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Nutritional Supplement (NT Factor™) Restores Mitochondrial Function and Reduces Moderately Severe Fatigue in Aged Subjects

Michael Agadjanyan, PhD[1], Vitaley Vasilevko, PhD[1], Anahit Ghochikyan, PhD[1], Paul Berns, MD[1], Patrick Kesslak, Ph.D.[2], Robert A. Settineri, MS[3], and Garth L. Nicolson, PhD[1*]

[1]The Institute for Molecular Medicine, Huntington Beach, CA 92649
[2]The Institute of Brain Aging and Dementia, University of California, Irvine, CA 92467
[3]Research Consultant, Nutritional Therapeutics, Inc., Hauppauge, NY

ABSTRACT

Objective: Decreased mitochondrial function is a characteristic of aging and fatigue. Here we determined if mild to moderately severe fatigue in a group of aged subjects (mean age >60 years), as defined by the validated Piper Fatigue Scale (PFS), can be significantly improved by use of a glycophospholipid dietary supplement, NT Factor™ (NTF). In addition, we determined if mitochondrial function, as defined by transport of the redox dye Rhodamine-123, is reduced in aging subjects with mild to moderately severe fatigue, and if this can be reversed with NTF supplementation in concert with improvement in fatigue scores.

Methods: Participants who described a condition consistent with mild to moderately severe fatigue, as defined by the PFS, were examined by research nurse and completed a PFS survey form. The PFS rates fatigue from a score of 0 (no fatigue) to 10 (severe fatigue). Respondents who fulfilled the entry requirements were admitted to the study when their self-reported fatigue severity scores were rated as mild to moderately severe, and their fatigue could not be explained by an obvious clinical condition. Blood leukocytes were isolated for analysis of mitochondrial function by transport of Rhodomine-123, and the subjects were provided by open label the study product NTF. Twenty of the respondents (mean age= 68.9±4.18) completed the first part of the study on NTF for 12 weeks, and 16 of these subjects who agreed to discontinue the product also completed a wash-out period for an additional 12 weeks. Fatigue and mitochondrial function were determined every four weeks during the study.

Results: There was a time-dependent reduction in overall fatigue in ten moderately fatigued subjects (average score 5.75±0.62, range 4.09-8.45) while on supplement but not in ten mildly fatigued subjects (average score 1.42±0.2, range 1.0-2.55). More specifically, after four weeks of NTF the average score of moderately fatigued subjects was reduced to 4.59 (20.2% reduction, $p<0.005$). Further use of NTF for a total of eight or twelve weeks decreased the overall average score of moderately fatigued subjects to 3.80±0.41 (33% reduction, $p<0.001$) or 3.71±0.48 (35.5% reduction, $p<0.001$), respectively, whereas in mildly fatigued subjects the fatigue scores were not significantly different.

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

Analysis of mitochrondrial function indicated that four and eight weeks of NTF use in moderately fatigued subjects increased function by 15% and 26.8%, respectively, and restored mitochondrial function to levels similar to those found in young adults. Further use of NTF for a total of 12 weeks did not increase mitochondrial function as measured by the Rhodamine-123 assay. Some subjects were monitored 12 weeks after discontinuing use of NTF. Fatigue and mitochondrial function in moderately fatigued subjects were found to be intermediate between the initial findings and the results found at eight or 12 weeks of supplement use, indicating that continued use of NTF would be necessary to maintain lower fatigue scores and maintain mitochondrial function.

Conclusions: The dietary supplement with NTF reduced significantly moderate fatigue as measured by the Piper Fatigue Scale and significantly increased mitochondrial function in aged subjects.

INTRODUCTION

The most common complaint of patients in general medical practice is fatigue,[1] and in fact, chronic fatigue is reported by 20% of all patients seeking medical Care.[1,2] Many well-known medical conditions are associated with chronic fatigue,[3] and it is often an important secondary condition in many clinical diagnoses. Loss of energy and the symptom of fatigue often precede a clinical diagnosis, and this may be one reason that it is so commonly reported by patients seeking medical care.

