

## **Blood homocysteine and fasting insulin levels are reduced and erythrocyte sedimentation rates increased with a glycopospholipid-vitamin formulation: a retrospective study in older subjects**

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### **Abstract**

**Background:** Elevations in Homocysteine (Hcys) levels in the blood have been correlated with increased risk for coronary heart disease and stroke, loss of cognition and memory, and other chronic medical conditions.

**Objective:** A retrospective study was initiated to determine if Hcys levels and other blood markers were altered in subjects taking an oral functional food supplement containing a mixture of phosphoglycolipids (NT Factor®) and vitamins.

**Methods:** Thirty-five patients (28 females, 7 males, Av. Age=60.7±9.6 years) who had used the functional food Advanced Physician's Formula™ with NTFactor® in tablet form each day were enrolled in a retrospective study on blood chemistry. This retrospective study followed a prospective study on the use of the same supplement to reduce fatigue in patients with chronic fatigue. Participants were patients with chronic fatigue syndrome (myalgic encephalomyelitis) or other fatiguing illnesses. Subjects had blood drawn over a 6-month period, and routine blood testing was performed. In this laboratory study the results were analyzed for differences, and statistical analyses were performed.

**Results:** All participants responded in the study and showed an average reduction of 31.8% in Hcys levels (from 10.85±0.42 to 7.40±0.42 μmol/L; t-test, p<0.001; Wilcoxon, p<0.001). Women responded better than men: women (from 11.06±0.50 to 8.67±0.82 μmol/L, 34.4% reduction, t-test, p< 0.001; Wilcoxon, p<0.001) versus men (from 10.80±0.51 to 7.01±0.47 μmol/L, 21.6% reduction, t-test, p< 0.0862). Differences were also found in fasting insulin levels (from 12.80±3.11 to 5.30±1.77 μIU/mL, 58.6% reduction, t-test, p<0.005) and erythrocyte sedimentation rate (ESR). ESR increased from 10.5±2.21 to 20.19±3.20 mm/hr (92.2% increase, t-test, p<0.0314; Wilcoxon, p<0.0154). Other tests were not significantly different after 6

months of supplement, there were no side effects from the test supplement, and none of the participants had any cardiovascular events during the study.

**Conclusions:** The test formulation was an effective method to reduce Hcys and fasting insulin blood levels and increase ESR rates in older subjects without adverse effects.

**Keywords:** Lipid Replacement Therapy, NT Factor®, fatigue, homocysteine, blood insulin, erythrocyte sedimentation rate, vitamin B complex

## **BACKGROUND:**

Elevations in Homocysteine (Hcys) levels in the blood have been correlated with increased risk for coronary heart disease (CHD) and stroke, loss of cognition and memory, and other chronic medical conditions [1-4]. For example, Hcys can be high in chronic renal failure, hypothyroidism, pernicious anemia, systemic lupus, certain methionine genetic alternations and deficiencies in folic acid, vitamin B6 and B12 or in heavy smokers or excess drug use [5]. But it is its role as an independent risk factor for arteriosclerotic and thromboembolic diseases, including myocardial infarction, cerebral infarction and deep-vein thrombosis that makes it so important as a blood marker [6-8].

## **INTRODUCTION:**

Recent clinical trials have shown the effectiveness of Lipid Replacement Therapy (LRT) plus antioxidants in the treatment of certain clinical disorders and conditions, such as chronic fatigue and fatiguing illnesses [9, 10]. This dietary approach to replace damaged cellular lipids with undamaged (unoxidized) lipids ensures proper function of cellular structures, such as cellular and organelle membranes [10-12]. LTR has proven to be an effective method to prevent Reactive Oxygen Species (ROS)-associated changes in function and for use in the treatment of various chronic conditions [9-12]. Chronic or intractable fatigue that is not reversed by sleep occurs naturally during aging and in many degenerative diseases [13, 14]. Chronic fatigue is the most common complaint of patients seeking general medical care in North America [13, 14], and it is associated with many normal activities, such as sports and physical training as well as certain occupations [15-17].

In the design of clinical trials to reverse chronic fatigue various LRT products have been utilized [10]. For example, one of these clinical trials used a formulation that included NT Factor® and a combination of vitamins and other ingredients [20]. After this trial was completed, participants continued receiving the test supplement for at least 6 months. This allowed us to continue following the patients during routine clinic visits where basic health assessments were undertaken as well as laboratory tests, such as routine blood chemistry. Surveying the results of several patients suggested that the levels of Hcys were declining with continued use of the test supplement. Thus we decided to retrospectively examine the blood levels of Hcys and other blood components in subjects that continued on LRT.

