JANA ARTICLE

Draft

Effect of Combination Pantethine, Plant Sterols, Green Tea Extract, Delta-tocotrienol and Phytolens on Lipid Profiles in Patients with Hyperlipidemia

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ABSTRACT

Background: Hyperlipidemia is a major risk factor for cardiovascular disease (CVD), which is the number one cause of mortality and morbidity in Western civilization.

Objective: We assessed the effects of a two-month program using a combination of nutraceutical agents known to improve lipid profiles in hyperlipidemic individuals.

Design: In an open-label thirty participants study, hyperlipidemic patients consumed a nutraceutical combination of a daily total of 900 mg pantethine, 800 mg plant sterols (from soybean), 600 mg green tea extract (containing 50% epigallocatechin gallate EGCG), 75 mg delta-tocotrienols (from annatto seed) and 5 mg Phytolens® (extract of lentil husks) (nutraceutical combination provided by Biotics Research Corporation). Participants were 18 - 80 years of age, not taking lipid lowering drugs or lipid lowering nutraceutical supplements. Patients were instructed not to change their diets, levels of exercise, smoking habits, caffeine intake and prescription medications during the study. All labs were drawn after a twelve-hour fast except for water, and included CBC, CHEM 12, LPP™ (Lipoprotein Particle Profile, SpectraCell Laboratories) and HS CRP.

Results: After two months of supplementation, laboratory tests showed statistically significant improvements in plasma total cholesterol (TC) ($p < 0.0001$), low density lipoprotein (LDL)($p < 0.003$), very low-density lipoprotein (VLDL)($p<0.05$), small dense LDL particles type III ($p < 0.01$) and type IV ($p < 0.02$), and diastolic blood pressure ($p < 0.05$).

Conclusions: The non-pharmacological treatment of hyperlipidemic individuals with a nutraceutical supplement containing pantethine, plant sterols, green tea extract, delta-tocotrienols and Phytolens® improves lipid profiles and diastolic blood pressure as measured at the end of a two-month trial.

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INTRODUCTION

Cardiovascular disease (CVD) remains the leading cause of death throughout the Western world and the second most common cause of death worldwide.[1] Hyperlipidemia has been identified as a major modifiable risk factor for CVD. Except when the concentration of triglycerides is elevated, most of the cholesterol in the plasma is carried by low density lipoproteins. The causal role of LDL particles in the pathogenesis of CVD is well established, as is the clinical benefit of lowering LDL in high risk patients.[2]

On the basis of size and density, LDL particles can be divided into large, buoyant particles and small dense particles.[3] Small dense particles of LDL have been demonstrated to have a greater atherosclerotic risk. Cholesterol testing has historically evaluated cardiovascular risk by measuring HDL (good) or LDL (bad). Small dense LDL particles are thought to easily penetrate the arterial endothelium and cause plaque formation. The National Institutes of Health in their publication, "*National Cholesterol Education Program*" states, "Atherogenic dyslipidemia is defined by elevation of serum triglycerides, presence of small LDL particles and low HDL-cholesterol. For clinical purposes, elevated triglycerides (≥ 150mg/dL) plus low HDL cholesterol (< 40 mg /dL) defined dyslidemia."[4] Advanced cholesterol testing technology can accurately measure both the density and number of lipoprotein particles. Individuals who may not appear to have a CVD risk using normal lipid panel testing methods may actually be at risk if they have high levels of small particle LDLs. Austin demonstrated that an LDL subclass distribution characterized by a preponderance of small, dense LDL particles was associated with a risk of myocardial infarction.[5] Up to fifty percent of persons who have heart attacks also have normal cholesterol.

A small dense LDL particle appears to be particularly atherogenic because it is more easily oxidized than the large buoyant LDL particles. Oxidized LDL particles bind more readily to the arterial wall. LDL accumulation in the artery wall and the subsequent formation of modified particles are taken up by macrophages in an unregulated manner, which contributes to lipid-laden cell formation. Oxidative modifications in the atherosclerotic lesions, along with other processes, contribute to the weakening of fibrous caps, subsequently resulting in plaque rupture.[6] Endothelial dysfunction has been shown to be independently related to the degree of dyslipidemia in type 2 diabetes.[7]

Endothelial dysfunction and vascular smooth muscle cell dysfunction are the initiating and perpetuating factors in essential hypertension. Endothelial dysfunction precedes intimal thickening and clinical atherosclerosis.[8] Management of the inflammation of the vasculature via a reduction in oxidative stress from low density LDL may improve dysregulation of blood pressure.