Fatigue is thought to be a multidimensional sensation with many possible causes and no universally accepted definition. Piper et al.[4] described fatigue as a multi-component sensation with behavioral, affective, sensory and cognitive components. They also designed a simple measurement model that combined multiple fatigue-associated elements into an overall fatigue score.[4]

At the cellular level fatigue is involved with cellular energy systems that for the most part are found in the mitochondria. Damage to cellular mitochondria can impair the abilities of cells to produce ATP and reduced NAD, and this occurs naturally with aging. Major targets of mitochondrial damage are phospholipid/protein membranes and mitochondrial DNA.[5-7] For example, damage of phospholipids in mitochondrial membranes by free radicals can affect membrane integrity, fluidity and transmembrane potentials, resulting in loss of energy production by the electron transport chain and its associated components. During the aging process mitochondria suffer damage to their membranes and DNA, and this is thought to contribute to or even be a cause of the aging process.[8, 9]

Preventing cell membrane damage and loss of membrane integrity are important in prevention of loss of cellular energy. One method that has been used to replace damaged mitochondrial membrane phospholipids is replacement therapy, and this has been accomplished, in part, by replacement of damaged lipids using a dietary supplement containing polyunsaturated phosphatidylcholines and other phospholipids and fatty acids that are essential structural and functional components of all biological membranes.[10-12]

In previous studies a dietary supplement, NT Factor™ (NTF) in a vitamin and mineral mixture (Propax™), was used to reduce chemotherapy-induced fatigue, nausea, vomiting and other side effects associated with chemotherapy.[10] NTF was also used to protect from hearing loss associated with aging and prevent mitochondrial membrane potential changes and mitochondrial DNA deletions that occur with aging[11]. We used Propax plus NTF in a pilot study with severely fatigued, aged subjects to reduce fatigue, as measured by the Piper Fatigue Scale (PFS). We found that fatigue was reduced 33%, from severe to moderate fatigue, after eight weeks of using Propax™ containing NTF[12]. The present study was initiated to examine the effects of NTF 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[13], improved with administration of NTF in concert with improvements in fatigue scores.

SUBJECTS AND METHODS

Subjects: Participants were prescreened on the basis of an initial phone conversation to determine whether their symptoms were consistent with persistent, intractable fatigue, or merely an intermittent condition linked to their work or lifestyle. Those who described a condition consistent with the definition of fatigue as defined in the Piper Fatigue Scale (PFS)[4] were mailed a survey form. This instrument defines fatigue as an unusual sense of tiredness that is not usually relieved by either a good night's sleep or by rest. The completed, returned surveys were then scored as described previously.[12]

After the initial PFS survey, participants aged 60 years and older with an overall fatigue score of 1 to 7 were examined by a research nurse and admitted to this pilot study if their fatigue could not be explained by a pre-existing clinical condition. The participants were divided into two groups: score 1-4 or mild fatigue and score 4-7 or moderate fatigue on a scale of 1-10 (0 = no fatigue, 1-4 = mild fatigue, 4-7 = moderate fatigue, 7-10 = severe fatigue).

There were 20 respondents who fully completed the study that had an average age of 68.9±4.8, with a range of 61-77. There were seven men whose average age was 67.6±3.15, with a range of 61-71 and thirteen women whose average age was 69.5±4.61, with a range of 62-77. All of the subjects were from Southern California.

Subjects were asked if they used any prescription medications. Nine participants or 45% used prescription medications (Table 1). However, of the nine subjects in Table 1 who indicated persistent, intractable fatigue, only four used more than one medication. All who listed depression as a diagnosis were on antidepressants, and of the four hypothyroid respondents three were on Armour Thyroid supplementation.

Table 1. Medications used by study participants (n=20)

Medications Represented by Categroy
Anti-neoplastic: 1
Asthma/allergies: 4
Anti-inflammatory/non steroidal: 2
Thyroid replacement: 4
Female hormone replacement: 2
Anti-depressant: 4
Anti-acids/H_2-blocker: 3
Anti-hypertensive: 4
Pain medications: 5

Study Design: Subjects signed an informed consent document and were admitted into the study with mild (1-4 on the PFS) or moderate fatigue (4-7 on the PFS). Each participant was given instructions to use three tablets of NT Factor™ twice daily. The identity of the product that the participants were to take during the trial was not identified on the label, and it was given to subjects in plain bottles with instructions clearly marked on the label. Their blood was taken for analysis, and they were provided a four-week supply of NTF and told to return after the fourth week of using the product. If a blood chemistry panel (Chem-20) indicated that the subject had values outside the normal range, they were excluded from the study. All subjects repeated the PFS assessment at the end of the fourth, eighth and 12th week when they returned for collection of blood. After the 12th week, the participants stopped using NTF. These subjects returned after the 24th week (12 week wash-out period). At that time blood was drawn, the participants completed their PFS questionnaires, and all of the forms were checked for verification, completion and scoring accuracy.[12]

