

Lipid replacement/antioxidant therapy for anti-aging, fatigue and restoration of mitochondrial function

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INTRODUCTION

Aging results in the loss of cellular function, and this is thought to be related to a variety of changes in cells and tissues. Several of the most important changes in the nuclei and organelles that occur during aging are related to accumulated oxidative damage due to reactive oxygen species (ROS)¹. Critical targets of ROS are the genetic apparatus and cellular membranes^{1,2}, and in the latter case oxidation can affect lipid fluidity and membrane permeability^{3,4}. One of the most important changes caused by accumulated ROS damage during aging is oxidation of mitochondria and loss of electron transport function, and this appears to be directly related to mitochondrial membrane lipid peroxidation¹, which can induce permeability changes in mitochondria and loss of transmembrane potential².

I will concentrate on discussing recent clinical trials that have shown the effectiveness of lipid replacement therapy (LRT) plus antioxidants in the treatment of

certain clinical disorders and conditions as well its use in anti-aging supplements. LRT is not just the dietary substitution of certain lipids with proposed health benefits; it is the actual replacement of damaged cellular lipids with undamaged lipids to ensure proper structure and function of cellular structures, mainly cellular and organelle membranes⁵. Damage to membrane lipids can impair fluidity, electrical properties, enzymatic activities and transport functions of cellular and organelle membranes¹⁻⁵.

During LRT lipids should be protected from oxidative and other damage, and this is also necessary during storage as well as during ingestion, digestion, and absorption in vivo. LRT should result in delivery of high concentrations of unoxidized, undamaged membrane lipids in order to reverse the damage and restore function to oxidized cellular membranes. Combined with antioxidant supplements, LTR has proven to be an effective method to prevent aging-associated changes in certain cellular activities and

functions and for use in the treatment of certain clinical conditions⁵.

BENEFITS OF LIPID SUPPLEMENTS AND LIPID TRANSPORT

Lipid dietary mixtures have been used to improve general health^{6,7}, and they have also been used as an adjunct in the treatment of various clinical conditions, such as the use of n-3 fatty acids in cardiovascular diseases and inflammatory disorders⁷⁻⁸. Although not every clinical study has found health benefits from lipid supplementation¹¹, most studies have documented the value of dietary supplements that favor certain types of lipids over others, such as when n-3 polyunsaturated fatty acids (mainly fish- or flaxseed-derived) are favored relative to n-6 lipids⁶⁻¹⁰.

Lipid supplementation works, because cellular lipids are in dynamic equilibrium in the body, and this is why LRT is possible⁵. Orally ingested lipids diffuse to the gut epithelium and are bound and eventually transported into the blood and lymph using specific carrier lipoproteins and also by nonspecific partitioning and diffusion mechanisms^{12,13}. Within minutes, lipid molecules are transported from gut epithelial cells to endothelial cells, then excreted into and transported in the circulation bound to lipoproteins and blood cells where they are generally protected from oxidation^{13,14}. Once in the circulation, specific lipoprotein carriers and red blood cells protect lipids throughout their passage and eventual deposition onto specific cell membrane receptors where they can be taken into cells via endosomes and by diffusion¹⁵. After binding to specific cell surface receptors that bring the lipids into cells, lipid transporters in the cytoplasm deliver specific lipids to cell organelles where they are taken in by specific transport proteins,

ABSTRACT

Decreased mitochondrial function and loss in the efficiency of the electron transport chain are related to aging and fatigue. Lipid Replacement Therapy (LRT) along with antioxidants can circumvent membrane damage during aging and replace and restore mitochondrial and other cellular membrane functions via delivery of replacement lipids in their unoxidized, undamaged states. Using aged subjects (>65 years) recent clinical trials have shown the benefit of LRT plus antioxidants in restoring mitochondrial electron transport function and reducing fatigue. In these trials mitochondrial function was restored to levels found in young adults in parallel with reductions in fatigue. These studies indicate the anti-aging and anti-fatigue benefits of LRT plus antioxidants in protecting mitochondrial and other cellular membranes from oxidative and other damage and preventing loss of function. In a clinical study we determined if mitochondrial function is reduced in aging subjects with mild to moderately severe fatigue, and if this can be reversed with a nutritional supplement (NTFactor™) that replaces damaged mitochondrial lipids along with antioxidants. There was a significant time-dependent reduction in overall fatigue in moderately or severely fatigued subjects while on the supplement. Analysis of mitochondrial function indicated that four and eight weeks of NTFactor use in moderately or severely fatigued subjects significantly increased function by 15% and 26.8%, respectively, restoring mitochondrial function to levels similar to those found in young adults. The results indicate that LRT plus antioxidants can significantly reduce moderate to severe fatigue in aging subjects and restore mitochondrial function to levels found in young adults.

partitioning, and diffusion¹⁶. The concentration gradients that exist from the gut during the digestion of lipids to their absorption by gut epithelial cells and their transfer to blood and then tissues are important in driving lipids into cells. Similarly, damaged lipids can be removed by a similar reverse process that may be driven by lipid transfer proteins and by enzymes that recognize and degrade damaged lipids and remove them¹⁶.

