



Lipid Replacement as an Adjunct to Therapy for Chronic Fatigue, Anti-Aging and Restoration of Mitochondrial Function

Garth L. Nicolson, PhD ^{1,2}

¹ Professor of Molecular Pathology, The Institute for Molecular Medicine, Huntington Beach, CA

² Professor of Integrative Medicine, Capital University of Integrative Medicine, Washington, DC

A Peer-Reviewed Journal on Nutraceuticals and Nutrition

Mark Houston, MD
Editor-in-Chief

ISSN-1521-4524

Lipid Replacement as an Adjunct to Therapy for Chronic Fatigue, Anti-Aging and Restoration of Mitochondrial Function

Garth L. Nicolson, PhD* ^{1, 2}

¹ Professor of Molecular Pathology, The Institute for Molecular Medicine, Huntington Beach, CA

² Professor of Integrative Medicine, Capital University of Integrative Medicine, Washington, DC

ABSTRACT

Lipid replacement therapy (LRT) has been used along with other strategies, such as antioxidant therapy, to replace damaged or oxidized cellular lipids that accumulate during aging and in various clinical conditions. Differing from traditional lipid nutritional supplementation, LTR replacement lipids are protected from oxidation and damage during storage, ingestion and digestion. Important lipids that require constant replacement are phospholipids, glycopospholipids and other lipids that make up cellular and organelle membranes, especially mitochondrial membranes. Decreased mitochondrial function and loss in the efficiency of the electron transport chain are related to aging and fatigue. Oxidative damage to mitochondria, mainly from Reactive Oxygen Species (ROS), results in peroxidation of cellular and mitochondrial lipids, proteins and DNA, but it is ROS damage to mitochondrial membrane lipids that may cause the most rapid loss of mitochondrial function. LRT along with antioxidants can circumvent ROS membrane

damage and replace and restore mitochondrial and other cellular membrane functions via delivery of replacement lipids in their unoxidized, undamaged states. Recent clinical trials have shown the benefit of LRT plus antioxidants in restoring mitochondrial electron transport function and reducing fatigue. In aging subjects mitochondrial function was restored to levels found in young adults in consort with reductions in fatigue, suggesting the anti-aging and anti-fatigue benefits of LRT plus antioxidants in protecting mitochondrial and other cellular membranes from oxidative and other damage and preventing loss of function.

INTRODUCTION

The use of natural lipids for dietary support and even therapy for various medical conditions has a long and rich history and will not be dealt with in this brief commentary. Instead 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 membranes. This constitutes the most important functional use of lipids in our bodies. Damage to membrane lipids can impair fluidity, electrical properties, enzymatic activities and transport functions of cellular and organelle membranes.¹⁻³

* Correspondence:

Garth L. Nicolson, PhD
The Institute for Molecular Medicine
15162 Triton Lane
Huntington Beach, CA 92649
Phone: 1-714-903-2901; Fax: 1-714-379-2082
Email: gnicolson@immed.org
Website: www.immed.org

An important difference between simple lipid dietary supplementation and LRT is that the lipids in LRT must be protected from oxidative and other damage during storage and during the ingestion, digestion and absorption processes in vivo. Thus LRT should result in delivery of high concentrations of unoxidized, undamaged lipids, and this is important in reversing the damage and restoring function to (partially 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 conditions.

n-3 LIPID SUPPLEMENTS AND CHRONIC ILLNESSES

In the past several years different sources of lipid dietary mixtures have been used to improve general health or for more specific uses, such as in the treatment of cardiovascular diseases and inflammatory disorders.⁴⁻¹⁰ Although not every clinical study has found health benefits from supplementing specific lipids in the diet,^{7,11} most studies have documented the value of dietary supplements that favor certain types of lipids over others. The most common substitution is the dietary administration of lipids where n-3 polyunsaturated fatty acids (mainly fish- or flaxseed-derived) are favored relative to n-6 lipids.⁴⁻¹⁰

