Chronic Fatigue, Aging, Mitochondrial Function and Nutritional Supplements

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Abstract

Intractable fatigue is the most common complaint of patients seeking medical care, and in most patients it is a chronic condition that is not reversed by sleep or rest. Although fatigue is a complex phenomenon, it has been defined recently as a multi-component sensation. It is related to aging, decreased mitochondrial function and loss in the ability of mitochondria in cells to produce high-energy molecules for cellular functions. Also, it is known that oxidative damage to mitochondria, mainly from Reactive Oxygen Species or ROS, resulting in modifications in mitochondrial lipids, proteins and DNA, is related to aging. Certain natural dietary products and supplements can reduce oxidative damage and replace high energy molecules or restore mitochondrial function. Recent clinical trials have shown the benefit of dietary supplements in restoring mitochondrial function and reducing fatigue. In aging subjects mitochondrial function was restored to levels found in young adults in consort with reductions in fatigue, suggesting the anti-aging and anti-fatigue benefits of protecting mitochondria and cells from oxidative and other molecular damage, by lipid replacement and antioxidant use.

What is Fatigue?

The most common complaint of patients seeking medical care from general medical practitioners is fatigue or loss of energy, and in fact, chronic fatigue (intractable fatigue lasting more than 6 months and not reversed by sleep) is reported by approximately one quarter of all patients seeking medical care.[1,2] Many medical conditions are associated with chronic fatigue, such as respiratory, coronary, skeletal-muscular and bowel conditions as well as various cancers and infections,[3,4] and chronic fatigue is often an important secondary condition in many clinical diagnoses. Loss of energy and the symptom of fatigue often precede and are usually related to clinical diagnoses, and this may be the most important reason that it is so commonly reported by patients seeking medical care.[5]

Fatigue has been in the medical literature for hundreds of years in many forms and indicated by several different historical terms, but it has been only recently that fatigue has been defined and attempts made to determine the extent of fatigue and its possible causes. Although we now know much more about fatigue, its universal definition remains to be determined. It is thought to be a multidimensional sensation with many possible causes.[1,2] Most patients understand fatigue as a loss of energy and inability to perform even simple tasks without exertion.

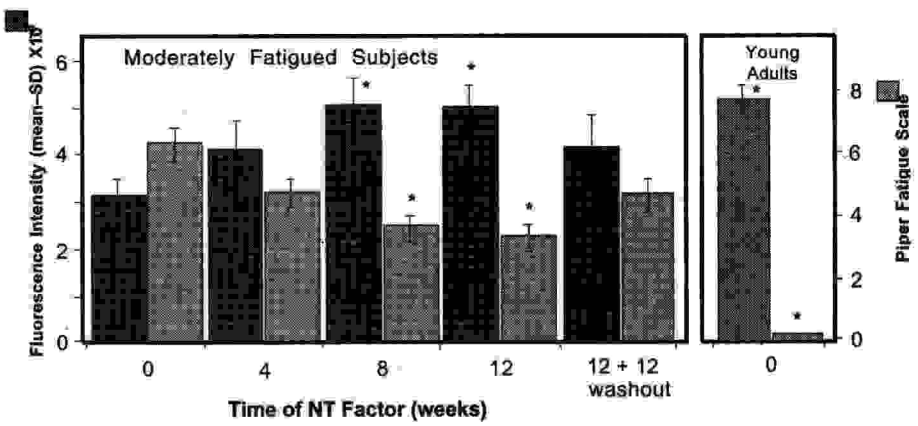
Recently Piper et al.[4] described fatigue as a multi-component sensation with behavioral (interference with normal activities), affective (how fatigue is described), sensory (feelings associated with fatigue) and cognitive (mood, memory and thinking) components. They also designed a simple measurement tool for assessing fatigue that combined multiple fatigue-associated elements into an overall fatigue score.[4,5] We have successfully used this validated instrument in clinical studies on aging subjects to determine their fatigue responses to various dietary supplements.[6,7]

Fatigue at the Cellular Level – Role of Mitochondria

At the cellular level fatigue is involved with cellular energy systems that for the most part are found in the mitochondria. Mitochondria are specialized semi-autonomous cellular organelles with their own lipid membranes, enzymes and DNA genetic information, and they degrade and convert sugars and lipids to energy that is stored in high-energy molecules (ATP, NADH, etc.) using oxygen and a system called the mitochondrial electron transport chain. The electron transport chain is responsible for oxidative phosphorylation, the principal source of high-energy molecules in every cell. Although mitochondria appear to be semi-autonomous, separate units within our cells; in fact, they are completely dependent functionally on many proteins and enzymes that are made by other parts of the cell and encoded by nuclear DNA.

