Impact of simvastatin, niacin, and/or antioxidants on cholesterol metabolism in CAD patients with low HDL

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Abstract The HDL Atherosclerosis Treatment Study (HATS) demonstrated a clinical benefit in coronary artery disease patients with low HDL cholesterol (HDL-C) levels treated with simvastatin and niacin (S-N) or S-N plus antioxidants (S-N+A) compared with antioxidants alone or placebo. Angiographically documented stenosis regressed in the S-N group but progressed in all other groups. To assess the mechanism(s) responsible for these observations, surrogate markers of cholesterol absorption and synthesis were measured in a subset of 123 HATS participants at 24 months (on treatment) and at 38 months (off treatment). Treatment with S-N reduced desmosterol and lathosterol levels (cholesterol synthesis indicators) 46% and 36% ($P < 0.05$), respectively, and elevated campesterol and β-sitosterol levels (cholesterol absorption indicators) 70% and 59% ($P < 0.05$), respectively, relative to placebo and antioxidant but not S-N+A. Treatment with antioxidants alone had no significant effect. Combining S-N with antioxidants reduced desmosterol and lathosterol by 37% and 31%, and elevated campesterol and β-sitosterol levels by 54% and 46%, but differences did not attain significance. Mean change in percent stenosis was positively associated with a percent change in lathosterol ($r = 0.26$, $P < 0.005$) and negatively associated with a percent change in β-sitosterol ($r = -0.21$, $P < 0.01$). These data suggest that changes in stenosis were attributable, in part, to changes in cholesterol metabolism.—Matthan, N. R., A. Giovanni, E. J. Schaefer, B. G. Brown, and A. H. Lichtenstein. **Impact of simvastatin, niacin, and/or antioxidants on cholesterol metabolism in CAD patients with low HDL. *J. Lipid Res.* 2003.** 44: **800–806.**

Supplementary key words desmosterol • lathosterol • campesterol • β-sitosterol • cholesterol synthesis • cholesterol absorption • statins • niacin • coronary artery disease

Epidemiological studies have reported a positive relationship between total cholesterol and LDL cholesterol (LDL-C) levels, and a negative relationship between HDL-C levels and risk of developing coronary artery disease (CAD) (1–6). This has resulted in the initiation of several large-scale primary (7, 8) and secondary (9–13) prevention statin trials aimed at lowering serum total and LDL, and raising HDL-C levels. The results have demonstrated a strong beneficial effect on coronary morbidity and mortality (2, 14). Additionally, it has been suggested that the use of antioxidants alone or in combination with lipid-lowering therapy would confer further benefit via improvements in LDL oxidation status (15, 16).

To test these hypotheses, several large-scale randomized trials have been conducted comparing either an individual (17–21) or a combination (22, 23) of antioxidants. However, results fail to support the efficacy of antioxidants in preventing vascular events. For example, the Heart Protection Study (22), conducted in the United Kingdom over a 5 year period, assessed the independent effects of simvastatin (40 mg) or antioxidant vitamins (600 mg vitamin E, 250 mg vitamin C, and 20 mg β-carotene daily) versus a placebo in 20,536 high-risk individuals. Statin treatment resulted in a 20–30% reduction in rates of myocardial infarction, stroke, and revascularization over a 5 year period. In contrast, the antioxidant regimen showed no cardiovascular benefit and, in fact, produced a small but significant increase in plasma triglyceride and LDL-C levels, with a nonsignificant trend toward a 5% increase in cardiovascular disease mortality. The HDL Atherosclerosis Treatment Study (HATS) examined the impact of simvastatin and niacin (S-N) therapy with or without antioxidants in CAD patients with low HDL-C levels (23, 24). Subjects were randomized to take a placebo, antioxidants (800 IU vitamin E, 1,000 mg vitamin C, 25 mg β-carotene, and 100 μg of selenium per day), S-N plus antioxidants (S-N+A), or S-N. The reductions in total, LDL-C, and triglycerides levels were comparable in the S-N and S-N+A groups, whereas the elevation in HDL-C was consistently higher in the S-N than in the S-N+A group.

