (Porter, 1990). For lipid peroxidation to occur, lipid molecules and pro-oxidants must be activated. For instance, the activation of oxygen requires the presence of transition metals known to increase with age (Spiteller, 2001). A search for documentation on increased lipid peroxidation during aging resulted in consistent findings that in aged conditions,

organisms' lipids are indeed more oxidized compared with younger states. For example, the quantification of pentane in exhaled air, as a marker for oxidized lipids, reported by Matsuo et al. (1993) gives most convincing evidence from their longitudinal measurements of in vivo lipid peroxidation, where measurements were made on the same rats throughout their life span. In the same study, these investigators also were able to show that calorie-restricted (CR) rats produced much less pentane throughout their life, indicating the suppressive action of CR on lipid peroxidation.

Accumulated evidence attributes increased lipid peroxidation during aging to several factors, increased and more oxidizable polyunsaturated fatty acids such as 22:6, increased oxidative stress due to reduced anti-oxidant levels, increased production of reactive species including reactive aldehydes and increased pro-oxidant transition metals. Since the aging process is characterized as time-dependent, progressive changes in biological systems, the age-related modification of lipid structures should be considered as one of the major contributors to functional deficits and the age-dependent diseases observed in aged organisms, such as those found by Pepe et al. (1999), where changes of PUFA patterns during aging were correlated with cardiac mitochondrial lipid composition.

One of the major components of mitochondrial lipids, cardiolipin (CL), contains a relatively high and very long-chain PUFA, making the membrane susceptible to oxidative modification. It has been suggested (Crompton, 2004) that the close-association of mitochondrial adenine nucleotide translocase (ANT) with the negatively charged head groups of CL could be interrupted by lipid peroxidation, and thereby destabilize ANT molecules. A new role for CL is further defined in apoptosis-related studies (Iverson and Orrenius, 2004). Accumulating evidence indicates that the release of cytochrome *c* from mitochondria, as what happens during apoptosis, may be modulated by CL and that the reduced CL levels that occur with aging weaken the binding of cytochrome *c* to the inner mitochondrial membrane. This would facilitate the release of cytochrome *c* into the mitochondrial intermembrane space. The nature of the cardiolipin–cytochrome *c* interaction was recently reviewed by Iverson and Orrenius (2004).

5. Age-related changes in membrane lipids

The dynamic, asymmetric nature of biological membranes is characterized by a thermodynamically stable, fluid superstructure consisting of phospholipid (PL), cholesterol, proteins and carbohydrates (Vereb et al., 2003). Various subclasses of PLs function as the backbone of the membrane infrastructure. Many changes have been detected in these membrane constituents during aging, particularly changes in the phospholipid profile as indicated by several studies analyzing membrane PLs. The reduction of the total amount PL, the composition of its subclasses and the asymmetrical distribution across membrane bilayers, are all reported to cause instability that leads to dysfunctional membranes (Schroeder, 1984). A reduction in the total amount of PL membrane is one of earliest signs of membrane alteration during aging. In rat liver, the total PL content is shown to decrease progressively. Hegner (1980) reported a 20% reduction between 2.5 and 26 months of age.

Cholesterol, another important membrane constituent that functions as a membrane stabilizer, is known to increase during aging, and is generally accepted as an underlying cause of age-related membrane rigidity, e.g., the higher the cholesterol levels, the more rigid the membranes. However, this rather simplistic view was disputed based on the findings that the ratio of cholesterol to PLs is a more accurate indicator of lipid physiochemical interactions. However, as will be discussed in a later section, a change in the Chol/ PL ratio is not likely the factor responsible for age-related membrane rigidity. Data clearly demonstrate that age-related membrane rigidity is influenced predominantly by the amount of lipid hydroperoxide and the extent of lipid peroxidation occurring in the membranes (Choe et al., 1995).

One of the significant membrane changes occurring during aging is its fatty acid composition. It was found that with age, an interesting shift occurs in the fatty acid profile toward the reduction of lipid peroxidation by replacing more oxidizable fatty acids with less oxidizable and more stable fatty acids (Laganiere and Yu, 1993; Pieri, 1997). Specifically, highly peroxidizable very long-chained polyunsaturated fatty acids, such as arachidonic (20:4), docosapentanoic (22:5) or docosahexanoic (22:6) acids, are replaced with fatty acids having fewer, less oxidizable double bonds, like linoleic (18:2) and linolenic (18:3) acids; yet, these PUFA are able to maintain a stable membrane structure (Laganiere and Yu, 1989).

This compositional adjustment seems very clever from the standpoint of the organism's defense mechanism against oxidative insult without compromising membrane fluidity. This innate protective mechanism against the attack of free radicals and many other pro-oxidants in nature seems to have developed through the evolutionary process, because long-lived species like humans have fewer very long-chain PUFA and more 18:2 or 18:3 fatty acids compared to birds or rodents, which have much shorter life spans (Pamplona et al., 1998). This protective mechanism that counteracts incessant oxidative stress to sustain membrane integrity has been viewed as a basic survival strategy. These findings are in accord with what was found in centenarians whose erythrocyte plasma membranes showed reduced susceptibility to peroxidation (Rabini et al., 2002).

