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Lipid Replacement and Antioxidant Nutritional Therapy for Restoring Mitochondrial Function and Reducing Fatigue in Chronic Fatigue Syndrome and other Fatiguing Illnesses*

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ABSTRACT. Evidence in the literature indicates that diminished mitochondrial function through loss of efficiency in the electron transport chain caused by oxidation occurs during aging and in fatiguing illnesses. Lipid Replacement Therapy (LRT) administered as a nutritional supplement with antioxidants can prevent oxidative membrane damage, and LRT can be used to restore mitochondrial and other cellular membrane functions via delivery of undamaged replacement lipids to cellular organelles. Recent clinical trials using patients with chronic fatigue have shown the benefit of LRT plus antioxidants in restoring mitochondrial electron transport function and reducing moderate to severe chronic fatigue. These studies indicate the benefits of LRT plus antioxidants in reducing fatigue and preventing loss of mitochondrial function, most likely by protecting mitochondrial and other cellular membranes from oxidative and other damage and removing damaged lipids by lipid replacement. In one clinical study we determined if mitochondrial function is reduced in subjects with mild to severe chronic fatigue, and if this can be reversed with NTFactor®, a nutritional supplement that replaces damaged cellular lipids. Using the Piper Fatigue Scale there was a significant time-dependent reduction in overall fatigue in moderately or severely fatigued subjects while on the dietary supplement for 4-8 weeks. Analysis of mitochrondrial function indicated that four and eight weeks of the dietary supplement in moderately or severely fatigued subjects significantly increased mitochondrial function. Similarly, chronic fatigue syndrome patients administered antioxidants plus LRT also show reductions in fatigue. The results indicate that LRT plus antioxidants can significantly reduce moderate to severe chronic fatigue and restore mitochondrial function. Dietary use of unoxidized membrane lipids plus antioxidants is recommended for patients with moderate to severe chronic fatigue.

KEYWORDS. lipids, antioxidants, therapy, dietary supplement, fatigue, mitochondria, chronic fatigue syndrome

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INTRODUCTION

One of the most important changes in tissues and cells that occurs during aging and chronic degenerative disease is accumulated oxidative damage due to cellular reactive oxygen species (ROS). ROS are oxidative and free radical oxygen- and nitrogen-containing molecules, such as nitric oxide, oxygen and hydroxide radicals and other molecules [1]. Critical targets of ROS are the genetic apparatus and cellular membranes [1,2], and in the latter case oxidation can affect lipid fluidity, permeability and membrane function [3,4]. Similar changes occur in fatiguing illnesses, such as chronic fatigue syndrome (CFS), where patients show increased susceptibility to oxidative stress and peroxidation [5,6]. One of the most important changes caused by accumulated ROS damage during aging and in fatigue is loss of electron transport function, and this appears to be directly related to mitochondrial membrane lipid peroxidation [1], which can induce permeability changes in mitochondria and loss of transmembrane potential and oxidative phosphorylation [1,2].

We will concentrate this brief review on recent clinical trials that have shown the effectiveness of lipid replacement therapy (LRT) plus antioxidants in the treatment of certain clinical disorders and conditions, such as chronic fatigue [7]. 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 membranes [7]. Damage to membrane lipids can impair fluidity, electrical properties, enzymatic activities and transport functions of cellular and organelle membranes [1-6].

During LRT lipids must 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 must 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 ROS-associated changes in certain cellular activities and functions and for use in the treatment of certain clinical conditions [7].

HEALTH BENEFITS OF LIPID SUPPLEMENTS

Mixtures of lipids introduced as dietary supplements have been used to improve general health [8,9], and they have also been used as an adjunct therapy in the treatment of various clinical conditions, for example, the use of n-3 fatty acids in cardiovascular diseases and inflammatory disorders [9-12]. Although not every clinical study has found health benefits from lipid dietary supplementation [13], 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 lipids [8-12].

Cellular lipids are in dynamic equilibrium in the body, and this is why LRT is possible [7]. Orally ingested lipids diffuse to the gut epithelium and are bound and eventually transported into the blood and lymph using specific carrier alipoproteins and also by nonspecific partitioning and diffusion mechanisms [14,15]. 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 [16,17]. 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 diffusion [17]. 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, partitioning, and diffusion [18]. 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 them [18].

