

Aging

Your research has focused on oxidative stress and aging. What is oxidative stress and what role does it play in the aging process?

A: We are very interested in experimentally testing the so-called "free radical theory of aging". This theory, one of over 300 theories advanced to explain aging, states that our cells are constantly bombarded by free radicals, and that some of these free radicals (or oxidants) can evade the vast array of antioxidant defenses and damage important biological molecules, such as DNA. Some of this damage would be expected to accumulate over our life-span and eventually cause cell and organ dysfunction, a loss in the ability to respond to environmental stress, age-related diseases (cancer, Alzheimer's disease, or cardiovascular problems) and ultimately lead to death of the individual. This theory is compelling because it encompasses so many of the things that we know to occur, both in our cells and during aging. For instance, we know that we must constantly consume oxygen in order to carry out normal cellular metabolism. On a cellular basis, oxygen is mostly used in organelles called mitochondria. These organelles have been called our cells' "power-plants" because this is where raw fuels that we eat (proteins, carbohydrates, and fats) are converted into a useful form of energy (adenosine tri-phosphate, or ATP) that the cell uses to carry out necessary chemical processes. This conversion process requires oxygen, which is used by the mitochondrion as an "electron acceptor". Consequently, this vital energy production would grind to a halt if we did not constantly breathe oxygen. Even though oxygen consumption is vital for our very survival there are unfortunate consequences for using oxygen. Oxygen can be converted into very deleterious forms called reactive oxygen species (ROS), which include free radicals, the same compounds that can be formed during a nuclear explosion. It now appears that ROS constantly arise as by-products of normal metabolism. Thus, using oxygen as a central part of our metabolism is really a double-edged sword-we need it to

provide energy to carry out normal metabolic processes, but we are also constantly converting some of the oxygen that we breathe into ROS that damage important biomolecules and eventually lead to cell and organ dysfunction.

Q: *Do you think aging then might be due partly to improper or incomplete repair of some of this damage caused by free radicals?*

A: Researchers have observed that certain components of the cellular "house-keeping machinery", including repair systems, tend to decline with age. Therefore, it appears to me that aging affects key components that significantly increase oxidative stress. First, cells don't seem to handle oxygen as efficiently, which increases free radical production. Second, our antioxidant defenses decline, and, finally, the repair systems tend to decline. All this result in greater oxidative stress to our bodies, leading to a vicious cycle of more oxidative damage that, in turn, result in loss of cell functions.

Q: *There has been a lot of media attention recently on telomeres and the role they may play in aging, cancer, and other pathologies. What is your opinion of the role of the telomere in the aging process?*

A: As a scientist, I was initially intrigued with these findings. Telomeres are the ends of the chromosomes that shorten when the cell divides. There has been speculation that after a certain number of cell divisions telomere losses is so acute that the cell can no longer divide and then it dies. This suggests that we have a finite number of cell divisions before the organ is no longer able to renew itself. However, if you look at this phenomenon a little more closely, some problems with this theory become apparent. For instance, the cells in many of our organs simply do not divide. There are tissues in our bodies called "post-mitotic", such as the heart and brain, in which cells quit dividing after development. Does that mean that our brains and hearts are senescent before we reach puberty? I do not think so!

Q: *Could one examine this issue experimentally by comparing rates of aging in different organs or tissues within an organism?*

A: Well, that has been tried, but it is a very difficult experiment to do. Not all cells of a given organ are dividing at any one time, so you have a very small number of cells that are dividing. How do you follow that one cell amidst the millions of other cells in order to observe how many times it divides? While the telomere theory of aging has some adherents, there are more scientists who think that the telomere may be a very important phenomenon in cancer, which is a subset of aging, but not a fundamental mechanistic explanation for aging. It certainly could not explain aging for all tissues.

Q: *How does the concept of telomere truncation fit in with the so called Hayflick number, which was described by Leonard Hayflick as the finite number of cell doublings that will occur in cell culture before cells senesce and die?*

A: To some extent, the telomere theory has resurrected the Hayflick model. Hayflick observed that normal cells in culture will divide for a certain number of times and then stop. Although this is very reminiscent of telomere loss, it is not exactly the same phenomenon. First of all, tissue culture conditions do not mimic what happens in a living animal. Secondly, a number of changes occur in cultured cells that are independent of telomere truncation that could result in the same phenotypic phenomenon of loss of cell division. So this phenomenon is called "replicative senescence", which does not adequately mimic all the varied changes that occur in our body during aging.

Q: *Is this why you have chosen to study whole organisms, such as rats, and intact cells from their organs to understand the cellular and molecular changes that occur in aging?*

A: Yes. This is why we rely on animal models to study aging. Compared to cell cultures, it is a much more difficult experimental system to use, but we get a better glimpse as to what is actually occurring in our cells as we get older. Presently, we are using rodent models to carry out our work because a lot is known physiologically about these animals, and they mimic what happens to humans as we

age. An old rat is about 2 1/2 to 3 years old, so we do not have to wait too long to get results. We can test rather easily how micronutrients affect the "health span", as well as the life span, of an animal. By doing so we hope to gain insights into what's happening in humans.

Q: *What do you mean by health span?*

A: Health span is a term that describes how long a person is healthy, enjoying life, doing what one wants to be doing thanks to good health. This is in contrast to "life span", which refers to how long a person lives.

Q: *Health span sounds very similar to a concept Linus Pauling promulgated many years ago. He was interested in extending the period of healthfulness in an individual. Once a population reaches the end of its life span, the death rate would be fairly rapid- not a gradual deterioration over a long period of time.*

A: Yes, indeed. Sociological studies have found that, given a choice to live longer with serious illnesses or live a shorter life and maintain health and vitality as a trade-off, people tend to opt for the latter. If there was a third option of living longer and healthier lives, I think that we all would choose that!

