Original Research

Complex Multivitamin Supplementation Improves Homocysteine and Resistance to LDL-C Oxidation

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Objective: We previously reported in an open-label pilot trial that a 24-ingredient multivitamin formula favorably influenced homocysteine concentration and LDL-C oxidation indices following 24 weeks of supplementation. Our current aim was to more thoroughly examine this same formula in a randomized, placebo-controlled, clinical study.

Methods: We examined 182 participants for selected plasma vitamin concentrations and clinically relevant variables including homocysteine, lipids and LDL-C oxidation indices at baseline and six months.

Results: We found no significant differences between groups for any parameter at baseline. Following six months of vitamin supplementation, we observed elevations in plasma concentrations of vitamin B6 (as pyridoxal 5′-phosphate; PLP), vitamin B12, folate, vitamin C, vitamin E and β-carotene ($p < 0.0001$), all of which were significantly greater than respective placebo group changes ($p < 0.0001$). Homocysteine decreased in the treatment (8.38 ± 2.9 *vs.* 6.93 ± 2.5 μmol/L; $p < 0.0001$) and placebo group (8.17 ± 3.0 *vs.* 7.42 ± 2.2 μmol/L; $p < 0.0001$) from baseline to six months, respectively, with reductions in the treatment group being greater than placebo ($p < 0.008$). LDL-C oxidation indices were also improved as LDL-C oxidation rate was decreased (-0.39 μmol/min/g protein; $p < 0.0003$) and LDL-C lag time increased (11.3 min; $p < 0.003$) in supplemented participants. Further analysis also showed that LDL-C oxidation rate was lower ($p < 0.0007$) and LDL-C lag time longer ($p < 0.0001$) for the vitamin group than placebo treatment after six months.

Conclusion: We conclude that a multi-ingredient vitamin formula with antioxidant properties has measurable effects on homocysteine and LDL-C oxidation indices.

INTRODUCTION

Dietary guidelines encourage individuals to ingest at least five servings of fruits and vegetables per day to meet recommended daily intakes [1]. However, recent survey evidence suggests that only 20% to 30% of the US population meets this goal and that food preparation, storage and reheating may decrease vitamin activity from 10% to 30% [2,3]. All of these factors, either alone or in combination, may cause suboptimal vitamin deficiency states where individuals are placed at risk for chronic diseases such as cardiovascular disease and cancer [4]. This is possibly why approximately 50% of the US populace ingests vitamins and minerals to supplement poor eating habits [5]. A classic example of this phenomenon is exhibited in portions of the population whose homocysteine concentrations are elevated, yet fall to more acceptable levels after a few weeks of supplementation with folate and vitamins B12 and B6 [6–10]. Recent observations from a cohort study examining folate status and total homocysteine concentrations showed that homocysteine concentrations were 10% lower among those in the study group who used additional supplements that increased folate and B-vitamin concentrations beyond food fortification alone [11]. This type of observation is important, as high concentrations of plasma homocysteine are a risk factor for vascular disease and arteriosclerosis [12–14].

In a recent review, recommendations were made that the problem of suboptimal vitamin ingestion could be easily overcome through the purchase of "brand-name" multivitamins for an annual investment of about $10 to $30 [4]. Others also hold this opinion [15]. The authors further state that they are "aware of no evidence that multivitamins differ in bioavailability." While we are not in disagreement with this premise, the Dietary

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Supplement and Health Education Act does not require supplement companies to demonstrate absorption characteristics/bioavailability or product efficacy. Therefore, consumers must take purported health benefits somewhat on faith, rather than clinical evidence. Independent consumer advocacy laboratories also show that the reported vitamin concentration in some products varies widely and is often less than advertised label claims [16]. Thus, even if absorption is not the issue *per se*, nutrient bioavailability will certainly be affected by availability of ingredients claimed to be in the product. We propose that a better solution is to validate the efficacy of individual products directly.

