ORIGINAL RESEARCH

Mitochondrial Dysfunction and Chronic Disease: Treatment With Natural Supplements

Garth L. Nicolson, PhD

ABSTRACT

Loss of function in mitochondria, the key organelle responsible for cellular energy production, can result in the excess fatigue and other symptoms that are common complaints in almost every chronic disease. At the molecular level, a reduction in mitochondrial function occurs as a result of the following changes: (1) a loss of maintenance of the electrical and chemical transmembrane potential of the inner mitochondrial membrane, (2) alterations in the function of the electron transport chain, or (3) a reduction in the transport of critical metabolites into mitochondria. In turn, these changes result in a reduced efficiency of oxidative phosphorylation and a reduction in production of adenosine-5'-triphosphate (ATP). Several components of this system require routine replacement, and this need can be facilitated with natural supplements. Clinical trials have shown the utility of using oral replacement supplements, such as L-carnitine, alpha-lipoic acid (α-lipoic acid [1,2-dithiolane-3-pentanoic acid]), coenzyme Q_{10} (CoQ_{10} [ubiquinone]), reduced nicotinamide adenine dinucleotide (NADH), membrane phospholipids, and other supplements. Combinations of these supplements can reduce significantly the fatigue and other symptoms associated with chronic disease and can naturally restore mitochondrial function, even in long-term patients with intractable fatigue. (*Altern Ther Health Med.* 2014;20 (suppl 1):18-25.)

Garth L. Nicolson, PhD, is founder, president, and research professor in the Department of Molecular Pathology at The Institute for Molecular Medicine in Huntington Beach, California.

Corresponding author: Garth L. Nicolson, PhD
E-mail address: gnicolson@immed.org

Mitochondrial dysfunction, characterized by a loss of efficiency in the electron transport chain and reductions in the synthesis of high-energy molecules, such as adenosine-5'-triphosphate (ATP), is a characteristic of aging, and essentially, of all chronic diseases.[1-4] These diseases include neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Friedreich's ataxia[1,2,4,5]; cardiovascular diseases, such as atherosclerosis and other heart and vascular conditions[6,7]; diabetes and metabolic syndrome[8-10]; autoimmune diseases, such as multiple sclerosis, systemic lupus erythematosus, and type 1 diabetes[11-14]; neurobehavioral and psychiatric diseases, such as autism spectrum disorders, schizophrenia, and bipolar and mood disorders[15-19]; gastrointestinal disorders[20,21]; fatiguing illnesses, such as chronic fatigue syndrome and Gulf War illnesses[22-24]; musculoskeletal diseases, such as fibromyalgia and skeletal muscle hypertrophy/atrophy[25-27]; cancer[28,29]; and chronic infections.[30,31]

It is well known among researchers that mitochondrial genetic or primary mitochondrial disorders contribute to mitochondrial dysfunction as well as secondary or acquired degenerative disorders.[32] This review will concentrate on nongenetic or acquired mechanisms that could explain mitochondrial dysfunction and their replacement treatment with natural supplements and combinations of natural supplements, including vitamins, minerals, enzyme cofactors, antioxidants, metabolites, transporters, membrane-type phospholipids, and other natural supplements.

MITOCHONDRIAL MOLECULAR DYSFUNCTION

Mitochondrial dysfunction arises from an inadequate number of mitochondria, an inability to provide necessary substrates to mitochondria, or a dysfunction in their electron transport and ATP-synthesis machinery. The number and functional status of mitochondria in a cell can be changed by (1) fusion of partially dysfunctional mitochondria and mixing of their undamaged components to improve overall function, (2) the generation of entirely new mito-

chondria (fission), and (3) the removal and complete degradation of dysfunctional mitochondria (mitophagy).[33] These events are controlled by complex cellular processes that sense the deterioration of mitochondria, such as the depolarization of mitochondrial membranes or the activation of certain transcription pathways.[34,35]

The ability of cells to produce almost all high-energy molecules like ATP is directly related to the ability of mitochondria to (1) convert the energy of metabolites to reduced nicotinamide adenine dinucleotide (NADH) and (2) transfer electrons from NADH to the electron transport chain and eventually to molecular oxygen while pumping protons from the mitochondrial matrix across the inner mitochondrial membrane to the intermembrane space. This process creates a transmembrane proton gradient (Δp) and an electrochemical gradient ($\Delta\psi_m$) across the mitochondrial inner membrane.[36,37] The transmembrane potential created by the proton gradient then uses ATP synthase to flow protons back across the inner mitochondrial membrane and employs the energy from this process to drive adenosine diphosphate (ADP) phosphorylation to ATP.[36,38]

A consequence of the electron transport process is the production of reactive oxygen species (ROS), highly reactive free radicals that are produced as a by-product of oxidative phosphorylation. The main sources of ROS and the related reactive nitrogen species (RNS) are mitochondria, and these free radicals can damage cellular lipids, proteins, and DNA.[39-41] However, some mechanisms can neutralize ROS/RNS; dismutase enzymes and antioxidants can control excess amounts of ROS/RNS.[42,43] In addition to creation of ROS/RNS, the electron transport process can induce uncoupling proteins, resulting in a controlled leak of protons back across the proton gradient of the inner mitochondrial membrane into the mitochondrial matrix.[36,37] This leak results in reduced ATP production while it still consumes excess oxygen.[43]

In the presence of a controlled proton leak, excess oxygen consumption and the resulting ROS production can result in inappropriate damage to mitochondrial membrane lipids,[41,42] such as the very ROS/RNS-sensitive cardiolipin, an inner mitochondrial membrane phospholipid.[44] Oxidative damage to the cardiolipin and other membrane phospholipids in the inner mitochondrial membrane can result in increased proton and ion leakage back across the inner membrane into the mitochondrial matrix and partial loss of the electrochemical gradient. Cardiolipin is also an important component of the electron transport chain, providing stability for the cytochrome/enzyme complexes in the inner mitochondrial membrane.[44] Once adversely damaged by ROS/RNS, oxidized cardiolipin instigates loss of electron-transport function.[45]

Cellular antioxidant defenses usually maintain ROS/RNS levels at concentrations that prevent excess oxidation of cellular molecules.[46-48] Cellular antioxidant defenses that are endogenous are mediated by glutathione peroxidase, catalase, and superoxide dismutase, among other enzymes.[48,49] Also, some dietary antioxidants with a low molecular weight can affect antioxidant status.[50,51] Some of these dietary antioxidants have been used as natural preventive agents to shift the excess concentrations of oxidative molecules down to physiological levels that can be maintained by endogenous antioxidant systems.[52]

Table 1. An Incomplete List of Ingredients/Agents That Medical Practitioners Have Used or Suggested for Treatment of Mitochondrial Dysfunction[a]

Category	Examples
Vitamins	Vitamins C, D and E, thiamine, riboflavin
Minerals	Magnesium, calcium, phosphate
Lipids	Membrane phospholipids, unsaturated fatty acids
Metabolites	Creatine, pyruvate
Cofactors	CoQ_{10}, α-Lipoic acid, NADH, nicotinic acid
Transporters	L-Carnitine, membrane phospholipids
Antioxidants	CoQ_{10}, α-lipoic acid, NADH, glutathione
Enzyme inhibitors	α-Lipoic acid, dichloroacetate
Herbs	Curcumin, schisandrin

Abbreviations: NADH = reduced nicotinamide adenine dinucleotide; CoQ_{10} = coenzyme Q_{10} (ubiquinone); α-lipoic acid = alpha-lipoic acid.

