A PLACEBO-CONTROLLED TRIAL OF A PROPRIETARY LIPID-LOWERING NUTRACEUTICAL SUPPLEMENT IN THE MANAGEMENT OF DYSLIPIDEMIA

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Received September 9, 2016 – Accepted November 22, 2016

There is an ever growing emergence in the popularity of patient-driven care. As this health and wellness model grows, inquiries into diet, lifestyle, and supplemental approaches will continue to become a focal point for the healthcare consumer. Because of this, the aim of this study is to determine the tolerability, and overall effectiveness of a proprietary multi-ingredient lipid-lowering supplement in subjects with dyslipidemia. Forty participants were recruited for a single-center, double-blind randomized, placebo-controlled trial. Study participants were recruited between December 2014 and March 2015. Initial screening included a physical examination, renal and hepatic function, serum lipid, serum electrolytes, complete blood counts, and urine analysis. The 40 participants were randomly assigned to receive either the proprietary multi-ingredient lipid-lowering supplement (PMILLS) n= 20 or placebo n= 20. The trial consisted of a screening visit, a two-week run-in, and a four-month treatment period. Samples were taken at baseline, one month and four months of treatment. Results from the trial showed that the PMILLS significantly reduced total cholesterol (TC), low density lipoprotein (LDL-C), very low density lipoprotein (VLDL-C), oxidized LDL (oxLDL), Apo-lipoprotein B, triglycerides (TG), LDL particle number (LDL-P), heart rate, and diastolic blood pressure compared to placebo at one month and four months. The PMILLS significantly increased high density lipoprotein (HDL) particle number (HDL-P), and low density lipoprotein (LDL) particle size from dense type III and IV to larger type I and II LDL particle, compared to placebo at one month and four months. In addition, the PMILLS significantly reduced high sensitivity C-reactive protein (hs-CRP), tumor necrosis alpha (TNF-α), and interleukin 6 (IL-6) within the treatment group from baseline. There were no adverse effects noted in the treatment group after four months of supplementation. The present study demonstrates this PMILLS improves all relevant lipid parameters, such as particle numbers and particles sizes, as well as showing a significant reduction in inflammatory markers linked to cardiovascular health. With such combined changes in lipids, lipid sub-fractions, and inflammation, which are considered among the most effective means of reducing coronary heart disease (CHD), this PMILLS represents a new addition to safe and effective lipid-modifying strategies.

Key words: dyslipidemia, nutraceutical supplements, coronary heart disease, cardiovascular disease, vascular inflammation, LDL cholesterol, HDL cholesterol, triglycerides

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0393-974X (2016)

To the Editor,

Dyslipidemia is considered to be one of the top five risk factors for coronary heart disease (CHD) and cardiovascular disease (CVD) along with hypertension, diabetes mellitus (DM), smoking, and obesity (1). Although there are an infinite number of vascular insults, there are only three finite responses of the cardiovascular tissue to these insults. The mechanisms by which certain lipids induce vascular damage are complex, from a pathophysiologic viewpoint; but still can be easily categorized into responses of inflammation, oxidative stress, and vascular immune dysfunction (2-4). These endpoints lead to endothelial dysfunction (EnD), vascular smooth muscle dysfunction (VSMD), cardiac myocyte dysfunction (CMD), and lastly CHD. Management of dyslipidemia with supplemental nutrients has been infrequently reviewed (5-7). Further preclinical and clinical studies are required to understand the present role of these natural agents in the management of dyslipidemia and subsequent sequelae (5-7). To date, there have been clinical trials that have shown significant improvement in both CHD and serum lipids with supplementation. Smaller studies involving nutrient therapies have also demonstrated reductions in surrogate vascular markers with decreases in carotid intimal medial thickness, carotid artery obstruction, plaque progression, and coronary artery calcium score as well as improvement in generalized atherosclerosis and EnD (5-9). It is important that the treatments for dyslipidemia, and subsequent vascular disease, address not only clinical lipid parameters, but also the various mechanisms by which lipids induce CVD. The combination of diet and mechanistically targeted nutritional supplementation have the potential to significantly modulate lipoprotein particle characteristics, improve HDL functionality, reverse cholesterol transport (RCT), and cholesterol efflux capacity. In addition, inflammation, oxidative stress, and vascular immune responses can be favorably influenced. In several clinical trials, surrogate cardiovascular markers, CHD and CVD have been reduced by optimal nutrition and/or administration of several nutritional supplements, including red yeast rice (RYR), curcumin, berberine, and phytosterols. Many of the ingredients in the PMILLS formula have been shown to reduce inflammation, and ingredients like RYR have been shown to reduce CHD and myocardial infarction (6). With that in mind, a combined regimen of dietary modification and nutritional supplements represents a viable alternative for patients who are statin-intolerant, cannot take other drugs for the treatment of dyslipidemia, or prefer non-drug therapies. Moreover, this combined approach targets numerous molecular pathways and networks known to be involved in the development of atherosclerosis and CHD.

