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Age-related Hearing Loss and its Association with Reactive Oxygen Species and Mitochondrial DNA damage*

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Age-related hearing loss, known as presbycusis, is characterized by the progressive deterioration of auditory sensitivity associated with the aging process and is the leading cause of adult auditory deficiency in the USA. Presbycusis is described as a progressive, bilateral, high-frequency hearing loss that is manifested on audiometric assessment by a moderately sloping pure tone audiogram. Approximately 23% of the population between 65 and 75 years of age, and 40% of the population older than 75 years of age are affected by this condition. It was estimated in 1980 that 11% of the population was 76 years or older and this number is expected to almost double by the year 2030. When one considers that the population over 65 years of age is experiencing the most accelerated development of hearing loss, the potential socioeconomic ramifications are staggering. Curiously, the frequency of presbycusis varies across different societies. This discrepancy has been attributed to many factors including genetics, diet, socioeconomic factors, and environmental variables. The purpose of this article is to review the various molecular mechanisms underlying presbycusis and to offer insights into potential methods of mitigating the effects of aging on hearing impairment *Key words: aging, mitochondrial DNA mutations, presbycusis, reactive oxygen species.*

INTRODUCTION

Aging is associated with a wide array of physiological, biochemical, and molecular changes including progressive DNA damage, deterioration of mitochondrial function, reduction of cellular water concentrations, ionic changes, vascular insufficiency, and decreased elasticity of cellular membranes. Studies have proposed that many factors contribute to this process, such as: altered vascular characteristics; reduced red blood cell velocity and vascular plasticity; increased vascular permeability (1, 2); reductions in oxygen and nutrient delivery and waste elimination (3–6); genetic mutations; and a significant presence of reactive oxygen species (ROS), otherwise known as free radicals. Concurrent with the increase in ROS is a decreased production or function of the endogenous enzymes that protect the cell against ROS damage including superoxide dismutase, catalase and glutathione peroxidase. These ROS have been shown to contribute in part to producing mitochondrial DNA (mtDNA) damage by causing deletions in the mitochondrial genome. These deletions are an important component of the age-associated diminution in auditory sensitivity. Specific deletions are known to be directly proportional to aging such as the common aging deletion in humans, which is 4977 base pairs in length (4834 base pairs in rats). When mtDNA

deletions reach a certain level, the mitochondria become bioenergetically inefficient. This inefficiency can be measured through reductions in specific enzymes (cytochrome oxidase (COX), succinate dehydrogenase (SDH), nicotinamide adenine dinucleotide (NADH)), as well as by using flow cytometry to measure reductions in mitochondrial membrane potentials (MMP) (Fig. 1), which serves as a marker of mitochondrial function, and by the measurement of specific deletions or point mutations in the mitochondrial DNA genome. Fig. 1A and 1B are typical histograms of cell fluorescence in young and old rat lymphocytes (10^6) incubated with 0.5 nM DiOC₆. Preliminary results revealed a two- to threefold greater intensity of DiOC₆ staining of peripheral blood lymphocytes (PBL) of young compared with old rats (Fig. 1C). Reductions in mitochondrial function and increases in mtDNA deletions have been identified within heart, brain, liver and skeletal muscle samples in aged rats, mice, monkeys and humans (7–11).

Aging thus involves the growing accumulation of metabolic and physiologic changes associated with an increasing susceptibility to disease. There are four main sources for failure of biological systems that may in part contribute to the aging processes.

1. Gene repression: essentially, this is a systematic, and probably programmed, age-dependent gene deterioration. Semsei et al. have demonstrated that increased intracellular potassium results in chromatin condensation with a consequent decline

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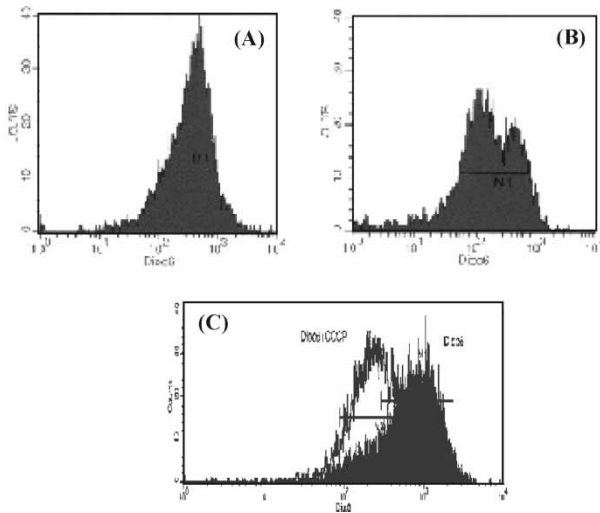