[a]Modified from Kerr.[32]

MITOCHONDRIAL DYSFUNCTION AND FATIGUE

Mitochondrial dysfunction is directly related to excess fatigue. Fatigue is considered a multidimensional sensation that is perceived to be a loss of overall energy and an inability to perform even simple tasks without exertion.[53,54] Although mild fatigue can be caused by a number of conditions, including depression and other psychological conditions, moderate to severe fatigue involves cellular energy systems.[53,54] At the cellular level, moderate to severe fatigue is related to loss of mitochondrial function and diminished production of ATP.[54-56] Intractable fatigue lasting more than 6 months that is not reversed by sleep (chronic fatigue) is the most common complaint of patients seeking general medical care.[53,57] Chronic fatigue is also an important secondary condition in many clinical diagnoses, often preceding patients' primary diagnoses.[57,58]

As a result of aging and chronic diseases, oxidative damage to mitochondrial membranes impairs mitochondrial function.[59-61] As an example, individuals with chronic fatigue syndrome present with evidence of oxidative damage to DNA and lipids,[61,62] such as oxidized blood markers[63] and oxidized membrane lipids,[64] that is indicative of excess oxidative stress. These individuals also have sustained, elevated levels of peroxynitrite due to excess nitric oxide, which can also result in lipid peroxidation and loss of mitochondrial function as well as changes in cytokine levels that exert a positive feedback on nitric oxide production.[65]

NATURAL SUPPLEMENTS AND MITOCHONDRIAL DYSFUNCTION

A number of natural supplements have been used to treat nonpsychological fatigue and mitochondrial dysfunction.[32,54,66] These supplements include those containing vitamins, minerals, antioxidants, metabolites, enzyme inhibitors and cofactors, mitochondrial transporters, herbs, and membrane phospholipids (Table 1).[32] Although several natural supplements have

been used to reduce fatigue, few are considered truly effective.[67] This article will discuss some of the most promising supplements and conclude with combinations of specific supplements that have been used to treat intractable chronic fatigue and improve mitochondrial function.

α-Lipoic Acid

Alpha-lipoic acid (α-lipoic acid [1,2-dithiolane-3-pentanoic acid]) is a potent antioxidant, transition metal-ion chelator, redox transcription regulator, and anti-inflammatory agent.[68] It acts as a critical cofactor in mitochondrial α-keto acid dehydrogenases, and thus it is important in mitochondrial, oxidative-decarboxylation reactions.[69,70] Clinically, α-lipoic acid has been used as an oral supplement in the treatment of complications associated with diabetes mellitus, and according to a review by Shay et al,[70] it has been shown to bring about improvements in various diabetes-associated neuropathies, inflammation, and vascular health. In model cellular systems, these effects have been attributed mainly to α-lipoic acid having signal-transduction effects on gene regulation and on glucose uptake and metabolism as well as its antioxidant effects.[71]

As a result of aging and in many chronic diseases, certain sphingolipids—especially ceramides, and in particular, short-chain ceramides—accumulate in mitochondria due to hydrolysis of sphingomyelin by sphingomyelinase, and eventually, this accumulation retards electron transport activity.[72,73] Ceramide accumulation in mitochondria is especially damaging to cardiac tissue. In aging rodents, α-lipoic acid has been used to lower ceramide levels in vascular endothelial cells of cardiac muscle through inhibition of sphingomyelinase activity, resulting in restoration of mitochondrial glutathione levels and increasing electron transport function.[74]

As mentioned above, in diabetes α-lipoic acid has been used extensively to reduce diabetic complications, such as sensorimotor polyneuropathies.[75] One 4-year, blinded study demonstrated that some neuropathic impairments improved significantly on α-lipoic acid (but not nerve conduction attributes), showing the antioxidant's clinical utility and the safety of long-term treatment with α-lipoic acid for diabetic patients.[76]

Given as an oral supplement, α-lipoic acid is rarely present in tissues above micromolar levels; thus, it is unlikely to be directly involved as an important primary cellular antioxidant.[70] However, its ability to increase cellular glutathione levels is an important antioxidant property, and this increase is accomplished by regulating glutathione synthesis and thus ameliorating oxidative stress.[77] This antioxidant can affect the regulation of the nuclear transcription factor NF-κB, and thus, it can cause widespread transcriptional effects, resulting in the attenuation of production of free radicals and cytotoxic cytokines.[78]

As a transition metal chelater, α-lipoic acid can remove excess copper, iron, and other metals that are involved in chronic diseases, such as hemochromatosis, end-stage renal failure, and Alzheimer's and Parkinson's diseases, and it is a potential therapeutic agent for prevention or mitigation of heavy metal poisoning.[68] It also improves cognitive function as well as mitochondrial function, suggesting a link between oxidative damage to mitochondria and cognition.[79] The use of α-lipoic acid for chronic fatigue has not yet been studied in controlled clinical trials, but its widespread use as a safe supplement (usually 200-600 mg/d)[70] to support mitochondrial function and reduce oxidative stress has justified its incorporation into various supplement mixtures.[70,76,78]

L-Carnitine

L-carnitine (3-hydroxy-4-*N*-trimethylaminobutyrate) is a naturally occurring fatty acid transporter found in all species of mammals. It is directly involved in the transport of fatty acids into the mitochondrial matrix for subsequent β-oxidation, but it also functions in removal of excess acyl groups from the body and in the modulation of intracellular coenzyme A (CoA) homeostatasis.[80,81] Because of its importance in fatty acid oxidation and CoA and acyl-CoA homeostasis, L-carnitine is usually maintained within relatively narrow concentration limits; thus, dietary supplementation is important to maintain optional L-carnitine concentrations within cells.[81] Indeed, L-carnitine deficiency disorders are associated with reduced mitochondrial function, insulin resistance, and coronary artery disease.[82-84]

The important role of L-carnitine in mitochondrial metabolism has spurred the use of L-carnitine supplements to potentially improve physical performance.[85] The rationale is that increased reliance on fat as the principle substrate for energy production during extreme exercise should reduce the need for carbohydrates and delay the depletion of carbohydrate stores and that these changes should increase overall energy production and reduce exercise-induced fatigue. Transport of fatty acids into mitochondria also requires increased levels of L-carnitine, and thus, indicates a need for dietary supplementation of L-carnitine. However, studies have shown that increasing oral L-carnitine supplementation, even for 2 to 3 weeks prior to extreme exercise, did not increase carnitine content in skeletal muscle. Therefore, it is unlikely that this supplementation alters muscle metabolism during extreme exercise.[86,87]