MATERIALS AND METHODS

Study design

This clinical trial was designed to examine the overall effects of the proprietary lipid-lowering nutraceutical on various lipids and inflammatory markers (Fig. 1). The study was a randomized, double-blind, randomized, placebo-controlled, single-center trial consisting of a screening visit, a two-week run-in, and a four-month treatment period. Written informed consent was obtained from each patient. The study was conducted in accordance with the principles of the Declaration of Helsinki. Study participants were recruited between December 2014 and March 2015 from the Hypertension Institute in Nashville, Tennessee, USA. Initial screening included taking a medical history, physical examination, renal and hepatic function, serum lipid concentrations, serum electrolytes, complete blood counts, and urine analysis. The 40 patients were randomly assigned to receive (double-blind) either proprietary multi-ingredient lipid-lowering supplement (PMILLS) or placebo. Randomization was performed centrally and was concealed. All 40 participants completed the study and none were lost after randomization.

Study goal

Primary aim: Reduction of LDL-P, LDL-C, apolipoprotein B (ApoB), TG, and total cholesterol level.

Secondary aim: Reduction of VLDL particle size and number, increases in HDL, HDL size, and HDL-P, changes in lipoprotein(a) (Lp(a)), oxLDL, changes in hs-CRP, myeloperoxidase (MPO), other inflammatory markers, cardiac and vascular function, evaluation of endothelial

function, heart rate variability (HRV), augmentation index (AI), small and large arterial compliance, and central arterial pressure. All vascular testing was non-invasive.

Participants

Subjects eligible for enrollment were either male or female, ages 18 to 80 years, with fasting dyslipidemia (LDL 130-250 mg/dL) based on initial screening with direct LDL measurements. Participants who were statin-intolerant or who refused to take statins or any other lipid-lowering drug for any reason were primarily recruited.

The exclusion criteria were: myocardial infarction, coronary artery bypass grafting, percutaneous transluminal coronary angioplasty, or stent within five years, known clinical CHD symptoms, or clinical angina; history of cerebrovascular accident; creatinine over 2.5 mg/dL; known allergy or sensitivity to any components of the nutritional supplement used; (v) chronic liver disease with AST, ALT, alkaline phosphatase over 1.5 x normal; known cancer within two years; clinical congestive heart failure (systolic or diastolic CHF); type I or type 2 DM; pregnancy or nursing; and women of child-bearing age not on an approved and accepted contraceptive.

Participants were randomized to receive PMILLS (one double packet, twice daily; for detailed ingredients see Table I), or a placebo prepared in indistinguishable capsules. A dose reduction to one double packet daily was suggested whenever constipation lasting more than two weeks occurred. The treatment capsules and placebo capsules were provided by Thorne Research, Inc. (Sandpoint, Idaho, USA).

Clinical and biochemical measurements

Blood samples were taken from the study participants after a 12-hour fast, except for the consumption of water and regular medications, which were allowed to be taken prior to each visit. Laboratory tests were performed at baseline and variable times depending on the lab specified. Date of birth, smoking, alcohol consumption, and past medical history were assessed. Height and weight (light clothes and without shoes), and seated blood pressure (measured on the patient's non-dominant arm supported at heart level) were determined by a senior physician or a qualified clinical nurse research specialist, using the American Heart Association (AHA) criteria for accurate blood pressure measurement. All patients were required to maintain current weight, nutrition program, diet, exercise regimen, alcohol intake, smoking, caffeine intake, and prescription medications (excluding lipid-lowering agents) during the study. This was to help reduce variability of effect in the placebo arm, given that alterations in lifestyle during the trial could potentially have positive effects on