Oral administration of n-3 polyunsaturated fatty acids has been beneficial in various clinical conditions. This includes reduction in risk of coronary heart disease¹¹⁻¹⁴ and death due to cardiac arrest,¹⁵⁻¹⁷ age-associated macular degeneration,¹⁸ asthma,¹⁹ ulcerative colitis,^{20,21} Crohn's disease,²² IgA nephropathy,^{23,24} rheumatoid arthritis,^{25,26} diabetes mellitus,^{27,28} various malignancies^{29,30} and other conditions. Discrepancies and conflicting results in some clinical studies on the health benefits of n-3 polyunsaturated fatty acids could be the result of insufficient care in the storage, preservation, dose and administration of the dietary lipid mixtures.³¹

INGESTED LIPIDS ARE QUICKLY ADSORBED AND TRANSPORTED TO TISSUES

Lipids such as those found in various cellular compartments are in dynamic equilibrium in the body, and this is why LRT is possible. Orally ingested lipids diffuse to the gut epithelium and are bound and eventually transported into the blood and lymph using specific (carrier lipoproteins) and nonspecific (partitioning and diffusion) mechanisms.³²⁻³⁴ 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.^{34,35} 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.³⁶ Inside the cells, lipid transporters deliver specific lipids to cell organelles where they are taken in by specific transport proteins and by partitioning and diffusion.³⁷ Once undamaged lipids such as phosphatidylethanolamine are transported to mitochondria, they can be used to synthesize other lipids, such as phosphatidylserine. This system works efficiently, probably due to the concentration gradients that exist from the gut during the digestion of lipids to their absorption by gut epithelial cells and their transfer to the blood, to the tissues, and ultimately to the cells' interior. 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.³⁸

FATIGUE, AGING AND OXIDATIVE DAMAGE TO MITOCHONDRIA

Many medical conditions are associated with fatigue, including respiratory, coronary, musculoskeletal, and bowel conditions as well as various cancers and infections.^{39,40} Chronic fatigue (intractable fatigue lasting more than 6 months that is not reversed by sleep) is the most common complaint of patients seeking medical care.^{41,42} It is an important secondary condition in many clinical diagnoses, often preceding and is related to patients' diagnoses.^{42,43} 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.^{40,43} Most patients understand fatigue as a loss of energy and inability to perform even simple tasks without exertion. Using the Piper Fatigue Scale measurement tool that combines multiple fatigue-associated elements into an overall score fatigue has been quantitated as a multi-component sensation.^{40,43} We have successfully used the Piper Fatigue Scale in clinical studies on aging subjects who complain of fatigue to determine their responses to LRT plus antioxidants.^{44,45}

The complex phenomenon called fatigue is involved with cellular energy systems found primarily in the mitochondria. Damage to cellular mitochondria can impair the abilities of cells to produce high-energy molecules, such as ATP and NADH. This occurs naturally with aging and during chronic illness, mainly by the build up of damaged mitochondrial components that impair function. 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 (enzymes) and DNA. Once oxidized, these cellular molecules are structurally and sometimes functionally changed. Major targets of cellular ROS damage are mitochondria and nuclei, mainly their phospholipid/protein membranes and DNA.^{3,46-49} Damage to the former results in alterations in membrane fluidity and electrical properties,

whereas damage to protein enzymes and deletions or modifications in DNA structure can result in alterations in enzyme activities and gene expression.

Mitochondria themselves produce some ROS as a consequence of oxidative phosphorylation,⁵⁰ but excess ROS production throughout our lifetimes can result in accumulation of mitochondrial and nuclear damage. To counter this, cellular free-radical-scavenging enzymes neutralize excess ROS and repair enzymes reverse ROS-mediated damage.⁵⁰ Although some ROS production is important in triggering cell proliferation, gene expression and destruction of invading microbes,⁵¹ with aging, ROS damage accumulates because antioxidant enzymes and enzyme repair mechanisms along with biosynthesis cannot restore or replace enough ROS-damaged molecules.^{3,46,47} Disease and infection can also result in similar damage that exceeds the abilities of cellular systems to neutralize, repair, or replace damaged molecules.^{3,50}