The next obvious question is whether there are any functional consequences from altered membrane lipid composition, and there is some such evidence: one is the effect of mitochondrial membrane fatty acid composition on mitochondrial permeability and another concerns is proton conductance. A recent report by Brand et al. (2003), who measured the proton conductance of mitochondria isolated

from birds with different body masses, found that proton conductance correlates strongly with body size, noting a shift in the fatty acid composition of the inner membrane. Liver mitochondrial phospholipids from larger birds contained less PUFA and more monounsaturated fatty acids compared with those from smaller birds. Thus, PUFA content positively correlated with proton conductance in these animals. Penzo et al. (2002) reported that mitochondrial membrane fatty acid double bonds increases membrane depolarizing effects, while effect of saturated fatty acids is negligible. These authors suggested that mitochondrial depolarization is likely due to the opening of the permeability transition pore rather than the direct actions of fatty acids on energy coupling.

6. Protective effects of calorie restriction

Gerontologists acknowledge calorie restriction (CR), the reduced intake of calories, as the most effective anti-aging intervention known in laboratory organisms. The consensus is that CR exerts diverse anti-aging actions by modulating multiple factors, including redox-responsive transcription factor and gene expression involved in both physiological and pathological processes, and thereby extends life span. Although the precise mechanisms of CR are yet to be established, one of the strongest possibilities points toward its modulation of oxidative stress (Yu, 2004). A well-covered review by Merry (2002) on the possible mechanisms underlying CR's anti-aging action describes several major issues involved in oxidative stress, mitochondrial bioenergetics and hormonal regulation of membrane fatty acid composition.

If oxidative stress, indeed the cause of the redox imbalance, that interrupts cellular homeostatic mechanisms, and thereby leads to aging, as proposed by the oxidative stress of theory of aging, any intervention that suppresses oxidative stress would exhibit an anti-aging effect. One approach tried was the use of various compounds with a known anti-oxidant property. The outcome of many feeding experimentations resulted in an average mean life span extension of 20% (Yu, 1996). Dietary anti-oxidants were assumed to build up cellular defenses. Yet, this assumption was incorrect, as we know of the complex cellular defense systems and the biochemistry of anti-oxidants' gut absorption. It is unfortunate that these feeding experimentations were limited solely to life span determination without measuring other aging parameters. A better study with an improved design is by Heidrick's group, who fed mice with 2-mercaptoethanol (2-ME) (Heidrick et al., 1984). In addition to life span extension (both mean and maximum were extended approximately 20%), they measured age-related lymphocyte function and lipid peroxidation. Based on mortality rate, these investigators concluded that 2-ME supplementation did not alter the rate of aging, but delayed the onset of disease with age. Although encouraging, this outcome was far less than the 50% extension in both mean and maximum life spans achieved by CR (Kristal and Yu, 1994; Yu, 1996). Many investigators consider that the extension of maximum life span is only achievable by the retardation of the aging process not by ameliorating age-related disease processes. Experimental laboratory interventions, including those using physical exercise, have succeeded in extending only mean life span, without influencing maximum life span.

It was a matter of finding a critical test to evaluate whether CR has any influence on the modulation of the oxidative status in aged animals, because if CR has no suppressive effects on oxidative status, the basic tenet of the oxidative stress theory of aging would be discredited. As it turned out from numerous findings, CR is consistently shown to have both efficient and broad anti-oxidative effects against a wide spectrum of oxidative stress in various organism models, contrasted to any other interventions tried earlier. The question is then, how does CR exert such powerful anti-oxidant effects. The answer can be found in CR's broad efficacy in utilizing multiple strategic approaches in protecting cellular redox balance against oxidative stress during aging (Cho et al., 2003). Just as all aerobic organisms are endowed with a variety of defense mechanisms to guard against oxidative threats, CR is shown to utilize multiple defense strategies to fight against reactive species and various pro-oxidants, including transition metals like Fe (Cook and Yu, 1998; Levenson and Tassabehji, 2004), to suppress oxidative challenge, while boosting the reductive capacities, such as cellular GSH levels during aging (Kim et al., 2002).