Table I. *Ingredient list of the proprietary lipid supplement.*

Each double packet contains:	
Ingredients	Dosage
Phytosterol esters	800 mg
Aged Garlic Extract (bulb) (*Allium sativum*) (Kyolic®)	600 mg
Red Yeast Rice (*Monascus purpureus*)	500 mg
Curcumin Phytosome (*Curcuma longa* extract (root)/ Phosphatidylcholine complex)	250 mg
Green Tea Phytosome (*Camellia sinensis* extract (leaf) / Phosphatidylcholine complex)	250 mg
N-Acetyl-L-Cysteine	250 mg
Berberine HCL (from Indian Barberry extract (root) (*Berberis aristata*)	200 mg
Deglycyrrhizinated Licorice (DGL) extract (root)	100 mg
Trans-Reseveratrol	40 mg
Quercetin Phytosome (*Sophora japonica* concentrate (leaf) / Phosphatidylcholine complex)	50 mg
Lycopene (from tomato, *Lycopersicon esculentum*)	4 mg

lipid and inflammatory parameters. Potential medication interactions with the treatment arm were monitored and, if present, were documented in the adverse event case report. Medications known to effect serum lipids were prohibited. Nutritional supplements known to effect lipids were not permitted. All such medications or supplements were required to be discontinued at least one month prior to study initiation and a steady state lipid profile was obtained and repeated prior to study entry.

Statistical analysis

This study was designed in accordance with a predetermined statistical analysis plan. The statistical analysis was performed in a blinded fashion by an independent third party that was not involved in the design, implementation or data collection.

A sample size of 20 patients in each of the two study groups, with a dropout rate up to 10%, was planned to provide a 90% power to detect a 15% or greater (with 95% confidence intervals, $p<0.05$ for significance) reduction in the primary end point variables in the PMILLS compared with the placebo group after one and four months at the end of the study. Statistical analysis was performed using the SAS 4.0 system (SPSS Inc., Chicago, IL), and data are presented as mean standard error (SE). Logarithmic transformation was used for interleukin-6 (IL-6) and hs-CRP because of the high degree of skewing.

Within-group comparisons were performed with paired sample *t*-tests to evaluate differences from baseline in each group. Independent-sample *t*-test and the analysis of covariance (ANCOVA) analysis with a model that included the baseline value of the dependent variable as a covariate were also used for comparison between groups.

RESULTS

Baseline characteristics

The data of 40 participants were analyzed, as shown in Table II. The baseline variables are not significantly different between the PMILLS and placebo groups.

Primary outcomes

Compared to the participants who received placebo (n=20, female=9, male=11), those receiving the PMILLS (n=20, female =9, male=11), had a significant reduction in total cholesterol, LDL-C, and VLDL-C at both one and four months. Total cholesterol decreased from 242.55 mg/dL to 214.16 mg/dL at four months with the PMILLS but increased from 246.65 mg/dL to 258.58 mg/dL with placebo (Fig. 2A). LDL-C fell from 166.2 mg/dL to 133.73 mg/dL with the PMILLS group, versus a slight increase from 164.1 mg/dL to 165.9 mg/dL with placebo (Fig. 2B). VLDL-C decreased from 21.62 mg/dL to 15.84 mg/dL with the PMILLS group, and increased from 26.58 mg/dL to 27.94 mg/dL with placebo (Fig. 2C). The ANCOVA analysis showed that serum total cholesterol, LDL-C, and VLDL-C concentrations in the proprietary PMILLS were significantly reduced compared to the changes in the placebo group ($p<0.0001$, $p<0.001$, $p<0.0001$, respectively). Serum total cholesterol, triglycerides, and LDL-C concentrations were reduced from one month ($p<0.0001$) compared with the baseline and maintained significance at four months, indicating that the PMILLS becomes effective as early as one

Table II. *Baseline and 4-month clinical characteristics of the proprietary lipid supplement and placebo groups. a= between group comparisons b- within group comparisons. *p <0.05*