Materials and Methods: The supplement product, NT Factor™ (Nutritional Therapeutics, Inc., Hauppauge, NY), is a proprietary vitamin, mineral and nutrient complex containing an exogenous source of polyunsaturated phosphatidylcholine and other membrane phospholipids (Table 2).[12] The participants took the product twice daily for 12 weeks. Each four weeks the participants returned the product container for determination of compliance. After 12 weeks the participants discontinued use of the product, and 12 weeks later they were retested.

The PFS is composed of 22 numerically scaled questions rated from 0 (no fatigue) to 10 (severe) fatigue. These items measure four dimensions of subjective fatigue: behavioral/severity (6 items); affective/meaning (5 items); sensory (5 items); and cognitive/mood (6 items). These are used to calculate the four sub-scale/dimensional scores and the total fatigue scores. The standardized alpha (Cronbach's alpha) did not drop below 0.90 for any of the subscales, and the standard alpha for the entire scale of 22 questions was 0.96, indicating excellent reliability for an established instrument.[14]

Mitochondrial function was determined by transport and reduction of the dye Rhodamine-123 as described previously[13]. Peripheral blood mononuclear cells (PBMC) were isolated from whole blood using a Ficoll-Hypaque gradient by centrifugation at 1,800 rpm for 30 min in a clinical centrifuge at room temperature. PBMC were stained with 2.0 or 10 μM of Rhodamine-123 (Sigma Chemical, St. Louis, MO) in phosphate-buffered saline (PBS) in the dark for 15 min at 37^{0}C. To remove unbound dye prior to flow cytometric analysis the cells were washed twice by centrifugation in ice-cold PBS and re-suspended in cold PBS. Non-viable cells were excluded from analysis using a light scatter gate established by staining with a propidium iodide (Sigma Chemical) solution. Data was collected on Rhodamine-123 fluorescence using an argon ion laser tuned at 488 nm (FACScan, Becton Dickinson, Mountain View, CA) and analyzed using CellQuest software (Becton Dickinson). Since the data using 2.0 or 10 mM of Rhodamine-123 were similar, only the data using the 10 mM dose was reported. Results from the mitochondrial staining with Rhodamine-123 were analyzed using a repeated measures analysis of variance (ANOVA) and Bonferroni/Dunn post-hoc test for specific group differences (young control, mild versus moderate fatigue, treatment times, washout, etc.).

RESULTS

NTF improved the overall fatigue scores of moderately fatigued subjects as measured by the PFS (Table 3). The initial PFS group average (mean) fatigue score was 5.75±0.6, and after four weeks of NTF this improved to 4.59±0.5 or a 20.2% reduction in fatigue. After eight and 12 weeks of NTF the PFS fatigue scores of the moderately fatigued group improved to 3.8±0.6 (33% reduction) and 3.71±0.65 (35.5% reduction), respectively (Table 3). These changes were significant ($p<0.001$). By sex, the total PFS mean score improved in moderately fatigued subjects after taking NTF for four weeks by 15.3% in women and 23.5% in men. After eight and 12 weeks, fatigue improved in women by 27.5% and 32.3%, respectively, and in men by 40.5% and

Table 2. Components of NT Factor™

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:

Glycophospholipids: polyunsaturated phosphatidylcholine, other polyunsaturated phosphatidyl lipids, glycolipids and essential fatty acids, including omega-3 and omega-6 fatty acids.

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.