L-carnitine supplementation has been successfully used in clinical disorders that are characterized by low concentrations of L-carnitine or impaired fatty acid oxidation, such as diabetes, sepsis, renal disease, and cardiomyopathy.[80] For example, in a small study of 18 individuals with congestive heart failure and 12 placebo controls, propionyl-L-carnitine supplementation resulted in increased peak heart rate (mean of 12%), exercise capacity (mean of 21%), and peak oxygen consumption (mean of 45%) in the treatment group.[88]

An important antiaging use of L-carnitine has been to increase the rate of mitochondrial oxidative phosphorylation that naturally declines as a result of aging. This decline impairs energy production while it increases production of ROS/RNS. Feeding old rats acetyl-L-carnitine was found to reverse age-related decreases in L-carnitine levels while it increased fatty acid metabolism. It also reversed the age-

related decline in cellular glutathione levels and improved the complex IV activity of muscle mitochondria.[89]

Although dietary supplementation with L-carnitine and its various derivatives appears to be safe (doses up to 2 g/d)[90] and potentially useful in increasing mitochondrial function, researchers have not performed the necessary multiple clinical trials to show its effectiveness in most age-related chronic illnesses (other than diabetes and cardiovascular diseases). One exception was a randomized, controlled clinical trial on 70 centenarians who were treated with L-carnitine for 6 months. These participants were generally found to have muscle weakness, decreasing mental health, impaired mobility, and poor endurance. By the end of the study, the treated group showed significant improvements in physical fatigue, mental fatigue, and fatigue severity. They also showed reductions in total fat mass, increased muscle mass, and an increased capacity for physical and cognitive activity through reduced fatigue and improved cognitive function.[91] Other trials on alcoholism, hepatic encephalopathy, coronary heart diseases, Peyronie's disease, cerebral ischemia, and infertility indicated that administration of L-carnitine can have positive effects on signs and symptoms of these conditions.[90]

CoQ_{10}

Coenzyme Q_{10} (CoQ_{10} [ubiquinone]) is a key cofactor and component of the mitochondrial electron transport chain and one of the most widely used natural supplements.[32,92] It is also a strong antioxidant in its reduced form, and it can affect the expression of certain genes involved in cell signaling, metabolism, and transport.[92,93] However, the main role of CoQ_{10} is its involvement in the transfer of electrons along the multiple complexes of the mitochondrial electron transport chain.[92,94] Clinically, it has been used in doses up to 1200 mg per day, but most studies used lower doses.[92]

Because CoQ_{10} is an important component of the mitochondrial oxidative phosphorylation system, its supplementation in individuals with reduced levels should result in increased energy production and reduced fatigue.[92,94] A systematic review of the effects of CoQ_{10} in physical exercise, hypertension, and heart failure by Rosenfeldt et al[95] revealed that six out of 11 published studies showed modest improvements in exercise capacity in the participants given dietary CoQ_{10}. In 8 of the studies, which examined the effects of CoQ_{10} on hypertension, a mean decrease occurred in blood pressure: systolic, a mean decline of 16 mm Hg; and diastolic, a mean decline of 10 mm Hg. In the review, nine randomized trials that examined the use of CoQ_{10} in participants with heart failure showed nonsignificant trends toward increased injection fraction and reduced mortality. Rosenfeldt et al[95] performed their own 3-month, randomized, placebo-controlled trial on the effects of oral CoQ_{10} in 35 patients with heart failure and found that participants in the CoQ_{10} arm, but not in the control arm, showed significant improvements in symptoms.[95] The study also showed a trend toward improvements in mean exercise times.[95]

The effects of administration of oral CoQ_{10} during physical exercise have also been examined. In a blinded, crossover trial, 17 healthy participants received CoQ_{10} or a placebo for 8 days, and their performance was then evaluated twice at fixed workloads on a bicycle ergometer for 2 hours, with a 4-hour rest between the tests.[96] The participants on CoQ_{10} were able to achieve higher work outputs and had less fatigue sensations, and their need for a recovery period was alleviated compared to the placebo group.[96]

Clinically, CoQ_{10} has been used to reduce symptoms and progression in various neurodegenerative diseases.[92,94] In studies using Alzheimer's disease models, CoQ_{10} administration significantly delayed brain atrophy and typical β-amyloid-plaque pathology.[97,98] In a randomized, placebo-controlled, 16-week clinical trial on 98 Alzheimer's participants who took an oral mixture of CoQ_{10}, vitamins C and E, and α-lipoic acid, the test arm showed significant reductions in oxidative-stress markers but did not show significant changes in cerebrospinal fluid (CSF) markers related to β-amyloid or tau pathology.[99]

In Parkinson's disease, individuals generally show increased oxidized-to-total CoQ_{10} ratios as well as significant increases in markers of oxidative damage in the CSF, which can be partially reversed with CoQ_{10} administration.[100] In individuals with early Huntington's disease, the Huntington Study Group's trial showed that CoQ_{10} administration for 30 months slowed the usual decline in total functional capacity, but the differences did not reach statistical significance.[101] Finally, in a multicenter, placebo-controlled, phase II trial with amyotrophic lateral-sclerosis patients, CoQ_{10} did not significantly modify functional decline over a 9-month period,[102] and Mathews et al[103] did not find CoQ_{10} plus several vitamins to be effective in individuals with genetic mitochondrial diseases.

NADH

NADH functions as a cellular redox cofactor in over 200 cellular redox reactions and as substrate for certain enzymes.[104,105] Humans universally require NADH, and its deficiency results in pellagra, which is characterized by dermatitis, diarrhea, dementia, and eventually death.[104] In the mitochondria, NADH delivers electrons from metabolite hydrolysis to the electron transport chain, but in its reduced form, it can also act as a strong antioxidant. The usual route of dietary supplementation has historically been via NADH precursors, such as niacin, nicotinic acid, or nicotinamide, but recently, microcarriers have been used to stabilize oral NADH so that it can be directly ingested in small doses and absorbed in the gastrointestinal system. This supplementation turns out to be more effective than large oral doses of NADH, as in some studies that used up to 50 mg/kg/d. At that size of dose, the NADH is prone to oxidation and degradation, and such supplementation is generally considered to be ineffective.[106]

In neurodegenerative diseases, oxidative damage is extensive,[1,2] and various mitochondrial antioxidants have been used to treat disease and delay progression.[1-6,32] In

Alzheimer's disease, one study showed that stabilized oral NADH could improve cognitive functioning and dementia[106]; however, another clinical trial showed no evidence of improvements in cognition or dementia using oral NADH.[107] In a controlled trial with 26 Alzheimer's participants who were given stabilized NADH or placebo for 6 months, Dermin et al found that that the test group had significantly better performance scores than the placebo group in verbal fluency and visual construction and showed a trend toward increased performance on abstract verbal reasoning.[108] However, the study provided no evidence of better performance for measures of attention or memory or on scores of dementia severity.