	Proprietary Lipid Supplement			Placebo			
	Before	After	P Value[b]	Before	After	P Value[b]	P Value[a]
No. (Male/Female)		20 (11/9)			20 (11/9)		
Age (Yr)		62 ± 6			58 ± 7		
Body Weight (lb)	168.4 ±26.5	165.3 ±24.7	0.87	170.2 ±31.5	171.4 ±29.7	0.79	0.84
BMI	26.76 ±2.41	26.49 ±2.53	0.91	26.98 ±3.05	27.01 ±2.99	0.81	0.75
Systolic blood pressure (mm Hg)	135.7 ± 4.92	130.9 ±3.62	**0.098***	136.6 ± 4.57	135.1 ±5.72	0.82	0.41
Diastolic blood pressure (mm Hg)	72.3 ±2.09	69.4 ±1.79	**0.001***	75.05 ±2.01	74.78± 2.14	0.79	**0.04***
Heart beat	66.5±2.24	63±1.99	**0.001***	67.5 ±3.1	70.8±2.4	0.38	**0.009***
HbA1C (%)	5.59 ±0.41	5.63 ±0.62	0.92	5.81 ±0.57	5.77±0.48	0.65	0.69

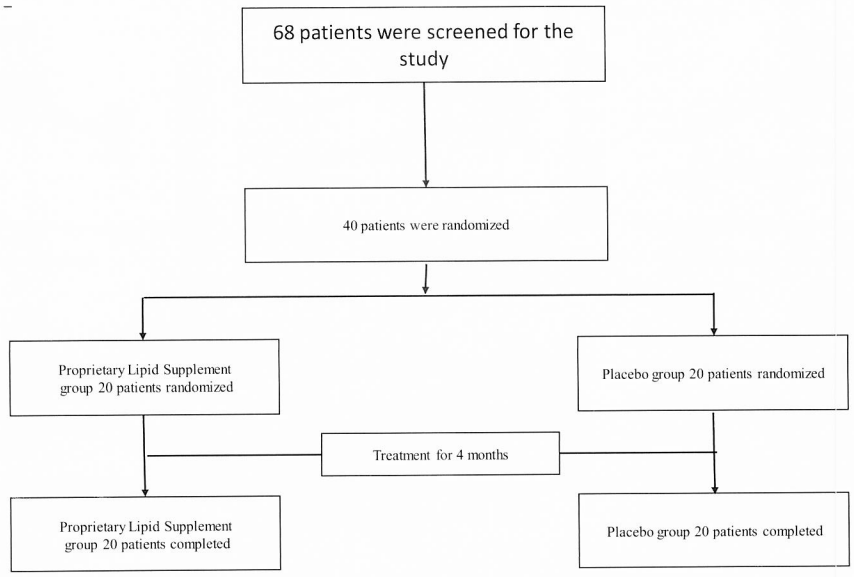

Fig. 1. *Clinical study design.*

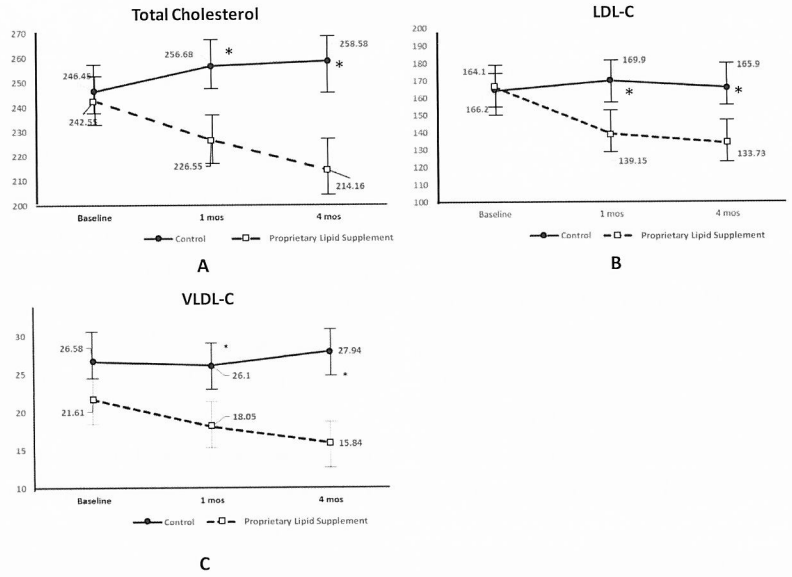

Fig. 2. *Comparison of cholesterol parameters in the proprietary lipid supplement and placebo group.* ***A)*** *Total cholesterol (1-month, *: p=0.039; 4-month, *: p= 0.0041);* ***B)*** *LDL-C (1-month, *:p=0.029; 4-month, * p=0.0016);* ***C)*** *VLDL-C (1-month, *:p=0.016; 4-month, *p=0.0057).*

month and has continuing effects.