Fig. 1. Mitochondrial membrane potential (MMP) was studied by flow cytometry. 3,3'-Dihexyloxacarbocyanine (DiOC₆), which is used for the quantitative measurement of MMP, is a lipophilic, cationic, fluorescent probe. Peripheral blood lymphocytes (PBL) of young (6 months) and old (24 months) (10⁶) rats were equilibrated with very low concentrations (0.5 nM) of DiOC₆. (A) and (B) Typical histograms of cell fluorescence in young and old rat lymphocytes (10⁶) incubated with 0.5 nM DiOC₆. There is a two- to threefold greater intensity of DiOC₆ staining of PBL of young compared with old rats (C). The mean fluorescence of cells treated with and without carbonyl cyanide m-chlorophenylhydrazone (CCCP) (20 μM) is shown in (C). Cells treated with CCCP consisted of low fluorescence, about 30% of control.

in RNA synthesis (12). This general decline in protein synthesis may in part account for the age-dependent decrease in gene expression, leading to the turning off of specific biological systems. Hence, a threshold exists beyond which homeostasis begins to fail.

2. Intracellular communication: this is based upon the fact that cells require specific spatial arrangement which provides for the rapid transfer of molecules to neighboring cells. Any change in this spatial arrangement may lead to breakdown in cellular communication with resultant tissue aging, disease and death. It is known that mutations in connexin-26, a gap junction protein, are responsible for a major form of non-syndromic recessive deafness and a more limited form of non-syndromic dominant hearing loss (13). It is possible that a similar mechanism may be involved in age-related hearing loss.
3. Waste accumulation: over time there are significant increases in waste products. The ability of cells to handle and process these products progressively decreases, hence, an increase in accretional defects,

i.e. intima thickening, lipofuscin accumulation, and damaged collagen.

4. Age-related depletional defects: over time there is progressive breakdown of cellular function. When this accumulates with age, entire organ systems lose their normal function, often leading to disease states as well as normal physiologic aging (14).

There are many hypotheses in the current literature providing explanations for senescence. Three of the most convincing theories are the telomerase theory of aging, the dysdifferentiation hypothesis of aging and the membrane hypothesis of aging (MHA; also referred to as the 'mitochondrial clock theory' of aging).

The telomerase theory of aging suggests that there is a reduction in telomere length over time. The end of a chromosome is made up of a structure called the telosome. The tip of the telosome is a region of DNA repeat sequences and associated proteins collectively known as the telomere. It is hypothesized that DNA transcription and replication are affected by position effects mediated by the telomere. Reduction in the length of the telomere and alterations in its chromatin assembly may explain the instability that occurs during senescence as well as the immortalization process *in vitro* (15). In tumor-derived cell lines, telomeres are maintained by the ribonucleoprotein enzyme, telomerase. However, telomerase activity is repressed in practically all normal human somatic cells. Thus, there is an inherent problem of end-replication creating a tendency toward progressive telomere shortening. Cumulatively, this predilection leads to limited replicative capacity, chromatin instability and eventually, cellular senescence. Viral oncogenes or certain somatic mutations can block cellular aging probably by activating telomerase. Telomere shortening is therefore effectively prevented and this appears to be an important mechanism for sustaining the cellular growth of tumors (16). Low-level telomerase activity has also been demonstrated in normal human T and B cells, which was also shown to decrease with aging (17). Although many aspects of telomerase activity remain undefined, it has been hypothesized that the balance between telomere shortening and telomerase activity may underlie cellular aging processes. Therefore, although unproven, changes in telomeres may also predispose to the development of presbycusis.