## **SUBJECTS AND METHODS:**

**Subjects.** Thirty-five patients (28 females, 7 males, Av. Age=60.7±9.6 years) from a previously approved clinical trial [18] that had used the functional food Advanced Physician's Formula™

(Table 1) in tablet form each day were enrolled in a follow-on retrospective study on blood chemistry. Participants were patients with chronic fatigue or other fatiguing illnesses [18]. Participants had blood drawn at various times over a 6-month period, and routine blood testing was performed. In this retrospective laboratory study the results were analyzed for differences, and statistical analyses were performed (see below). Subjects were asked if they used any prescription medications to see if this might exclude them from the study, as determined previously [19], and their general health was assessed by a physician [13, 14].

**Table 1.** Test Supplement (Advanced Physicians Formula™ with NT Factor® and vitamins)

Component	Amount Per Serving	% Daily Value*
NT Factor® <sup>#</sup> (phospholipids)	4,000 mg	**
Vitamin E (as d- $\alpha$ -tocopheryl succinate, mixed tocopherols)	50 IU	167
Thiamin (Vitamin B-1) (as thiamine HCl)	3.75 mg	250
Riboflavin (Vitamin B-2)	4.25 mg	250
Niacin (Vitamin B-3) (as niacinamide, niacin)	100 mg	500
Vitamin B-6 (as pyridoxine HCl)	10 mg	500
Folate (as folic acid)	800 mcg	200
Vitamin B-12 (as methylcobalamin, cyanocobalamin)	1,000 mcg	16,667
Biotin	750 mcg	250
Pantothenic acid (as d-calcium pantothenate)	25 mg	250
Calcium (as dicalcium phosphate, carbonate, pyruvate, Borogluconate, d-calcium pantothenate)	400 mg	40
Phosphorus (as calcium phosphate)	125 mg	13
Magnesium (as magnesium oxide)	125 mg	31
Methylsulfonylmethane (OptiMSM™)	364 mg	**
Alpha-keto-glutaric acid	300 mg	**
L-Carnitine L-tartrate (as L-carnipure®)	225 mg	**
L-Tyrosine	150 mg	**

\*Daily values are based on a 2,000 calories per day diet

\*\*Daily values not established

<sup>#</sup>NT Factor® is a patented (U.S. Patent 8,877,239 B2) proprietary blend of food and food components: phosphoglycolipids (polyunsaturated phosphatidylcholine, glycolipids and other polyunsaturated phosphatidyl nutrients), *Bifido* and *Lactobacillus* bacteria (freeze-dried and microencapsulated in viable form), growth media (bacterial growth factors and food, including rice bran extract, arginine, beet root fiber, black strap molasses, glycine, magnesium sulfate, para-amino benzoate, leek, pantethine, taurine, garlic, calcium borogluconate, potassium citrate, spirulina, bromelain, natural vitamin E, calcium ascorbate,  $\alpha$ -lipoic acid, oligosaccharides, vitamin B-6, niacinamide, riboflavin, vitamin B-12, folic acid, inositol, calcium pantothenate, chromium picolinate). NT Factor® is a registered trademark of Nutritional Therapeutics Inc., Hauppauge, NY.

**Methods.** Subjects that had previously signed an informed consent document and completed a clinical trial on the use of a LRT supplement to reduce fatigue [18] were subsequently enrolled in

a retrospective study on blood chemistry. Blood samples were taken at routine visits to the Tustin Longevity Center, Tustin, California under similar conditions of fasting and time of day and were subjected to analysis. The results were compared to visits during the previous clinical trial and subsequent visits after the trial was completed and just before starting the test supplement. Hcys was determined with an enzymatic cycling assay [21] using a kit from Diazyme Laboratories (San Diego, CA). Fasting insulin was determined by a solid-phase, two-site chemoluminescent method on an Immulite 1000 analyzer according to the manufacturer’s instructions (Siemens Medical, Diagnostics Products Corporation division, Los Angeles, CA) [22]. Erythrocyte ESR determinations were performed with a modification of the Westergren method [23] using the Sediplast ESR system (Polymedco, Cortlandt Manor, NY). Routine blood chemistry was performed as described previously [18].

**Statistics.** Data were analyzed by ANOVA, with significance defined as  $p < 0.05$ . Further data analysis was performed with Tukey test and Wilcoxon signed rank analysis, with significance defined as  $p < 0.05$ . The standardized alpha (Cronbach’s alpha) was used to confirm reliability and internal consistency of the data [18].