LDL-P decreased from 1,114/dL to 1,058.1/dL (p=0.0492; Fig. 3A) at four months in the PMILLS group, while no change was observed in the placebo group (1,149.1/dL *vs* 1,147.2/dL).

Total LDL-III and IV particle number (LDL-P), an important indicator of LDL particle size, decreased to 392±44 nmol/L in the PMILLS group, significantly lower than the 576±59 nmol/L in the placebo group (p=0.0027).

After treatment, oxLDL was significantly decreased in the PMILLS group, (51.65 mg/dL versus 42.6 mg/dL, p= 0.021); whereas, no change was observed in the placebo group (55.95 mg/dL *vs* 53 mg/dL, p=0.89) (Fig. 3D). Similar between-group changes were also observed for Apo-lipoprotein

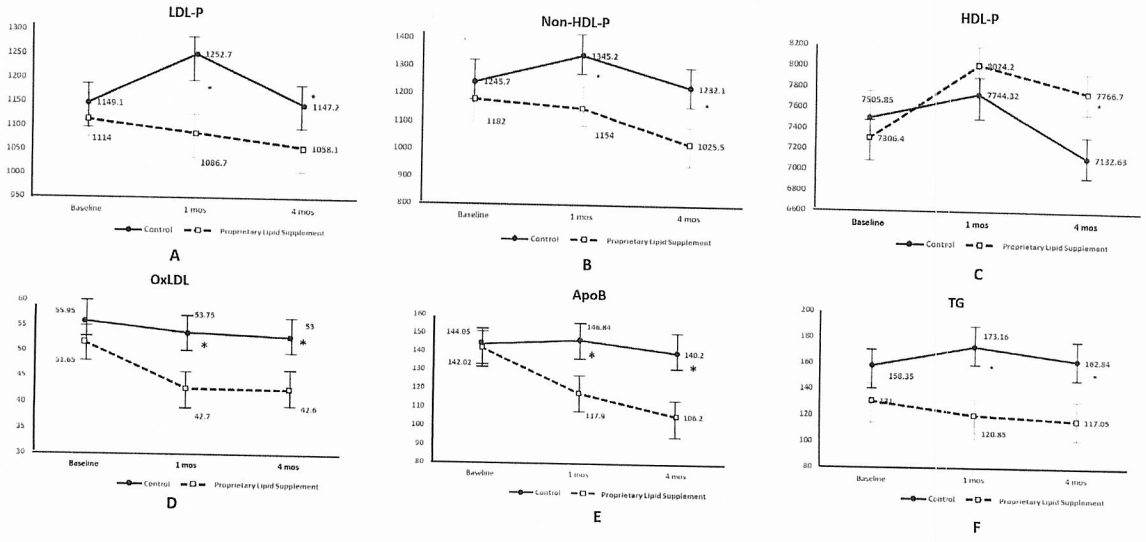

Fig. 3. *Comparison of lipid particle parameters in the proprietary lipid supplement and placebo group.* ***A)*** *LDL-P (1-month, *: p=0.0059; 4-month, *: p= 0.0372);* ***B)*** *non-HDL-P (1-month, *:p=0.0031; 4-month, *p=0.0421);* ***C)*** *HDL-P (4-month, *p=0.0018).* ***D)*** *oxLDL (1-month, *: p=0.0019; 4-month, *: p= 0.0323);* ***E)*** *ApoB (1-month, *:p=0.0055; 4-month, * p=0.021);* ***F)*** *TG (1-month, *p=0.014; 4-month, *p=0.0019).*

B and TG (Fig. 3 E and F), (p=0.0029 and 0.014, respectively).

Secondary outcomes

Diastolic blood pressure decreased from 72.4±9 mmHg to 68.1±11 mmHg in the PMILLS group, while increasing from 72.3±8 mmHg to 73.9±9 mmHg with placebo (see Table II) (p=0.047). No changes in systolic blood pressure were observed. Supplementation with the PMILLS group, was also associated with decreased heart rate, from 67.5±6 to 62.4±7 (p=0.0093), which was significantly lower compared to the placebo group (Table II).