Since mitochondria are considered a hub of oxidative stress, and because of their high oxygen consumption, they have been the subject of many studies on oxidative damage (Shigenaga and Ames, 1994) and its attenuation by CR (Yu, 1996). In a special issue of *Aging Cell* (see vol. 3, 2004) entitled, ''Mitochondria and aging—facts and fancies,'' authors who are focusing on issues of bioenergetics and membrane potential highlight many unsettled and controversial topics related to mitochondrial aging. Among the interesting papers, two are more relevant to our discussion on CR. One, by Merry (2002), covers oxidative stress and mitochondrial function with aging and the effect of CR by critically evaluating many outstanding issues about oxidative stress and how CR modulates it and what is known about CR's effects on oxidatively stressed, aged mitochondria. A second paper by Speakman et al. (2004) describes a study in which mice with a high metabolic rate and greater mitochondrial uncoupling have extended life spans. These authors extend earlier investigations by Brand (2000), who showed mitochondrial inefficiency in aging develops from mitochondrial uncoupling, which is likely due to protons leaking through the mitochondrial inner membrane. The physiological significance of this uncoupling was taken, in part, as a safety mechanism for mitochondrial defense against a possible membrane potential collapse and free radical over-production. Interestingly, in CR animals, both membrane potential and mitochondrial uncoupling were

shown to be modulated in effort to protect mitochondrial integrity (Merry, 2002). In a more recent paper (Lambert and Merry, 2004), hydrogen peroxide, measured as mitochondrial ROS production, was suppressed by CR. What is more intriguing about their findings is that CR's suppressive action against hydrogen peroxide production was partially reversed by 2 weeks of insulin treatment. It would be important to delineate underlying mechanisms of insulin's counteraction against CR by exploring the insulin's effect on defense enzymes and nitric oxide synthase activation (Merry, 2002).

Recent information on gene profiling data on aged animals showed a substantial modulation during aging and by CR paradigm (Cao et al., 2001). Of particular relevance to oxidative stress is the up-regulation of redox-sensitive and pro-inflammatory genes such as IL-2, IL-6 and TNFα that are attenuated by CR. The biochemical analyses on anti-inflammatory action of CR on various transcriptional factors like NF-κB, AP-1 and PPARs were reported recently (Kim et al., 2002; Sung et al., 2004). The increase in the inflammatory gene expression strongly indicates the dysregulated cellular signaling pathways, making the organism to be more vulnerable to other insults or perturbants. Based on the molecular events regarding the CR's regulation of altered genes, molecular inflammation hypothesis of aging (Chung et al., 2001) was proposed to signify the physiological consequence of the age-related inflammatory status that acts as a transitional phase converging to the chronic diseased state.

CR, as a powerful modulator of oxidative stress, (Yu and Chung, 2001), can effectively reduce the risk for the inflammatory condition by turning off activated transcription factors, and thereby induce resistance to age-related chronic diseases. Recent data on CR's suppression of the pro-inflammatory adhesion molecule activation that is known to be associated with various age-related disease processes, further illustrate such a possibility (Zou et al., 2004). Many medical researchers now hold a view that major chronic diseases such as cardiovascular disease, dementia, osteoarthritis, cancer, diabetes and neurodegeneration are in fact inflammation-related procecesses (Tracy, 2003). Increased several key clinical cytokine markers for inflammation found in aged people further support this view (Franceshi and Bonafe, 2003).

A salient point of the inflammation hypothesis is the possibility that the organism's overall, systemic redox status being responsible for age-related disease rather than the failure of any specific function. In the literature, oxidatively damage by free radicals is generally accepted as a causative factor for many diseases. However, evidence for the exact molecular processes or the identity of responsible entities is mostly lacking, and the ability to obtain such evidence is technically limited. In view of the lack of evidence at present on the specific components and factors responsible for age-related diseases, it would make more a sense that the uncontrolled systemic activation of various pro-inflammatory genes under oxidative stress may have a more extended impact on the pathogenesis of most age-related chronic diseases than free radicals. Therefore, CR's ability to maintain a proper redox balance is likely the key-determining factor in interrupting the link between normal aging and the pathological process, as proposed in this inflammation hypothesis of aging (Chung et al., 2000).

7. Concluding remarks

The current enthusiasm for rapid development in the exciting area of molecular biology tends to overshadow the fundamental importance of a dynamic, functional biological membrane that controls all cellular trafficking, including into the nuclear membrane. In this brief review, various aspects of membrane alterations by lipid peroxidation during aging were described. Arguments were made to emphasize that lipid peroxidation by-products could be more potent and damaging than free radicals. Although age-related damages to mitochondrial function are not likely to be the biologic clock of an organism, nonetheless, the mitochondrion, as a prime model of a membranous superstructure, can show us how altered membranes could modify functionality. In the final section of this review, the activation of age-related redox-responsive pro-inflammatory transcription factors were described as a cross-talk link between normal aging and pathological processes. This review also discussed oxidative stress in its ability to modify specific proteins, DNA and lipids, while strongly highlighting its powerful ability to elicits an overall, systemic reaction by turning on redox-sensitive cellular signal pathways, and thereby raising the risk and vulnerability to pathogenic insult. There are many outstanding, unsolved issues on aging and oxidative stress, and the time is long overdue for more intensive studies on the identification of oxidative species, technical improvements in quantitative in vivo measurements of oxidative modifications, and the establishment of more reliable physiological markers for redox status and the molecular clues that associate increased disease incidence with increased oxidative stress. It is hoped that some of these questions and issues may be partially answered in this review.

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