FATIGUE, AGING AND OXIDATIVE DAMAGE TO MITOCHONDRIA

Intractable or chronic fatigue lasting more than 6 months that is not reversed by sleep is the most common complaint of patients seeking medical care^{17,18}. It is also an important secondary condition in many clinical diagnoses and occurs naturally during aging^{18,19}. The phenomenon of fatigue has only recently been defined as a multidimensional sensation, and attempts have been made to determine the extent of fatigue and its possible causes^{17,19}. Most patients understand fatigue as a loss of energy and inability to perform even simple tasks without exertion. Many medical conditions are associated with fatigue, including respiratory, coronary, musculoskeletal, and bowel conditions as well as infections and cancers¹⁹⁻²¹.

Fatigue is involved with cellular energy systems found primarily in the cells' mitochondria. Damage to mitochondrial components, mainly by oxidation, can impair their ability to produce high-energy molecules such as ATP and NADH. This occurs naturally with aging and during chronic illnesses¹. During aging the production of Reactive Oxygen Species (ROS), made up of oxidative and free radical oxygen- and nitrogen-containing molecules, such as nitric oxide, oxygen and hydroxide radicals and other molecules, can cause oxidative stress and cellular damage, resulting in oxidation of lipids, proteins and DNA^{22,23}. When oxidized, these molecules are structurally and sometimes functionally changed. The major targets of ROS damage are mitochondria, mainly their phospholipid-containing membranes, and cellular and mitochondrial DNA^{1,22,23}.

Excess ROS production throughout our lifetimes can result in accumulation of mitochondrial and nuclear damage^{1,23,24}. On the other hand, cellular free-radical scavenging enzymes neutralize excess ROS and repair the enzymes that reverse ROS-mediated damage²⁵. Although some ROS production is important in triggering cell proliferation, gene expression and destruction of invading microbes²⁶, with aging ROS damage accumulates^{1,22,23}. When this occurs, antioxidant enzymes and enzyme repair mechanisms along with biosynthesis cannot restore or replace enough ROS-damaged molecules^{1,22,27-29}. Disease and infection can also result in oxidative damage that exceeds the abilities of cellular systems to repair and replace damaged molecules^{5,24}.

PREVENTING ROS-MEDIATED DAMAGE WITH ANTIOXIDANTS

The reduction of cellular and mitochondrial membrane and DNA damage and loss of membrane integrity are important in preventing loss of cellular energy and regulating cellular life span²⁸. This can be accomplished, in part, by neutralizing ROS with various antioxidants or increasing free-radical scavenging systems that neutralize ROS. Thus dietary antioxidants and some accessory molecules, such as zinc and certain vitamins, are important in maintaining antioxidant and free-radical scavenging systems. In addition to zinc and vitamins, there are at least 40 micronutrients required in the human diet³⁰, and aging increases the need to supplement these to prevent age-associated damage to mitochondria and other cellular elements. Antioxidant use alone, however, may not be sufficient to maintain cellular

components free of ROS damage. Therefore, LRT is important in replacing ROS-damaged lipids⁵.

Dietary antioxidant supplementation has partially reversed the age-related declines in cellular antioxidants and mitochondrial enzyme activities and prevented mitochondria from most age-associated functional decline. For example, rodents were fed diets supplemented with antioxidants, and the antioxidants were found to inhibit the progression of certain age-associated changes in cerebral mitochondrial electron transport chain enzyme activities^{31,32}. Thus animal studies have shown that antioxidants can partially prevent age-associated changes in mitochondrial function. However, antioxidants alone cannot completely eliminate ROS damage to mitochondria, and this is why LRT is an important addition to antioxidant supplementation.

Antioxidants may also modify the pathogenic processes of certain diseases^{5,33}. For example, antioxidant administration has been shown to have certain neuroprotective effects³⁴.

The dietary use of antioxidants has been shown to prevent age-associated mitochondrial dysfunction and damage, inhibit the age-associated decline in immune and other functions and prolong the lifespan of laboratory animals^{5,34-36}.

PRECLINICAL STUDIES USING LIPID REPLACEMENT THERAPY

LTR replaces damaged cellular and mitochondrial membrane phospholipids and other lipids using dietary supplements containing polyunsaturated phosphatidylcholines and other phospholipids, glycopospholipids and fatty acids that are essential structural and functional components of all biological membranes⁵. One such LRT dietary supplement is called NTFactor[®], and it has been used successfully in animal and clinical lipid replacement studies. NTFactor's encapsulated lipids are protected from oxidation in the gut and can be absorbed and transported into tissues without undue damage. NTFactor[®] contains a variety of components, including glycopospholipids and other lipids, nutrients, probiotics, vitamins, minerals and plant extracts (Table 1).

Table 1. Components of NTFactor[®] (from ref. 5).