Subjects, materials and methods: A two-month open label trial was conducted supplementing thirty hyperlipidemic patients with a nutraceutical product. Patients selected for the study had cholesterol values ranging initially from 175 to 337 mg/dL. There were eleven (11) males and nineteen (19) females ranging from thirty to eighty-one years of age. To enroll in the study, participants could not be taking lipid lowering drugs or lipid lowering nutraceutical supplements. The participants were examined at baseline and at one and two months, and were requested to maintain their present weight, diets, exercise levels, alcohol intake, smoking habits, caffeine intake and prescription medications during the study. All labs were drawn after a twelve-hour fast (except for water), and included CBC, CHEM 12, LPP™ (SpectraCell Laboratories) and high sensitivity c-reactive protein (HS CRP). Lipid-Sirt™ (Biotics Research Corporation) was taken as two servings per day in capsule form. Compliance was measured by capsule counts at each visit.

Patients consumed two servings daily for two months. Two servings consisted of eight capsules of Lipid-Sirt™ per day and provided 900 mg of pantethine, 800 mg of plant sterols from soybean, 600 mg of green tea extract (containing 300 mg epigallocatechin gallate), 75 mg of delta-tocotrienol from annatto seed and 5 mg Phytolens® (Biotics Research Corporation).

Statistical analysis: A repeated measures t-test (one group pre-tested and post-tested) was employed. The t-test was compared to the means of the two groups post-test. (Table 1)

DISCUSSION

This study clinically demonstrated that a combination of nutraceutical agents could lower blood lipids associated with an increase risk for cardiovascular disease in patients with known lipid disorders. Total cholesterol, low-density lipoprotein cholesterol (LDL), dense LDL III, dense LDL IV and VLDL were all lowered in this two-month study. Diastolic blood pressure also improved [$p < 0.045$]. The only modification in the patient's lifestyle was the inclusion of a nutraceutical product.

The nutraceutical ingredients used in the study were a combination of plant sterols from soy, green tea extract, delta-tocotrienols and Phytolens.® All of these ingredients are natural products and have not been reported to have the side effects associated with conventional agents such as statins, which are routinely used to lower LDL cholesterol. Lowering LDL concentrations has been well documented in numerous epidemiological studies and clinical trials to reduce coronary mortality and morbidity.[9] The National Cholesterol Education Program (NCEP) report identifies LDL cholesterol as the primary target of cholesterol lowering therapy.[10] Every 1% decrease in LDL cholesterol results in a 2% decreased risk of CHD.[11] According to the guidelines, patients with established CHD and CHD risk equivalents should be considered high risk. The goal of LDL lowering therapy in high risk patients should be < 100 mg/dL.

The second group of risk patients, those with a multiple of (2+) risk factors, had previously had a guideline of reaching an LDL level of < 130 mg/dl. These guidelines have been changed based upon ten-year risk factors derived from the Framingham studies. Finally, persons with 0 to 1 risk factor are recommended to have LDL cholesterol to be < 160 mg/dL. The five major risk factors for CHD are high blood pressure, dyslipidemia, diabetes mellitus, obesity and smoking. According to the NCEP report, fifty percent of the people who have had a heart attack have normal choles-

Table 1. Results: Change from baseline to month two for collected variables. All subjects (N = 30) overall average results.

Lab Test	N	Mean baseline	One Month	Two Months	% Change	p-value
CRP	29	4.33	3.33	5.01	15.7	0.4674
Total cholesterol	28	237	222	204	-13.9	**0.0007**
TRIG	28	173	170	164	-5.3	0.7556
DENSE LDL III	28	232	199	174	-25.0	**0.0066**
DENSE LDL IV	28	88	84	71	-19.3	**0.0160**
GLUCOSE	29	101	100	98	-3.0	0.9653
HDL	28	52	50	46	-11.5	**0.0007**
HDL 2B	28	1789	1741	1601	-10.5	**0.0137**
LDL	28	156	146	134	-14.1	**0.0027**
LP (A)	21	35.43	40.98	45.49	12.4	0.0926
Lipoprotein						
VLDL	28	114	100	91	-20.2	0.0457
RLP	28	189	186	165	-12.7	0.1399
PROTEIN C-REACT	17	0.85	0.3	0.29	-34.0	0.3811
METABO. SYND	28	1.17	0.088	0.75	-36.0	0.2580
Vital Signs						
Weight	29	184	184	177	-3.8	0.9278
Waist	29	38	38	37	-2.7	0.9862
Systolic BP	29	131	130	128	-2.3	0.9200
Diastolic BP	**29**	**81**	**75**	**75**	**-7.4**	**0.0448**

Lipid and protein values are in mg/dL.

terol. Those individuals with apparently normal risk levels of LDL may actually still be at risk for CVD, which may be elucidated by advanced cholesterol testing.