Stabilized, oral NADH has also been used to ameliorate the symptoms of Parkinson's disease. In a preliminary, open-label clinical trial, Birkmayer et al examined the effects of IV and oral NADH in over 800 individuals with Parkinson's disease.[109] They found that 19.3% of participants showed a 30% to 50% decrease in disability; 58.8% had moderate (10%-30%) improvement; and 21.8% did not respond to the therapy ($P < .01$). Younger patients with a shorter duration of disease had a much better chance of responding and showing more significant improvements than older patients or patients with a longer duration of disease. The oral form was comparable to IV NADH in its effects.[109] When they repeated this type of trial, however, Dizdar et al did not find statistically significant improvements in Parkinson's disease rating scores in participants treated with NADH, and differences were also not found in CSF clinical markers associated with Parkinson's disease severity.[110]

Stabilized, oral NADH has also been used to reduce symptoms in patients with chronic fatigue. One such study on individuals with chronic fatigue syndrome was designed to treat participants with stabilized, oral NADH or placebo for 4 weeks in a crossover trial.[111] Of these participants, 8 of 26 (30.7%) responded positively to the microencapsulated NADH compared with 2 of 26 (8%) in the placebo arm ($P < .05$).[111] Clearly an effect occurred but only in a subset of participants in the trial; thus, these results were not considered significant by others.[112] A clinical trial that compared oral, stabilized NADH to psychological/nutritional therapy for 31 individuals with chronic fatigue syndrome revealed that stabilized NADH alone reduced fatigue in the first 4 months of a 12-month trial. After the first 4 months, however, symptom scores were similar in the 2 arms of the trial.[113] In another study, stabilized NADH was given orally for 2 months to treat individuals with chronic fatigue syndrome.[114] This treatment resulted in decreases in anxiety and in maximum heart rate after a stress test, but Alegre et al found few or no differences in the functional impact on fatigue, quality of life, sleep quality, exercise capacity, or functional reserve.[114] Thus stabilized NADH alone has shown mixed results in various diseases and disorders, and not every patient responded to the oral, stabilized supplement.

Table 2. Oral Membrane Phospholipid Supplementation and Fatigue in Chronically Ill Patients[a]

Participants/Patients	n	Avg Age	Avg Time on LRT	PFS Fatigue Reduction (%)	Reference
Chronic fatigue	34	50.3	8 wk	40.5[c]	Ellithorpe et al[117]
Aging, chronic fatigue	20	68.9	12 wk	35.5[b]	Agadjanyan et al[55]
Chronic fatigue syndrome (and/or fibromyalgia syndrome)	15	44.8	8 wk	43.1[b]	Nicolson & Ellithorpe[116]
Aging, fatigue	67	57.3	1 wk	36.8[b]	Nicolson et al[118]
Chronic illnesses	58	55.0	8 wk	30.7[b]	Nicolson et al[119]

Abbreviations: Avg = average.

[a]Modified from Nicolson and Settineri.[54]
[b]$P < .001$ compared to no supplement.
[c]$P < .0001$ compared to no supplement.

Membrane Phospholipids

The dietary replacement of mitochondrial membrane phospholipids (lipid replacement therapy [LRT]) using food-derived molecules to remove damaged, mainly oxidized, membrane lipids in mitochondria and other cellular organelles has proved very effective at increasing mitochondrial function and reducing fatigue.[10,23,54,55] To some degree, antioxidant supplements can reduce ROS/RNS levels and prevent some oxidation of mitochondrial membrane phospholipids, but antioxidants alone cannot repair the damage already done to cells, and in particular, to cells' mitochondrial inner membranes.[29,55,115]

The use of oral membrane phospholipids plus antioxidants in doses ranging from 500 to 2000 mg per day has been effective in the treatment of certain clinical conditions, such as chronic fatigue and fatiguing illnesses.[55,67,115-117] LRT results in the actual replacement of damaged membrane phospholipids with undamaged (unoxidized) lipids to ensure proper function of cellular and especially mitochondrial membranes.

Oral membrane phospholipids can increase mitochondrial function and decrease fatigue in chronic fatigue syndrome, fibromyalgia syndrome, and other fatiguing conditions, including natural aging (Table 2). For example, a mixture of membrane phospholipids and vitamins (Propax with NT Factor) was used by Ellithorpe et al[117] in a study on aging individuals with severe chronic fatigue and was found to reduce their fatigue by 40.5% in 8 weeks. In these studies, fatigue was monitored using the Piper Fatigue Scale (PFS) to measure clinical fatigue and quality of life.[58] In a subsequent crossover study, the effects of membrane phospholipids on fatigue and mitochondrial function in patients with moderate-to-severe, chronic fatigue was initiated.[55] Oral administration of NT Factor for 12 weeks resulted in a 35.5% reduction in fatigue and 26.8% increase in mitochondrial function, whereas after a 12-week washout period, fatigue increased and mitochondrial function decreased back toward control levels.[55] Similar findings on fatigue reduction were observed in individuals with chronic fatigue syndrome and fibromyalgia syndrome who were given oral membrane phospholipids (NT Factor).[116] Using a new formulation of NT Factor plus vitamins, minerals, and other supplements in

individuals with moderate chronic fatigue resulted in a 36.8% reduction in fatigue within 1 week (Table 2).[118]

COMBINATION ORAL SUPPLEMENT TO REDUCE FATIGUE

In a 2-month trial of the treatment of long-term intractable fatigue in patients with a variety of diagnoses, the author and several colleagues combined membrane phospholipids (2000 mg/d), CoQ_{10} (35 mg/d), microencapsulated NADH (35 mg/d), L-carnitine (160 mg/d), α-ketoglutaric acid (180 mg/d), and other nutrients into an oral supplement (ATP Fuel) to treat fatigue and mitochondrial dysfunction.[119,120] The 58 participants in this trial had moderate-to-severe, intractable fatigue for an average of >17 years and had been to an average of >15 practitioners without resolution of their fatigue. The study included 30 individuals with chronic fatigue syndrome; 17 with chronic Lyme disease; 16 with other fatiguing illnesses, including fibromyalgia syndrome and Gulf War illness; 4 with autoimmune disease, including rheumatoid arthritis; 2 with cancer; and 2 with diabetes. These patients had tried many drugs and supplements (average >35) to reduce their fatigue without success.