**RESULTS:**

When analyzed after 6 months of test supplement, all participants in the study responded and showed an average 31.8% in Hcys levels (from  $10.85 \pm 0.42$  to  $7.40 \pm 0.42$   $\mu\text{mol/L}$ ; t-test,  $p < 0.001$ ; Wilcoxon,  $p < 0.001$ ) (Table 2). Women responded better than men: women (from  $11.06 \pm 0.50$  to  $8.67 \pm 0.82$   $\mu\text{mol/L}$ , 34.4% reduction, t-test,  $p < 0.001$ ; Wilcoxon,  $p < 0.001$ ) versus men (from  $10.80 \pm 0.51$  to  $7.01 \pm 0.47$   $\mu\text{mol/L}$ , 21.6% reduction, t-test,  $p < 0.0862$ ). Differences were also found in fasting insulin levels (from  $12.80 \pm 3.11$  to  $5.30 \pm 1.77$   $\mu\text{IU/mL}$ , 58.6% reduction, t-test,  $p < 0.005$ ) and erythrocyte sedimentation rate (ESR). ESR increased from  $10.5 \pm 2.21$  to  $20.19 \pm 3.20$  mm/hr (92.2% increase, t-test,  $p < 0.0314$ ; Wilcoxon,  $p < 0.0154$ ). There were also non-significant increases in iron and ferritin found at the end of the study.

**Table 2.** Blood chemistry values of participants before and 6-months after taking the test supplement

Blood Meas.	Time=0 Av. $\pm$ SEM	Time=6 mo Av. $\pm$ SEM (% change)	Statist. Signif.
Hcys	$10.85 \pm 0.42$ $\mu\text{mol/L}$	$7.40 \pm 0.41$ (-34.4%)	$p < 0.001$
Fast. Insulin	$12.80 \pm 3.1$ $\mu\text{IU/mL}$	$5.30 \pm 1.77$ (-58.6%)	$p < 0.005$
ESR	$10.5 \pm 2.21$ mm/hr	$20.19 \pm 3.20$ (+92.2%)	$p < 0.003$
BUN	$17.63 \pm 4.4$	$17.26 \pm 4.6$	NS
GGT	$33.01 \pm 5.9$	$34.8 \pm 8.4$	NS
ALP	$61.2 \pm 3.5$	$65.06 \pm 5.1$	NS
AST/ALT	$22.81 \pm 1.3$	$24.03 \pm 2.8$	NS
CRP	$2.81 \pm 2.4$	$2.02 \pm 2.8$	NS
HDL	$64.2 \pm 3.8$	$62.3 \pm 2.8$	NS
LDL	$115.7 \pm 6.4$	$119.1 \pm 7.5$	NS
Cholesterol	$199.5 \pm 8.4$	$201.8 \pm 7.9$	NS

Blood Meas.	Time=0 Av.±SEM	Time=6 mo Av.±SEM (% change)	Statist. Signif.
Chol/HDL	3.18±0.3	3.14±0.5	NS
Triglyceride	98.1±9.1	100.6±9.8	NS
Sodium	138.9±0.4	138.6±0.3	NS
Potassium	4.14±0.05	5.5±1.2	NS
Iron	80.5±9.5	92.0±11.5	NS
Ferritin	39.2±14.9	45.0±12.3	NS
Creatine	0.78±0.04	0.83±0.03	NS

ALP, alkine phosphatase; BUN, blood urea nitrogen; CRP, c-reactive protein; ESR, erythrocyte sedimentation rate; GGT, gama-glutamyl transpeptidase; HDL, high-density lipoprotein; Hcys, homocysteine; LDL, low-density lipoprotein; AST/ALT, alanine aminotransferase

Other tests in the chemistry panel were not significantly different after 6 months of supplement (Table 2). In addition, there were no side effects from the test supplement, and none of the participants had any cardiovascular events during the study.