Mitochondria from aging animals show higher levels of accumulated ROS damage to mitochondrial membranes, enzymes and DNA than found in young animals.^{3,51} At the molecular level, damage to phospholipids and other lipids in mitochondrial membranes by ROS free-radicals can affect membrane integrity, fluidity and transmembrane electrical potentials, resulting in damage to the electron transport chain and its associated components and loss of function.^{3,50} Young cells and organisms can cope with ROS since they possess high levels of free-radical scavenging systems that neutralize ROS, such as superoxide dismutase and glutathione reductase. They also have a higher capacity to repair or replace damage caused by ROS. With aging these homeostatic systems naturally decline and can be overwhelmed by ROS and oxidative stress.^{51,52} Since the aging process results in mitochondria accumulating ROS damage to their membranes, enzymes and DNA, this is thought to contribute to or even be a cause of the aging process.^{3,47,51-53}

MANAGING ROS-MEDIATED DAMAGE WITH ANTIOXIDANTS

Reducing cellular and mitochondrial membrane and DNA damage and loss of membrane integrity are important in preventing loss of cellular energy and regulating cellular life span.^{3,54} This can be done, in part, by neutralizing ROS with various antioxidants or increasing free-radical scavenging systems that neutralize ROS. Dietary antioxidants and some accessory molecules, such as zinc and certain vitamins, are important in maintaining free-radical scavenging systems, biosynthetic capacity, membranes, enzymes and DNA. There are at least 40 micronutrients required in the human diet,⁵⁵ and aging increases the need to supplement these in a normal diet to prevent age-associated declines in mitochondrial and other cellular functions. Although very important, antioxidant use alone may not be sufficient to

maintain cellular components free of ROS damage. This is why LRT is important in replacing ROS-damaged lipids along with antioxidant use to prevent further oxidation.

In animal studies the effects of reducing ROS have been dramatic in aging and disease models. For example, in rodents there are age-dependent losses in antioxidants and antioxidant vitamins as well as reductions in glutathione and levels of antioxidant enzymes.⁵⁶ In an aged rat study, the effects of alpha-lipoic acid and other dietary antioxidants on the levels of cellular antioxidants, such as reduced glutathione and vitamins C and E, levels of mitochondrial membrane lipid peroxidation and activities of mitochondrial electron transport and accessory enzymes, have been investigated and found to decrease but not eliminate ROS damage to the electron transport chain.⁵⁷ Thus dietary antioxidant supplementation partially reversed the age-related declines in cellular antioxidants and mitochondrial enzyme activities and prevented mitochondria from most age-associated functional decline. In another study rats were fed diets supplemented with coenzyme Q₁₀, alpha-lipoic acid, melatonin, or alpha-tocopherol for a six-month period. They found that the antioxidant mixture could inhibit the progression of certain age-associated changes in cerebral mitochondrial electron transport chain enzyme activities.^{58,59} Thus animal studies have shown that antioxidants can prevent, at least in part, age-associated changes in mitochondrial structure and function. However, antioxidants alone cannot completely eliminate ROS damage to mitochondria, and this is why LRT is an important adjunct to antioxidant administration.

In addition to the aging-associated oxidative changes in mitochondrial enzymes and lipids, mitochondrial DNA also accumulates oxidative damage during the aging process.^{3,51-54,60,61} To prevent this, antioxidants have also been useful, such as vitamins C and E, coenzyme Q₁₀, sulfur-containing antioxidants and plant antioxidant extracts.^{62,63} Age-associated damage to mitochondrial DNA may affect their ability to function due, in part, to a loss in ability to synthesize and replace critical mitochondrial enzymes.

Antioxidants may also affect the pathogenic processes of certain diseases.^{50,60} The experimental dietary use of antioxidants can prevent age-associated mitochondrial dysfunction and damage, inhibit the age-associated decline in immune and other functions and prolong the lifespan of laboratory animals.^{3,56-59,63,64}

ANIMAL STUDIES USING LIPID REPLACEMENT THERAPY AND ANTIOXIDANTS

Another method used to reverse damage to tissue membranes is to replace damaged cellular and mitochondrial membrane phospholipids and other lipids using dietary supplements containing polyunsaturated phosphatidylcholines and other phospholipids, glycerophospholipids and fatty acids

that are essential structural and functional components of all biological membranes.^{44,45} One such LRT dietary supplement is called NT Factor,TM and it has been used successfully in animal and clinical lipid replacement studies. Its encapsulated lipids are protected from oxidation in the gut by the inclusion of antioxidants and can be absorbed and transported into tissues without undue damage.^{44,45} NT Factor contains a variety of components (Table 1), including glycopospholipids and other lipids, antioxidants, nutrients, probiotics, vitamins, minerals and plant extracts.⁴⁴