We recently reported in an open-label, proof-of-concept pilot trial that participants supplementing with a complex multivitamin formula (e.g., 24-ingredient, Table 1) were capable of significantly elevating plasma concentrations of vitamins B6, B12, folic acid, vitamin C, vitamin E and beta-carotene. Clinical markers associated with cardiovascular disease (CVD) examined during this trial also showed significant reductions in homocysteine concentration, increases in LDL-C oxidation lag time and decreases in LDL-C oxidation rate [17]. The primary ingredients of this formula include vitamins A, D, C, E and K, as well as folic acid and vitamins B6 and B12. An elegant review of many of these ingredients has been detailed elsewhere [4]. Though the pitfalls of such a study design are obvious, the aim of our current investigation reported here is to examine this same formula in a more vigorous clinical follow-up trial. As the benefits of these vitamins are broad, we have chosen to focus this report on those indices specifically related to coronary heart disease. To accomplish this, we have examined the plasma concentrations of vitamins B6, B12, C, folic acid and E, as well as several clinical markers associated with CVD risk including homocysteine, LDL-C oxidation rate and LDL-C oxidation lag time.

Table 1. Percent Daily Value Quantities of each Ingredient for the Total Number of Servings (6 capsules) Ingested Daily during the Study

Ingredient	Amount	Percent Daily Value
[Addition 5] Mixed retinols and pro-vitamin A carotenoids obtained from Dunaliella salina algae (d. salina) and containing natural mixed beta carotene (3 mg)	5,000 IU	100
Vitamin C (as ascorbic acid)	1,000 mg	1,111
Vitamin D (as cholecalciferol)	400 IU	200
Vitamin E (as d-α-tocopherol succinate)	800 IU	1,818
Vitamin K	25 mcg	30
Thiamine (as thiamine hydrochloride)	3 mg	250
Riboflavin (B2)	10 mg	769
Niacinamide	20 mg	125
Vitamin B6 (as pyridoxine hydrochloride)	25 mg	2941
Folic acid	800 mcg	200
Vitamin B12 (as cyanocobalamin)	400 mg	6,666
Biotin (as d-biotin)	300 mcg	1000
Pantothenic Acid (as d-calcium pantothenate)	10 mg	200
Iodine (potassium iodide)	150 mcg	100
Magnesium (mg oxide)	400 mg	100
Zinc (zinc oxide)	15 mg	136
Selenium (rice bran chelate)	100 mcg	182
Copper (copper gluconate)	2 mg	200
Chromium (chromium amino acid chelate)	100 mcg	287
Potassium (potassium phosphate)	400 mg	10
Choline (choline bitartrate)	500 mg	100
Lycopene (tomato fruit)	10 mg	*
Lutein	6 mg	*
Co-Enzyme Q-10 (ubiquinone)	50 mg	*

* Daily value not established.

METHODS

Participants

Participants were 182 men and women 24 to 79 years of age who volunteered for this investigation conducted at The Cooper Institute (Dallas, TX USA). The trial group comprised individuals from the surrounding Dallas community who were recruited by radio, television and newspaper advertisements. Before study initiation, all participants signed an informed written consent approved by The Cooper Institute Review Board for possible risks of the investigation.

Inclusion Criteria and Pretrial Screening

Inclusion criteria for this study necessitated that male and female participants have a homocysteine concentration not less than 8.0 and 7.0 μmol/L, respectively. All participants agreed to maintain their current diet and exercise habits during the trial and to have abstained from taking vitamin or mineral supplements for six weeks prior to screening. We excluded pregnant or lactating women from participation. Postmenopausal women both on and off hormone replacement therapy (HRT) were accepted into the trial; however, we asked those on HRT to remain on their current medication and dosage schedule and notify us if the regimen was changed. Participants currently on standard medical therapy (for conditions such as hypertension, hypercholesterolemia, diabetes, arthritis or other chronic diseases) were allowed to enter the study if they agreed to remain on their current therapy during the trial.

Participants were excluded from the trial if their BMI was <18.5 or >34.9, they had recently donated blood (<3 months) or failed to agree not to donate blood during the trial. Also excluded were participants with elevated blood pressure, total cholesterol, LDL-cholesterol or fasting plasma glucose requiring immediate drug therapy according to national guidelines (JNC VI, Adult Treatment Panel II, and American Diabetes Association). Participants were also excluded if they planned to move from the area during the trial, smoked more than two packs of cigarettes, consumed alcoholic beverages exceeding

an average of three drinks per day or consumed coffee in excess of three cups per day.