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Regression of stenosis (0.4%) was documented in the S-N group whereas progression was documented in all other groups (3.9% with placebo, 1.8% with antioxidant, and 0.7% with S-N+A). These data suggested that the use of antioxidants (S-N+A) may diminish the beneficial effects observed with angiography in the S-N group, independent of plasma lipoprotein levels. However, the mechanisms responsible for these effects have yet to be elucidated.

Plasma cholesterol concentrations are regulated by feedback mechanisms between the endogenous (synthesis) and exogenous (diet and intestinal absorption) pathways. Data suggest that the efficiency of cholesterol absorption and the rate of cholesterol synthesis are key factors in regulating plasma lipoprotein levels and, consequently, the risk of developing CAD. A recent report has suggested that despite similar serum cholesterol concentrations, postmenopausal women with CAD had enhanced absorption and reduced synthesis rates of cholesterol relative to the control women (25). These and other observations (26, 27) suggest that cholesterol metabolism may play a more important role in CAD development than previously recognized.

The aim of the present study was to quantify the level of selected noncholesterol sterols (reflecting cholesterol absorption efficiency and synthesis rates) in a subset of 123 HATS participants and to relate these data to changes in disease progress and lipoprotein profiles. The intent was to better understand the mechanism of action of the lipid modifying and/or antioxidant treatments.

METHODS

Study subjects and experimental design

Details relating to the HATS population and interventions have been published previously (23, 24). The study was approved by the Human Subject Review Committee of the University of Washington, and informed consent was obtained from all subjects before entering the study. Work performed for the current report was approved by the Human Investigation Review Committee of Tufts University and New England Medical Center. Briefly, all enrolled subjects had at least 50% stenosis of one coronary artery or three 30% coronary lesions. By design, all subjects had low HDL-C levels (men <0.9 mmol/l and women <1.0 mmol/l) and LDL-C levels <3.6 mmol/l. All subjects received counseling regarding lifestyle modification and smoking cessation, as well as professional training in moderate exercise. Subjects were randomized to receive either a placebo, antioxidants (800 IU vitamin E, 1,000 mg vitamin C, 25 mg β-carotene, and 100 μg of selenium per day), S-N+A, or S-N. The present study population included 123 out of the original 160 CAD patients; patients for whom plasma was available both on (at 24 months) and off (at 38 months) treatment. For this subpopulation, mean change in stenosis development was 3.5%, 1%, 0.5%, and −0.4% in the placebo, antioxidant, S-N+A, and S-N groups, respectively. Based on power calculations, the present sample size was adequate to detect at a 90% level statistically significant differences between and within groups for each cholesterol absorption and synthesis marker. Estimates of standard deviation and mean difference were derived from previous work in our laboratory and by comparison with data from similar studies published in the literature.

The average (±SD) dosage of simvastatin and niacin in the S-N group was 13 ± 6 mg/day and 2,287 ± 866 mg/day, and in the S-N+A group was 12 ± 4 mg/day and 2,353 ± 1,042 mg/day, respectively. Given the intent to treat design of the study, subjects in the placebo (n = 3) and antioxidant (n = 4) groups whose LDL-C levels increased to >3.6 mmol/l were given 10 mg/day of simvastatin. Analyses performed with and without these subjects did not significantly alter the results, hence they were not excluded from participation in the cholesterol metabolism arm of the study.