NT Factor has been used to produce an anti-aging effect in aged laboratory animals. In 18- to 20-month-old rats, Seidman et al⁶⁵ found that NT Factor 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 NT Factor group. These results were highly significant ($p < 0.005$). They also found that NT Factor preserved cochlear mitochondrial function as measured in a Rhodamine-123 transport assay, increasing mitochondrial function by 34%. In these experiments, Rhodamine-123 is transported into mitochondria where it is chemically reduced to its fluorescent form only under conditions where mitochondria are fully functional.⁶⁶ NT Factor also prevented a common aging-related mitochondrial DNA deletion (mtDNA⁴⁸³⁴) found in the cochlear of aging rats.⁶⁵ Thus LRT plus antioxidants was successful in preventing age-associated hearing loss and mitochondrial damage in an animal model for aging.

CLINICAL STUDIES USING LIPID REPLACEMENT THERAPY AND ANTIOXIDANTS

LRT plus antioxidants 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 (PropaxTM) in cancer patients to reduce the effects of cancer therapy, such as chemotherapy-induced fatigue, nausea, vomiting, and other side effects associated with chemotherapy.⁶⁷ In a twelve-week double-blinded, cross-over, placebo controlled, randomized trial on cancer patients receiving chemotherapy, Propax supplementation resulted in improvement from fatigue, nausea, diarrhea, impaired taste, constipation, insomnia and other quality of life indicators.⁶⁷ The majority (64%) of the patients in this study reported significant reductions in chemotherapy-induced side effects, and 29% experienced no overall worsening of chemotherapy side-effects. Following cross-over to the supplement containing the Propax, patients reported rapid improvement in nausea, impaired taste, tiredness, appetite, sick feeling and other indicators associated with chemotherapy.⁶⁷

We have used Propax plus NT Factor in an LRT study with severely fatigued, aged subjects (>60 years-old) with a variety of clinical diagnoses to reduce fatigue, as measured by the Piper Fatigue Scale.^{40,43} We found that fatigue was reduced approximately 40%, from severe to moderate fatigue, after eight weeks of using Propax containing NT Factor. The results were highly significant ($p < 0.0001$).⁴⁵ A

Table 1. Components of NT FactorTM and their proposed functions (modified from ref. 44).

<p>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:</p>
<p>Glycophospholipids:</p> <p>polyunsaturated phosphatidylcholine, other polyunsaturated phosphatidyl lipids and glycolipids. <i>Proposed purpose:</i> repair and maintenance of membrane lipids.</p>
<p>Probiotics:</p> <p><i>Bifido bacterium</i>, <i>Lactobacillus acidophilus</i>, and <i>Lactobacillus bacillus</i> in a freeze-dried, microencapsulated form with appropriate growth nutrients. <i>Proposed purpose:</i> supports digestion, gut epithelium and the immune system.</p>
<p>Food Supplements, Vitamins, and Growth Medium:</p> <p>bacterial growth factors to support probiotic growth, including defatted rice bran, arginine, beet root fiber extract, blackstrap 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 B₆, niacinamide, riboflavin, inositol, niacin, calcium pantothenate, thiamin, vitamin B₁₂, folic acid, chromium picolinate. <i>Proposed purpose:</i> antioxidants support lipids from oxidation, growth medium supports probiotics and gut epithelium, vitamins support general health and the immune system, and food supplements support lipids from enzymatic digestion and oxidation.</p>

more recent LRT plus antioxidant study was initiated to examine the effects of NT Factor 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 NT Factor.⁴⁴ Using NT Factor for eight or twelve weeks resulted in a 33% or 35.5% reduction in fatigue, respectively. The results were highly significant ($p < 0.001$) and were obtained using the Piper Fatigue Scale for measuring fatigue.⁴⁴