Without the proper functioning of mitochondria, our cells must depend on anaerobic sources of metabolism to produce high-energy molecules from starches and sugars, resulting in the production of lactic acid as a byproduct

Figure 1. Piper Fatigue Scores and mitochondrial function of moderately fatigued subjects (>60 years-old) before, during and after use of NTFactor. Fatigue was determined using the Piper Fatigue Scale. Mitochondrial function was determined by cytofluorographic analysis of 10μM Rhodamine-123 incorporation into the mitochondrial membranes of blood monocytes isolated from fatigued subjects. Blood samples were taken at 0, 4, 8, 12 weeks and 12 weeks on NTFactor plus 12 weeks off NTFactor (12+12 washout). *p<0.001 compared to study subjects at time=0.



of sugar metabolism. Everyone at one time or another, has noticed what happens when we over-exert physically and cannot provide enough oxygen for our mitochondria, and our cells must resort to sources such as anaerobic metabolism to produce high-energy molecules such as ATP for our muscles. Eventually our muscles cramp due to the build-up of lactic acid and other metabolites. Thus our mitochondria are our most important sources of high-energy molecules for building and maintaining cellular functions in an oxygen environment.

Oxidative Damage to Mitochondria and Aging

Damage to cellular mitochondria can impair the abilities of cells to produce high-energy molecules, and this occurs naturally with aging, mainly by the buildup of oxidative damage to mitochondrial molecules. During aging the production of Reactive Oxygen Species or ROS, made up of oxidative and free radical molecules, such as nitric oxide, oxygen and hydroxide radicals and other oxidative molecules, can cause oxidative stress and cellular damage, resulting in oxidation of lipids, proteins (enzymes) and DNA in cells. Once oxidized, these cellular molecules can be deactivated or structurally and functionally changed. Major targets of cellular ROS damage are mitochondria and nuclei, mainly their phospholipid/protein membranes and DNA,[8-11] resulting in damage to membrane lipids and protein enzymes and deletion or modification of DNA.

ROS production and damage to mitochondria and nuclei occur throughout our lifetimes, but we have natural cellular systems that neutralize excess ROS and repair ROS-mediated damage. Although some ROS production is actually important in triggering cell proliferation and gene expression, with aging ROS damage *accumulates*. For example, cellular antioxidant enzymes normally neutralize excess ROS and enzyme repair mechanisms, or biosynthesis systems restore ROS-damaged molecules or replace them. However, when the concentration of ROS far exceeds the ability of cells to neutralize ROS or repair or replace ROS-mediated alterations, molecular damage accumulates within cells. Typically this occurs in aged animals and humans, but disease and infection can also result in similar damage that exceeds the abilities of cellular systems to neutralize, repair or replace damaged molecules.

In contrast to mitochondria isolated from young animals, mitochondria from aging animals show higher levels of accumulated ROS damage to mitochondrial membranes, enzymes and DNA.[12] At the molecular level, damage to phospholipids and other lipids in mitochondrial membranes by ROS free-radicals can affect membrane integrity, membrane fluidity and transmembrane electrical potentials, resulting in loss of energy production by the electron transport chain and its associated components. This occurs because the functional status of the mitochondrial electron transport chain is dependent on the integrity of mitochondrial membranes and maintenance of an electrical potential across the membranes.