Q: *In your experimental work on rats, you have done a lot of work with liver cells. What made you select the liver as an organ of special interest?*

A: The liver is a good model in some important respects and in others it is not so good. We use the liver as an "anchor organ" so that we can compare and contrast its age-related changes to those in other organs, such as the heart and the brain. The liver is a very large organ, so there is a lot of material to use in order to do many concurrent experiments. This allows us to build up a good profile of the age-related changes that occur in this vital organ. We can test the free radical theory of aging more easily than in other, smaller organs of the body. The liver is the major detoxification organ, and liver cells

can still divide. Therefore, we can compare them with “post-mitotic” tissue that no longer divides, such as the heart and the brain.

Q: *What differences in liver cell function do you see during the aging process?*

A: In general terms, we and other investigators have found that the aging process causes us to lose the ability to respond to environmental stresses. This includes oxidative stress (free radicals) or other types of stresses like toxin exposure. We can place different types of stresses on the liver to see how these cells respond. We are also interested in doing this in other organs. For instance, the heart is exquisitely sensitive to energy status; therefore we can place a burden on the heart by simply making it beat faster.

Q: *What kinds of experiments have you carried out with rat liver cells to assess differences between young and old rats and how they respond to environmental stresses?*

A: One of our premises is that the mitochondria become more dysfunctional as we age. As I mentioned before, the mitochondria are specialized organelles in the cell that convert raw fuel from our diet into a usable form of energy currency, ATP. We have observed that this conversion process in old animals is no longer very efficient. The mitochondria are not able to utilize raw fuels, especially fatty acids, very well. This causes the cell to try to compensate for mitochondrial decay. This compensation may take the form of neglecting some cell functions in order to maintain more vital systems that the cell must maintain to survive. This is what we have been looking at very closely in the liver cell and in the liver as a whole. We have found that, due to mitochondrial decay, overall cellular metabolism slows. This may be the cell’s attempt to compensate for mitochondrial decline. We have examined how this metabolic decline affects the cell’s ability to cope with everyday stresses by using a toxin called tertiary butyl-hydro-peroxide to determine whether cells from old animals are more susceptible to toxic insult. We recently showed that cells from old animals are twice as susceptible to this toxin as cells from young

animals. Since this toxin specifically targets the mitochondria, we can now see that age-related mitochondrial decay may make us much more prone to a variety of toxicological and environmental insults. Of course, damage to the mitochondria by toxins also affects their ability to produce energy.

Q: *You have described a number of problems associated with mitochondrial dysfunction or increased mitochondrial damage due to exposure to toxins. What kinds of strategies have you found that can either prevent or reverse some of these types of insults to the mitochondria?*

A: Our goals are not only to understand the extent and precise nature of mitochondrial dysfunction with age and how that relates to the free radical theory of aging, but also to understand how dietary micronutrients may either mask, maintain, or improve mitochondrial function in the aging animal. We want to relate these dietary effects to the health span of animals and, hopefully, to humans. We are trying to identify the micronutrients that directly affect mitochondria. In order to do this we first have to understand what micronutrients decline with age and how this decline affects the mitochondrial function. We have found several that decline, including carnitine, which is an amino acid that transports fatty acids from the diet into the mitochondria to be converted into ATP. Carnitine levels decline by more than 50% in old animals compared to young animals. Therefore, the mitochondria are starved of dietary fuel because it simply cannot be brought in to be burned. We have also found that a phospholipid--cardiolipin -declines dramatically by about 50% in old animals. Cardiolipin is very important for overall maintenance of mitochondrial function. We have also observed that mitochondria have lost certain antioxidants to a significant degree, including glutathione and vitamin C. The decline in both of these antioxidants is more pronounced in the mitochondria than in the rest of the cell. This tells us two things: we have loss of micronutrients like carnitine and cardiolipin that can directly affect mitochondrial metabolism and vital antioxidants are lost. This means that mitochondria may be even more vulnerable to free radical insult.

What can we do about this? Well, when we feed animals a compound called acetyl-L-carnitine (a more bioavailable derivative of carnitine also called ALCAR), their cells are repleted with this important substance. This results in dramatic metabolic effects. For example, many of the indicators of mitochondrial dysfunction disappear after only one month of dietary ALCAR supplementation. Metabolically, the animals are much more active, they have improved short-term memory as assessed by special tests, and we see an improvement in their overall physical activity. We see a very significant improvement in a number of metabolic parameters that would lead us to believe that these animals are simply doing much better on the supplemented diet. We have also attacked the problem associated with increased mitochondrial performance in the old animals-an increased oxidative insult to the mitochondria from oxygen free radicals-by providing a relatively obscure antioxidant called lipoic acid to the animals in their diet. Lipoic acid is a naturally occurring antioxidant found in green leafy vegetables, as well as in meat, that is required for the production of energy from glucose. Hence, lipoic acid can directly affect metabolism and in its free form is a very potent antioxidant, even more powerful than vitamin C. Lipoic acid also “spares” vitamin C and other antioxidants. We fed lipoic acid to rats and found that it can completely reverse the age-related loss of vitamin C and glutathione, an important endogenous cellular antioxidant. So lipoic acid not only acts as an antioxidant in its own right, it also repletes very important antioxidants like vitamin C. We are now exploring these functional mechanisms, which are quite complex. We think that lipoic acid may improve the uptake of vitamin C into tissues as well as increase glutathione synthesis in cells.