In the LRT/antioxidant trial with moderately fatigued patients, reductions in fatigue paralleled significant gains in mitochondrial function.⁴⁴ In fact, there was good correspondence between reductions in fatigue and gains in mitochondrial function. After only eight weeks of NT Factor, mitochondrial function was significantly improved ($p < 0.001$). Interestingly, after twelve weeks of NT Factor use, mitochondrial function was found to be similar to that of young, healthy adults.⁴⁴ After twelve weeks of NT Factor use, subjects discontinued the supplement for an additional twelve weeks, when their fatigue and mitochondrial function were again measured. After the twelve-week wash-out period, fatigue and mitochondrial function were intermediate between the initial starting values and those found after eight or twelve weeks, indicating that continued use of the supplement is probably required to maintain lower fatigue scores and show improvements in mitochondrial function.⁴⁴ The results indicate that LRT/antioxidants can significantly improve and even restore mitochondrial function and improve fatigue scores in aging human subjects.

CHRONIC FATIGUE, MITOCHONDRIAL FUNCTION AND DEGENERATIVE DISEASE

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,^{45,46,54} but oxidation and damage of mitochondrial lipids in membranes are thought to be among the most important causes.^{3,54,68} Oxidation of membrane lipids results in modification of membrane fluidity and the electrical potential barrier across mitochondrial membranes, essential elements in the proper functioning of the electron transport chain.^{3,54,68} 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 health. At the biochemical level fatigue is related to the metabolic energy available to tissues and cells. Thus the integrity of cellular and intracellular membranes, especially in the mitochondria, is critical to cell function and energy metabolism. When mitochondrial membrane glycopospholipids, phospholipids, fatty acids, and other essential lipids are damaged by oxidation, they must be

repaired or replaced in order to maintain the production of cellular energy to alleviate fatigue.

The decline of cellular energy production with aging appears to be due, in part, to mitochondrial lipid peroxidation by ROS and the failure to repair or replace damaged molecules at a rate that exceeds their damage. Membrane damage and subsequent mitochondrial dysfunction by ROS can also lead to modifications (especially mutations and deletions) in mitochondrial DNA (mtDNA). The mitochondrial theory of aging proposes that the development of chronic degenerative diseases is the result, in part, of accumulated mtDNA mutations and deletions and oxidative damage to mitochondrial membranes over time.^{3,54,61,68,69} Indeed, these studies have linked the development of certain chronic diseases with the degree of mitochondrial membrane lipid peroxidation and mtDNA damage. Thus the damage to mtDNA and mitochondrial membranes seems to be involved in the etiology of age-associated degenerative diseases leading to changes in the expression of genes important for cell survival as well as those that control aging.⁶⁹ Restoration of mitochondrial membrane integrity, fluidity and other properties are essential for the optimal functioning of the electron transport chain and oxidative generation of ATP and NADH. Declines in energy production with aging and disease coupled with increases in oxidative stress can change gene expression programs and activate cellular apoptosis programs.⁷⁰ Apoptosis can also be attenuated with the administration of n-3 polyunsaturated fatty acids.⁷¹

The ability to control membrane lipid peroxidation and DNA damage likely play a major role in the aging process and the development of age-related degenerative diseases.^{3,60,72} LRT has proven to be a valuable tool in helping maintain mitochondrial function, and along with combined antioxidant use LRT should be an important part of anti-aging strategies as well as strategies used to treat various age-associated degenerative diseases and conditions.

REFERENCES

1. Nicolson GL, Poste G, Ji T. Dynamic aspects of cell membrane organization. *Cell Surface Rev.* 1977;3:1-73.
2. Subczynski WK, Wisniewska A. Physical properties of lipid bilayer membranes: relevance to membrane biological functions. *Acta Biochim Pol.* 2000;47:613-625.
3. Shigenaga MK, Hagen TM, Ames BN. Oxidative damage and mitochondrial decay in aging. *Proc Nat Acad Sci USA.* 1994;91:10771-10778.
4. Harris WS. n-3 fatty acids and lipoproteins: comparison of results from human and animal studies. *Lipids.* 1996;31:243-252.
5. Connor WE. Importance of n-3 fatty acids in health and disease. *Am J Clin Nutr.* 2000;71:S171-S178.
6. Nordoy A, Marchioli R, Arnesen H, Videback J. n-3 polyunsaturated fatty acids and cardiovascular diseases. *Lipids.* 2001;36:S127-S129.