Most of the cholesterol in the plasma is carried in LDL, except when the concentration of triglycerides is high. LDL particles can be classified based upon size and density. Large particles of LDL are more buoyant and have a size of 29.0 nm. Small dense particles of LDL can be divided into subclasses by analytical methods using ultracentrifugation or by gradient gel electrophoresis. Small dense LDL particles have a particle size as small as 22 nm. Small dense LDL have several characteristics that have been linked to atherosclerosis. Compared to buoyant LDL particles, small dense LDL particles have a longer residence time in the plasma, exhibit enhanced oxidizability and binding to arterial proteoglycans, and have been shown to be more permeable through the endothelial barrier.[12]

The longer residence time of small dense LDL in plasma is postulated to be due to a reduced exposure to LDL receptors. The smaller particle size of the small dense LDL is more similar to the pore size of that between the endothelial cells lining the vascular walls. Austin found in a case-control study that individuals characterized by a preponderance of small dense LDL particles had a threefold risk of myocardial infarction, independent of age, sex and relative weight.[13] Mohty examined 102 explanted aortic stenosic valves and found that an increased proportion of circulating small dense LDL particles was associated (1) with a faster progression of stenosis and (2) with an increased content of oxidized LDL and the degree of inflammation in the aortic valve.[14] This study concluded that small dense LDL was an independent risk factor of stenosis. Gardner investigated the association of small dense LDL and the incidence of fatal and nonfatal coronary artery disease (CAD), and in a prospective population-based study, reported that LDL size was smaller in CAD cases than in controls ($p < 0.01$)[15]

Our study is the first to document a reduction of small dense LDLs by a combination of nutraceutical ingredients. Several studies have shown that statins have minimal or no effect on the size of LDL particles.[16] Niacin has been shown to lower dense LDL concentration, shifting the distribution to larger particles.[17] Niacin is often used in patients who have failed to lower their LDL levels to desirable levels with conventional statin use. Guidelines by the National Cholesterol Education Program now place a greater emphasis on small dense lipoprotein cholesterol particles for the management of atherogenic dyslipidemia.

Pantethine is a natural disulfide form of pantethine, and is a precursor of the coenzymatic form of vitamin B5 (pantothenic acid). Pantethine has been used in Asia and

Europe as a lipid lowering agent for over thirty years. In a double blind, randomized, placebo controlled, cross over study, 900 mg of pantethine daily for six weeks has been reported to reduce LDL by 15%.[18] The mechanism by which pantethine lowers cholesterol is not understood. Pantethine's metabolite cysteamine may decrease the hepatic synthesis of cholesterol by inhibiting HMG-CoA reductase. Conversely, pantothenic acid exhibits no lipid altering properties.

Tocotrienols down regulate HMG-CoA reductase activity, which is the rate limiting activity in the mevalonate pathway that contributes to the synthesis of cholesterol. Annato seed extract contains only gamma (10%) and delta (90%) tocotrienol. Other plant oils such as palm oil, rice bran oil and barley contain tocotrienols but also contain tocopherols. Tocopherols, in particular alpha tocopherol, inhibit tocotrienol's down regulation of HMG-Co A reductase. Tan has reported that small daily doses (75 -100 mg) of delta/gamma tocotrienols isolated from Annato reduced cholesterol, LDL and triglycerides by 15 - 20%.[19] Unlike statins that can lower coenzyme Q10 levels, tocotrienols have been reported to increase coenzyme Q10 levels by up to 25%.[20] Tocotrienols are highly lipophilic compounds, which are absorbed via the lymph system, attaining peak plasma levels in three hours. There is a clear dose-dependent cholesterol reduction associated with tocotrienols intake.

Green tea consumption has been associated with a reduction in mortality due to all causes and to cardiovascular disease. Imai observed that increased consumption of green tea was associated with decreased serum concentrations of total cholesterol, (P for trend < 0.001), triglycerides, and very low lipoprotein cholesterol.[21] Green tea polyphenols, such as epigallocatechin gallate (EGCG), significantly increase plasma antioxidant activity in vivo.[22] EGCG is able to recycle vitamin E as an antioxidant. The recycling of antioxidants [oxidation /reduction] by the free radical electron transfer between antioxidants allows antioxidants to protect against lipid peroxidation in both membranes and LDL. Because LDL oxidation increases the risk of atherogenic process, antioxidant protection of LDL particles is fundamental for preventing CVD.