Young cells and young organisms can cope with ROS since they possess high levels of free-radical scavenging systems that neutralize ROS, such as the enzymes superoxide dismutase and glutathione reductase. They also have a high capacity to repair or replace damage caused by ROS. With aging this system can decline or be overwhelmed by ROS and oxidative stress.[12,13] Since the aging process results in mitochondria suffering accumulated ROS damage to their membranes and DNA, this is thought to contribute to or even be a cause of the aging process.[9,12,13] It is also important in fatigue, as will be shown below. In animals caloric restriction has been used to extend longevity, and this also reduces oxidative stress and oxidative damage to tissue mitochondria.[14]

Reducing ROS-mediated Damage

Reducing cellular and mitochondrial membrane and DNA damage and loss of membrane integrity are important in preventing loss of cellular energy and regulating cellular life span.[15] This can be done by neutralizing ROS with various antioxidants or increasing free-radical scavenging systems that neutralize ROS. Some common dietary antioxidants are shown in Table 1 along with some accessory molecules that are important in maintaining free-radical scavenging systems, biosynthetic capacity, immune systems and other important cellular functions. Although this list is incomplete, the antioxidants and accessory substances shown in Table 1 have been commonly used as anti-aging supplements as well as substances to help prevent or lessen the effects of various chronic and degenerative diseases. There are at least 40 micronutrients required in the human diet,[16] and aging increases the need to supplement these in a normal diet to prevent age-associated declines in mitochondrial and other cellular functions.

In animal studies the effects of reducing ROS have been dramatic in aging and disease models. For example, in rodents there are age-dependent losses in antioxidants, such as vitamins C and E, as well as reductions in reduced glutathione and the levels of antioxidant enzymes.[16,17] Using aged rats the effects of alpha-lipoic acid and other dietary antioxidants on the levels of cellular antioxidants, such as reduced ➤

Table 1. Some common antioxidants and accessory molecules used as dietary supplements (incomplete list)

Vitamin C (ascorbic acid, buffered)
Vitamin E (alpha-tocopherol, other tocopherols, tocotrienols)
Coenzyme Q10
Alpha-Lipoic Acid (dihydrolipoate)
N-acetyl cysteine (also S-allyl cysteine, S-allyl cercaptocysteine)
Carotenoids/Oxycarotenoids (beta carotene, lycopene, lutein)
Flavonoids (quercetin, procyanidins, flavonols)
Proanthocyanidins
Selenium

Important Accessory Molecules
Vitamin B3 (niacin)
Vitamin B6 (pyridoxine hydrochloride)
Vitamin B12 (cyanocobalamin)
Vitamin B2 (riboflavin)
Folic Acid (folate)
Melatonin
Magnesium
Zinc

glutathione and vitamins C and E, levels of mitochondrial membrane lipid peroxidation and activities of mitochondrial electron transport and accessory enzymes were investigated.[18] Supplementation with antioxidants reduced mitochondrial lipid peroxidation, decreased levels of ROS and increased amounts or activities of certain electron transport enzymes. These authors found that dietary antioxidant supplementation reversed the age-related declines in cellular antioxidants and mitochondrial enzyme activities and prevented mitochondria from age-associated functional decline.

In another study rats were fed diets supplemented with coenzyme Q10, alpha-lipoic acid, melatonin or alpha-tocopherol for a six-month period. They found that antioxidants could inhibit the progression of certain age-associated changes in cerebral mitochondrial electron transport chain enzyme activities.[19] Similar results in rats using dietary coenzyme Q10 and other antioxidants were found in Japan.[20] Thus animal studies have shown that antioxidants can prevent the aging-associated changes in mitochondrial structure and function.

In addition to the aging-associated oxidative changes in mitochondrial enzymes and lipids, mitochondrial DNA also accumulates oxidative damage during the aging process.[12,13,21] To prevent this antioxidants have also been useful, such as vitamins C and E, coenzyme Q10, sulfur-containing antioxidants and plant antioxidant extracts.[22,23] Age-associated damage to mitochondrial DNA may affect their ability to function due to a loss in the ability to synthesize and replace critical mitochondrial enzymes.