7. Butcher G, Hengstler HC, Schindler P, Meier C. n-3 polyunsaturated fatty acids in coronary heart disease: a meta-analysis of randomized controlled trials. *Am J Med.* 2002;112:298-304.
8. Belluzzi A. n-3 fatty acids for the treatment of inflammatory bowel diseases. *Proc Nutr Soc.* 2002; 61:391-393.
9. Calder PC. Dietary modification of inflammation with lipids. *Proc Nutr Soc.* 2002; 61:345-358.
10. Grimble RF. Nutritional modulation of immune function. *Proc Nutr Soc.* 2001;60:389-397.
11. Angerer C, Stork W, von Schacky S. Effect of dietary supplementation with omega-3 fatty acids on progression of atherosclerosis in carotid arteries. *Cardiovasc Res.* 2002;54:183-190.
12. Schmidt J, Skou EB, Christensen HA, Dyerberg JH. N-3 fatty acids from fish and coronary artery disease: implications for public health. *Public Health Nutr.* 2000;3(1):91-98.
13. Hu JE, Bronner FB, Willett L, Stampfer WC, Rexrode MJ, et al. Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. *JAMA.* 2002;287:1815-1821.
14. Kinsella RA, Lokesh JE, Stone B. Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: possible mechanisms. *Am J Clin Nutr.* 1990;52:1-28.
15. Siscovick LH, Raghunathan DS, King TE, Weinmann I, et al. Dietary intake and cell membrane levels of long-chain n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. *JAMA.* 1995; 274:1363-1367.
16. Hu WC, Manson FB, Willett JE. Types of dietary fat and risk of coronary heart disease: a critical review. *J Am Coll Nutr.* 2001;20:5-19.
17. Bucher G, Hengstler HC, Schindler P, Meier C. N-3 polyunsaturated fatty acids in coronary heart disease: a meta-analysis of randomized controlled trials. *Am J Med.* 2002;112:298-304.
18. Seddon W, Rosner JM, Sperduto B, Yannuzzi RD, et al. Dietary fat and risk for advanced age-related macular degeneration. *Arch Ophthalmol.* 2001;119:1191-1199.
19. Peat JK. Prevention of asthma. *Eur Respir J.* 1996;9:1545-1555.
20. Aslan G, Triadafilopoulos A. Fish oil fatty acid supplementation in active ulcerative colitis: a double-blind, placebo-controlled, crossover study. *Am J Gastroenterol.* 1992; 87:432-437.
21. Stenson W, Cort WF, Rodgers D, Burakoff J, DeSchryver Kecskemeti R, et al. Dietary supplementation with fish oil in ulcerative colitis. *Ann Intern Med.* 1992; 116: 609-614.
22. Belluzzi M, Brignola A, Campieri C, Pera M, et al. Effect of an enteric-coated fish-oil preparation on relapses in Crohn's disease. *N Engl J Med.* 1996; 334:1557-1560.
23. Donadio JP, Larson Jr JV, Bergstralh TS, Grande EJ, et al. A randomized trial of high-dose compared with low-dose omega-3 fatty acids in severe IgA nephropathy. *J Am Soc Nephrol.* 2001; 12:791-799.