We initially screened 1,275 potential participants by phone, with 447 qualifying to initiate the study. After completing an initial blood assessment, 259 participants remained eligible to participate, but 20 of these participants withdrew prior to randomization. Of the remaining 239 participants who formally entered the trial, 57 withdrew from the trial after initiating treatment. The time that elapsed between the screening and the first blood sample was less than two weeks. The initial screening for homocysteine was performed at the Cooper Clinic Clinical Laboratory because of its proximity. However, once successful screening had taken place, all blood assessments used as dependent variables were examined at an independent and blinded laboratory. Following a successful screening procedure, participants donated fasting blood samples to assess various indices (see below) before we gave them a 24-ingredient multivitamin/mineral formula (Cooper Complete, Dallas, TX USA) for 24 weeks (Table 1). A scientific advisory board assisted in the development of this formula based on an evidence-based, systematic review of the literature and their personal research performed in three nutrition research centers in the USA.

Treatment and Assessment Indices

Each participant consumed three tablets twice a day for six months and was verbally instructed to take them with a meal. During each visit, all participants returned their pill bottles, and a pill count was undertaken to ensure compliance. Blood assessments were obtained in a fasting condition (>12 hours) for a variety of parameters inclusive of a blood lipid profile, blood glucose concentration, muscle, kidney and hepatorenal indices (Chem-16), homocysteine, LDL-C oxidation rates (i.e., lag time and oxidation rate) and blood vitamin concentrations. Each blood collection consisted of obtaining approximately 50 mL of blood that was divided into one serum separator vacutainer (10 mL) and four K3 EDTA tubes (≈40 mL). All samples were subsequently spun within three minutes of venous collection in a cold centrifuge at 1200 rpm for 12 minutes. Separated plasma and red blood cells were divided into four cryovials and placed in a −80°C freezer. Blood lipid profiles, fasting blood glucose concentration and muscle and hepatorenal indices were analyzed immediately at the Cooper Clinic (Dallas, TX USA) via a Dimension RXL analyzer (Oxford, Connecticut USA). The Cooper Clinic Lab is a College of American Pathologists certified lab. All frozen samples were shipped frozen on dry ice via overnight express to various labs for subsequent analyses (see below).

Homocysteine, Folate and Vitamins B12 and B6. Blood samples were obtained to determine concentrations of homocysteine, folate, vitamin B12 and pyridoxal 5′-phosphate (the active circulating form of vitamin B6). Total homocysteine concentration in plasma was determined by high-performance liquid chromatography with fluorometric detection [18]. Plasma folate was measured by a microbial (Lactobacillus casei) assay in a 96-well plate [19,20]. Plasma pyridoxal 5′-phosphate was measured by the tyrosine decarboxylase apoenzyme method and plasma vitamin B12 was measured by radioassay (Quantaphase II, Bio-Rad, Hercules, CA USA) [21]. Coefficients of variation for these assays were 8% for homocysteine, 13% for folate, 16% for pyridoxal 5′-phosphate and 7% for vitamin B12.

Vitamin E (α-tocopherol), Vitamin C (ascorbate), β-Carotene and LDL-C Oxidation Indices. Additional blood was also obtained for plasma α-tocopherol, ascorbate and β-carotene concentrations. Samples for plasma ascorbate were deproteinized with ice-cold 10% metaphosphoric acid and centrifuged. The supernatant was purged with nitrogen and stored below −20°C in foil-covered tubes. Plasma ascorbate concentrations were determined spectrophotometrically after derivatization with 2,4-dintrophenylhydrazine. The concentrations of α-tocopherol and β-carotene were measured in plasma and LDL-C following extraction by reverse-phase high-performance liquid chromatography [22]. The plasma concentrations of both α-tocopherol and β-carotene were standardized to total plasma lipids as described [23].

Two indices of oxidation were used in this study. First, the lipid peroxide content of oxidized LDL-C was measured by a modification of the thiobarbituric acid-reactive substances (TBARS) [24]. TBARS activity was expressed as malondialdehyde equivalents using freshly diluted 1,1,3,3-tetramethoxypropane as the standard. Second, the amount of conjugated dienes formed during LDL-C oxidation was determined by measuring the absorbance of LDL-C against a PBS blank at 234 nm following a 1:4 dilution of the samples in PBS [25]. Research has shown that dilution of an oxidized LDL-C sample to 1:2, 1:4 and 1:8 displays linearity and excellent recovery; the data are expressed as the increase in conjugated dienes over baseline (A234) [26]. The rate of LDL-C oxidation was determined from the propagation phase of the time-course curve using a spline function. We determined lag phase by drawing a tangent to the slope of the propagation phase and extrapolating it to the horizontal axis [26]. The lag time constitutes the interval from zero time to the intersection point.