Antioxidants may also affect the pathogenic processes of certain diseases. In a mouse model for Amyotrophic Lateral Sclerosis (ALS) or Lou Gehrig's Disease, a neurodegenerative disease that results in brain motorneuron death, dietary coenzyme Q10 significantly increased lifespan and provided some neuroprotective effects, including decreased loss of nerve mitochondria.[24] The experimental dietary use of antioxidants can prevent age-associated mitochondrial dysfunction and damage, inhibit the age-associated decline in immune function and prolong the lifespan of laboratory animals.[25]

Clinical Studies on Antioxidants

There are few clinical studies, unfortunately, that have used the information from animal research to investigate the role of multiple dietary antioxidants in human aging and disease. Of course, one of the problems facing researchers who conduct clinical trials is the widespread use of vitamins and antioxidants by the general population that could affect such trials. Although the results obtained from controlled animal studies are backed up by studies *in vitro* using cultured human cells,[26] there have been only a few clinical trials that directly address the role of antioxidants in preventing mitochondrial damage during aging and disease. Major problems in designing and conducting such trials are that it is extremely unlikely that a single or even a few antioxidants can produce significant effects and prevent aging-associated changes or affect pathogenic processes and the problem that each individual may have optimum levels of antioxidants that could be suboptimal for others. Also, the number of various different antioxidant combinations and concentrations that could be used in controlled clinical trials is daunting.

Nonetheless, there have been clinical trials that have found some interesting results. One of the few well controlled clinical studies on antioxidants examined their role in preventing ultraviolet (UV) damage to skin cells in 100 young and aged healthy subjects.[27] Damage was measured by the UV-induced accumulation of oxidized lipids and reductions in natural antioxidants, such as vitamin E and coenzyme Q10. They found age-associated increases in oxidized lipids and decreases in natural antioxidants, and UV irradiation worsened these in a dose-dependent manner but this could be prevented, in part, by increasing antioxidant concentrations through dietary intervention.

Clinical research has just begun to examine the use of combinations of antioxidants in dietary supplements in reducing increased oxidative stress found in aging. For example, in one study various formulas containing mixtures of dietary antioxidants were studied for their effects on oxidative stress using a method that detects metabolic derivatives of ROS action. Using healthy volunteers they compared the effects of low-dose combinations of (1) zinc, selenium, vitamin A (as retinol acetate), beta-carotene, vitamin E (as alpha-tocopheryl acetate) and L-cysteine, (2) citrus bioflavonoids, vitamin C (as L-ascorbic acid), coenzyme Q10 and vitamin B-6 (as pyridoxine hydrochloride) and (3) a combination of dietary formulations 1 and 2. The formulations were administered in a cross-over study where subjects received placebo and then test samples or the converse. They found that formulations 1 and 3 significantly reduced ROS metabolic derivatives in most of the subjects but formulation 2 did not. Future studies will have to expand the list of potential antioxidants and determine more optimal doses of antioxidants for dietary use, but it may be necessary to individualize such formulations to reach optimal antioxidant combinations in each individual.

Table 2. Components of NTFactor™

NTFactor™ is a nutrient complex that is extracted and prepared using a proprietary process. In addition, nutrients, vitamins and probiotic microorganisms are added to the preparation. It contains the following ingredients:

Glycophospholipids and Other Lipids: *polyunsaturated phosphatidylcholine, other polyunsaturated phosphatidyl lipids and glycolipids.*

Probiotics: *Bifido bacterium, Lactobacillus acidophilus and Lactobacillus bacillus* in a freeze-dried, microencapsulated form with appropriate growth nutrients.

Food Supplements, Vitamins and Growth Medium:
Bacterial growth factors to support probiotic growth, including defatted rice bran, arginine, beet root fiber extract, black strap molasses, glycine, magnesium sulfate, para-amino-benzoate, leek extract, pantethine (bifidus growth factor), taurine, garlic extract, calcium borogluconate, artichoke extract, potassium citrate, calcium sulfate, spirulina, bromelain, natural vitamin E, calcium ascorbate, alpha-lipoic acid, oligosaccharides, vitamin B-6, niacinamide, riboflavin, inositol, niacin, calcium pantothenate, thiamin, vitamin B-12, folic acid, chromium picolinate.