CHRONIC FATIGUE 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 [19,20]. It is also an important secondary condition in many clinical diagnoses and occurs naturally during aging [19,20]. 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 [21-23]. 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 cancer [7,20-23].

Fatigue is related to cellular energy systems found primarily in the cells' mitochondria. Damage to mitochondrial components, mainly by ROS oxidation, can impair their ability to produce high-energy molecules such as ATP and NADH. This occurs naturally with aging and during chronic illnesses, where the production of ROS can cause oxidative stress and cellular damage, resulting in oxidation of lipids, proteins and DNA [24,25]. When oxidized, these molecules are structurally and sometimes functionally changed. Important targets of ROS damage are mitochondria, mainly their phospholipid-containing membranes, and cellular and mitochondrial DNA [1,24,25].

Excess ROS production throughout our lifetimes can result in accumulation of mitochondrial and nuclear damage [1,24-26]. Opposed to this, cellular free-radical scavenging enzymes neutralize excess ROS and repair the enzymes that reverse ROS-mediated damage [25,26]. Although some ROS production is important in triggering cell proliferation, gene expression and destruction of invading microbes [27,28], with aging ROS damage accumulates [1,24-26]. When this occurs, antioxidant enzymes and enzyme repair mechanisms along with biosynthesis cannot restore or replace enough ROS-damaged molecules [1,24,28-30]. Disease and infection can result in oxidative damage that exceeds the abilities of cellular systems to repair and replace damaged molecules [6,24,27], and this is also the situation in fatiguing illnesses [5,6].

In CFS patients there is evidence of oxidative damage to DNA and lipids [reviewed in 5,6] as well as the presence of blood markers, such as methemoglobin, that are indicative of excess oxidative stress [31]. Fulle et al. [32] found oxidative damage in the DNA and membrane lipids from muscle biopsy samples obtained from CFS patients. They also found increases in antioxidant enzymes, such as glutathione peroxidase, and suggested that this was an attempt to compensate for excess oxidative stress in CFS. Pall [33] has proposed that CFS patients have sustained elevated levels of the RNS peroxynitrite due to excess nitric oxide and that this results in lipid peroxidation and loss of mitochondrial function as well as changes in cytokine levels that exert a positive feedback on nitric oxide production. In addition to mitochondrial membranes,

mitochondrial enzymes, such as succinic dehydrogenase and cis-aconitase, are inactivated by peroxynitrite, and this could also contribute to loss of mitochrondrial function [34,35]. Also, cellular molecules that could counteract the excess oxidative capacity of ROS/RNS, such as glutathione and cysteine, have been found in lower levels in CFS patients [36].

PREVENTING ROS/RNS-MEDIATED DAMAGE WITH ANTIOXIDANTS

Reversal of damage of cellular and mitochondrial membranes as well as DNA are important in preventing loss of cellular energy [5,29,30,37]. This can be accomplished, in part, by neutralizing ROS/RNS with various antioxidants or increasing free-radical scavenging systems that neutralize ROS/RNS. Thus dietary antioxidants and some accessory molecules, such as zinc and certain vitamins, are important in maintaining antioxidant and free-radical scavenging systems [reviewed in 5]. In addition to zinc and vitamins, there are at least 40 micronutrients required in the human diet [38], 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 membrane lipids [7].

In animal studies 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, in rodents fed diets supplemented with antioxidants the antioxidants were found to inhibit the progression of certain age-associated changes in cerebral mitochondrial electron transport chain enzyme activities [39,40]. 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 dietary supplementation [7].

Dietary antioxidants may also modify the pathogenic processes of certain diseases [5,7,33,41]. For example, antioxidant administration has been shown to have certain neuroprotective effects [42]. 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 [5,7,42-44].

PRECLINICAL STUDIES USING LIPID REPLACEMENT THERAPY

LTR replaces damaged cellular and mitochondrial membrane phospholipids and other lipids that are essential structural and functional components of all biological membranes [7]. One such LRT dietary supplement is NTFactor®, and this supplement has been used successfully in animal and clinical lipid replacement studies [45,46]. NTFactor's encapsulated lipids are protected from oxidation in the gut and can be absorbed and transported into tissues without undue damage. NTFactor(r) contains a variety of components, including phospholipids, glycophospholipids and other lipids, nutrients, probiotics, vitamins, minerals and plant extracts (Table 1).