Biochemical analyses

Lipids and lipoproteins were measured using standardized techniques (28–30). The technique for determining mean change in percent proximal stenosis (at baseline and 36 months) has been described previously (23). Concentrations of squalene, the cholesterol precursor sterols, and phytosterols were quantified using a gas chromatography (GC) method similar to that previously described (26). An internal standard containing 150 μl of 5 α-cholestane was added to 1 ml of plasma and saponified with 0.5 M methanolic KOH for 1 h at 100°C, followed by the addition of 2.5 ml of distilled water and 3 ml petroleum ether. The tubes were vortexed, centrifuged at 1,500 *g* for 15 min, the upper layer containing the nonsaponifiable materials transferred into clean glass tubes, and the extraction procedure repeated twice. Combined extracts were dried down under nitrogen and resuspended in 1 ml of chloroform. A sample volume of 2 μl was injected into a GC equipped with a flame ionization detector (Perkin Elmer-Autosystem GC) using a 30 m capillary column (SAC-5, Supelco, Bellefonte, PA). Injector and detector temperatures were 300°C and 310°C, respectively. A multi-ramp oven temperature program was used. The initial temperature was 150°C, held for 1 min, and then increased to 295°C at a rate of 20 degrees/min. After 30 min, the temperature was further increased to 315°C at a rate of 20°C/min for 5 min. Peaks of interest were identified by comparison with authentic standards (Supelco, Bellefonte, PA). Since the noncholesterol sterols are transported in plasma by lipoproteins, their concentration has been expressed relative to the concentration of total cholesterol (mmol/mol of cholesterol) to correct for the differing number of lipoprotein acceptor particles.

Statistical analysis

All analyses were performed using the SAS software (SAS version 8, SAS Institute Inc., Cary NC). $P < 0.05$ was considered to indicate statistical significance. The lipid, lipoprotein, and noncholesterol sterol values obtained off and on treatment for each patient were compared within groups using paired Student's *t*-tests. Differences among groups were determined using one-way ANOVA followed by Scheffe's post-hoc comparisons. Associations between noncholesterol sterols and lipid and lipoprotein data, as well as primary angiographic endpoint data (mean change in percent proximal stenosis) were determined using Spearman's rank correlation. Prior to statistical analysis, the distribution of each variable was assessed for normality, and if necessary appropriate transformations were performed. Untransformed data are presented in text and tables as means ± SE.

RESULTS

Baseline values

Mean age, body mass index, gender distribution, plasma lipid, and lipoprotein levels were similar among treatment groups (**Table 1**). The placebo, S-N+A, and S-N groups had seven smokers each, compared with the 10 in

TABLE 1. Baseline characteristic of HDL Atherosclerosis Treatment Study participants (mean ± SE)

Variable	Placebo n = 30	Antioxidant n = 34	S-N+A n = 29	S-N n = 30
Age (yrs)	53 ± 1.5	54 ± 1.6	56 ± 1.5	53 ± 1.8
Gender	2 F/28 M	5 F/29 M	6 F/23 M	5 F/25 M
BMI (kg/m^2)	29 ± 0.7	28 ± 0.5	29 ± 0.9	29 ± 0.7
Smokers	7	10	7	7
Diabetics	10	4	9	4
Plasma cholesterol (mmol/l)				
Total cholesterol	5.1 ± 0.1	4.9 ± 0.1	5.2 ± 0.2	5.2 ± 0.2
LDL-C	3.3 ± 0.1	3.2 ± 0.1	3.3 ± 0.1	3.5 ± 0.2
HDL-C	0.8 ± 0.01	0.8 ± 0.03	0.8 ± 0.02	0.8 ± 0.01
Plasma triglycerides (mmol/l)	2.1 ± 0.2	2.1 ± 0.2	2.5 ± 0.3	2.2 ± 0.2

BMI, body mass index; S-N, simvastatin and niacin; S-N+A, S-N plus antioxidants.

the antioxidant group. The distribution of diabetic subjects was higher in the placebo and S-N+A groups compared with the antioxidant and S-N groups.

Within group treatment effects on plasma lipid, lipoprotein, and noncholesterol sterol levels

The mean (±SE) change in plasma lipid, lipoprotein (mmol/l), and noncholesterol sterol (10^2 × mmol/mol of cholesterol) levels off and on treatment are shown in **Table 2**. Total cholesterol and LDL-C levels were significantly yet unexpectedly reduced, and HDL-C levels elevated on treatment compared with off treatment in the placebo group. Similarly, a trend toward lower levels of the cholesterol synthesis markers and higher levels of the cholesterol absorption markers with placebo treatment was observed. Lipid and lipoprotein levels remained unchanged in the antioxidant group on and off treatment, as did markers of cholesterol synthesis and absorption.