Participants in this trial took the combination LRT supplement (ATP Fuel) for 8 weeks, and fatigue was scored using the Piper Fatigue Scale (PFS).[119] The PFS is a validated instrument that measures four dimensions of subjective fatigue: behavioral/severity, affective/meaning, sensory, and cognitive/mood.[58] The study used the instrument to calculate the four subscale or dimensional scores and the total fatigue scores. Participants had initial, total, mean PFS fatigue scores of 7.51 ± 0.29, and after 8 weeks of supplements, the mean scores improved to 5.21 ± 0.28, or a 30.7% reduction in fatigue (t test, $P < .0001$ and Wilcoxon signed-rank, $P < .0001$).[119]

PFS fatigue scores can be further dissected into 4 subcategories: (1) the behavior/severity subcategory, which deals with completing tasks, socializing, and engaging in sexual and other activities and with intensity or degree of fatigue; (2) the affective/meaning subcategory, which determines whether an individual finds the fatigue/tiredness to be pleasant or unpleasant, agreeable or disagreeable, protective or destructive, and normal or abnormal; (3) the sensory subcategory, which determines whether an individual feels strong or weak, awake or sleepy, refreshed or tired, and energetic or unenergetic; and (4) the cognitive/mood subcategory, which assesses whether an individual feels relaxed or tense, exhilarated or depressed, and able or unable to concentrate, remember, and think clearly. In the study being discussed in this section, all of these subcategories showed significant reductions by the end of the 8-week trial ($P < .0001$), indicating that significant improvements occurred for all subcategories of fatigue. For example, a 30.7% reduction ($P < .0001$) in severity/behavior of fatigue occurred, indicating a significant reduction in the intensity of fatigue and a significant increase in the ability to complete tasks, socialize, and engage in sexual and other activities. Also, a 28.0% improvement ($P < .0001$) occurred in mood and cognitive ability, such as the ability to concentrate, remember, and think clearly.[119]

Regression Analysis of Fatigue Data

To determine the trends in fatigue reduction as the result of participants' use of the combination LRT supplement (membrane phospholipids, CoQ_{10}, NADH, L-carnitine, α-ketoglutaric acid, and other ingredients) over the time of the trial, the author and colleagues conducted regression analyses of the data to determine if results were (1) consistent, (2) occurred with a high degree of confidence, and (3) could predict further reductions in fatigue.[119] The regression analysis of overall fatigue and of each of the subcategories of fatigue indicated significant and consistent downward trends in the fatigue data, suggesting that further reductions in fatigue would have been likely if the trial had been continued. The regression R^2 values for the various subgroups were (1) behavior/severity, 0.956; (2) affective meaning, 0.960; (3) sensory, 0.950; and (4) cognitive/mood, 0.980. Regression analysis of the overall fatigue yielded $R^2 = 0.960$. This finding indicated that a high level of confidence and reproducibility existed in the downward trends in all fatigue data. The combination LRT supplement was safe, and no safety issues came up during the trial.[119] Examination of scores from individuals with chronic fatigue syndrome, Lyme disease, or other diagnosis categories did not reveal major differences in overall fatigue or its reduction by the combination supplement.[119,120]

SUMMARY

Oral natural supplements containing membrane phospholipids, CoQ_{10}, microencapsulated NADH, L-carnitine, α-lipoic acid, and other nutrients can help restore mitochondrial function and reduce intractable fatigue in patients with chronic illnesses. The combination of these supplements can result in a safe and effective method to reduce fatigue and help restore quality of life.

AUTHOR DISCLOSURE STATEMENT

The author has received no financial benefit from and has no conflict of interest regarding the products discussed in this article.

REFERENCES

1. Swerdlow RH. Brain aging, Alzheimer's disease, and mitochondria. *Biochim Biophys Acta.* 2011;1812(12):1630-1639.
2. Reddy PH. Mitochondrial medicine for aging and neurodegenerative diseases. *Neuromolecular Med.* 2008;10(4):291-315.
3. Green DR, Galluzzi L, Kroemer G. Mitochondria and the autophagy-inflammation-cell death axis in organismal aging. *Science.* 2011;333(6046):1109-1112.
4. Reddy PH, Reddy TP. Mitochondria as a therapeutic target for aging and neurodegenerative diseases. *Curr Alzheimer Res.* 2011;8(4):393-409.
5. Karbowski M, Neutzner A. Neurodegeneration as a consequence of failed mitochondrial maintenance. *Acta Neuropathol.* 2012;123(2):157-171.
6. Victor VM, Apostolova N, Herance R, Hernandez-Mijares A, Rocha M. Oxidative stress and mitochondrial dysfunction in atherosclerosis: mitochondria-targeted antioxidants as potential therapy. *Curr Med Chem.* 2009;16(35):4654-4667.
7. Limongelli G, Masarone D, D'Alessandro R, Elliott PM. Mitochondrial diseases and the heart: an overview of molecular basis, diagnosis, treatment and clinical course. *Future Cardiol.* 2012;8(1):71-88.