Statistical Analysis

We calculated median and interquartile ranges for skewed variables and means and standard deviations for normally distributed variables by treatment group. Spearman correlations were computed to assess association between variables. The mean change in each variable was compared between treatment groups using ANCOVA with adjustment for age, body mass index, gender, HRT, statin use and baseline value. Results are reported as least squares adjusted means. Correlations were also performed to examine the relationship between changes in plasma vitamin levels and homocysteine, LDL-C lag time and

lag rate. All statistical analyses were performed using SAS Version 8.1. Statistical significance refers to a value of two-tailed $p < 0.05$.

RESULTS

Demographics

The characteristics for the treatment group (M ± SD) are age 50.4 ± 10.3 years, weight 76.7 ± 15.4 (kg), height 170.7 ± 9.9 cm and BMI of 26.2 ± 3.9 (kg/m^2). Forty-four percent of the treatment group were men, while 7% of the women in this group were taking hormone replacement therapy. Tobacco use was reported as 3% for this group. The characteristics for the placebo group are age 50.5 ± 9.5 years, weight 79.3 ± 15.7 (kg), height 174.4 ± 9.8 cm and BMI of 26.45 ± 4.1 (kg/m^2). Males composed 41% of the placebo group. Eleven percent of the women in this group were taking hormone replacement therapy, and 1% reported to be tobacco users. None of the participants successfully completing the trial stopped vitamin supplementation prior to trial entry. The ethnic partitioning for each group (treatment vs. placebo) was predominantly white (92% *vs.* 86%). Blacks (7% *vs.* 7%) and others (1% *vs.* 7%) composed the balance of each treatment group, respectively. Within the treatment group, 3% of the population had a history of diabetes, 0% had a history of heart attacks and 1% had a history of stroke. For the placebo group, the prevalence was 2%, 0% and 1%, respectively.

Blood Chemistry, Plasma Vitamin and Homocysteine Concentration

We observed no significant changes for total cholesterol, LDL-C, triacylglycerols, kidney function, hepatorenal function or hemoglobin A1C following treatment (Table 2). However, a significant decrease in HDL-C was noted for the placebo group (−0.06 mmol/L; [95% CI; −0.11, −0.01]). Our analysis did show significant elevations in the plasma concentrations of vitamin B6 (as pyridoxal 5′-phosphate), vitamin B12, folate, vitamin C, vitamin E and beta-carotene ($p < 0.0001$; Table 3). *Post-hoc* analysis demonstrated that all measured vitamin concentrations were significantly elevated in the treatment group at six months ($p < 0.0001$). No significant changes in these values were observed for the placebo condition. Between group analyses also showed that the corresponding increase in plasma vitamin concentrations were statistically greater than those in the placebo treatment. Significant changes in homocysteine were observed as both groups demonstrated a reduction in homocysteine concentration ($p < 0.0001$). Further analysis revealed that the reduction observed in the treatment group was greater than that observed in the placebo group ($p < 0.008$).

Only changes in plasma vitamin B12 concentration were significantly related to the changes in homocysteine (partial r, 0.062; $p < 0.004$). No significant relationships were found for vitamin B6 (partial r, 0.0002; $p < 0.60$), folate (partial r, 0.006; $p < 0.37$) or β-carotene (partial r, 0.023; $p < 0.08$).