The dysdifferentiation theory suggests that aging is a continuum of programmed differentiation leading to either a cessation of normal gene activity or a systematic activation of genes whose effects are deleterious to cellular function. Support for this

theory is provided by apoptosis (programmed cell death) studies in *Caenorhabditis elegans* (*C. elegans*) (earthworm). These experiments elegantly elaborated the genetic mechanisms responsible for controlling cell death. The maintenance of homeostasis for cellular metabolism and function consumes a large fraction of total body energy expenditure. This is engineered by the delicate balance between cellular proliferation and death. The *Bcl-2* gene, for example, exists in progenitor and long-lived cells and therefore appears to have a key function in the cells of the developing embryo. The gene appears to prevent oxidative damage to cellular organelles and lipid membranes. Studies have demonstrated that *Bcl-2* protects cells from the toxicity of H_2O_2 or t-butyl hydroperoxide in a dose-dependent manner (18, 19). *Bcl-2*-deficient mice demonstrate changes expected of more rapid cell death, including fulminant lymphoid apoptosis of systemic organs (20). *Bcl-2* also inhibits other types of apoptotic cell death, implying a common mechanism of lethality. Studies have demonstrated, for example, that *Bcl-2* protected cells from H_2O_2 and menadione-induced oxidative deaths (18, 21). Another protein that appears to operate as an accessory to *Bcl-2*, is a 21 kd protein referred to as *Bax*. In experiments by Hockenberry and colleagues, the suggested model of the ratio between *Bcl-2* and *Bax* appears to determine survival or death following an apoptotic stimulus. Specifically, elevated expression of *Bcl-2* appears to be preventative, while that of *Bax* favors the apoptotic process.

Lastly, the membrane hypothesis of aging states that aging is related to decreasing effectiveness of cellular protective and reparative mechanisms. This yields biochemical and metabolic errors, which progressively accumulate resulting in cell death (22). The membrane hypothesis of aging (MHA) further postulates that cellular senescence is attributable to the cross-linking action of free oxygen radicals within the cellular membrane. Additionally, ROS lead to lipid peroxidation, polysaccharide depolymerization, nucleic acid disruption, and oxidation of sulfhydryl groups leading to enzyme inactivation (23). Therefore, the MHA suggests that ROS-induced cell membrane structural damage is the primary mediator in cellular aging (24, 25).

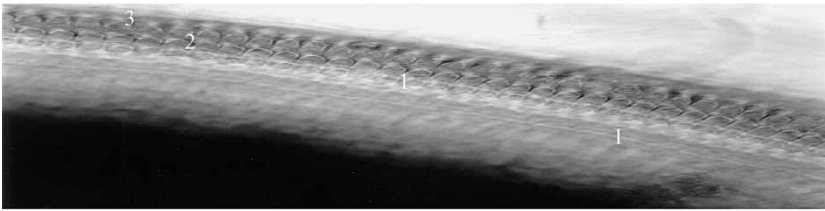
Careful analysis of the above mechanisms suggests that certain aspects of the three leading theories of aging may be interrelated. That is, free radical species lead to genetic and cellular alterations resulting in cellular dysfunction, and consequently senescence, and perhaps presbycusis. It is even more intriguing to realize that a trigger for the *Bax* gene are the ROS (26). Thus, a critical analysis of the prominent hypotheses of aging suggests that aspects of all three theories are likely to apply. Specifically, the generation

of ROS damages cellular integrity, which may lead to alterations in gene expression, including telomere shortening and activation of *Bax* genes, resulting in aging, presbycusis and ultimately death.

As presbycusis represents one aspect of aging, it is imperative thus to understand these molecular mechanisms of aging. In our laboratory, studies have focused on the mitochondrial theory of aging and its applicability to presbycusis. There are, however, several animal models that have been used to study presbycusis. These models have served to illuminate the various molecular mechanisms underlying age-related hearing loss. One such model is the C57BL/6J strain of mice, which demonstrates an age-dependent, high-frequency, sloping loss (27). The adult hearing loss (*Ahl*) gene, characterized as recessive and mapped to chromosome 10, has been identified in the C57 mouse strain and is the presumed cause of progressive hearing loss in this species. A proposed mechanism for this progressive hearing loss is that the *Ahl* gene mediates, through mRNA expression, a decrease in protective enzymes and therefore an increase in levels of oxidative stress within the period of time that the C57 mouse strain undergoes increased exposure to free radicals and its most significant diminution in hearing (28). More recently, the *Ahl* gene has also been implicated in noise-induced hearing loss (NIHL) (29).