Table 3. Piper Fatigue Scale: results of study and subscale values

Time (weeks)	0	4	8	12	12+12 washout
Moderate Fatigue	Mean (%)‡	Mean (%)	Mean (%)	Mean (%)	Mean (%)
Overall Fatigue	**5.75 (0)**	**4.59^{+} (20.2)**	**3.80* (33.0)**	**3.71* (35.5)**	**4.53^{+} (21.2)**
Behavior/Severity	3.67 (0)	3.40 (7.6)	3.37 (8.2)	3.11^{+} (15.3)	3.00^{+} (18.2)
Affective/Meaning	6.67 (0)	4.24* (36.4)	4.20* (37.0)	3.82* (42.7)	5.17^{+} (22.5)
Sensory	6.18 (0)	5.26 (14.9)	3.84* (37.9)	3.82* (40.5)	5.46 (11.7)
Cognitive/Mood	5.78 (0)	4.48^{+} (22.5)	3.63* (37.2)	3.78* (34.6)	4.45^{+} (23.0)
Mild Fatigue	Mean (%)‡	Mean (%)	Mean (%)	Mean (%)	Mean (%)
Overall Fatigue	**1.62 (0)**	**1.60 (1.2)**	**1.57 (3.1)**	**1.53 (5.6)**	**1.52 (6.2)**
Behavior/Severity	1.43 (0)	1.48 (0)	1.42 (0)	1.46 (0)	1.40 (0)
Affective/Meaning	2.17 (0)	2.00 (7.8)	1.96 (9.7)	2.04 (6)	1.83 (15.6)
Sensory	2.33 (0)	2.02 (13.3)	1.84^{+} (21)	1.74^{+} (25.3)	1.89^{+} (18.8)
Cognitive/Mood	2.20 (0)	2.24 (0)	2.29 (0)	2.10 (0)	2.12 (0)

‡mean values (% difference)
$^{+}$p<0.005 (compared to baseline values)
*p<0.001(compared to baseline values)

42.9%, respectively. There were no significant differences between the results with men and women. In contrast to moderately fatigued subjects, however, NTF use did not have a significant effect on mild fatigue. The improvement in fatigue scores overall after 12 weeks of NTF in mildly fatigued subjects was only 5.6% (Table 3). As found previously with severely fatigued subjects,[12] age was not associated with the degree of change in fatigue in the moderately fatigued group using the NTF supplement. When subjects stopped using NTF, their fatigue scores increased. Twelve weeks after stopping NTF the moderately fatigued group had fatigue scores of 4.53±0.4 or 21.2% difference with the baseline value, whereas in the mildly fatigued group there were no significant differences in overall PFS fatigue scores (Table 3).

Using the PFS subscales the Behavioral/Severity scores improved in moderately fatigued subjects after 12 weeks of NTF an average of 15.3% (Table 3). The Affective/Meaning subscale improved by an average of 37% and 42.7% after NTF use for 8 and 12 weeks, respectively. The Sensory subscale revealed 37.9% and 40.5% average improvements for the group after 8 and 12

weeks NTF use, respectively. Finally, the Cognitive/Mood subscale showed average improvements of 37.2% and 32.9% after 8 and 12 weeks of NTF use, respectively (Table 3).

Mitochondrial function was measured in the various groups using the Rhodamine-123 assay. Since the data using 2 or 10 μM Rhodamine-123 were not significantly different, only the data using 10 μM dye was reported. The staining of mitochondria with Rhodamine-123 changed significantly throughout the course of treatment of both moderately and mildly fatigued subjects with NTF (Figures 1 and 2) ($F [4,76]=29.917$, $p<0.0001$). Post-hoc analysis with the Bonferroni/Dunn test for specific differences between groups indicated that after 8 and 12 weeks of NTF the results were significantly different from baseline ($p<0.0001$) and after washout for an additional 12 weeks ($p<0.001$); however, there was no significant difference between the use of NTF for 8 and 12 weeks ($p>0.4$). After 12 weeks of NTF the Rhodamine-123 mitochondrial assay yielded results similar to and not significantly different from those found in non-fatigued young adults that had not taken NTF (Figures 1 and 2). In the moderately fatigued group 8 or 12 weeks of NTF use resulted in significant differences ($p<0.001$) in mitochondrial function; however, there was no significant difference between the 8- and 12-week groups ($p>0.17$) (Figure 1). This amounted to an increase in mitochondrial function by 8.4%, 23.8% and 23.7%, respectively, after four, eight and 12 weeks of NTF use in moderately fatigued subjects (Figure 1). Some subjects were monitored 12 weeks after discontinuing use of NTF. Although still significantly different from baseline ($p<0.001$), mitochondrial function returned to intermediate values between baseline and the values at 8 or 12 weeks (Figures 1 and 2). When analyzed by sex, there were no significant differences between men and women in any of the groups.