8. Ma ZA, Zhao Z, Turk J. Mitochondrial dysfunction and beta-cell failure in type 2 diabetes mellitus. *Exp Diabetes Res.* 2012;2012:703538. doi:10.1155/2012/703538.
9. Joseph AM, Joanisse DR, Baillot RG, Hood DA. Mitochondrial dysregulation in the pathogenesis of diabetes: potential for mitochondrial biogenesis-mediated interventions. *Exp Diabetes Res.* 2012;2012:642038. doi:10.1155/2012/642038.
10. Nicolson GL. Metabolic syndrome and mitochondrial function: molecular replacement and antioxidant supplements to prevent membrane peroxidation and restore mitochondrial function. *J Cell Biochem.* 2007;100(6):1352-1369.
11. Ghafourifar P, Mousavizadeh K, Parihar MS, Nazarewicz RR, Parihar A, Zenebe WJ. Mitochondria in multiple sclerosis. *Front Biosci.* Jan 2008;13:3116-3126.
12. Mao P, Reddy PH. Is multiple sclerosis a mitochondrial disease? *Biochim Biophys Acta*. 2010;1802(1):66-79.
13. Fernandez D, Perl A. Metabolic control of T cell activation and death in SLE. *Autoimmun Rev*. 2009;8(3):184-189.
14. Maiese K, Morhan SD, Chong ZZ. Oxidative stress biology and cell injury during type 1 and type 2 diabetes mellitus. *Curr Neurovasc Res.* 2007;4(1):63-71.
15. Rossignol DA, Frye RE. Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis. *Mol Psychiatry*. 2012;17(3):290-314.
16. Palmieri L, Persico AM. Mitochondrial dysfunction in autism spectrum disorders: cause or effect? *Biochim Biophys Acta*. 2010;1797(6-7):1130-1137.
17. Prince JA, Harro J, Blennow K, Gottfries CG, Oreland L. Putamen mitochondrial energy metabolism is highly correlated to emotional and intellectual impairment in schizophrenics. *Neuropsychopharmacology*. 2000;22(3):284-292.
18. Marazziti D, Baroni S, Piccheti M, et al. Psychiatric disorders and mitochondrial dysfunctions. *Eur Rev Med Pharmacol Sci*. 2012;16(2):270-275.
19. Konradi C, Eaton M, MacDonald ML, Walsh J, Benes FM, Heckers S. Molecular evidence for mitochondrial dysfunction in bipolar disorder. *Arch Gen Psychiatry*. 2004;61(3):300-308.
20. Chitkara DK, Nurko S, Shoffner JM, Buie T, Flores A. Abnormalities in gastrointestinal motility are associated with diseases of oxidative phosphorylation in children. *Am J Gastroenterol*. 2003;98(4):871-877.
21. Di Donato S. Multisystem manifestations of mitochondrial disorders. *J Neurol.* 2009;256(5):693-710.
22. Norheim KB, Jonsson G, Omdal R. Biological mechanisms of chronic fatigue. *Rheumatology (Oxford).* 2011;50(6):1009-1018.
23. Nicolson GL, Nicolson NL, Berns P, Nasralla MY, Haier J, Nass M. Gulf War Illnesses: chemical, radiological and biological exposures resulting in chronic fatiguing illnesses can be identified and treated. *J Chronic Fatigue Syndr*. 2003;11(1):135-154.
24. Myhill S, Booth NE, McLaren-Howard J. Chronic fatigue syndrome and mitochondrial dysfunction. *Int J Clin Exp Med*. 2009;2(1):1-16.
25. Cordero MD, de Miguel M, Carmona-Lopez I, Bonal P, Campa F, Moreno-Fernandez AM. Oxidative stress and mitochondrial dysfunction in fibromyalgia. *Neuro Endocrinol Lett*. 2010;31(2):169-173.
26. Rabinovich RA, Vilaro J. Structural and functional changes of peripheral muscles in chronic obstructive pulmonary disease patients. *Curr Opin Pulm Med*. 2010;16(2):123-133.
27. Breeding PC, Russell NC, Nicolson GL. Integrative model of chronically activated immune-hormonal pathways important in the generation of fibromyalgia. *Br J Med Pract*. 2012;5(3):a524-a534.
28. Sotgia F, Martinez-Outschoorn UE, Lisanti MP. Mitochondrial oxidative stress drives tumor progression and metastasis: should we use antioxidants as a key component of cancer treatment and prevention? *BMC Med*. 2011;9:62-67.
29. Nicolson GL. Lipid replacement therapy: a nutraceutical approach for reducing cancer-associated fatigue and the adverse effects of cancer therapy while restoring mitochondrial function. *Cancer Metastasis Rev*. 2010;29(3):543-552.
30. Gabridge MG. Metabolic consequences of Mycoplasma pneumoniae infection. *Isr J Med Sci*. 1987;23(6):574-579.
31. Ashida H, Mimuro H, Ogawa M, et al. Cell death and infection: a double-edged sword for host and pathogen survival. *J Cell Biol*. 2011;195(6):931-942.
32. Kerr DS. Treatment of mitochondrial electron transport chain disorders: a review of clinical trials over the past decade. *Mol Genet Metab*. 2010;99(3):246-255.
33. Twig G, Shirihai OS. The interplay between mitochondrial dynamics and mitophagy. *Antioxid Redox Signal*. 2011;14(10):1939-1951.
34. Priault M, Salin B, Schaeffer J, Vallette FM, di Rago JP, Martinou JC. Impairing the bioenergetic status and the biogenesis of mitochondria triggers mitophagy in yeast. *Cell Death Differ.* 2005;12(12):1613-1621.
35. Lee J, Giordano S, Zhang J. Autophagy, mitochondria and oxidative stress: cross-talk and redox signaling. *Biochem J.* 2012;441(2):523-540.
36. Rich PR, Marechal A. The mitochondrial respiratory chain. *Essays Biochem*. 2010;47:1-23.
37. Nicholls DG. Mitochondrial ion circuits. *Essays Biochem*. 2010;47:25-35.
38. Divakaruni AS, Brand MD. The regulation and physiology of mitochondrial proton leak. *Physiology (Bethesda).* 2011;26(3):192-205.
39. Richter C, Park JW, Ames BN. Normal oxidative damage to mitochondrial and nuclear DNA is extensive. *Proc Nat Acad Sci USA*. 1998;85(17):6465-6467.
40. Stadtman E. Introduction to serial reviews on oxidatively modified proteins in aging and disease. *Free Radic Biol Med*. 2002;32(9):789.
41. Spector AA, Yorek MA. Membrane lipid composition and cellular function. *J Lipid Res*. 1985;26(9):1015-1035.