Q: *Dr. Ewan Cameron, the Scottish surgeon who collaborated closely with Linus Pauling for many years on the clinical use of vitamin C in cancer patients, found that many of his terminal patients suffering from severe lassitude who were given doses of vitamin C reported an increased sense of well-being and had more energy. When Cameron looked into this more closely he realized that vitamin C is required for the hydroxylation reaction that converts the dietary amino acid lysine to carnitine.*

A: The loss in vitamin C may actually be causing the loss of carnitine. And we would like to explore that in the future.

Q: *How applicable are your results with liver cells to other tissues or organs in the aging rat and to people?*

A: That is a good question. We have just been awarded a large NIH grant to explore these phenomena in heart function as well. We are rapidly setting up and doing these experiments. Again, in the heart and in the brain, we see a very marked age-related decline in vitamin C status. We are also seeing changes in mitochondrial function to a significant degree. So it looks like the phenomena we saw in the liver is almost a universal effect of aging. There are some differences in the rate of these changes in different organs, but it is clear that this is a general phenomenon of the aging process. Most importantly, whenever we do our dietary intervention studies with these micronutrients, we not only see effects in the liver but also systemic effects. When we put both ALCAR and lipoic acid into the diet, we also see an enhancement of short-term memory and cognitive function. That leads into the last part of your question. I think that these supplements may have an important health potential for humans not only in terms of energetics and vitality, but also as a way to slow short-term memory loss, frequently called mild cognitive impairment. Since we do not have any human clinical data yet, we cannot make any claims.

Q: *Is your strategy to provide sufficient information on the molecular and biochemical functions of some of these molecules in animals that would provide a platform for human studies?*

A: Absolutely. What we are trying to do right now is understand exactly how these micronutrients work in the body. We need to understand both the proper effective dosage and whether they are completely safe. I should add that we have only done short-term feeding studies, and the benefits may or may not be preserved in long-term studies. Micronutrients can powerfully affect overall

metabolism. Before going on to human clinical trials, we feel compelled to understand the ramifications of supplementation, including any potential safety problems with the use of these supplements. Therefore, I cannot recommend that people use them until this information is available.

Q: *Many safety reviews on vitamin C have been published over the years, and to date there does not seem to be any significant problem with large doses of vitamin C, except for perhaps in certain populations, such as those with hemochromatosis. Has not lipoic acid been used to treat diabetic neuropathy in Europe for some time?*

A: It has been used for 40 years as a treatment for type II diabetes in Germany in relatively high doses, but the pharmacokinetics and the safety data are not widely distributed—they are primarily held by pharmaceutical companies. I have not heard of any untoward effects of lipoic acid or ALCAR or carnitine. Carnitine is naturally made in our bodies and is also a component of red meat, and lipoic acid is found in green leafy vegetables, so we routinely ingest these substances in small amounts.

Q: *Are there any human diseases that might be exacerbated by these substances?*

A: Lipoic acid has been reported to cause hypoglycemia if not taken with food.

Q: *Dr. Roy Walford and others have popularized the idea of extending life span by caloric restriction. How does the concept of caloric restriction fit in with some of these other ideas that we have been talking about?*

A: Actually it fits in very well. Caloric restriction is a very harsh form of food restriction in which approximately 60% of the calories is restricted. Even though this diet is severe, the life span of an animal can increase by 30 to 40%. That means that humans would live 140 to 150 years! These animals are not nutritionally restricted or malnourished—they have everything they need for proper cell function. However, it seems that food restriction orients the body to

be extremely efficient. Caloric restriction also seems to maintain mitochondrial function much longer. The mitochondria become very efficient in utilizing oxygen, so there are not so many free radicals being generated. The general housekeeping machinery of the cell, the ability to respond to stresses of all types, and hormone levels are also maintained. Frankly, the major problem with caloric restriction is that it is impractical and unappealing. To achieve effects on life span similar to those seen in animals would require a very severely restricted diet-something that most people would not tolerate. Our research is designed to see if we can bring about much of the same phenomena of increased life span and health span that caloric restriction produces, but doing it instead by maintaining overall micronutrient levels. This is an example of orthomolecular medicine as defined by Linus Pauling. We want to give the right substances in the right amounts. Nutritional needs change throughout life. Some nutrients that young people normally get very well in the diet or make endogenously are simply not present to the extent needed in older people.

Q: *Can you provide us with other tantalizing glimpses about recent results and the work you plan to do in the near future?*

A: Well, as I said, we have been very pleased to have been awarded a large NIH grant to study the metabolic effects of lipoic acid and carnitine on heart function. I am also quite gratified that we have attracted a large number of people into the lab in order to pursue our goals. So our lab and procedures are really set up and working well. Currently, we are looking at the metabolic effects of carnitine and lipoic acid in hearts and heart tissue from animals. So far, our results are similar to or better than what we observed in our liver studies.

We have another completely serendipitous finding. It is been known that there is a large age-related increase in metal accumulation, especially iron, in our tissues. In some cases, this may be harmful because free iron is not used by the body very well and may help produce free radicals. In particular, Alzheimer's and Parkinson's

patients have higher levels of accumulated iron than people who are undergoing normal aging. We have found that lipoic acid supplementation reverses this increase in unbound iron. This opens the possibility that lipoic acid may be able to lower metal-related oxidant stress and slow the progression of Alzheimer's disease.