24. Donadio KE, Bergstralh JV, Offord EJ, Spencer KP, et al. A controlled trial of fish oil in IgA nephropathy. Mayo Nephrology Collaborative Group. *N Engl J Med.* 1994;331:1194-1199.
25. Kremer JM. Effects of modulation of inflammatory and immune parameters in patients with rheumatic and inflammatory disease receiving dietary supplementation of n-3 and n-6 fatty acids. *Lipids.* 1996;31:S243-S247.
26. Ariza MH, Mestanza Peralta R, Cardiel M. Omega-3 fatty acids in rheumatoid arthritis: an overview. *Semin Arthritis Rheum.* 1998;27:366-370.
27. Malasanos PW, Stacpoole TH. Biological effects of omega-3 fatty acids in diabetes mellitus. *Diabetes Care.* 1991;14:1160-1179.
28. Landgraf Leurs R, Drummer MM, Froschl C, Steinhuber H, et al. Pilot study on omega-3 fatty acids in type I diabetes mellitus. *Diabetes.* 1990;39:369-375.
29. Gogos F, Ginopoulos CA, Salsa P, Apostolidou B, et al. Dietary omega-3 polyunsaturated fatty acids plus vitamin E restore immunodeficiency and prolong survival for severely ill patients with generalized malignancy: a randomized control trial. *Cancer.* 1998;82:395-402.
30. Daly M, Weintraub JM, Shou FN, Rosato J, Lucia EF. Enteral nutrition during multimodality therapy in upper gastrointestinal cancer patients. *Ann Surg.* 1995;221:327-338.
31. Takahata PC, Monobe K, Tada K, Weber M. The benefits and risks of n-3 polyunsaturated fatty acids. *Biosci Biotechnol Biochem.* 1998;62:2079-2085.
32. Hajri T, Abumrad NA. Fatty acid transport across membranes: relevance to nutrition and metabolic pathology. *Annu Rev Nutr.* 2002; 22:383-415.
33. Schmitz G, Langmann T, Heimerl S. Role of ABCG1 and other ABCG family members in lipid metabolism. *J Lipid Res.* 2001;42:1513-1520.
34. Hamilton JA. Fatty acid transport: difficult or easy? *J Lipid Res.* 1998;39(3):467-481.
35. Fellmann P, Herve P, Pomorski T, Muller P, et al. Transmembrane movement of diether phospholipids in human erythrocytes and human fibroblasts. *Biochemistry.* 2000;39:4994-5003.
36. Conner SD, Schmid SL. Regulated portals of entry into the cell. *Nature* 2003;422:37-44.
37. Mansbach CM, Dowell R. Effect of increasing lipid loads on the ability of the endoplasmic reticulum to transport lipid to the Golgi. *J Lipid Res.* 2000;41: 605-612.
38. E. Bruce C, Chouinard RA, Tall AR. Plasma lipid transfer proteins, high-density lipoproteins, and reverse cholesterol transport. *Annu Rev Nutr.* 1998;18:297-330.
39. McDonald E, David AS, Pelosi AJ, Mann AH. Chronic fatigue in primary care attendees. *Psychol Med.* 1993;23:987-998.
40. Piper BF, Linsey AM, Dodd MJ. Fatigue mechanism in cancer. *Oncol Nursing Forum.* 1987; 14:17-23.