42. Spiteller G. Is lipid peroxidation of polyunsaturated acids the only source of free radicals that induce aging and age-related diseases? *Rejuvenation Res*. 2010;13(1):91-103.
43. Duchen MR, Szabadkai G. Roles of mitochondria in human disease. *Essays Biochem*. 2010;47:115-137.
44. Chicco AJ, Sparagna GC. Role of cardiolipin alterations in mitochondrial dysfunction and disease. *Am J Physiol Cell Physiol*. 2007;292(1):C33-C44.
45. Houtkooper RH, Vaz FM. Cardiolipin, the heart of mitochondrial metabolism. *Cell Mol Life Sci.* 2008;65(16):2493-2506.
46. Barber DA, Harris SR. Oxygen free radicals and antioxidants: a review. *Am Pharm*. 1994;NS34(9):26-35.
47. Sun Y. Free radicals, antioxidant enzymes, and carcinogenesis. *Free Radic Biol Med*. 1990;8(6):583-599.
48. Fridovich I. Superoxide radical and superoxide dismutases. *Annu Rev Biochem*. 1995;64:97-112.
49. Chandra Jagetia G, Rajanikant GK, Rao SK, Shrinath Baliga M. Alteration in the glutathione, glutathione peroxidase, superoxide dismutase and lipid peroxidation by ascorbic acid in the skin of mice exposed to fractionated gamma radiation. *Clin Chim Acta*. 2003;332(1-2):111-121.
50. Aeschbach R, Loliger J, Scott BC, et al. Antioxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. *Food Chem Toxicol.* 1994;32(1):31-36.
51. Schwartz JL. The dual roles of nutrients as antioxidants and prooxidants: their effects on tumor cell growth. *J Nutr*. 1996;126(4)(suppl):1221S-1227S.
52. Prasad KN, Cole WC, Kumar B, Prasad KC. Scientific rationale for using high-dose multiple micronutrients as an adjunct to standard and experimental cancer therapies. *J Am Coll Nutr*. 2001;20(5)(suppl):450S-453S.
53. Kroenke K, Wood DR, Mangelsdorff AD, Meier NJ, Powell JB. Chronic fatigue in primary care: prevalence, patient characteristics, and outcome. *JAMA*. 1988;260(7):929-934.
54. Nicolson GL, Settineri R. Lipid Replacement Therapy: a functional food approach with new formulations for reducing cellular oxidative damage, cancer-associated fatigue and the adverse effects of cancer therapy. *Funct Foods Health Dis*. 2011;1(4):135-160.
55. Agadjanyan M, Vasilevko V, Ghochikyan A, et al. Nutritional supplement (NTFactor) restores mitochondrial function and reduces moderately severe fatigue in aged subjects. *J Chronic Fatigue Syndr*. 2003;11(3):23-26.
56. Booth NE, Myhill S, McLaren-Howard J. Mitochondrial dysfunction and the pathophysiology of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS). *Int J Clin Esp Med*. 2012;5(3):208-220.
57. Morrison JD. Fatigue as a presenting complaint in family practice. *J Family Pract.* 1980;10(5):795-801.
58. Piper BF, Linsey AM, Dodd MJ. Fatigue mechanisms in cancer patients: developing nursing theory. *Oncol Nurs Forum*. 1987;14(6):17-23.
59. Wei YH, Lee HC. Oxidative stress, mitochondrial DNA mutation, and impairment of antioxidant enzymes in aging. *Exp Biol Med (Maywood)*. 2002;227(9):671-682.
60. Huang H, Manton KG. The role of oxidative damage in mitochondria during aging: a review. *Front Biosci*. May 2004;9:1100-1117.
61. Logan AC, Wong C. Chronic fatigue syndrome: oxidative stress and dietary modifications. *Altern Med Rev*. 2001;6(5):450-459.
62. Manuel y Keenoy B, Moorkens G, Vertommen J, De Leeuw I. Antioxidant status and lipoprotein peroxidation in chronic fatigue syndrome. *Life Sci*. 2001;68(17):2037-2049.
63. Richards RS, Roberts TK, McGregor NR, Dunstan RH, Butt HL. Blood parameters indicative of oxidative stress are associated with symptom expression in chronic fatigue syndrome. *Redox Rep*. 2000;5(1):35-41.
64. Fulle S, Mecocci P, Fano G, et al. Specific oxidative alterations in vastus lateralis muscle of patients with the diagnosis of chronic fatigue syndrome. *Free Radic Biol Med*. 2000;29(12):1252-1259.
65. Pall ML. Elevated, sustained peroxynitrite levels as the cause of chronic fatigue syndrome. *Med Hypotheses*. 2000;54(1):115-125.
66. Dimauro S, Rustin P. A critical approach to the therapy of mitochondrial respiratory chain and oxidative phosphorylation diseases. *Biochim Biophys Acta*. 2009;1792(12):1159-1167.
67. Chambers D, Bagnall AM, Hempel S, Forbes C. Interventions for the treatment management and rehabilitation of patients with chronic fatigue syndrome/myalgic encepthalomyelitis: an updated systematic review. *J R Soc Med*. 2006;99(10):506-520.
68. Smith AR, Shenvi SV, Widlansky M, Suh JH, Hagen TM. Lipoic acid as a potential therapy for chronic diseases associated with oxidative stress. *Curr Med Chem*. 2004;11(9):1135-1146.
69. Maczurek A, Hager K, Kenklies M, et al. Lipoic acid as an anti-inflammatory and neuroprotective treatment for Alzheimer's disease. *Adv Drug Deliv Rev*. 2008;60(13-14):1463-1470.
70. Shay KP, Moreau RF, Smith EJ, Smith AR, Hagen TM. Alpha-lipoic acid as a dietary supplement: molecular mechanisms and therapeutic potential. *Biochim Biophys Acta*. 2009;1790(10):1149-1160.
71. Henriksen EJ, Jacob S, Streeper RS, Fogt DL, Hokama JY, Tritschler HJ. Stimulation by alpha-lipoic acid of glucose transport activity in skeletal muscle of lean and obese Zucker rats. *Life Sci*. 1997;61(8):805-812.
72. Gudz TI, Tserng KY, Hoppel CL. Direct inhibition of mitochondrial respiratory chain complex III by cell-permeable ceramide. *J Biol Chem*. 1997;272(39):24154-24158.
73. Di Paola M, Cocco T, Lorusso M. Ceramide interaction with the respiratory chain of heart mitochondria. *Biochemistry.* 2000;39(22):6660-6668.