Q: *One last question concerns what many people have called the "antioxidant network". Some of the antioxidants that you have discussed we can obtain dietarily. Other antioxidants are enzymes that are made within the body, for example, superoxide dismutase and catalase. Jim Fleming at the Linus Pauling Institute of Science and Medicine in California worked with fruit flies, which are composed of post-mitotic cells. He and his colleagues were able to increase the amount of some of these endogenous antioxidant enzymes in flies, which resulted in increased resistance to stress as well as increased life span. Do you have any concluding thoughts about how these dietary or endogenous antioxidants collaborate?*

A: You are absolutely right. You can never talk about one compound in isolation when you are dealing with something as complex as a living cell or organism. Every one of these antioxidants—glutathione, vitamin C, vitamin E, lipoic acid, uric acid, antioxidant enzymes—works in conjunction. A deficiency in one may be compensated for by another. Our work suggests that with aging, there is a true loss of this antioxidant networking. Aging causes a breakdown of this whole important web with serious consequences to biomolecules, cells, and whole organisms, which then become more vulnerable to free radicals and other environmental stresses.

Dr Troy Hagen is conducting these studies at the Linus Pauling Institute.

Basic Mechanism Found In How Aging Blood Vessels Lose Their Elasticity

Researchers in the Linus Pauling Institute at Oregon State University have discovered a fundamental mechanism that causes aging blood

vessels to lose their elasticity -- a literal “hardening of the arteries” that is often a prelude to high blood pressure and cardiovascular disease.

An understanding of this mechanism, scientists say, provides an important new target for both drugs and dietary changes that might help prevent or treat atherosclerosis and heart disease. This is a leading cause of death around the world that, in some form, affects about 80 percent of older Americans.

The findings were just published in *Aging Cell*, a professional journal. The study was funded by the National Institute on Aging, National Center for Complementary and Alternative Medicine, and the American Heart Association.

“This could ultimately provide a new, fundamental and possibly inexpensive way to treat or prevent high blood pressure,” said Tory Hagen, an OSU associate professor of biochemistry and biophysics, and lead author on the study. “It is also a key to understanding the biological effects of inflammation, which increasingly seems to be implicated not only in heart disease but other chronic and neurologic diseases.”

The research, which was done in test tubes and animal models, needs to be confirmed in humans before it could form the basis for new therapies. But the fundamental findings reveal an important insight into how blood vessels change with age and lose much of their ability to relax, contract, and facilitate the circulation of blood in the body. Blood vessels in humans, like those of other animals, have vascular “smooth muscles” that can alternatively relax and contract to accommodate fluctuations in blood flow and volume. A thin layer of “endothelial cells” in the vessels serves, in part, as a sensor mechanism to help regulate this process. Proper function of the endothelial cells, in turn, is driven by specific enzymes and signaling pathways.

What has been known for some time is that blood vessels, as they age, lose much of their capacity to relax -- about half of that capacity, even in healthy vessels. If the vessels are narrowed by atherosclerotic

lesions the problem is further exacerbated. High blood pressure is often the result, which in turn can lead to heart attacks, strokes, and death.

Some of the most common high blood pressure medications, in fact, function by helping to address this loss of elasticity in blood vessels. The nitroglycerin pills used by many people with unstable angina provide an immediate boost of nitric oxide, which serves to relax blood vessels.

What has not been known is exactly why this “hardening” of the blood vessels occurs with age. “Basically, we have learned that in older blood vessels, the cellular signaling process is breaking down,” said Hagen. “The vessels still have the ability to relax much as they did when they were younger, but they are not getting the message.” A complex enzymatic process outlined in the new study explains how this “failure to communicate” occurs. An enzymatic reaction called “phosphorylation” which is essential to the signaling process loses about half of its effectiveness in aging blood vessels. This loss of phosphorylation is due to less activity in one enzyme, AKT that facilitates the process and excess activity of phosphatases, which reverse it.

The researchers also discovered that ceramides, one type of lipid, or fat, are primarily responsible for the excessive activity of phosphatases. In laboratory experiments with blood vessels from rats, they were able to inhibit ceramide synthesis.

“The laboratory studies were very compelling,” Hagen said. “We were able to make aging blood vessels behave as if they were young again.”

The strength of this approach is that it points the way to use diet to prevent the decline in blood vessel function with age, and to treat it, if necessary, through drugs.

“A compound we are already using showed the ability to lower ceramide levels and improve the cell signaling process, and this compound would be a good starting point for possible drug therapies,” Hagen said. “certain types of diet may help reduce this natural, age-related process.”

As is appropriate for many other disease concerns and health conditions, a diet that is heavy in fruits and vegetables seems to slow down the loss of blood vessel function. However, the scientists also are doing research with lipoic acid, a powerful antioxidant that is very promising and may ultimately show it could play a role as a dietary supplement to help address this problem.

This overall process, the researchers said, is linked to a low-grade, chronic inflammation that occurs with aging, in blood vessels and probably many other metabolic functions. Efforts to understand and address these inflammatory processes are some of the most promising areas of chronic disease prevention and treatment, they said.

Mitochondrial Restoration

Restoring Mitochondrial Function and Bio-Energetics

In 1956 Denham Harman, MD, introduced his groundbreaking paper on the Free Radical Theory of Aging. Over the years a number of scientists have elaborated on Dr. Harman’s original work, striving to establish a direct link between free radical damage and human aging, and age-related diseases.

Mitochondrial damage is one of the main causes of the age-related decline of cellular energy production (bio-energetic decline). In addition to being the principal source of energy for all cells, mitochondria (Fig. 1) are also the primary site of free radical production. Free radicals are highly reactive molecules that damage cellular structures such as membranes, proteins, and both nuclear and

mitochondrial DNA. Due to their proximity to the inner mitochondrial respiratory chain (Fig 2) which is also a primary source of free radical production and their limited capacity for self-protection and repair, mitochondrial DNA are particularly susceptible to free radical damage. Mitochondrial dysfunction is now well recognized as a cause of a number of diseases (Table 1), as well as aging itself.