NT Factor[®] 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:

Glycopospholipids: 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 Media: 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.

NT Factor is a registered trademark of Nutritional Therapeutics, Inc., PO Box 5963 Hauppauge NY 11788 Tel.: +1-800-982-9158

NT Factor[®] has been used for anti-aging studies in aged laboratory animals. In aged rodents, Seidman *et al.*³⁹ found that

NTFactor prevented hearing loss associated with aging and shifted the threshold hearing from 35-40 dB in control aged animals to 13-17 dB in the treatment group ($P < 0.005$). They also found that NTFactor preserved cochlear mitochondrial function as measured in a Rhodamine-123 transport assay⁴⁰, increasing mitochondrial function by 34%. NTFactor also prevented aging-related mitochondrial DNA deletions found in the cochlear³⁹. Thus LRT was successful in preventing age-associated hearing loss and mitochondrial damage.

CLINICAL STUDIES USING LIPID REPLACEMENT THERAPY

LRT has been successfully used in clinical studies to reduce fatigue and protect cellular and mitochondrial membranes from damage by ROS. For example, NT Factor has been used in a vitamin and mineral mixture (Propax®) in cancer patients to reduce the effects of cancer therapy, such as chemotherapy-induced fatigue, nausea, vomiting and other side effects associated with chemotherapy⁴¹. This double-blinded, cross-over, placebo-controlled, randomized trial on cancer patients receiving chemotherapy Propax® supplementation showed LRT improvement from fatigue, nausea, diarrhea, impaired taste, constipation, insomnia and other quality of life indicators⁴¹. Following cross-over to the Propax supplement, patients reported rapid improvement in nausea, impaired taste, tiredness, appetite, sick feeling and other quality of life indicators⁴¹. Propax plus NTFactor has been used in an LRT study with severely fatigued, aged subjects (>60 years-old with a variety of clinical diagnoses) to reduce fatigue³⁷. We found that fatigue was reduced approximately 40% ($P < 0.0001$), from severe to moderate fatigue, after eight weeks of supplementation with Propax. Recently we examined 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 and fatigue scores, improved with administration of NTFactor³⁸. Use of NTFactor for 8 or 12 weeks resulted in a 33% or 35.5% reduction in fatigue, respectively ($P < 0.001$)³⁸. In this clinical trial there was good correspondence between reductions in fatigue and gains in mitochondrial function. After only 8 weeks of LRT with NTFactor, mitochondrial function was significantly improved ($P < 0.001$), and after 12 weeks of NTFactor supplementation, mitochondrial function was found to be similar to that of young healthy adults³⁸. After 12 weeks of supplement use, subjects discontinued the supplement for an additional 12 weeks, and their fatigue and mitochondrial function were again measured. After the 12-week wash-out period fatigue and mitochondrial function were intermediate between the initial starting values and those found after eight or 12 weeks on supplement, indicating that continued LTR is probably required to show improvements in mitochondrial function and maintain lower fatigue scores³⁸. The results, however, indicate that in aging subjects LRT can significantly improve and even restore mitochondrial function and improve fatigue.

CONCLUSIONS

When mitochondrial function is impaired, the net energy available to cells is limited to the Krebs Cycle and anaerobic metabolism. There are a number of conditions and substances that can impair mitochondrial function, but oxidation and damage of mitochondrial membrane lipids are probably among the most important effects^{28,42}. Mitochondrial function appears to be directly related to fatigue, and when patients experience fatigue their mitochondrial function is inevitably impaired. Fatigue is a complex phenomenon determined by several factors,

including psychological health⁵, but at the biochemical level fatigue is related to the metabolic energy available to tissues and cells, mainly through mitochondrial electron transport. Thus the integrity of mitochondrial membranes is critical to cell function and energy metabolism. When mitochondrial membrane lipids are damaged by oxidation, they must be repaired or replaced in order to maintain the production of cellular energy to alleviate fatigue. During aging and in many diseases ROS-mediated accumulation of oxidized mitochondrial lipid occurs. The failure to repair or replace these damaged molecules at a rate that exceeds their damage results in gradual loss of function. The failure to repair or replace these lipids at a rate that exceeds their damage can result in loss of function. Mitochondrial membrane damage and subsequent dysfunction by ROS can also lead to an increased rate of mitochondrial DNA modifications (especially mutations and deletions). 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^{27,28,33,35}. The damage to mitochondrial membranes and DNA seems to also be involved in the etiology of age-associated degenerative diseases^{33,43}. Restoration of mitochondrial membrane integrity, fluidity and other properties are essential for the optimal functioning of the electron transport chain. The ability to control membrane lipid peroxidation and DNA damage will likely play an important role in attenuating the aging process and the development of age-related degenerative diseases^{33,35,44}. LRT plus antioxidants has proven to be a valuable tool in maintaining mitochondrial function and preventing fatigue, and it should be an important part of anti-aging and disease prevention strategies⁵.

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