Figure 1. Cytofluorographic analysis of 10 μM Rhodamine-123 incorporation into the mitochondrial membranes of blood monocytes isolated from moderately fatigued subjects. *$p<0.001$

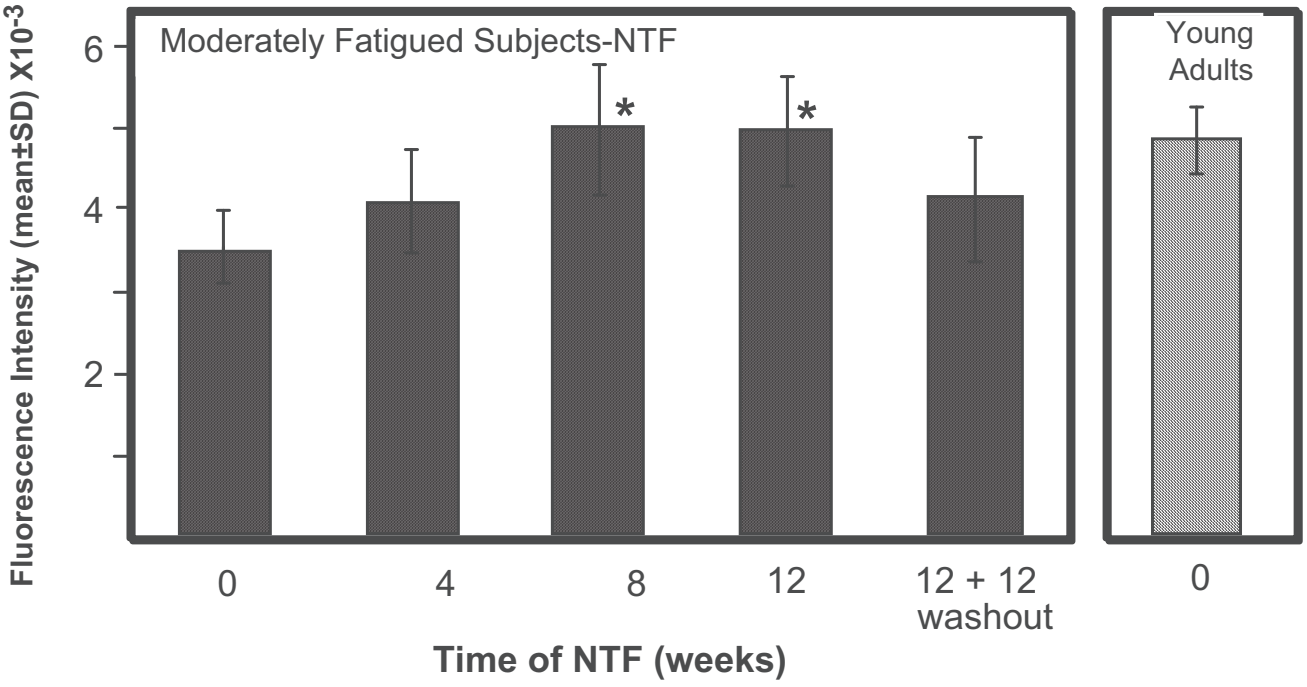
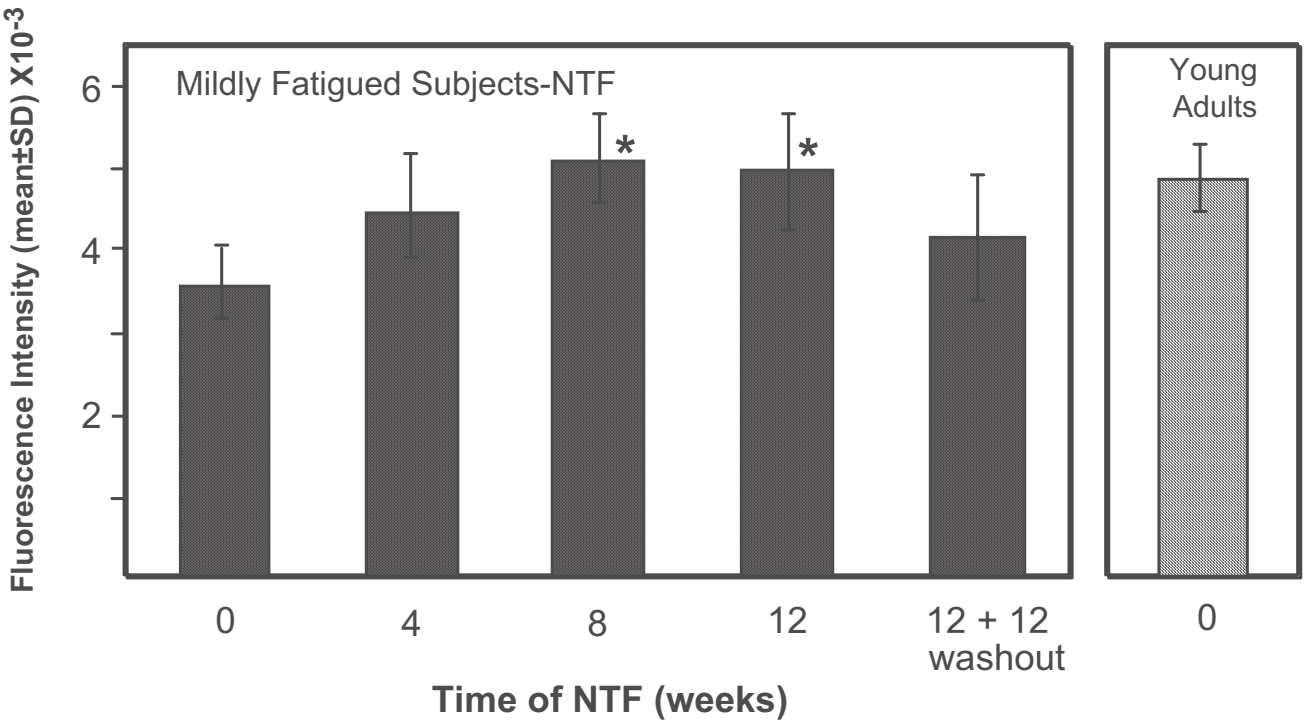


