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Polyunsaturated Phosphatidylcholine in NT Factor™ Improves Mitochondrial Function, Auditory Sensitivity and may Slow some of the Aging Processes

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The purpose of this study was to investigate the effects of polyunsaturated phosphatidylcholine (PPC) in NT Factor™* on aging, in general, and age-associated hearing loss, in specific. Additionally, this brief report supports that the Membrane Hypothesis of Aging (MHA) provides a plausible explanation for age-related hearing loss. According to this hypothesis, which is also known as the Mitochondrial Clock Theory of Aging, Reactive Oxygen Metabolites (ROM), are responsible for progressive insults on mitochondria and other cellular structures. Over an extended time period, these insults accumulate leading to a reduction in the energy generating capacity of the mitochondria, cellular demise and resultant senescence.

Soy lecithin is a source from which PPC can be extracted. These molecules are indispensable for cellular differentiation, proliferation and

regeneration. PPC are high energy functional and structural elements of all biological membranes. PPC plays a rate-limiting role in the activation of numerous membrane located enzymes, including superoxide dismutase and glutathione peroxidase, which are important antioxidants that protect cell membranes from damage by reactive active metabolites (ROM).

PPC are highly purified extracts of the semen of soybean, supplying the organism with nontoxic choline molecules with a high content in polyunsaturated fatty acids, in particular linoleic acid. These PPC correspond to the body's own PPC molecule. The physiologic functions of these phospholipids are related to the morphology of the biological membranes, the incorporation of these molecules into membranes and thus on the intact character of the structure of cell membranes.

There are several disease processes related to membrane damage for which clinical and pharmacological trials using PPC have been conducted. Effects of PPC on these various disorders have shown enhancement in cognitive performance of the aging brain, improvement of coronary, peripheral and cerebral blood flow, activation of liver metabolism and detoxification and promotion of gastrointestinal function by mucosal restoration. The current study was designed to investigate the effects of PPC on age related hearing loss by evaluating its ability to preserve mitochondrial function, protect mitochondrial DNA from oxidative damage and preserve auditory sensitivity.

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Harlan-Fischer 344 rats, 18-20 months of age, were used as the experimental subjects. The subjects were caged individually and maintained at 21 – 22°C in a 12:12 light-dark cycle. The experimental protocols were reviewed and approved by the Care for Experimental Animal Committee (CEAC) at the Henry Ford Health System. These protocols are in strict compliance with guidelines as established by the National Institute of Health.

NT Factor containing PPC was obtained from Nutritional Therapeutics (Smithtown, NY). A dose of 300mg/kg/day of NT Factor was supplemented to each subject, by adding it to the oral diet. The animals were divided randomly into two groups (n = 7 for each group). Group-1 served as the control, and group-2 as the experimental group.

At the onset of the study, Auditory Brainstem Responses were obtained to measure base-line hearing thresholds in all subjects. Age-associated changes in hearing sensitivities were then recorded at two-month intervals for six months. In order to assess age-related changes in mitochondrial function, mitochondrial membrane potentials were studied using flow cytometry. For this purpose, peripheral blood was obtained from each subject at the beginning and at the end of the protocol. At the conclusion, the subjects were euthanized according to NIH protocol, and tissue samples were obtained from brain and cochlea (stria vascularis and auditory nerve) to study mitochondrial DNA deletion associated with aging. This was achieved by amplifying the specific common aging mitochondrial deletion (4834-bp) by Polymerase Chain Reaction. DNA quantification was performed. The data obtained for each protocol was compared between the two groups and analyzed using ANOVA.

The effects of PPC on age-related hearing loss demonstrate a gradual age associated decline in hearing sensitivities at all the frequencies tested (3, 6, 9, 12 and 18 kHz). These results are comparable to previous studies that have shown similar results under similar experimental conditions. There was a statistically significant preservation of hearing noted in the treated subjects at all frequencies, which was observed at four and six months of treatment. Overall, there was a continued decline in hearing in the control subjects and a statistically significant protective effect of PPC on the experimental subjects ($p < .005$).

Mitochondrial membrane potentials were recorded by flow cytometry as a measure of the uptake of Rhodamine 123 by mitochondria. This probe is specific for mitochondria as it is selectively taken up by the mitochondrial membrane. The intensity of this uptake corresponds directly to the mitochondrial activity and hence membrane potential. The data obtained from the two groups were averaged and statistical analysis was performed using ANOVA. The

mean fluorescence intensity (MFI) in group-1 subjects measured 3190 and 2100 at the beginning and end of the study, respectively.

This, approximately, 30% decline in membrane potential with time was statistically significant ($p=0.003$). Conversely, the MFI in the experimental group remained essentially unchanged at 2990 from 3165 at the beginning of the study. This difference between the control and treated groups was statistically significant ($p<0.05$), demonstrating the protective effect of PPC supplementation on mitochondrial membrane potential.