LDL-C Oxidation Characteristics

Our analysis showed a significant treatment effect for each LDL-C oxidation index (Table 3; $p < 0.0001$). Specifically, LDL-C oxidation rate was decreased 0.39 μmol/min/g protein ($p < 0.0003$) and LDL-C lag time increased 11.3-minutes ($p < 0.003$) in the treatment group following six months of vitamin supplementation. These changes in LDL-C oxidation rate ($p < 0.0007$) and LDL-C lag time ($p < 0.0001$) were significantly

Table 2. Baseline Blood Chemistry (Mean; SD) and Subsequent Change in Standard Blood Chemistry Following Six Months of Vitamin Treatment

		n	Baseline		Change	[95% CI]	Vitamin *vs.* Placebo
			Mean	SD			
Glucose (mmol/L)	Treatment	74	5.09	1.08	0.25	[0.05, 0.45]	ns
	Placebo	78	5.12	1.21	0.25	[0.06, 0.44]	
Cholesterol (mmol/L)	Treatment	74	5.17	0.94	0.05	[−0.10, 0.20]	ns
	Placebo	78	5.38	1.06	−0.06	[−0.21, 0.08]	
HDL-C (mmol/L)	Treatment	74	1.48	0.37	0.01	[−0.05, 0.06]	ns
	Placebo	78	1.51	0.42	−0.06	[−0.11, −0.01]	
LDL-C (mmol/L)	Treatment	74	3.04	0.79	0.02	[−0.11, 0.14]	ns
	Placebo	74	3.16	0.90	−0.03	[−0.15, 0.10]	
Triacylglycerol (mmol/L)	Treatment	74	1.43	0.79	0.03	[−0.18, 0.24]	ns
	Placebo	78	1.64	1.45	0.00	[−0.21, 0.20]	
AST (SGOT; UL)	Treatment	74	23.45	8.73	0.58	[−3.70, 4.87]	ns
	Placebo	78	23.69	12.34	1.24	[−2.90, 5.38]	
ALT (SGPT; UL)	Treatment	74	21.97	14.89	4.37	[0.74, 8.00]	ns
	Placebo	78	20.54	10.79	0.88	[−2.63, 4.39]	
Hemoglobin A1C (Proportion of total hemoglobin)	Treatment	74	0.05	0.02	0.002	[−0.002, 0.005]	ns
	Placebo	78	0.05	0.02	0.002	[−0.001, 0.005]	

The mean change in each variable was compared between treatment groups using ANCOVA with adjustment for age, body mass index, gender, HRT, statin use, and baseline value. Results are reported as least squares adjusted means.

Table 3. Baseline Plasma Vitamin Characteristics, LDL Oxidation Indices (Mean; SD) and Subsequent Change in Homocysteine, Plasma Vitamin Concentration and Oxidation Indices Following Six Months of Vitamin Treatment

		Baseline			Change	95% CI	Vitamin *vs.* Placebo
		n	Mean	SD			
Homocysteine	Treatment	90	8.38	2.90	−1.45	[−1.82, −1.09]	< 0.008
(μmol/L)	Placebo	92	8.17	3.01	−0.75	[−1.11, −0.38]	
Vitamin B6	Treatment	90	80.54	73.60	219.75	[189.52, 249.97]	< 0.0001
(nmol/L)	Placebo	92	62.96	61.44	21.85	[−8.72, 52.42]	
Folate	Treatment	89	14.22	6.83	6.72	[5.28, 8.16]	< 0.0001
(nmol/L)	Placebo	90	13.85	7.55	1.15	[−0.3, 2.6]	
Vitamin B12	Treatment	90	411.42	141.79	225.56	[187.91, 263.21]	< 0.0001
(pmol/L)	Placebo	92	381.75	118.30	26.17	[−11.91, 64.25]	
Vitamin C	Treatment	86	0.61	0.32	0.37	[0.28, 0.45]	< 0.0001
(μmol/L)	Placebo	72	0.60	0.33	0.01	[−0.07, 0.09]	
Vitamin E	Treatment	87	23.35	8.01	23.37	[21.20, 25.53]	< 0.0001
(nmol/mmol/lipid)	Placebo	71	23.62	8.04	−0.83	[−3.03, 1.37]	
B-Carotene	Treatment	87	0.31	0.27	0.19	[0.15, 0.22]	< 0.0001

different from the placebo treatment. Plasma changes in vitamin E were significantly correlated with changes in LDL-C lag rate (partial r, 0.037; $p < 0.03$), but not LDL-C lag time (partial r, 0.025; $p < 0.07$).