Figure 2. Cytofluorographic analysis of 10 μM Rhodamine-123 incorporation into the mitochondrial membranes of blood monocytes isolated from mildly fatigued subjects. *p<0.001



DISCUSSION

Mitochondria are the most important source of cellular energy in our bodies. If their function is impaired, energy available to cells is limited to the Krebs Cycle. There are a number of conditions and substances that can impair mitochondrial function,[5-8] but oxidation and damage of mitochondrial lipids in membranes are among the most important causes of impairment of mitochondrial function. This may result in modification of the electrical potential barrier across the mitochondrial membranes that is essential in the electron transport chain generation of cellular energy molecules. The dietary supplement NTF used in this pilot study is a unique mixture of cellular lipids that is rich in phospholipids and glycophospholipids, and in particular, polyunsaturated phosphatidylcholine and other membrane lipids. It also contains essential fatty acids and other lipids that are important in mitochondrial function and cellular membrane health and probiotic microorganisms to aid in intestinal uptake.[12]

NTF has been used in clinical trials on cancer patients, and it has been shown to cause a substantial positive impact on fatigue. In a twelve week double-blinded, cross-over, placebo controlled, randomized trial on cancer patients receiving chemotherapy NTF in a vitamin-mineral supplement (Propax™) showed improvement from fatigue, nausea, diarrhea, impaired taste, constipation, insomnia and other quality of life indicators.[10] Most (64%) of the patients in the study reported significant improvement in these and other chemotherapy-induced side effects, and 29% experienced no overall worsening of side-effects. Following cross-over to the supplement containing NTF patients reported rapid improvement in nausea, impaired taste, tiredness, appetite, sick feeling and other indicators.

NTF has also demonstrated an anti-aging effect on hearing loss in aging rats. Using 18-20 month-old Harlan-Fisher 344 rats Seidman et al.[11] found that NTF prevented hearing loss associated with aging, shifting the threshold hearing from 35-40 dB in control aged animals to 13-17 dB in the test group. These results were found to be significant ($p<0.005$). They also found that NTF preserved cochlear mitochondrial function as measured in the Rhodamine-123 transport assay, increasing mitochondrial function by 34%. NTF also prevented the common aging-related mitochondrial DNA deletion (mtDNA4834) found in the cochlear of aging rats.

We also found an effect of Propax™ with NTF in a pilot trial designed to measure fatigue in aged patients

(>50 years-old) with a variety of common clinical conditions[12]. In these severely fatigued subjects (mean PFS scores = 7.9±0.82) NTF significantly reduced fatigue to moderate levels. After eight weeks of NTF there was a 40% reduction in overall fatigue (mean PFS scores = 4.7±2.01) as measured by the FPS instrument. These results are comparable to the data presented here for moderately fatigued subjects, where we found a 35.5% reduction in overall fatigue in moderately fatigued subjects after eight weeks use of NTF.