Tumor necrosis factor-alpha (TNF-α), hs-CRP, and IL-6 were measured in serum. Between-group analysis showed no significant difference between the PMILLS group, and the placebo group, which may be due to the high standard deviations. Pearson correlation was conducted, comparing baseline values of inflammatory markers with the change in inflammatory markers over time. In the PMILLS group, participants with the highest inflammatory markers at baseline showed the greatest response to intervention, which was highly significant (hs-CRP; p<0.001: TNF-α; p<0.001: IL-6; p<0.001) (Fig. 4). These effects were not observed in the placebo group. Myeloperoxidase (MPO) is another critical marker for oxidative stress and less so with inflammation. However, the MPO change was not significant between PMILLS and placebo groups or within the PMILLS group. This may be in part due to the relatively normal oxidative stress in the study patients. In addition, the duration of the study may have been too short to affect MPO. Using the EndoPAT medical device, endothelial cell function was measured in both the PMILLS group, and the placebo group.

Heart rate variability (HRV), augmentation index (AI) and small and large arterial compliance with Computerized Arterial Pulse Wave Analysis (CAPWA) were also measured, since they are major indicators of cardiovascular events. No significant changes were observed in any of the indicators, which may also be due to the short duration of the study, as changes in arterial compliance of both large and small arteries, as well as pulse wave velocity may take 6 to 12 months to improve. No significant changes in the EndoPAT score, a measure of endothelial dysfunction, occurred due to the relatively brief trial time.

Safety

During the study period, safety parameters including renal and hepatic function, serum electrolytes, blood counts, and urinary analysEs