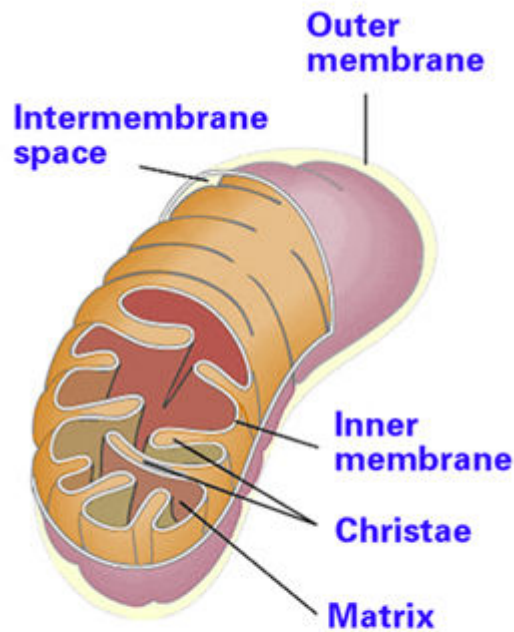


Fig. 1. Mitochondria are responsible for generating cellular energy, as well as producing cell-damaging free radicals.

Inner mitochondrial membrane

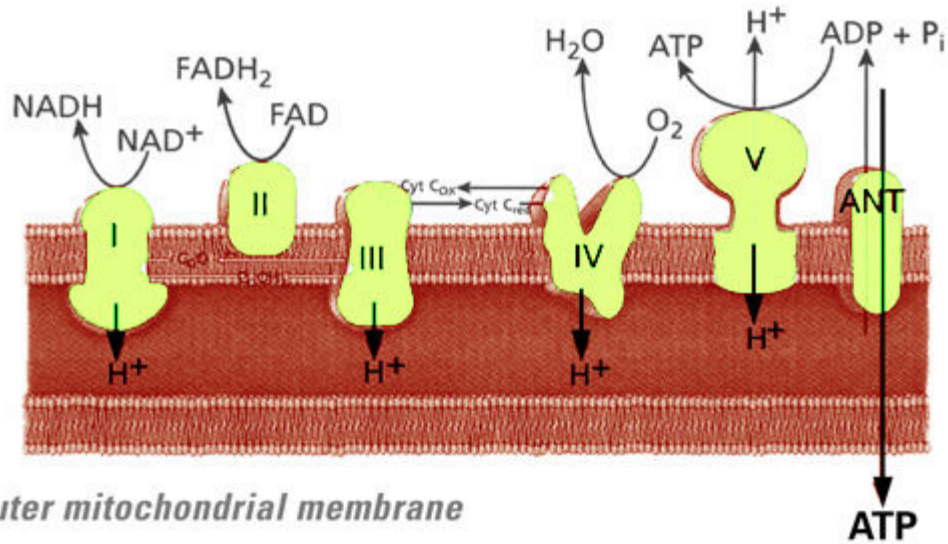


Fig. 2. Electron transport chain, by which oxidative phosphorylation takes place. **Complex I** (NADH dehydrogenase) accepts fuel from the citric acid cycle in the form of NADH, which donates electrons to the chain. **Complex II** (succinate dehydrogenase) accepts electrons from FADH₂ and passes them to **Complex III** (cytochrome-c reductase) via CoQ 10. The electron is then passed to **Complex IV** (cytochrome-c oxidase) via cytochrome C. **Complex V** (ATP synthase) converts adenosine diphosphate (ADP) to adenosine triphosphate (ATP)—the chemical energy “currency” of the cell. ATP can then be transported to where work needs to be done.

As evidence implicating mitochondrial dysfunction in the aging process continues to accumulate, the question becomes: What if anything, can we do about it?

Improving Mitochondrial Function

- Alzheimer’s disease
- Parkinson’s disease
- Essential hypertension
- Cardiomyopathy
- Congenital muscular dystrophy
- Immune (Hyper Thyroid)
- Fatigue & Exercise intolerance
- Huntington’s chorea

- Longevity (Aging)
- MELASL (Mitochondrial Encephalopathy, Lactic Acidosis, and Stroke-Like episodes)
- Deafness
- Diabetes
- Multiple symmetric lipomatosis
- Myalgias
- Myoglobinuria
- Myopathy syndromes
- Neoplasms (Cancer)
- Optic atrophy
- Rhabdomyolysis: mtDNA
- Sudden infant death (SIDS)
- Wilson's disease

Table 1. Diseases due to mitochondrial dysfunction.

Fortunately, a growing body of research suggests that a number of interventionist strategies may help to reduce mitochondrial damage, enhance mitochondrial repair, and restore mitochondrial energy-producing processes to more youthful levels. These strategies include lifestyle changes, such as diet and exercise, as well as supplementation with nutritional and pharmaceutical substances that may minimize age-related mitochondrial changes and enhance mitochondrial function.

CoQ10

Coenzyme Q10 is probably the most widely used cofactor for treating mitochondrial-related diseases. CoQ10 functions as the electron carrier in the inner mitochondrial membrane, transferring electrons from complexes I and II to complex III. In addition to increasing biosynthesis of ATP (the universal energy molecule), and acting as a potent free radical scavenger, CoQ10 also reduces lactic acid levels, improves muscle strength, and decreases muscle fatigability.

Idebenone

Idebenone is a CoQ10 analog that, while sharing some of CoQ10's properties, offers unique mitochondrial-protective benefits of its own. Idebenone is a powerful mitochondrial free radical quencher that reduces the ever-increasing damage to mitochondrial DNA that occurs with age. Idebenone has also been shown to be more effective than CoQ10 in the electron transport chain. Studies show that when cellular oxygen levels are low a condition that may occur periodically over a lifetime Idebenone is actually superior to CoQ10 for preventing free radical damage while helping cells maintain relatively normal ATP levels a property that is especially beneficial to brain and heart cells that may be rapidly damaged during low ATP production due to poor tissue oxygenation.