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Clinical Studies on High-Energy Molecules

Another method to increase the concentrations of high-energy molecules used by cells, such as ATP and NADH, is to administer these in dietary formulations. Unfortunately, this cannot be easily done with the very unstable, high energy phosphorylating molecule ATP, but it can be done with reduced NAD or NADH which can be converted inside cells to ATP. Although NADH can be administered in a dietary formulation, it is very unlikely that this alone is sufficient to reach cells intact at effective concentrations after oral administration. The reason for this is that NADH is quickly converted to low-energy forms in the gut and during transport in the blood.

To prevent breakdown of NADH, a stabilized oral form that can be absorbed by the gut without degradation has been devised called ENADA™ (www.enada.com). This form was used to assess the effects of NADH on 26 Chronic Fatigue Syndrome patients in a plabeco-controlled clinical trial of cross-over design where patients receive placebo or test samples for four weeks, then switch to one or the other midway during the trial for another four weeks after a four week wash-out period. In this trial 8 of 26 (31%) patients responded favorably to NADH in contrast to 2 of 26 (8%) to placebo. Response was measured by improvements in signs and symptoms reported by patients.[28] In a follow-up pilot study these same authors report that 72% of patients who used ENADA™ experienced some improvement in clinical signs and symptoms associated with fatigue. Unfortunately, these clinical trials did not use a validated fatigue assessment instrument to determine the effects of ENADA on fatigue, so it is difficult to actually determine how effective the product is in suppressing fatigue.

Animal Studies using Lipid Replacement Therapy

Another method that has been used to reverse damage to tissue mitochondria is to replace damaged mitochondrial membrane phospholipids and other lipids by replacement therapy. This has been accomplished by replacement of damaged lipids using a dietary supplement containing polyunsaturated phosphatidylcholines and other phospholipids and fatty acids that are essential structural and functional components of all biological membranes.[6,7] This dietary supplement is called NTFactor™ (www.NTFactor.com), and it has been used successfully in animal and clinical lipid replacement studies because the encapsulated lipids are protected from oxidation and can be picked-up and transported into tissue cells without undue oxidation.[6,7] NTFactor™ contains a variety of components, including glycolipids and other lipids, nutrients, probiotics, vitamins, minerals and plant extracts (Table 2).

Using NTFactor, an anti-aging effect has been demonstrated in aging rats. In 18-20 month-old rats Seidman et al.[29] found that NTFactor prevented hearing loss associated with aging, shifting the threshold hearing from 35-40 dB in control aged animals to 13-17 dB in the test group. These results were significant ($p<0.005$). They also found that NTFactor preserved cochlear mitochondrial function as measured in a Rhodamine-123 transport assay, increasing mitochondrial function by 34%. (Rhodamine-123 is transported into mitochondria where it is reduced only under conditions where mitochondria are fully functional)[30] NTFactor also prevented the common aging-related mitochondrial DNA deletion ($mtDNA^{4834}$) found in the cochlear of aging rats.[29] Thus lipid replacement in an animal model of aging was successful in preventing age-associated hearing loss and mitochondrial damage.

Clinical Studies using Lipid Replacement Therapy

Lipid replacement therapy has been successfully used in clinical studies to reduce fatigue and protect cellular and mitochondrial membranes from damage by ROS. For example, NTFactor has been used in a vitamin and mineral mixture (Propax™; www.propax.com) in cancer patients to reduce the effects of cancer therapy, such as chemotherapy-induced fatigue, nausea, vomiting and other side effects associated with chemotherapy.[31]

In a twelve-week double-blinded, cross-over, placebo controlled, randomized trial on cancer patients receiving chemotherapy Propax™ supplementation resulted in improvement from fatigue, nausea, diarrhea, impaired taste, constipation, insomnia and other quality of life indicators.[31] Most (64%) of the patients in the study reported significant improvement in these and other chemotherapy-induced side effects, and 29% experienced no overall worsening of side-effects. Following cross-over to the supplement, patients receiving the Propax supplement reported rapid improvement in nausea, impaired taste, tiredness, appetite, sick feeling and other indicators.