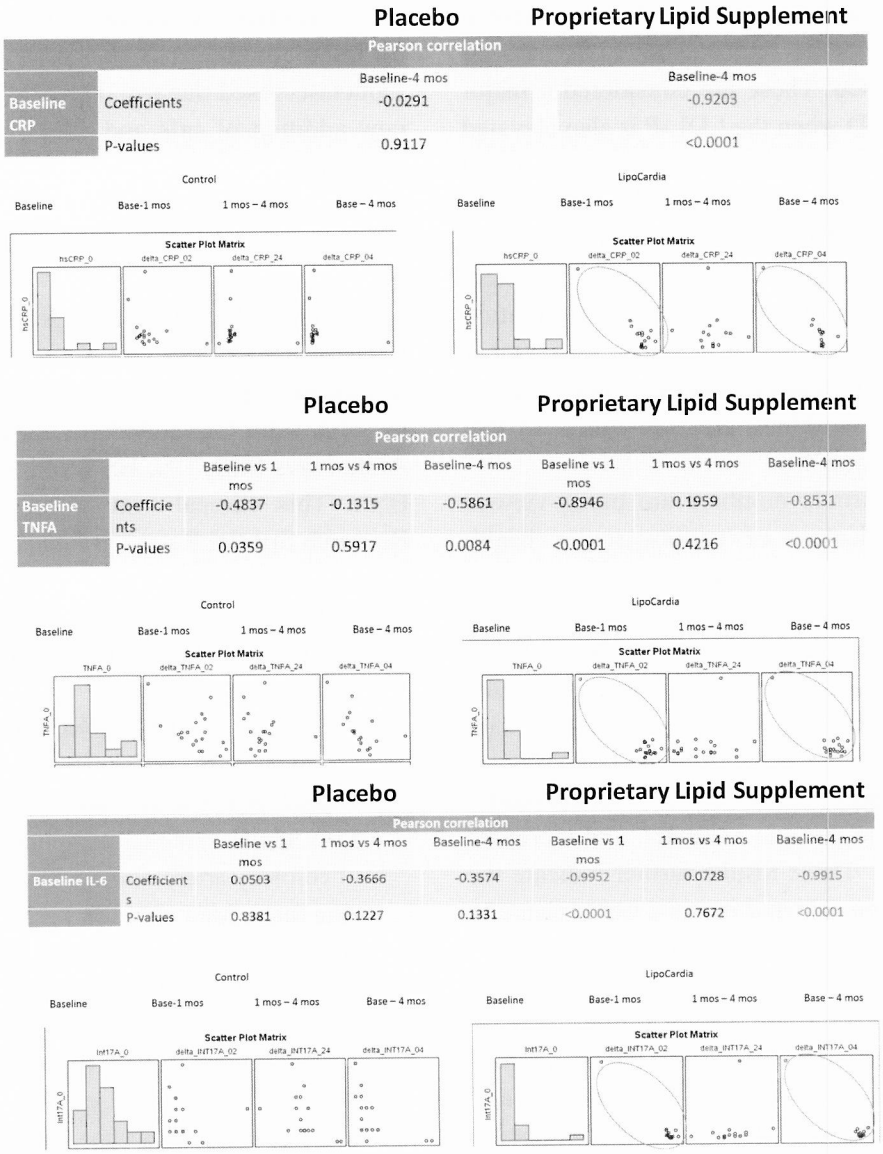

Fig. 4. *Comparison of inflammatory markers in the proprietary lipid supplement and placebo group. A) CRP; B) TNF-α; C) IL-6.*

were assessed. Adverse events were reported and recorded in case report form for each patient. No adverse events occurred in the treatment group or in the placebo group. No episodes of hypoglycemia were reported. Two of the recognized sides effects related to HMG-CoA inhibitor-based therapy are myopathy and serum coenzyme Q10 (CoQ10) reduction. None of the study participants reported any symptoms of myalgias or myopathy. Nor was there any significant reduction in serum CoQ10 levels at any time in the PMILLS group compared to the control group.

DISCUSSION

This PMILLS appears to be a safe, effective nutritional supplement for the management of dyslipidemia and its attendant inflammation. It offers an alternative to individuals who are statin intolerant or who refuse to take statins/other lipid-lowering drugs. This PMILLS significantly reduced all of the atherogenic particles implicated in CHD, such as LDL, LDL-P, Apo-lipoprotein B, VLDL, TG and oxLDL. LDL and LDL-P, the driving risks for CHD (6, 10), and achieved significant reductions of

19.5% ($p<0.001$) and 5% ($p=0.0492$), respectively, at one and four months. Small dense LDL particles were also decreased. These are considered a major risk factor for CHD when the LDL-P is also elevated (6, 10). Further contributing to a reduced CHD risk, HDL-P significantly increased by 6.3 percent. In addition, the PMILLS group had a significant reduction in primary inflammatory markers associated with vascular disease. This observation was more pronounced in participants with higher baseline levels ($p<0.001$). Finally, there was a reduction in diastolic blood pressure and heart rate in the PMILLS group.

Lipoprotein particle numbers are being viewed as emerging indicators of cardiovascular health (10), however, LDL-P has been largely ignored in previous studies. LDL particle size is also a critical indicator of cardiovascular risk. It has been shown that LDL particles of smaller size, especially LDL-III and LDL-IV, have a stronger association with stroke, CHD, and myocardial infarction The present study demonstrates that PMILLS successfully reduces lipoprotein particle numbers, and increases LDL particle size. These results further indicate the PMILLS protective role and opens a new avenue to explore the underlying molecular mechanism.

The limitations of this research can be resolved in future studies. Future study design should increase the number of participants alongside the duration of the trial, given the chronicity of dyslipidemia, and to ensure that the safety profile of the formulation continues to produce minimal adverse events. Future studies should also explore participants with a more complicated CVD picture, and if there are other effects to the standard of care (SoC) that could potentially lower therapeutic dosage, further optimizing the risk benefit profile for the SoC. This PMILLS was developed to target a number of molecular pathways and networks important in lipid-induced vascular disease, with ingredients considered to be safe, especially in the doses utilized (11-13). In this study, this PMILLS was found to improve all relevant lipid particle numbers and particles sizes, as well as improving diastolic blood pressure and inflammatory markers. Such combined changes in lipids, lipid sub-fractions, and inflammation are considered among the most effective means of reducing CHD, based on large clinical trials that have used HMG Co-A reductase-based therapy. This PMILLS represents a new addition of safe and effective lipid-modifying strategies.

ACKNOWLEDGEMENTS

We thank Dr. Michael Schmidt (Colorado State University, CO, USA) for his critical suggestions on this manuscript. Also thanks to the excellent clinical research team that included Laurie Hays RN, BSN, Anita Hays PhD, RN, BSN and Sissy Denton, RN, BSN. This clinical trial is supported by Thorne Research Special Research Fund.

DISCLOSURE: Drs Zhang, Rountree and Phipps are employed by Thorne Research Inc.

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