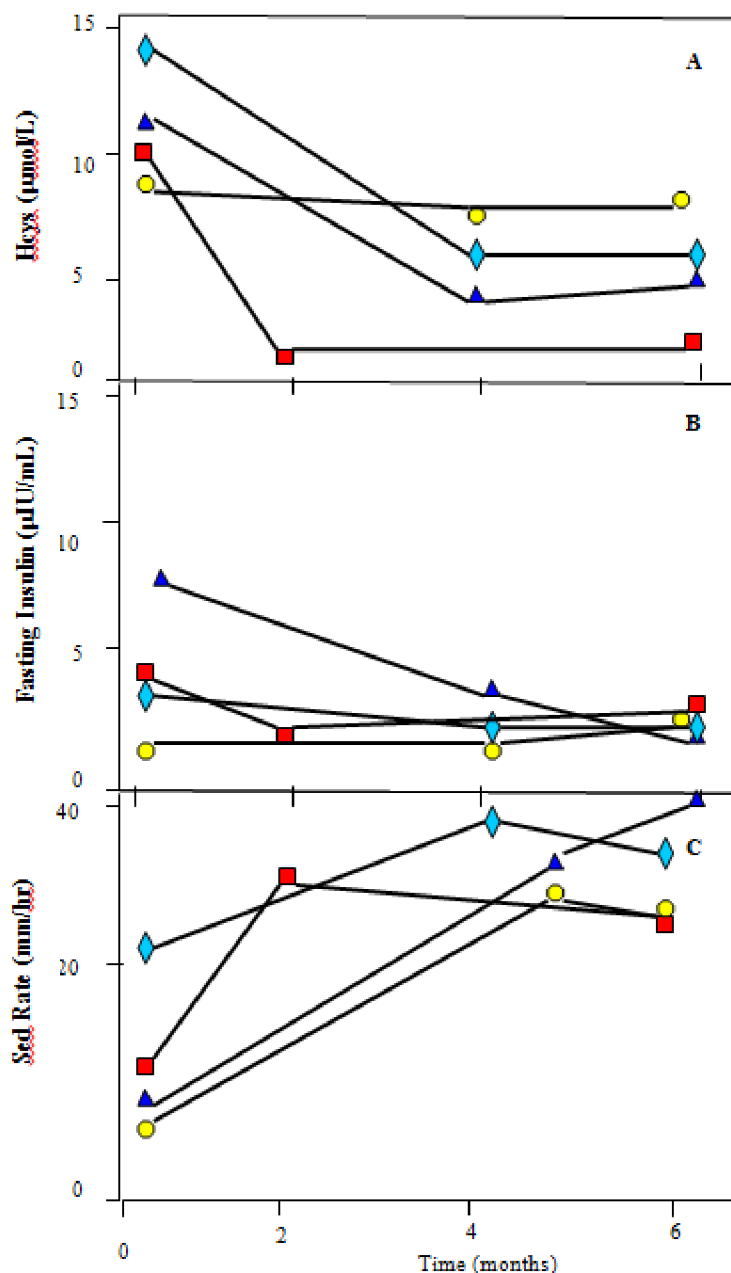
A few of the subjects in the study were analyzed at intermediate end-points (Figure 1). The changes in blood Hcys, fasting insulin and ESR were gradual during the course of the study, and not every participant showed significant changes in all three of these parameters.

## DISCUSSION:

Polyunsaturated glycopospholipids in the supplement in NT Factor® have been used successfully in animal and clinical lipid replacement studies [9-12, 18-20, 24]. In this formulation encapsulated lipids are protected from oxidation in the gut and can be absorbed and transported into tissues without oxidative damage [9, 10]. The NT Factor® lipids are primarily membrane phospholipids and phosphoglycolipids that are normally found in all vertebrate cellular membranes [12].

Mitochondrial dysfunction and the accumulation of damaged mitochondrial components have been linked to a wide variety of chronic, metabolic and degenerative diseases, cancer and aging [9, 11, 25, 26]. Lipid Replacement has been successfully used in clinical studies to reduce fatigue, increase mitochondrial function and protect cellular and mitochondrial membranes from oxidative damage [reviewed in 9-11]. In multiple clinical studies fatigue was reduced 35-43% by oral administration of NT Factor® [9-11, 18-20]. Even in severely fatigued patients with chronic fatigue syndrome or fibromyalgia, NT Factor® reduced fatigue by 43.1% [9]. In the study by Agadjadyan et al. [20] NT Factor® reduced fatigue 35.5% in aging adults and significantly improved mitochondrial function to a level that was similar to that found in young, healthy adults. Thus LRT has the potential to decrease the effects of aging on mitochondrial function and improve mitochondrial function in chronic diseases.

Here we examined the effects of LRT on blood chemistry in a retrospective study using a supplement that contained NT Factor®, vitamins and other ingredients [18]. We found that blood Hcys and fasting insulin levels were significantly reduced while ESR rates were significantly increased after 6 months on the test supplement.