74. Monette JS, Gomez LA, Moreau RF, et al. (R)-α-Lipoic acid treatment restores ceramide balance in aging rat cardiac mitochondria. *Pharmacol Res*. 2011;63(1):23-29.
75. Ziegler D, Nowak H, Kempler P, Vargha P, Low PA. Treatment of symptomatic diabetic polyneuropathy with the antioxidant alpha-lipoic acid: a meta-analysis. *Diabet Med*. 2004;21(2):114-121.
76. Ziegler D, Low PA, Litchy WJ, et al. Efficacy and safety of antioxidant treatment with α-lipoic acid over 4 years in diabetic polyneuropathy: the NATHAN 1 trial. *Diabetes Care*. 2011;34(9):2054-2060.
77. Yoshida K, Hirokawa J, Tagami S, Kawakami Y, Urata Y, Kondo T. Weakened cellular scavenging activity against oxidative stress in diabetes mellitus: regulation of glutathione synthesis and efflux. *Diabetologia*. 1995;38(2):201-210.
78. Goraca A, Huk-Kolega H, Piechota A, Kleniewska P, Ciejka E, Skibska B. Lipoic acid—biological activity and therapeutic potential. *Pharmacol Rep*. 2011;63(4):849-858.
79. Head E, Nukala VN, Fenoglio KA, Muggenburg BA, Cotman CW, Sullivan PG. Effects of age, dietary and behavioral enrichment on brain mitochondria in a canine model of human aging. *Exp Neurol*. 2009;220(1):171-176.
80. Marcovina SM, Sirtori C, Peracino A, et al. Translating the basic knowledge of mitochondrial functions to metabolic therapy: role of L-carnitine. *Transl Res*. 2013;161(2):73-84.
81. Reuter SE, Evans AM. Carnitine and acylcarnitines: pharmacokinetic, pharmacological and clinical aspects. *Clin Pharmacokinet*. 2012;51(9):553-572.
82. Newgard CB, An J, Bain JR, et al. A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. *Cell Metab*. 2009;9(4):311-326.
83. Koves TR, Ussher JR, Noland RC, et al. Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance. *Cell Metab*. 2008;7(1):45-56.
84. Shah SH, Hauser ER, Bain JR, et al. High heritability of metabolomic profiles in families burdened with premature cardiovascular disease. *Mol Syst Biol*. 2009;5:258. doi:10.1038/msb.2009.11.
85. Spriet LL, Perry CG, Talanian JL. Legal pre-event nutritional supplements to assist energy metabolism. *Essays Biochem*. 2008;44:27-43.
86. Brass EP. Supplemental carnitine and exercise. *Am J Clin Nutr*. 2000;72(2)(suppl);618S-623S.
87. Wachter S, Vogt M, Kreis R, et al. Long-term administration of L-carnitine to humans: effect on skeletal muscle carnitine content and physical performance. *Clin Chim Acta*. 2002;318(1-2):51-61.
88. Anand I, Chandrashekhan Y, De Giuli F, et al. Acute and chronic effects of propionyl-L-carnitine on the hemodynamics, exercise capacity and hormones in patients with congestive heart failure. *Cardiovasc Drugs Ther*. 1998;12(3):291-299.
89. Hagen TM, Moreau R, Suh JH, Visioli F. Mitochondrial decay in the aging rat heart: evidence for improvement by dietary supplementation with acetyl-L-carnitine and/or lipoic acid. *Ann NY Acad Sci*. April 2002;959:491-507.
90. Acetyl-L-carnitine: monograph. *Altern Med Rev*. 2010;15(1):76-83.
91. Malaguarnera M, Cammalleri L, Gargante MP, Vacante M, Colonna V, Motta M. L-carnitine treatment reduces severity of physical and mental fatigue and increases cognitive functions in centenarians: a randomized and controlled clinical trial. *Am J Clin Nutr*. 2007;86(6):1738-1744.
92. Littarru GP, Tiano L. Clinical aspects of coenzyme Q10: an update. *Nutrition*. 2010;26(3):250-254.
93. Groneberg DA, Kindermann B, Althammer M, et al. Coenzyme Q10 affects expression of genes involved in cell signalling, metabolism and transport in CaCo-2 cells. *Int J Biochem Cell Biol*. 2005;37(6):1208-1218.
94. Orsucci D, Mancuso M, Ienco EC, LoGerfo A, Siciliano G. Targeting mitochondrial dysfunction and neurodegeneration by means of coenzyme Q10 and its analogues. *Curr Med Chem*. 2011;18(26):4053-4064.
95. Rosenfeldt F, Hilton D, Pepe S, Krum H. Systematic review of effect of coenzyme Q10 in physical exercise, hypertension and heart failure. *Biofactors*. 2003;18(1-4):91-100.
96. Mizuno K, Tanaka M, Nozaki S, et al. Antifatigue effects of coenzyme Q10 during physical fatigue. *Nutrition*. 2008;24(4):293-299.
97. Li G, Jack CR, Yang XF, Yang ES. Diet supplement CoQ10 delays brain atrophy in aged transgenic mice with mutations in the amyloid precursor protein: an *in vivo* volume MRI study. *Biofactors*. 2008;32(1-4):169-178.
98. Yang X, Dai G, Li G, Yang ES. Coenzyme Q10 reduces beta-amyloid plaque in an APP/PS1 transgenic mouse model of Alzheimer's disease. *J Mol Neurosci*. 2010;41(1):110-113.
99. Galasko DR, Peskind E, Clark CM, et al; Alzheimer's Disease Cooperative Study. Antioxidants for Alzheimer's disease: a randomized clinical trial with cerebrospinal fluid biomarker measures. *Arch Neurol*. 2012;69(7):836-841.
100. Isobe C, Abe T, Terayama Y. Levels of reduced and oxidized coenzyme Q10 and 8-hydroxy-2'-deoxyguanosine in the cerebrospinal fluid of patients with living Parkinson's disease demonstrates that mitochondrial oxidative damage and/or oxidative DNA damage contributes to the neurodegenerative process. *Neurosci Lett*. 2010;469(1):159-163.
101. Huntington Study Group. A randomized, placebo-controlled trial of coenzyme Q10 and remacemide in Huntington's disease. *Neurology*. 2001;57(3):397-404.
102. Kaufmann P, Thompson JL, Levy G, et al; QALS Study Group. Phase II trial of CoQ10 for ALS finds insufficient evidence to justify phase III. *Ann Neurol*. 2009;66(2):235-244.
103. Matthews PM, Ford B, Dandurand RJ, et al. Coenzyme Q10 with multiple vitamins is generally ineffective in treatment of mitochondrial disease. *Neurology*. 1993;43(5):884-890.
104. Penberthy WT. Nicotinamide adenine dinucleotide biology and disease. *Curr Pharm Des*. 2009;15(1):1-2.
105. Kirkland JB. Niacin status, NAD distribution and ADP-ribose metabolism. *Curr Pharm Des*. 2009;15(1):3-11.
106. Birkmayer JG. Coenzyme nicotinamide adenine dinucleotide: new therapeutic approach for improving dementia of the Alzheimer type. *Ann Clin Lab Sci*. 1996;26(1):1-9.
107. Rainer M, Kraxberger E, Haushofer M, Mucke HA, Jellinger KA. No evidence for cognitive improvement from oral nicotinamide adenine dinucleotide (NADH) in dementia. *J Neural Transm*. 2000;107(12):1475-1481.
108. Demarin V, Podobnik SS, Storga-Tomic D, Kay G. Treatment of Alzheimer's disease with stabilized oral nicotinamide adenine dinucleotide: a randomized, double-blind study. *Drugs Exp Clin Res*. 2004;30(1):27-33.
109. Birkmayer JG, Vrecko C, Volc D, Birkmayer W. Nicotinamide adenine dinucleotide (NADH)—a new therapeutic approach to Parkinson's disease: comparison of oral and parenteral application. *Acta Neurol Scand Suppl*. 1993;146:32-35.
110. Dizdar N, Kagedal B, Lindvall B. Treatment of Parkinson's disease with NADH. *Acta Neurol Scand*. 1994;90(5):345-347.
111. Forsyth LM, Preuss HG, MacDowell AL, Chiazze L Jr, Birkmayer GD, Bellanti JA. Therapeutic effects of oral NADH on the symptoms of patients with chronic fatigue syndrome. *Ann Allergy Asthma Immunol*. 1999;82(2):185-191.
112. Colquhoun D, Senn S. Is NADH effective in the treatment of chronic fatigue syndrome? *Ann Allergy Asthma Immunol*. 2000;84(6):639-640.
113. Santaella ML, Font I, Disdier OM. Comparison of oral nicotinamide adenine dinucleotide (NADH) versus conventional therapy for chronic fatigue syndrome. *P R Health Sci J*. 2004;23(2):89-93.
114. Alegre J, Rosés JM, Javierre C, Ruiz-Baqués A, Segundo MJ, de Sevilla TF. Nicotinamide adenine dinucleotide (NADH) in patients with chronic fatigue syndrome [in Spanish]. *Rev Clin Esp*. 2010;210(6):284-288.
115. Nicolson GL. Lipid replacement as an adjunct to therapy for chronic fatigue, anti-aging and restoration of mitochondrial function. *J Am Nutraceutical Assoc*. 2003;6(3):22-28.
116. Nicolson GL, Ellithorpe R. Lipid replacement and antioxidant nutritional therapy for restoring mitochondrial function and reducing fatigue in chronic fatigue syndrome and other fatiguing illnesses. *J Chronic Fatigue Syndr*. 2006;13(1):57-68.
117. Ellithorpe RR, Settineri RA, Nicolson GL. Pilot study: reduction of fatigue by use of a dietary supplement containing glycophospholipids. *J Am Nutraceutical Assoc*. 2003;6(1):23-28.
118. Nicolson GL, Ellithorpe RR, Ayson-Mitchell C, Jacques B, Settineri R. Lipid replacement therapy with a glycophospholipid-antioxidant-vitamin formulation significantly reduces fatigue within one week. *J Am Nutraceutical Assoc*. 2010;13(1):10-14.
119. Nicolson GL, Settineri R, Ellithorpe R. Lipid replacement therapy with a glycophospholipid formulation with NADH and CoQ10 significantly reduces fatigue in intractable chronic fatiguing illnesses and chronic Lyme disease patients. *Int J Clin Med*. 2012;3(3):163-170.
120. Nicolson GL, Settineri R, Ellithorpe R. Glycophospholipid formulation with NADH and CoQ10 significantly reduces intractable fatigue in Western blot-positive chronic Lyme disease patients: preliminary report. *Funct Foods Health Dis*. 2012;2(3):35-47.