In the current study we utilized moderately (PFS scale=4-7) and mildly fatigued (PFS scale=1-4) subjects but only found a significant effect on fatigue in the moderately fatigued group of either sex. There could be a number of reasons for this observation, but it is unlikely that the only contribution to fatigue in these patients is mitochondrial function. Fatigue is a complex phenomenon, and it may be determined by several factors, including psychological health of the subjects. Also, in the mildly fatigued patients differences are difficult to determine because of the nature of the measuring instrument, and it might be unreasonable to expect significant differences in subjects that score very low initially on the PFS.

Our subjects were not randomly chosen for this study; they were recruited using a health talk radio program in the Los Angeles, CA region. The only criteria was that they were older than 50 years-old, mildly to moderately fatigued (using the PFS scales), and their fatigue could not be explained by an underlying clinical condition. Subjects were given a physical examination by a research nurse, and their blood was analyzed using a standard chemistry profile for possible clinical problems. This procedure did eliminate some prospective subjects from the trial. The subjects that qualified for the trial used NTF supplement for 12 weeks, and most of them then went off product for an additional 12 weeks to see if they would return to baseline fatigue and mitochondrial function values. Another potential problem was the number of participants with mild or moderate fatigue used in the study. Ideally, we would have liked to have a larger number of participants, but a number of factors prevented this, including the cost of the study.

Fatigue is related to the metabolic energy available to an individual and ultimately to the many cells that perform their myriad of functions. The integrity of cell and intracellular membrane structures, especially in the mitochondria, is critical to cell function and energy production.[15-17] NTF provides cells and mitochondria with the glycophospholipids, fatty acids and other essential lipids to repair and replace membrane components needed for maintenance of cell and mitochondrial function necessary in the production of cellular energy to combat fatigue.

The decline of energy production with aging may be due, in part, to mitochondrial lipid peroxidation by reactive oxygen species. Membrane damage and subsequent mitochondrial dysfunction 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. Indeed, some studies have linked the development of certain chronic diseases with the degree of mitochondrial membrane lipid peroxidation and mtDNA damage. Thus the damage to mtDNA and mitochondrial membranes seems to be involved in the etiology of age-associated degenerative diseases leading to changes in the expression of genes important for cell survival as well as the phenomenon of aging itself.[18] Restoration of mitochondrial membrane integrity and fluidity are essential for the optimal functioning of the electron transport chain.[19] Declines in energy production with aging coupled with an increase in oxidative stress can modify membrane lipids and increase mitochondrial membrane permeability and activate cellular death programs (apoptosis). Together these factors likely play a major role in the aging process and they also affect the development of age-related degenerative diseases.[20]

The first outward sign of cellular deterioration may be fatigue. As the phospholipid structure of the mitochondrial membrane loses fluidity and becomes more porous at lipid/protein interfaces in the membrane, the membrane potential is affected and less able to maintain the electron transport process. In addition, the electron transport chain increases the production of Reactive Oxygen Species (ROS), free radicals that can further damage mitochondrial membranes and mtDNA. Although there is always some inherent mitochondrial membrane leakage and damage, this is usually repaired, unless the rate of repair is exceeded by the rate of oxidative damage.[21]

Finally, since ROS are highly implicated in age-associated mtDNA damage, we tried to determine the age-dependent accumulation of a particular 4977 bp mtDNA deletion in platelets of patients. The 83 bp fragment of deleted mtDNA was detected in all samples including control blood from young volunteers. The amount of the deletion in blood cells did not show an age-dependent increase, and differences in amounts (quantity was estimated by fluorescence intensity of ethidium bromide stained DNA in agarose gel) of deletion were detected in blood before and after NTF use (data not shown). The 4977 bp deletion in mtDNA is known to accumulate with age in various human postmitotic tissues, such as brain, heart and skeletal muscle. Different groups have tried to use blood cells as a possible model for screening the accumulation of mtDNA mutations; however, the results have so far been contradictory. Biagini et al.[22] as well as the other groups[23-26] failed to

detect this particular deletion in blood or platelets both from young and old individuals, whereas Meissner et al.[23] demonstrated that this deletion is detectable in blood cells, but the amount is substantially lower than in postmitotic tissues. Also Meissner et al.[23] did not show an age-dependent increase in the 4977 bp deletion in mtDNA. Our data are in good accordance with the assertion of Meissner et al.[23] that the accumulation of 4977 bp mtDNA deletion in blood cells is not age-dependent, and this might be explained by higher turnover rate of blood cells.