**Figure 1.** Time course of (A) Hcys, (B) fasting insulin and (C) ESR in four patients. The determinations are given as single test results over time.

Before our study the participants had an average Hcys level of  $10.85 \pm 0.42 \mu\text{mole/L}$ , which is slightly above the level ( $10.8 \mu\text{mole/L}$ ) that predicts the development of heart failure [27]. After 6 months on the test supplement Hcys levels were significantly reduced to  $7.40 \pm 0.42 \mu\text{mol/L}$ , well below the level that can predict coronary problems in adults [27]. Although these subjects were only followed for 6 months, there were no cases of coronary heart problems, strokes or other issues that could be related to elevated blood Hcys, even though the average age of this group was above 60 years. Although women in the study responded slightly better to the test supplement than men in terms of reduced blood Hcys levels, there may not have been

enough men in the study to conclude, in general, that women respond to the test supplement better than men.

The test supplement used in the present study contained B-vitamins, and the long-term use of high doses of B-vitamins, above those used in the test supplement, have been shown to have a modest effect on CHD and stroke [28]. The independent effect of B-vitamins in the test supplement could not be assessed in the current study, but it is doubtful that the results found here were due simply to the presence of B-vitamins. In a high-dose vitamin B study that examined Hcys levels over several years of use the levels of Hcys were reduced an average of 2.2  $\mu\text{mol/L}$  [29], which is less than that achieved with the test supplement used in the current study (an average reduction of 3.45  $\mu\text{mol/L}$ ).

Changes were also found in fasting insulin levels. There was a reduction in average fasting insulin level from  $12.80 \pm 3.1$  to  $5.30 \pm 1.77$   $\mu\text{IU/mL}$  (58.6% reduction) after 6 months on the test formulation. Increased fasting insulin levels are positively associated with CHD and stroke, especially in women [30, 31]. In a Canadian study the average blood fasting insulin levels of 13  $\mu\text{IU/mL}$  or above correlated with an 8-fold higher heart attack risk than an average level of 9.3  $\mu\text{IU/mL}$  [32]. The average fasting insulin levels for U.S. women is 8.4  $\mu\text{IU/mL}$  and for men, 8.8  $\mu\text{IU/mL}$  [33]; therefore, the test supplement reduced the level of fasting insulin to below the average for women and men in the U.S. population.

In addition to reductions in Hcys and fasting insulin, subjects in the trial showed an average increase in erythrocyte ESR rates, an independent risk marker for CHD [34] and especially CHD mortality in men [35]. This test is less impressive in predicting cardiovascular disease in women [36]. ESR rates are also modified in chronic inflammation, tissue injury, collagen diseases and in many malignancies [37]. This test detects changes in the presence of acute phase reactants but it is not dependent on them to show changes [38]. Nonetheless, ESR rates were significantly increased during the trial from an average of  $10.5 \pm 2.21$  to  $20.19 \pm 3.20$  mm/hr (92.2% increase). Interestingly, we did not find significant changes in C-reactive protein, a measurement that generally, but not always [38], parallels changes in ESR. The reason for this may be related to changes in erythrocyte membrane lipid composition that allow erythrocytes to be more flexible—thus increasing ESR values without affecting erythrocyte-erythrocyte aggregation. Support for this hypothesis comes from studies where polyunsaturated n-3 fatty acids were supplemented for 3 months in patients with cystic fibrosis. In parallel with changes in erythrocyte lipid composition, ESR rates were found to be significantly increased [39]. Indeed, the rheological properties of human red blood cells can change, depending on the amounts of saturated fats and cholesterol in the diet [40].

Finally, there were no cardiovascular events during the 6-month retrospective trial, and the test supplement was well tolerated. In fact, there were no incidents of adverse responses to the test supplement during this study. This indicates that the test supplement was a safe and effective method to improve blood markers that predict future CHD. The test supplement also reduced fatigue, as measured during the first prospective part of the trial. Fatigue was reduced by a mean of 36.8% during the first week of the original trial [18], and although fatigue was not measured at 6 months in this retrospective study, participants indicated that their lower fatigue levels were maintained while on the test supplement.

**Abbreviations Used:** CHD, coronary heart disease, ESR, erythrocyte sedimentation rate; Hcys, homocysteine; LRT, Lipid Replacement Therapy; ROS, reactive oxygen species

**Competing Interests:** The authors have no financial interests or conflicts of interest.

**Authors' Contributions:** All authors contributed to this study.

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