Acetyl-L-Carnitine

Mitochondrial changes that occur with age include alteration of mitochondrial membrane potential (mitochondrial membrane potential of old rats is known to decline by about 40 percent compared with young animals); a reduction in membrane levels of cardiolipin (an important phospholipid that serves as a cofactor for a number of critical mitochondrial transport proteins); a reduction in Coenzyme Q10 levels (an important factor in the electron transport chain); and a decrease in the concentration of carnitine (an important factor in the beta-oxidation of fatty acids).

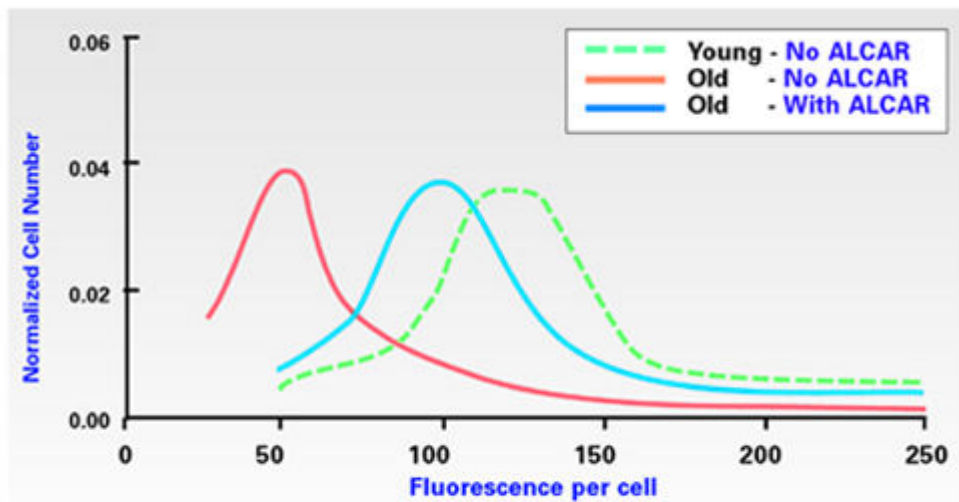


Fig. 3. Acetyl-L-Carnitine (ALC) reverses the age-associated decline in mitochondrial membrane potential, as measured by R123 Fluorescence (Hagen, et al, 1998).

Dietary supplementation might reverse some of these age-related mitochondrial changes. It was demonstrated that ALC restores mitochondrial membrane potential (Fig. 3) and cardiolipin levels (Fig. 4) of old mice to that of young animals, facilitates fatty acid transport into mitochondria, and increases overall cellular respiration. The researchers also noted that ALC enhances cognitive performance, increased production of neurotransmitters, and restores levels of certain hormone receptors to more youthful levels. They concluded that ALC reverses many aspects of age-related cellular dysfunction, principally through maintenance of mitochondrial function.

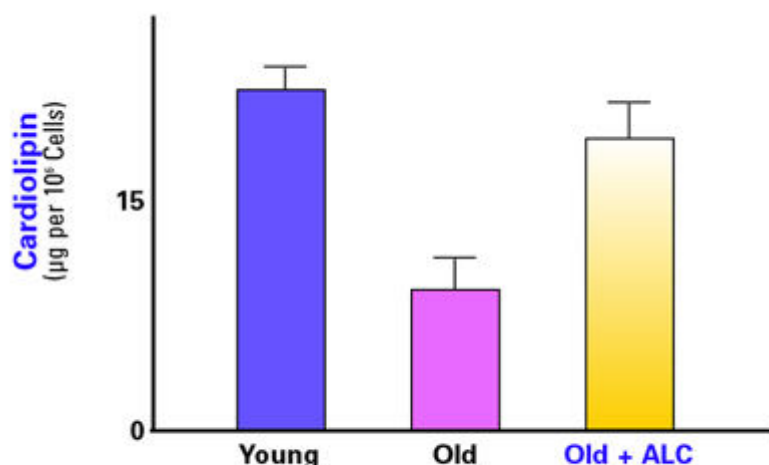


Fig. 4. Acetyl-L-Carnitine (ALC) restores mitochondrial membrane cardiolipin levels to that of young animals (Hagen, et al, 1998).

N-Acetyl Cysteine

As previously noted, a major cause of mitochondrial dysfunction is due to changes that take place in the respiratory chain where oxidative phosphorylation occurs. A team of researchers in the Department of Biochemistry and Biophysics at the University of Kalyani in India studied the effects of N-Acetyl Cysteine (NAC) on key elements of the respiratory chain. They administered NAC to mature (40 week-old) rats. After 20 weeks of treatment they found that the activities of Complex I, IV and V were significantly higher in the treated rats compared to the controls. NAC also helped to maintain levels of the important mitochondrial antioxidant, glutathione, as well as prevented cell death in ‘in vitro’ studies. In other in vitro studies, NAC protected cells from programmed cell death (PCD) also known as apoptosis by promoting oxidative phosphorylation, mitochondrial membrane integrity, and mitochondrial homeostasis.

(R) Alpha Lipoic Acid

Dr. David Horrobins group at Trinity College, Dublin, Ireland, found that a diet supplemented with alpha lipoic acid reversed a number of age-related changes in the brains of rats. These changes included: (1)

increased activity of the antioxidant enzymes, superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH px); and (2) decreased production of free radicals.