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REFERENCES

1. Kroenke K, Wood DR, Mangelsdorff AD, Meier NJ, Powell JB. Chronic fatigue in primary care. Prevalence, patient characteristics, and outcome. *JAMA.* 1988; 260: 929-934.
2. Morrison JD. Fatigue as a presenting complaint in family practice. *J Family Pract* 1980; 10: 795-801.
3. McDonald E, David AS, Pelosi AJ, Mann AH. Chronic fatigue in primary care attendees. *Psychol Med.* 1993; 23: 987-998
4. Piper BF, Linsey AM, Dodd MJ. Fatigue mechanism in cancer. *Oncol Nursing Forum.* 1987; 14: 17-23.
5. 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.
6. Wei YH, Lee HC. Oxidative stress, mitochondrial DNA mutation and impairment of antioxidant enzymes in aging. *Exp Biol Med.* 2002; 227:671-682.
7. Spector AA, Yorek MA. 1985. Membrane Lipid composition and cellular function. *J Lipid Res*. 1985; 26:10105.
8. Harman D. Aging: A theory based on free radical and radiation chemistry. *J Gerontol.* 1956; 2: 298-300.
9. Xu D, Finkel T. A role for mitochondria as potential regulators of cellular life span. *Biochem Biophysics Res Commun* 2002; 294:245-248.
10. 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.
11. 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.
12. Ellithorpe RR, Settineri R, Nicolson GL. Pilot Study: Reduction of fatigue by use of a dietary supplement containing glycophospholipids. *JANA* 2003; in press.
13. 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.
14. Nunnally JC. 1978. *Psychometric Theory* (2nd ed.) New York: McGraw-Hill, pp. 117-123.
15. Zeisel SH, In Hanin I, Popell G, (eds.) 1996. Phospholipids, biochemical pharmaceutical and analytical considerations. New York: Plenum Press, pp. 219-231.
16. Conlay LA, Wurtman RJ, Blusztajn K, Coviella IL, Maher TJ, Evoniak GE. *N Engl J Med*. 1986; 175: 892.
17. Johns DR. 1995. Seminars in medicine of Beth Israel Hospital, Boston: Mitochondrial DNA and Disease. *N Engl J Med.* 1995; 333: 638-44.
18. Kowald A. The mitochondrial theory of aging: do damaged mitochondria accumulate by delayed degradation? *Exp Gerontol* 1999; 34:605-612.
19. Paradies G, Petrosillo G, Pistolese M, Ruggiero F. Reactive oxygen species affect mitochondrial electron transport complex I activity through oxidative cardiolipin damage. *Gene* 2002; 286:135-141.
20. Lin M, Simon D, Ahn C, Lauren K, Beal MF. High aggregrate burden of somatic mtDNA point mutations in aging and Alzheimer's disease brain. *Human Mol Genet* 2002; 11:133-145.
21. 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.
22. Biagini G, Pallotti F, Carraro S, Sgarbi G, Pich MM, Lenaz G, Anzivino F, Gualandi G, Xin D.
23. Mitochondrial DNA in platelets from aged subjects. *Mech Ageing Dev* 1998; 101:269-275.
24. Meissner C, Mohamed SA, Klueter H, Hamann K, von Wurmb N, Oehmichen M. Quantification of mitochondrial DNA in human blood cells using an automated detection system. *Forensic Sci Int* 2000; 113:109-112.
25. Lee HC, Oang CY, Hsu HS, Wei YH. Deletion in blood mitochondrial DNA in Kearns-Sayre syndrome. *Biochim Biophys Acta* 1994; 1226:37-43.
26. Poulton J, Deadman ME, Ramacharan S, Gardiner RM. Germ-line deletions of mtDNA in mitochondrial myopathy. *Am J Hum Genet* 1991; 90:649-653.
27. Smith OP, Hamm MJ, Woodward CE, Brockington M. Pearson's marrow/pancreas syndrome: haematological features associated with deletion and duplication of mitochondrial DNA. *Br J Haemotol* 1995;90:469-472.