DISCUSSION

We demonstrated two primary findings in this placebo-controlled investigation with respect to six months of multivitamin supplementation. First, we showed that six months of supplementation was capable of significantly elevating plasma concentrations of vitamin B6 (as pyridoxal 5′-phosphate), vitamin B12, folic acid, vitamin E, vitamin C and β-carotene. This observation supports the premise that a multi-ingredient formula is capable of adequate absorption and maintenance of plasma vitamin concentrations. Complementing these elevations was a significant change in three clinical markers associated with CVD risk including a reduction in homocysteine and LDL-C oxidation rate, the latter being mirrored by an increase in LDL-C lag time. These results confirm our previous pilot trial and are particularly salient to a discussion regarding the efficacy of vitamin supplementation [17].

Following six months of supplementation, we observed significant increases in plasma concentrations of vitamin B6, plasma folate and vitamin B12 in multivitamin treated participants, all of which are associated in some degree to circulating homocysteine concentrations [8–10,27]. The increase in plasma vitamin concentrations observed during our trial is matched by a 1.45 μmol/L decrease in homocysteine concentration for treated participants. Though the placebo group also showed a significant reduction in homocysteine, it was not of the same magnitude and was significantly less than the treatment group. In this regard, we cannot discount regression to the mean. However, we did attempt to control for this phenomenon by employing a screening of subjects prior to trial entry. In addition, the nature of the subject pool is such that each individual was already "skewed" compared to a broader population by having elevated homocysteine. It is interesting to note that no other parameter changed in similar fashion (i.e., LDL-oxidation indices). These data confirm our previous trial showing a 1.20-μmol/L reduction in homocysteine after three months of supplementation and are consistent with previous research showing an inverse relationship between folic acid, vitamins B6 and B12 and homocysteine [8–10,27]. This is clinically important, as several cohort studies have shown retrospectively and prospectively that low plasma folate and B vitamin concentrations are associated with CHD [28,29].

One of the most compelling reports examining folate and vitamin B status showed that grain products fortified with folic acid were associated with a substantial improvement in folate status and a decreased homocysteine concentration [11]. Perhaps the most intriguing feature of this report is the observation that homocysteine concentrations were an additional 10% lower among those who used vitamin supplements *versus* those who did not. The authors attributed the difference in homocysteine to higher vitamin B12 and B6 concentrations in participants using B vitamin supplements [11]. Furthermore, previous research in men and women receiving supplementation with increasing doses of folic acid has shown that the usual dependency of homocysteine on folate ingestion diminishes with supplementation and that vitamin B12 becomes the main determinant of plasma homocysteine concentration [30]. This finding suggests that fortification with folic acid *and* vitamin B12, rather than folic acid alone, may be more effective at lowering homocysteine concentrations. We believe that this observation is clinically relevant because it shows that homocysteine can be further improved via supplementation in a population already consuming food products fortified with folic acid [11]. For example, treated participants in our trial increased baseline plasma folate concentrations from 14.22 ± 6.83 nmol/L to 20.75 ± 8.26 nmol/L after six months of

supplementation. Despite this increase in folate concentration, the strongest association between changes in homocysteine and the changes in individual plasma vitamin concentrations observed in our study was vitamin B12. It is conceivable that our population had sufficient dietary folate, yet was marginal in vitamin B12 status. Therefore, it seems prudent to recommend that adequate folic acid and vitamin B12 are included in a supplement routine if the goal is to reduce homocysteine concentration. Though we cannot conclude that our supplement will decrease the incidence of CHD, similar findings to ours support the idea that folic acid and vitamin B supplementation reduces risk factors associated with several chronic diseases including CHD [27], colon cancer in men and women [31,32] and breast cancer in women regularly consuming alcohol [33].

Our second clinical finding is the observation that our formulation improved two indices of LDL-C oxidation. Specifically, treated participants in our trial showed a 12% increase in LDL-C oxidation lag time and an 18% decrease in LDL-C oxidation lag rate. The results for LDL-C lag time in this trial are similar to those in our first uncontrolled trial. However, our overall data from our first trial for LDL-C lag rate was longer at three months and six months of supplementation [17]. Nonetheless, positive alterations in LDL-C oxidation have been associated with vitamin E, vitamin C and lycopene [34,35]. In our present study, we measured plasma concentrations of vitamins E and C, but not lycopene. However, lycopene has been shown to be easily absorbed and work synergistically with vitamin E relative to LDL-C oxidation [36]. Evidence has also shown that vitamin C attenuates LDL-C oxidation [37] and may complement the effects of vitamin E [38]. This latter point is still a matter for debate as Huang *et al.* recently demonstrated that supplementation with vitamin C and vitamin E alone reduced lipid peroxidation to a similar extent. However, supplementation with a combination of vitamins C and E conferred no benefit beyond that of either vitamin alone [39], and the weight of current evidence suggests that vitamin E is the more potent progenitor of LDL-C oxidation—an effect that may be dose dependent [40].