In other studies, cochlear microphonic (CM) sensitivity has been shown to vary with age, with a gradual decrease in sensitivity from 12 to 24 months of age (30). Rat cochlear action potential (CAP) sensitivity has also been shown to decrease with age, and changes in CAP waveform with age have been shown (31). In a study of rat auditory brainstem response (ABR), delay in conduction times and waveform amplitude decreases were noted to occur in older animals (32). Our laboratory has studied groups of rats varying in age and has documented evidence of presbycusis using changes in cochlear microcirculation, electrophysiology, molecular biology and histology (33, 34). Furthermore, histologic analysis of presbycusis subjects demonstrate age-related hair cell degeneration and loss (35). Human studies reveal a preferential loss of outer hair cells (OHCs) (36, 37). Loss of OHCs is greatest in the first half of the basal turn (36, 38). This correlates tonotopically with the high-frequency loss seen in human presbycusis. In the rat, age-related hair cell loss is also predominantly OHC in nature. In a study of elderly rats, inner hair cell (IHC) losses ranged from 3.1% to 9.2%, while OHC losses ranged from 7.4% to 46.8%. The IHC loss was greatest in the upper apex while OHC losses were greatest at the basal turn (39). While there are species-specific variations in cochleotopic patterns of hair cell loss, the rat is an appropriate aging model (30, 32, 39) (Fig. 2). Several

Photomicrograph of cochlea from a young rat



Photomicrograph of cochlea from old rat

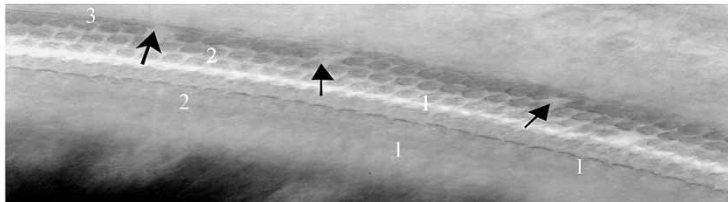


Fig. 2. Photomicrograph ($40\times$) of the cochlea from a representative animal. It shows a surface mount preparation of the basillar membrane of the right organ of Corti with osmium tetroxide staining from a 4-month old (A) and an 18-month old (B) rat. Outer hair cell rows are shown by 1, 2, and 3. '1' denotes the single inner hair cell row. Loss of hair cells is denoted by arrows. No obvious hair cell pathology is noted in (A). Hair cell loss is shown in (B).

human studies have also demonstrated accelerated hearing loss in patients with Alzheimer's disease and other cognitive and neurodegenerative disorders (40–42). There is a genetic predilection for Alzheimer's disease and perhaps these findings can be extrapolated to auditory dysfunction as well. As the formation of reactive oxygen species (ROS) and their subsequent effects on mitochondrial integrity are an integral mechanism in presbycusis, it is important to consider these processes in greater detail.

REACTIVE OXYGEN SPECIES (ROS)

Reactive oxygen species (ROS) contain an unpaired number of electrons, making them chemically reactive and extremely toxic to cellular and subcellular structures. ROS have been implicated in more than 100 clinical conditions (43). They are produced *in vivo* during mitochondrial respiration, as well as via auto-oxidation of chemical and biological molecules. ROS are also environmental contaminants and can be formed from ionizing and ultraviolet radiation. The most common ROS are superoxide anion (O_2^-), hydroxyl radical (OH^-), hypochloride (OCL^-) and nitric oxide (NO^-). Not only is the pathogenic role of ROS becoming acceptable in the realm of human disease, but their contribution as active participants in determining disease activity is also becoming increasingly recognized (44). Despite this primacy, ROS are notoriously difficult to quantify in biological systems. However, the use of modern assay techniques has made this less of a limitation (43).

There is extensive support in the literature for the protective effects of antioxidants and ROS scavengers. For example, tocopherols decrease the process of

atherosclerosis and delay death from myocardial infarction, presumably by inhibiting lipid peroxidation (45). Carotenoids, such as β -carotene and other plant pigments, may also have preventive effects against cancer and cardiovascular disease (45–47).

Available data also suggest that many of the pathological correlates of Alzheimer's disease are precipitated by oxidative stress-induced mechanisms (48–50). Similarly, Parkinson's disease has also been associated with oxidative stress, increased lipid peroxidation, reduced levels of glutathione (GSH), high concentrations of iron, and free radical generation via auto-catalytic mechanisms within neuromelanin containing catecholaminergic neurons (51, 52). Recent studies have further demonstrated that aging in the central nervous system is directly correlated with oxidative stress. In the harlequin mouse model, mutations in the apoptosis-inducing factor (AIF) gene resulted in greater susceptibility to ROS-induced damage (53). A lack or down-regulation of AIF in neuronal cells renders them more sensitive to peroxides and to a much larger extent vulnerable to progressive increases in ROS-induced damage. This observation provides a molecular mechanism by which free radical damage can lead to neuronal death.