41. Kroenke K, Wood DR, Mangelsdorff AD, et al. Chronic fatigue in primary care. Prevalence, patient characteristics, and outcome. *JAMA*. 1988;260:929-934.
42. Morrison JD. Fatigue as a presenting complaint in family practice. *J Family Pract*. 1980;10:795-801.
43. Piper BF, Dribble SL, Dodd MJ, et al. The revised Piper Fatigue Scale: psychometric evaluation in women with breast cancer. *Oncol Nursing Forum*. 1998;25:667-684.
44. Agadjanyan M, Vasilevko V, Ghochikyan, et al. Nutritional supplement (NT Factor) restores mitochondrial function and reduces moderately severe fatigue in aged subjects. *J Chronic Fatigue Syndr*. 2003; 11(4):in press.
45. Ellithorpe RR, Settineri R, Nicolson GL. Pilot study: reduction of fatigue by use of a dietary supplement containing glycerophospholipids. *JANA*. 2003;6(1):23-28.
46. Richter C, Par JW, Ames B. Normal oxidative damage to mitochondrial and nuclear DNA is extensive. *Proc Nat Acad Sci USA*. 1998; 85:6465-6467.
47. Wei YH, Lee HC. Oxidative stress, mitochondrial DNA mutation and impairment of antioxidant enzymes in aging. *Exp Biol Med*. 2002;227:671-682.
48. Spector AA, Yorek MA. 1985. Membrane lipid composition and cellular function. *J Lipid Res*. 1985;26:101-105.
49. Harman D. Aging: a theory based on free radical and radiation chemistry. *J Gerontol*. 1956; 2:298-300.
50. Halliwell B. Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment. *Drugs Aging*. 2001;18:685-716.
51. Chen D, Cao G, Hastings T, et al. Age-dependent decline of DNA repair activity for oxidative lesions in rat brain mitochondria. *J Neurochem*. 2002;81:1273-1284.
52. Oslewacz HD. Genes, mitochondria and aging in filamentous fungi. *Ageing Res Rev*. 2002; 1:425-442.
53. Barja G. Endogenous oxidative stress: relationship to aging, longevity and caloric restriction. *Ageing Res Rev*. 2002;1:397-411.
54. Xu D, Finkel T. A role for mitochondria as potential regulators of cellular life span. *Biochem Biophys Res Commun*. 2002;294:245-248.
55. Ames BM. Micronutrients prevent cancer and delay aging. *Toxicol Lett*. 1998;102:1035-1038.
56. De AK, Darad R. Age-associated changes in antioxidants and antioxidative enzymes in rats. *Mech Ageing Dev*. 1991;59:123-128.
57. Arivazhagan P, Ramanathan K, Panneerselvam C. Effect of DL-alpha-lipoic acid on mitochondrial enzymes in aged rats. *Chem Biol Interact*. 2001; 138:189-198.
58. Sharman EH, Bondy SC. Effects of age and dietary antioxidants on cerebral electron transport chain activity. *Neurobiol Aging*. 2001;22:629-634.
59. Sugiyama S, Yamada K, Ozawa T. Preservation of mitochondrial respiratory function by coenzyme Q₁₀ in aged rat skeletal muscle. *Biochem Mol Biol Int*. 1995;37:1111-1120.
60. Lin M, Simon D, Ahn C, Lauren K, Beal MF. High aggregate burden of somatic mtDNA point mutations in aging and Alzheimer's disease brain. *Human Mol Genet*. 2002;11:133-145.
61. Sastre J, Pallardo FV, Garcia de la Asuncion J, Vina J. Mitochondria, oxidative stress and aging. *Free Radical Res*. 2000;32(3):189-198.
62. Kagan T, Davis C, Lin L, Zakeri Z. Coenzyme Q₁₀ can in some circumstances block apoptosis, and this effect is mediated through mitochondria. *Ann NY Acad Sci*. 1999;887:31-47.
63. Matthews RT, Yang L, Browne S, et al. Coenzyme Q₁₀ administration increases brain mitochondrial concentrations and exerts neuroprotective effects. *Proc Natl Acad Sci USA*. 1998;95:8892-8897.
64. Miquel, J. Can antioxidant diet supplementation protect against age-related mitochondrial damage? *Ann NY Acad Sci*. 2002; 959:317-347.
65. Seidman M, Khan MJ, Tang WX, Quirk WS. Influence of lecithin on mitochondrial DNA and age-related hearing loss. *Otolaryngol Head Neck Surg*. 2002;127:138-144.
66. Kim MJ, Cooper DD, Hayes SF, Spangrude GJ. Rhodamine-123 staining in hematopoietic stem cells of young mice indicates mitochondrial activation rather than dye efflux. *Blood*. 1998;91:4106-4117.
67. Colodny L, Lynch K, Farber C, Papish S, et al. Results of a study to evaluate the use of Propax to reduce adverse effects of chemotherapy. *JANA*. 2000;2:17-25.
68. Paradies G, Petrosillo G, Pistolesse M, Ruggiero F. Reactive oxygen species affect mitochondrial electron transport complex I activity through oxidative cardiolipin damage. *Gene*. 2002;286:135-141.
69. Kowald A. The mitochondrial theory of aging: do damaged mitochondria accumulate by delayed degradation? *Exp Gerontol*. 1999;34:605-612.
70. Koboska J, Coskun P, Esposito L, Wallace DC. Increased mitochondrial oxidative stress in the Sod2(+/-) mouse results in age-related decline of mitochondrial function culminating in increased apoptosis. *Proc Nat Acad Sci USA*. 2001;98:2278-2283.
71. Fernandes G, Chandrasekar B, Luan X, Troyer DA. Modulation of antioxidant enzymes and programmed cell death by n-3 fatty acids. *Lipids*. 1996;S9:1-6.
72. Johns DR. Seminars in medicine of Beth Israel Hospital, Boston: mitochondrial DNA and Disease. *N Engl J Med*. 1995;333:638-44.