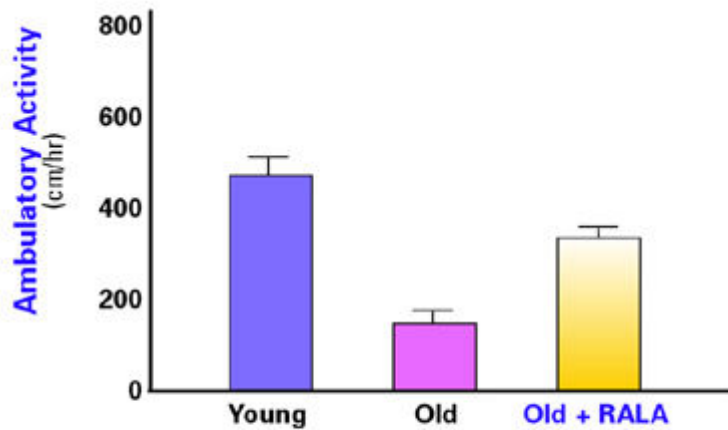


Fig. 5. R-Alpha Lipoic Acid supplementation increases ambulatory activity in old rats. This figure illustrates distance traveled in 8 hours before and after supplementation with RALA for 2 weeks. Old animals usually demonstrate about 1/3 of the "Ambulatory activity" (i.e., "moving around") of young animals. This also illustrates the dramatic improvement over a short period of time with RALA supplementation (Hagen, et al, 1999).

Dr. Tory Hagen and associates at the University of California evaluated the mitochondrial-resuscitating properties of an even more effective form of lipoic acid (R)-alpha lipoic acid. They gave (R)-alpha lipoic acid to young and old rats for two weeks, and found that mitochondrial oxygen consumption of the old rats treated with (R)-alpha lipoic acid was completely restored to the level of young, unsupplemented rats. The researchers also found that (R)-alpha lipoic acid, like ALC, increased mitochondrial membrane potential of old rats by up to 50 percent, compared to unsupplemented old rats.

In addition, they found that animals treated with (R)-alpha lipoic acid demonstrated twice the activity of the untreated old animals again, demonstrating a partial reversal of age-associated changes (Fig. 5).

(R)-alpha lipoic acid supplementation also increased mitochondrial

glutathione and vitamin C in old animals to levels higher than those of young animals (Fig. 6), indicating (R)-alpha lipoic acids ability to reverse the age-associated decline in low molecular weight antioxidants, therefore reducing the risk for oxidative damage that occurs with aging.

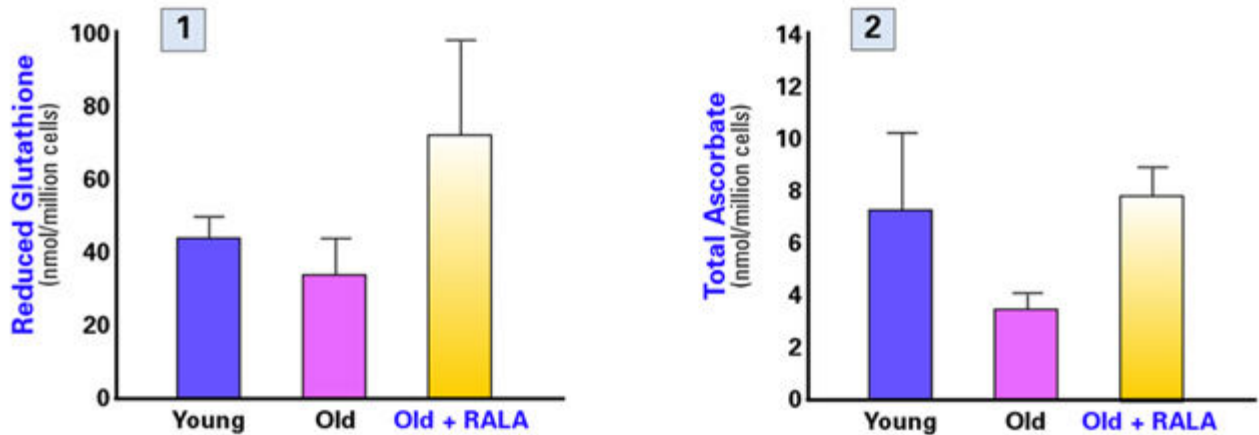


Fig. 6. Cellular and mitochondrial glutathione (1) and vitamin C (2) levels increase after RALA supplementation. Note that RALA raises levels of both of these low molecular weight antioxidants to levels that are even higher than young animals (Hagen, et al, 1999).

Hagen and his colleagues concluded that (R)-alpha lipoic acid supplementation improves mitochondrial function in old rats, alleviates some of the age-related loss of metabolic activity, increases ATP synthesis and aortic blood flow, and increases glucose uptake. Furthermore, (R)-alpha lipoic acid appears to be about ten times more potent than the more commonly available form of lipoic acid. The researchers further concluded that (R)-alpha lipoic acid supplementation may be a safe and effective means to improve general metabolic activity and increase antioxidant status.

Omega Three Fatty Acids

Mitochondrial calcium levels increase and mitochondrial membrane cardiolipin content decreases with aging. Scientists at the National Institute on Aging found that omega-3 fatty acids from fish oils are cardio-protective in aging animals, in that they minimized the

increase in mitochondrial calcium content, prevented the decrease in cardiolipin content, and increased levels of phosphatidylcholine.

Dr. Salvatore Pepe of the Alfred Hospital Cardiac Surgical Research Unit in Melbourne, Australia, reported similar findings. Dr. Pepe demonstrated that an omega-3 rich diet directly increases mitochondrial membrane cardiolipin concentrations, increases the ratio of mitochondrial membrane omega-3 to omega-6, and increases tolerance of the heart to ischemia and reperfusion.