Many intrinsic enzyme systems protect cells from oxidative damage. These include superoxide dismutase (SOD), glutathione peroxidase, and the glutathione transferases, and catalase. Additionally, a variety of small molecules in the human diet are required for antioxidant defenses. Experimentally and clinically, it is well known that a primary source of ROS generation is through oxidative phosphorylation, ischemia/reperfusion or prolonged hypoperfusion, such as that seen in myocardial infarction, cerebrovascular accidents,

aging, and possibly sudden sensorineural hearing loss and presbycusis. It is clear that in the aging cochlea there is a significant reduction of blood supply and the ongoing need for energy generation through oxidative phosphorylation (2). Thus, these two processes, as well as others, allow for the generation of ROS within the cochlea.

MITOCHONDRIAL DNA DELETIONS (MTDNA DEL) AND HEARING LOSS

Mitochondria are unique organelles possessing their own DNA as well as their own enzymatic constituents to allow for transcription and translation of genetic information into proteins. Each mtDNA codes for a complete set of ribosomal (rRNA) and transfer RNA (tRNA). Additionally, mtDNA codes for 13 of the approximately 60 polypeptides necessary for oxidative phosphorylation. These include seven of the 25 subunits of respiratory complex I (ND1-4, 4L, 5, and 6)(NADH-ubiquinol-oxidoreductase), one of the approximately nine subunits (cyt b) of respiratory complex III (ubiquinol-cytochrome c oxidoreductase), three of the 13 subunits of respiratory complex IV (COI, COII, and COIII) (cytochrome c oxidase), and two of the 12 subunits of respiratory complex V (ATP6 and 8) (ATP synthase). The remaining subunits of these complexes are encoded by nuclear DNA (54–56).

It has been proposed that mitochondrial genomic mutations may be a major cause of human diseases. In 1959, the first patient with a mtDNA deletion as a cause of their illness was reported by Ernster. Luft is often credited with recognizing the importance of mitochondrial medicine in 1962. Mitochondrial mutations and subsequent cytoplasmic segregation contribute to neuromuscular disorders such as Kearns–Sayre/chronic external ophthalmoplegia plus syndrome (57–60), mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) (61), subacute necrotizing encephalomyopathies (SNE, Leigh syndrome), progressive neuronal degeneration of childhood (Alpers syndrome) (59), and myoclonic epilepsy associated with ragged-red muscle fibers (MERRF) (54, 62). Interestingly, up to 67% of patients with mtDNA disorders also manifest sensorineural hearing loss (63).

Mitochondrial DNA deletions with subsequent segregation and enrichment of the deletions throughout life are contributing factors in the aging process (9, 64, 65). MtDNA deletions in the human heart are age-dependent (9), and these mitochondrial mutations accumulate progressively until death. Cells that accumulate large numbers of mitochondrial mutations become bioenergetically deficient. This compromised

energy state explains their important role in many human diseases (57). MtDNA deletions accumulate preferentially in post-mitotic tissues such as the inner ear, retina, skeletal muscle and brain. This appears to account for the increased susceptibility of these tissues to hypoxia, ischemia and aging. Richter has shown that extensive oxidative damage occurs to mitochondrial and nuclear DNA, and has identified the presence of 8-hydroxydeoxyguanosine (OH⁸dG) in mtDNA and nuclear DNA (nDNA). This compound is formed by excited oxygen species and is considered a marker for DNA damage. Mitochondria treated with prooxidants have an increased level of OH⁸dG. This increased level may be caused by several factors, including extensive oxygen metabolism, inefficient DNA repair and lack of mitochondrial histones. Studies have shown that increasing amounts of ROS occur as a function of age, which lead to an increase in membrane peroxide content and, thus, rapidly exceeds the capacity of homeostatic protection (66). Thus, there is extensive support that mtDNA deletions accumulate with age and disease (67–69).