The effect of vitamin E (α-tocopherol) on LDL-C oxidation kinetics has been thoroughly reviewed elsewhere [40]. In brief, vitamin E is postulated to prevent atherosclerotic disease via its antioxidant effects, attenuation of smooth muscle proliferation and/or a reduction in platelet adhesion, and of arterial plaque accumulation [41–43]. Despite these physiologic benefits, the clinical benefit of secondary prevention has not been shown, even when vitamin E is taken in high doses [44,45]. However, all of these studies have been relatively short in length and deal with individuals with known metabolic disease (i.e., high risk). There is also an issue for a potential dose dependency relationship, where one shorter trial has demonstrated a reduction in deaths from coronary artery disease and nonfatal myocardial infarctions [46].

An equally important issue is that mortality stemming from atherosclerotic disease often takes place over several decades and is affected by oxidative stress. Further, the occurrence of atherosclerotic plaque at the carotid bifurcation has been found to be inversely associated with vitamin E intake [42]. Thus, vitamin E may have more influence on the disease process and be appropriate for primary prevention with longer investigations and use, as high vitamin E intake has been shown to decrease LDL-C oxidation and inhibit lesion progression [40,43]. In keeping with this finding, we observed an increase in LDL-C oxidation lag time and a decrease in LDL-C oxidation rate following six months of supplementation using a formula containing 800 IU of vitamin E and 1,000 mg of vitamin C per day. These effects are similar to those noted by Jialal and Grundy, who showed that the combined supplementation of α-tocopherol (400 IU/day) plus ascorbate (1,000 mg/day) and beta carotene (30 mg/day) resulted in the two-fold prolongation of the oxidative lag phase and a 40% decrease in the oxidation rate [25]. Interestingly, several studies have shown attenuation in atherosclerotic progression when vitamin E and C are taken together [47–49].

A final point to consider in our study is that some trials using β-carotene show an increased risk of lung cancer among smokers [50]. However, this effect has not been observed in all trials. The obvious implication is that β-carotene supplementation is contraindicated among smokers. It is also important to observe that the dose in our formula (3-mg) is much smaller than those observed in other trials (20–25 mg) noting this adverse effect [50]. Our vitamin A was composed of mixed β-carotene and other carotenoids, primarily lutein and zeazanthin, ingredients which have shown in serum studies to have an inverse relationship to breast cancer [51,52].

CONCLUSION

In accordance with our previous findings, our current trial has shown that a 24-ingredient multivitamin formula is capable of elevating and maintaining plasma concentrations of several vitamins, as well as favorably altering several risk factors associated with the incidence of CHD. Whether this type of formulation will reduce associated morbidities and mortalities is currently a matter for continued investigation. Our study has several strengths such as its randomized, placebo-controlled, double-blinded design. We had excellent data on monthly health status including changes in medications or smoking habits. We kept a monthly pill count and had baseline and follow-up measures of plasma vitamin concentrations to assure compliance with study protocol in both the treatment and placebo groups.

We also believe that it is important for the reader to distinguish between the potential for long-term prophylaxis via a nutrient support intervention designed to overcome suboptimal vitamin intakes and inadvertently assigning vitamin supplements the role of a pharmacological treatment. Our evidence suggests that it is biologically plausible to alter processes

associated with disease progression and cause. Therefore, the context of each investigation should be kept in mind as "low risk" groups are seldom examined clinically (i.e., placebo controlled studies), but rather rely on epidemiological evidence. In this regard, only long-term follow up studies will be capable of addressing the issue of whether additional multivitamin supplementation will be beneficial in decreasing the incidence and mortality associated with certain chronic diseases.

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