NTFactor® has also been used for studies in laboratory animals. In aged rodents, Seidman et al. [47] 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 assay [48], increasing mitochondrial function by 34%. NTFactor® also prevented aging-related mitochondrial DNA deletions found in the cochlear [47]. Thus LRT was successful in preventing age-associated hearing loss and mitochondrial damage in rodents.

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/RNS [45,46]. For example, NTFactor® 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 chemotherapy [49]. 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 indicators [49]. Following cross-over to the Propax® supplement, patients reported rapid improvement in nausea, impaired taste, tiredness, appetite, sick feeling and other quality of life indicators [49].

Propax® containing NTFactor® has been used in a dietary LRT study with severe chronic fatigued patients to reduce their fatigue [45]. Using the Piper Fatigue Scale [23] we found that fatigue was reduced approximately 40.5% ($P<0.0001$), from severe to moderate fatigue, after eight weeks of supplementation with Propax(r) containing NTFactor® (Table 2). Recently we 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 and fatigue scores, improved with administration of NTFactor® [46]. Use of NTFactor® for 8 or 12 weeks resulted in a 33% or 35.5%

reduction in fatigue, respectively (P<0.001) (Table 2) [46]. 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 adults [46]. 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 dietary LTR is probably required to show improvements in mitochondrial function and maintain lower fatigue scores [46]. The results indicate that in moderately to severely fatigued subjects dietary LRT can significantly improve and even restore mitochondrial function and significantly improve fatigue. Using the Piper Fatigue Scale our unpublished data on a small number of CFS (and/or Fibromyalgia Syndrome) patients indicates that LRT plus antioxidants for 8 weeks reduces moderate to severe fatigue by 43.1% (Table 2).

SUMMARY

When mitochondrial function is impaired, such as during moderate to severe fatigue, 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 peroxidation and damage of mitochondrial membrane lipids are probably among the most important effects [35,39,50]. Mitochondrial function appears to be directly related to fatigue, and when patients experience moderate to severe fatigue their mitochondrial function is inevitably impaired. Fatigue is a complex phenomenon determined by several factors, including psychological health [22,23], 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, including fatiguing illnesses, ROS/RNS-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 impaired mitochondrial function.

Mitochondrial membrane damage and subsequent dysfunction by ROS/RNS 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 oxidative damage to mitochondrial membranes and DNA over time [29,30,41,43]. The damage to mitochondrial membranes and DNA seems to also be involved in the etiology of age-associated degenerative diseases [41,51]. 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 development of age-related degenerative diseases [41,43,52]. Dietary 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 treatment strategies for CFS and other fatiguing illnesses [7].

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TABLE 1. Components of NTFactor®, a dietary LRT supplement [7].

NT Factor® is a nutrient complex that is extracted and prepared using a proprietary process that protects lipids from oxidation. In addition, nutrients, vitamins and probiotic microorganisms are added to the preparation. It contains the following ingredients:

Glycophospholipids: polyunsaturated phosphatidylcholine, other polyunsaturated phosphatidyl lipids, glycolipids and other lipids such as cardiolipin and sterol lipids.

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.

NTFactor® is a registered trademark of Nutritional Therapeutics, Inc., P.O. Box 5963, Hauppauge, NY 11788 (Tel: +1-800-982-9158), Website: www.propax.com

TABLE 2. Effects of NTFactor®, a dietary LRT supplement, on Piper Fatigue Scale scores.

Subjects/patients	N	Average age	Time on NTFactor®	Piper Fatigue Scale fatigue reduction (%)	Reference
Chronic fatigue	34	50.3	8 wks	40.5**	45
Chronic fatigue	20	68.9	12 wks	35.5*	46
CFS (and/or FMS‡)	15	44.8	8 wks	43.1*	--

***P*<0.0001, **P*<0.001 compared to data without supplement
‡ Fibromyalgia Syndrome, 5/15