Deafness is clearly shown to have an association with mtDNA mutations. It has been suggested that mitochondrial diseases should be considered in cases of progressive sensorineural hearing loss, especially those associated with multisystem involvement (70, 71). A 10.4 kb mtDNA deletion has been identified in association with maternally transmitted diabetes and deafness, without ophthalmoplegia or mitochondrial myopathies, which were the hallmark of mtDNA deletion syndromes (72). Other studies have identified mutations in the tRNA Leu(UUR) gene in a large pedigree with maternally inherited diabetes mellitus type II and deafness (73). A 3243 point mutation (A→G) has been demonstrated in a patient with sensorineural deafness without diabetes (74). Several human studies have demonstrated an association of mitochondrial DNA mutations and presbycusis, including a study which demonstrated that older patients with presbycusis had a higher frequency of the common aging deletion (4977 bp) compared with similar aged patients without presbycusis (75). It has been demonstrated, using human archival temporal bones, that 14 of 17 aged patients with presbycusis had the 4977 bp deletion compared with 8 of 17 control patients with normal hearing. This difference was statistically significant ($p < 0.05$) (76).

Through experiments conducted in our laboratory, various nutrient compounds have been identified that enhance mitochondrial function by their ability to protect and repair age-induced damage to cochlear mtDNA (Fig. 3). One such compound is acetyl L-carnitine. In one study involving Fischer rats, it was noted that auditory thresholds actually improved over

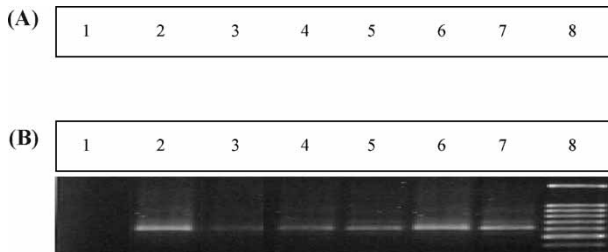


Fig. 3. (A) Data for the identification of ND1-16S rRNA from stria vascularis. This represents a 601 bp product and verifies the integrity of the PCR and the presence of mitochondrial DNA. Lane 1: negative control; lane 2: placebo; lane 3: diet restriction; lane 4: vitamin C; lane 5: vitamin E; lane 6: melatonin; lane 7: lazaroïd; lane 8: molecular weight standards. (B) Data identifying the 4834 bp deletion (common aging deletion) from stria vascularis. This represents a 597 bp product. Lane 1: negative control; lane 2: placebo; lane 3: diet restriction; lane 4: vitamin C; lane 5: vitamin E; lane 6: melatonin; lane 7: lazaroïd; lane 8: molecular weight standards. The DNA concentration was normalized to 150 ng for all samples.

the course of 6 weeks, suggesting a strong positive role for these compounds (77) (Fig. 4). In another study, our laboratory investigated the effects of lecithin on aging and age-associated hearing loss in rats by measuring hearing sensitivities using auditory brain-stem responses (ABR). In addition, mitochondrial function, as a measure of aging, was assessed by determining mitochondrial membrane potentials. This was achieved using flow cytometry and by amplifying mitochondrial DNA deletions associated with aging

using PCR. Harlan-Fischer rats, aged 18–20 months ($n = 14$), were divided into two groups. The experimental group was supplemented orally for 6 months with lecithin, a purified extract of soybean phospholipid (Nutritional Therapeutics, Allendale, NJ, USA). The data obtained were compared with the control group. ABR were recorded at 2-month intervals and showed significant preservation of hearing sensitivities in the treated subjects. Flow cytometry revealed mitochondrial membrane potentials to be significantly higher in the treated subjects, suggesting preserved mitochondrial function. Finally, the common aging mitochondrial DNA deletion (mtDNA⁴⁸³⁴) were amplified from brain and cochlear tissue, including stria vascularis and auditory nerve. This specific deletion was quantitatively found to be fewer in all tissues in the treated group compared with the controls. Based on these findings, these experiments support our hypothesis and provide evidence that lecithin may preserve cochlear mitochondrial function and protect from hearing loss associated with aging.

Caloric restriction has also been shown to retard the aging process. In a study conducted by Lee et al. (78), a control and calorie-restricted group of mice were compared over a 30-month period, and the results revealed that age-related changes in gene expression profiles were remarkably attenuated by caloric restriction. During aging there is a lower expression of metabolic and biosynthetic genes, and caloric restriction was noted to decrease the normal age-induced genetic alterations by causing a metabolic shift toward increased protein turnover and decreased macromole-