Niacinamide (Vitamin B3)

Drs. Christopher Driver and Angela Georgiou of the National Aging Research Institute in Australia tested the efficacy of Niacinamide to re-energize the bio-energy system of old fruit flies. After administering Niacinamide (250 mcg/ml of water) to the flies, they determined that Niacinamide ameliorated age-related changes in bio-energy and extended the lifespan of the flies by 15 percent.

Thiamine (Vitamin B1)

Large doses of thiamine (vitamin B1) have been used to stimulate NADH, which then augments oxidative phosphorylation at Complex I. Doses of 300 mg/day in patients with chronic external ophthalmoplegia (CPEO), resulted in normalization of blood levels of lactate and pyruvate.

Riboflavin (Vitamin B2)

Riboflavin (vitamin B2) functions as a cofactor in Complex I and II. Riboflavin in a dose of 100 mg/day improved exercise capacity in a patient with a mitochondrial myopathy due to a Complex I dysfunction.

Exercise

Elderly subjects tend to use more glucose and less fat during exercise than young subjects. However, endurance training increases muscle respiratory capacity, decreases glucose production and oxidation, and increases fat oxidation, thereby correcting or compensating to some

degree the age-related alterations in substrate oxidation and energy production.

One argument that is propounded by some scientists as an excuse for their sedentary lifestyles is the fact that exercise increases the production of free radicals. However, scientists at the Guang-zhou Institute of Physical Education in Canton, China, showed that endurance training actually increases the production of mitochondrial manganese superoxide dismutase (MnSOD) and glutathione peroxidase (GSH px), resulting in an overall increase in antioxidant activity and decrease in lipid peroxidation.

Ginkgo Biloba

Ginkgo biloba extract has been found by scientists in Spain to protect mitochondrial DNA (MtDNA) against oxidative damage and oxidation of mitochondrial glutathione. The Spanish researchers found that Ginkgo biloba extract also prevents age-related morphological changes in mitochondria of the brain and liver. They concluded that mitochondrial aging may be prevented by antioxidants, and that certain antioxidants are also able to prevent the impairment in physiological performance, particularly motor coordination, that occurs with aging.

Succinate

Succinate is a tricarboxylic acid (Krebs) cycle that donates electrons directly to Complex II. Succinates have been widely used for their alleged ability to enhance athletic performance especially in Russia. Dilman believed succinic acid was a non-specific cell receptor sensitizer. Several studies reported improvement in clinical conditions using six grams per day of sodium succinate. One patient with respiratory failure and a known mitochondrial defect of Complex I, IV, and V completely resolved on a regimen of 300 mg CoQ10 per day, and six grams of sodium succinate. Another patient with mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) improved dramatically when treated with six grams of sodium succinate alone. I think the use of succinates is even

more effective when a balance of several salts is used especially combinations of magnesium and potassium.

| Nutrients That Normalize Sites/ Causes of Mitochondrial Dysfunction | Co Q10 | Idebenone | ALC | NAC | Lipoic Acid | Omega 3 FAs | Vitamin B1 | Vitamin B2 | Vitamin B3 | Ginkgo Biloba | Succinic Acid | Exercise |
|------------------------------------------------------------------------|--------|-----------|-----|----------------|------------------|-------------|------------|------------|------------|---------------|---------------|------------------|
| Complex I | X | | | | | | X | X | | | X | |
| Complex II | X | | | | | | | X | | | X | |
| Complex III | X | | | | | | | | | | | |
| Complex IV | | | | X | | | | | | | X | |
| Complex V | | | | X | | | | | | | X | |
| Membrane Potential | | | X | | X | | | | | | | |
| Membrane Cardiolipin | | | X | | | X | | | | | | |
| Coenzyme Q10 Level | X | | X | | | | | | | | | |
| Carnitine Level | | | X | | | | | | | | | |
| ATP Biosynthesis | X | | | | X | | | | | | | |
| Fatty Acid Transport | | | X | | | | | | | | | |
| Cellular Respiration | | | X | X | X | | | | X | | | X |
| Antioxidant | X | | | X ¹ | X ^{2,3} | X | | | | X | | X ^{2,4} |
| Reduces Lactic Acid | X | | | | | | X | | | | X | |
| Improves Muscle Strength | X | | | | X | | | | | | | X |
| Decreases Muscle Fatiguability | X | | | | X | | | | | | | X |
| Improves Cognition | | X | X | | | | | | | | | |
| Increases Neurotransmitters | | | X | | | | | | | | | |
| Improves Receptor Sensitivity | | | X | | | | | | | | | |
| Increases Phosphatidylcholine | | | | | | X | | | | | | |
| Increases Pyruvic Acid | | | | | | | X | | | | | |

Table 2. Nutritional Normalization of Mitochondrial Bioenergetics. 1. Glutathione. 2. Glutathione Reductase. 3. Vitamin C. 4. Mn SOD.

Conclusion

Mitochondrial dysfunction has been identified as one of the principal causes of age-related bio-energetic decline. Although there is no single silver bullet or even combination of substances that will unfailingly resuscitate all aspects of aging mitochondria, anti-aging physicians and scientists have discovered a number of nutrients and prescription substances that alleviate or completely restore many aspects of mitochondrial failure. Some of these substances have been discussed in this article, and their sites of action and specific mitochondrial-resuscitating properties are summarized in Table II. Combinations of these nutrients, acting on multiple targets, may normalize mitochondrial function, increase cellular and systemic energy production, alleviate mitochondrial-related disease, and delay age-related decline in many organs and systems of the body.