We have used Propax plus NTFactor in a pilot study with severely fatigued, aged subjects (>60 years-old) with a variety of clinical diagnoses to reduce fatigue, as measured by the Piper Fatigue Scale.[4,5] We found that fatigue was reduced approximately 40%, from severe to moderate fatigue, after eight weeks of using Propax containing NTFactor. The results were highly significant ($p<0.0001$).[7]

A more recent study was initiated to examine the effects of NTFactor on fatigue in moderately and mildly fatigued subjects and to determine if their mitochondrial function, as measured by the transport and reduction of Rhodamine-123,[30] improved with administration of NTFactor in concert with improvements in fatigue scores. The results of this clinical trial are shown in Figure 1.[6] After eight or twelve weeks of NTFactor, there was a 33% or 35.5% reduction in fatigue, respectively. The results were highly significant ($p<0.001$) and were obtained using a validated instrument for measuring fatigue.

In the lipid replacement trial with moderately fatigued patients, reductions in fatigue paralleled the significant gains in mitochondrial function.[6] In fact, there was good correspondence between fatigue and mitochondrial function (Figure 1).[6] Mitochondrial function was significantly ($p<0.001$) improved by the use of NTFactor for eight weeks. Interestingly, after 12 weeks of NTFactor use mitochondrial function was found to be similar to that found in young, healthy adults (Figure 1).[6] After 12 weeks of NTFactor, subjects discontinued the supplement for 12 weeks and their fatigue and mitochondrial function were ➤

then measured. Their fatigue and mitochondrial function were intermediate between the starting values and those found on eight or 12 weeks of NTFactor, indicating that continued use of the supplement is likely required to maintain lower fatigue scores and show improvements in mitochondrial function. The results indicate that mitochondrial lipid replacement therapy can significantly restore mitochondrial function and improve fatigue scores in aging human subjects.

Mitochondrial Function, Fatigue and Degenerative Disease

Mitochondria are the most important source of cellular energy in our bodies. If their function is impaired, energy available to cells is limited to the Krebs Cycle. There are a number of conditions and substances that can impair mitochondrial function,[8-10] but oxidation and damage of mitochodrial lipids in membranes are among the most important causes of impairment of mitochondrial function.[32] This can result in modification of the electrical potential barrier across the mitochondrial membranes that is essential in the electron transport chain generation of cellular high-energy molecules.[32] Mitochondrial function appears to be directly related to fatigue, and as patients experience fatigue their mitochondrial function is likely to be impaired.

Fatigue is a complex phenomenon, and it may be determined by several factors, including psychological health of the subjects. At the biochemical level, fatigue is related to the metabolic energy available to an individual and ultimately, to the many cells that perform their myriad of functions. The integrity of cell and intracellular membrane structures, especially in the mitochondria, is critical to cell function and energy production. If mitochondrial membrane glycophospholipids, fatty acids and other essential lipids are damaged by oxidation, they must be repaired or replaced in order to maintain cell and mitochondrial functions necessary in the production of cellular energy to combat fatigue.

The decline of energy production with aging appears to be due, in part, to mitochondrial lipid peroxidation by ROS and the failure to repair or replace the damaged molecules. Membrane damage and subsequent mitochondrial dysfunction by ROS can also lead to modifications (especially mutations and deletions) in mitochondrial DNA (mtDNA). The mitochondrial theory of aging proposes that the development of chronic degenerative diseases is the result, in part, of accumulated mtDNA mutations and deletions and oxidative damage to mitochondrial membranes over time.[9,22,33] Indeed, these studies have linked the development of certain chronic diseases with the degree of mitochondrial membrane lipid peroxidation and mtDNA damage. Thus the damage to mtDNA and mitochondrial membranes seems to be involved in the etiology of age-associated degenerative diseases leading to changes in the expression of genes important for cell survival as well as the phenomenon of aging itself.[33]

Restoration of mitochondrial membrane integrity and fluidity are essential for the optimal functioning of the electron transport chain. Declines in energy production with aging and disease coupled with increases in oxidative stress can modify membrane lipids and increase mitochondrial membrane permeability and activate cellular death programs (apoptosis).[34] Together, these factors likely play a major role in the aging process and they also affect the development of age-related degenerative diseases.[21,35]

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