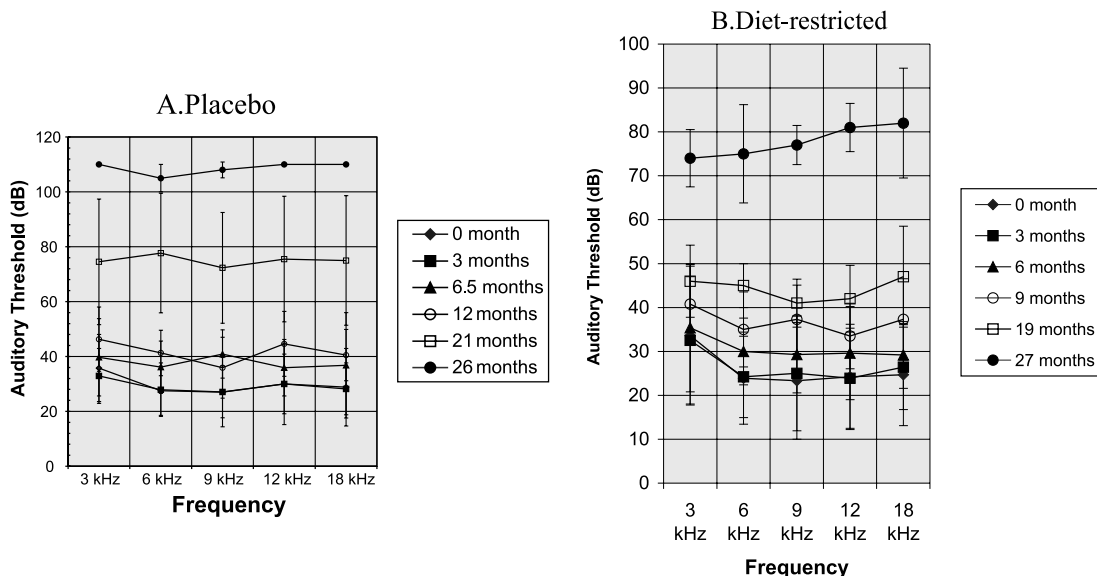


Fig. 4. Auditory threshold measurements in (A) placebo and (B) diet-restricted groups. Over the lifespan of 24–27 months, the thresholds shifted the least in diet-restricted groups. The difference between diet-restricted groups and placebo was statistically significant ($p < 0.01$).

cular damage, hence creating a more bioenergetically efficient cell. Another study by Lee et al. (79) demonstrated that caloric-restricted mice produced fewer proteins associated with the oxidative stress response and heat shock factors.

DISCUSSION

Presbycusis, or the progressive deterioration of hearing associated with aging, is the most common cause of adult auditory deficiency in the USA. This condition creates a significant burden not only for the sufferers, but also for those who communicate with them. The medical and socioeconomic costs are staggering, and with the expansion of the world population and the numbers of elderly individuals expecting to more than double by the year 2030, this problem is escalating. However, significant progress has been made to identify the molecular mechanisms of age-related hearing loss, and this in turn has led to research on methods of mitigating the adverse effects on the auditory system. Among the various mechanisms that are postulated to result in age-related hearing loss in the current literature, the one that is arguably the most intriguing, is the membrane hypothesis of aging (MHA). Also known as the mitochondrial clock theory of aging, the premise of this theory is that with aging, hypoperfusion of the cochlear tissue occurs, which leads to ischemia and the formation of ROS. These species are highly toxic substances that adversely affect the auditory neuroepithelia. More specifically, these ROS damage mitochondrial DNA (mtDNA), resulting in both the production of specific mtDNA deletions, as well as reducing the mitochondrial membrane potential (MMP). The resultant effect is to render the mitochondria bioenergetically inefficient. These specific mtDNA deletions are also known as the common aging deletion. Studies are increasingly supportive of this relationship between presbycusis and the common aging deletion.

The results of these studies have further led to investigations concerning the role of antioxidants, nutritional supplementation, and dietary restriction on hearing loss and the aging process in general. There is compelling evidence to suggest that the beneficial effect of these compounds is to slow down the 'mitochondrial clock', by decreasing the amount of mtDNA deletions and causing less of a reduction in mitochondrial membrane potential. Currently, our laboratory is investigating the role of resveratrol, a constituent of the red wine grape, in attenuating the age-induced reductions in auditory sensitivity; specifically, its antioxidant effect on the mitochondrial apparatus. This study will hopefully provide strong empirical evidence of an integrated hypothesis of

aging and presbycusis, with the ultimate goal of enhancing our understanding of the mechanisms that surround senescence, both as a phenomenon and as an intrinsic biological property.

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