For the mtDNA deletion tests, mtDNA from brain, stria vascularis and auditory nerve were studied. In order to verify the presence of mtDNA, the ND-1 16SrRNA segment was identified, which is a highly preserved region of the mitochondrial genome. Specific primers for this segment and for the common aging deletion were synthesized in our laboratory. Equal quantities of DNA were used in all samples for standardization purposes. The PCR products identified the ND-1 16SrRNA region (a control to verify the presence of mitochondrial DNA) by a 601bp product in all samples and the common aging deletion (4834 bp deletion) by a 598 bp product. This aging deletion was identified in five of the seven control subjects and four of the experimental subjects. Quantitative determination reveals a significantly lower ratio of this common aging deletion to the total mtDNA in the experimental subjects as compared to the control subjects. Based upon these findings we conclude that PPC has a protective effect on mitochondrial DNA damage and function.

ROM are known to play important roles in many biochemical reactions that are critical in maintaining normal cell functions. Increasing evidence indicates that ROM are also important mediators of several forms of tissue damage, such as injuries associated with inflammatory responses, ischemic injuries to tissues, injuries resulting from the intracellular metabolism of chemicals and drugs, coronary artery disease, cerebrovascular accidents, age-related hearing loss and aging. The primary *in vivo* source of ROM appears to be the mitochondrial electron transport system during oxidative phosphorylation (during the process of energy generation). There are many other sources of ROM production including; prostaglandin biosynthesis, environmental contaminants, cigarette smoking, ionizing radiation and poor dietary regimens.

ROM generation occurs from periods of prolonged relative hypoperfusion such as can be seen with arteriosclerosis and aging. It has been demonstrated that in the elderly population there is significantly decreased flow within the circulatory system in general,¹⁻⁵ and the inner ear, in specific.⁶⁻⁹

Prolonged periods of reduced blood flow such as those accompanying aging lead to the formation of tissue damaging ROM. ROM have been implicated in injury to polyunsaturated fatty acids in cell membranes resulting in the process of auto-oxidation which is of great importance in the pathogenesis of cell membrane damage. They have also been shown to be mediators of mitochondrial DNA damage including the generation of mitochondrial DNA deletions (mtDNA del). MtDNA del have been associated with cellular and tissue dysfunction, age-related hearing loss,¹⁹ senescence and death. This sequence of events is the foundation of the membrane hypothesis of aging (MHA).⁸⁻¹⁰

Phospholipids are integral structural components of all biological membranes with PPC and phosphatidylethanolamine being the pre-dominant types, quantitatively. They constitute the phospholipid bilayer structure of cellular membranes, which is responsible for membrane stability and cellular function. PPCs maintain and promote the activity of several membrane bound proteins and enzymes, including Na-K ATPase, adenylate cyclase and glutathione reductase. They are also known to be precursors of cytoprotective agents such as eicosanoids, prostaglandins and antioxidants.

These experiments suggest that NT Factor containing PPC may protect mitochondrial function by preserving the age-related decline in mitochondrial membrane potentials and hence their activity. Additionally, there was less mitochondrial DNA damage noted in the treated group. This may also explain the demonstrated effect of preservation of hearing loss associated with aging, by the ability of PPC to specifically up regulate cochlear mitochondrial function. There are many studies demonstrating the effects of mitochondrial metabolites on cognition and aging,^{11-18, 20-22} Additionally, recent work from our laboratory has shown that acetyl-L-carnitine and α -lipoic acid delay the progression of age-related hearing loss by protecting cochlear mitochondrial DNA from oxidative damage.²³ These results support the membrane hypothesis of aging and provide further evidence to support this theory as a possible explanation for age-related hearing loss. Thus, PPC may be one of many rational approaches to consider for the purpose of membrane preservation, enhanced mitochondrial function, reduction of age-associated mitochondrial DNA damage and slowing of some of the aging processes.

**NT Factor is comprised of defatted rice bran, arginine, beet root fiber, black strap molasses, glycine, magnesium sulfate, enriched polyunsaturated phosphatidylcholine (phospholipids), saponin (glycolipids), para-amino benzoate, leek, pantethine (bifidus growth factor), taurine, garlic, calcium*

borogluconate, omega-6 essential fatty acids, omega-3 essential fatty acids, artichoke, barley malt, potassium citrate, calcium sulfate, {barley malt removed 7/2002} spirulina, bromelain, natural vitamin E, calcium ascorbate, alpha-lipoic acid, oligosaccharides, B-6, niacinamide, riboflavin, inositol, niacin, calcium pantothenate, thiamin, B-12, bifidus, acidophilus, folic acid, chromium picolinate.

NT Factor™ is a registered trademark of Nutritional Therapeutics Inc. Smithtown, NY

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