Green tea catechins have also been shown in animal models to decrease the solubility of cholesterol micelles.[23] Green tea catechins have been shown to increase the fecal excretion of total fatty acids as well as bile.[24] EGCG has been shown to inhibit pancrelipases in vitro. EGCG inhibits the activity of the enzyme xanthine oxidase.[25] Xanthine oxidase inhibition has been shown to improve endothelial vasodilation in hypercholesterolemic persons.[26]

The cholesterol lowering effect of plant sterols is well documented in a number of studies. Labeling law allows products to carry a health claim for plant sterol: "Foods containing at least 0.4 grams per serving of vegetable oil sterols, eaten twice a day with meals for a daily intake of at least 0.8 grams, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease."[27] In the gut, plant sterols prevent the absorption of cholesterol from the gut lumen by competing with cholesterol for incorporation into micelles formed by bile salts. Via the bile, the liver secretes an average of 1,000 mg of cholesterol per day, of which up to 60% is reabsorbed.[28] The structure of cholesterol and sterols is very similar. Few sterols are actually absorbed from the gut. Blood levels of plant sterols are typically a hundredfold less than cholesterol. In plants, phytosterols are essential components of plant membranes. Sterols are commonly found in foods such as fruits, legumes, nuts, grains and cooking oils.

This study demonstrated a statistically significant reduction in HDL. Of the twenty-eight patients measured over two months, two had no changes in HDL, three patients had increased HDL, and twenty-three had decreased HDL. The LDL/HDL ratio in the study remained relatively constant [2.884 initial finding vs. 2.913 at two months]. The nutraceuticals used in the study have clinically been shown to either raise HDL (pantethine and sterols) or have no reported effect on HDL (tocotrienols, phytolens and green tea extract). Some patients using statin drugs also exhibit a reduction in HDL values.[29] Powell reported a decrease in HDL cholesterol ($p < 0.0001$) after examining the effects of a twenty-one-day nutritional intervention program, which included fruit and vegetable consumption, energy restriction and the addition of dietary supplements to twenty-eight patients.[30] In our study, three patients did not take any prescription drugs, five patients took one prescription per day, four patients took two prescriptions per day, and eighteen took three or more prescriptions per day.

This study reflected the need to monitor HDL in all hyperlipidemic patients taking lipid lowering drugs as well as nutraceutical products. Raising HDL with weight loss, exercise, diet and smoking cessation (lifestyle modifications) should be considered in any wellness program designed to improve lipid profiles. Some patients may also require the use of niacin to raise HDL values.[31]

Endothelial dysfunction and vascular smooth muscle dysfunction are initiating and perpetuating factors in essential hypertension.[32] Hypertension may be defined as a disease of the blood vessel in which vascular biology is dysfunctional. Over a two-month period of time, diastolic blood pressure improved by an average of 6 mm Hg ($p < 0.045$). Seventeen of twenty-eight patients improved diastolic pressure. None of the nutraceutical ingredients used in this study have been reported to reduce blood pressure in humans. Antonello demonstrated in an animal model that green tea extracts could prevent hypertension induced by angiotension, likely by scavenging superoxide anions.[33] Inhibition of xanthine oxidase could decrease superoxide anion production by green tea extracts.

Newaz has provided evidence that γ-tocotrienol reduces blood pressure in the spontaneously hypertensive rat model.[34] Nitric oxide synthase (NOS) was increased in all animals by the addition of γ-tocotrienol. Endothelial NOS produces nitric oxide (NO). NO is responsible for the acetylcholine mediated vascular relaxation. Since superoxide is known to react with NO and form peroxynitrite (ONOO-), the addition of both green tea extract and γ-tocotrienol could improve the production of NO by preventing its free radical degradation, which would lead to an improvement of vasodilation, hence improving diastolic blood pressure. More studies need to be done in this field to examine this possible effect, however, we demonstrate here that supplementation with a non-pharmacological agent improves both the lipid profiles and the diastolic blood pressure.

This is a short tem pilot study demonstrating the efficacy of this nutraceutical combination in patients with dyslipidema during the time period studied. Additional long term studies should be done to evaluate the long term efficacy of these natural compounds as well as potential adverse effects and reductions in cardiovascular outcomes.

FINANCIAL DISCLOSURE

Dr. Houston has received a research grant from Biotics Research Corporation, has served as a consultant to Biotics Research Corporation, and has received travel expenses and speaking fees from Biotics Research Corporation. William Sparks is employed by Biotics Research Corportion.

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