Treatment with S-N+A and S-N significantly reduced total cholesterol, LDL-C, and TG levels, and increased HDL-C levels on treatment compared with off treatment (Table 2). Levels of the cholesterol synthesis markers (squalene, desmosterol, and lathosterol) were significantly reduced, while levels of the cholesterol absorption markers (campesterol and β-sitosterol) were significantly increased with S-N treatment, regardless of the presence of antioxidants.

Among group treatment effects on levels of the noncholesterol sterols

There was no significant difference in off-treatment levels of the cholesterol synthesis and absorption markers among the four treatment groups. The mean percent change (on treatment vs. off treatment) in levels of the cholesterol synthesis and absorption markers relative to the concentration of total cholesterol is depicted in **Figs. 1** and **2**, respectively. As is observed, there were no significant differences in the percent change of cholesterol synthesis and absorption markers between the placebo and antioxidant groups. In the S-N group, levels of desmosterol and lathosterol were decreased by 46% and 36%, respectively, and these changes were significantly different from those observed in the placebo and antioxidant groups, but not the S-N+A group. Supplementation of S-N with antioxidants also caused a 37% and 31% reduction in desmosterol and lathosterol levels, respectively; however, the magnitude was reduced such that they were no longer significantly different from the changes seen in the placebo and antioxidant groups. A trend ($P = 0.07$) to-

TABLE 2. Fasting plasma lipids, lipoproteins and non-cholesterol sterols off and on treatment (mean ± SE)

Variables	Placebo		Antioxidant		S-N+A		S-N	
	Off	On	Off	On	Off	On	Off	On
				mmol/l				
Plasma lipids and lipoproteins								
Total cholesterol	5.2 ± 0.1^a	4.8 ± 0.1^b	5.2 ± 0.1	5.0 ± 0.1	5.0 ± 0.2^a	3.8 ± 0.2^b	5.1 ± 0.2^a	3.6 ± 0.1^b
LDL-C	3.4 ± 0.1^a	3.1 ± 0.1^b	3.4 ± 0.1	3.2 ± 0.1	3.2 ± 0.1^a	2.3 ± 0.1^b	3.4 ± 0.2^a	2.1 ± 0.1^b
HDL-C	0.8 ± 0.01^b	0.9 ± 0.04^a	0.9 ± 0.03	0.9 ± 0.03	0.8 ± 0.03^b	1.0 ± 0.03^a	0.9 ± 0.04^b	1.1 ± 0.05^a
Triglycerides	2.1 ± 0.1	2.0 ± 0.2	2.2 ± 0.2	2.4 ± 0.3	2.4 ± 0.2^a	1.6 ± 0.2^b	2.2 ± 0.3^a	1.4 ± 0.1^b
				10^2 × mmol/mol of cholesterol				
Plasma noncholesterol sterols								
Squalene	28 ± 3	32 ± 5	29 ± 3	31 ± 4	35 ± 4^a	25 ± 3^b	34 ± 4^a	25 ± 2^b
Desmosterol	101 ± 9	81 ± 7	100 ± 9	94 ± 9	95 ± 14^a	47 ± 7^b	89 ± 9^a	46 ± 5^b
Lathosterol	173 ± 14	137 ± 8	162 ± 13	143 ± 11	154 ± 11^a	99 ± 8^b	155 ± 13^a	92 ± 7^b
Campesterol	184 ± 10	206 ± 15	193 ± 17	200 ± 15	180 ± 13^a	259 ± 15^b	183 ± 13^a	284 ± 18^b
β-Sitosterol	134 ± 10	145 ± 12	132 ± 9	135 ± 11	125 ± 13^a	167 ± 14^b	120 ± 10^a	186 ± 13^b
Latho-camp ratio	1.0 ± 0.1	0.8 ± 0.1	1.0 ± 0.3	0.9 ± 0.1	0.9 ± 0.1^a	0.4 ± 0.1^b	1.1 ± 0.2^a	0.4 ± 0.1^b

Latho-camp ratio, lathosterol to campesterol ratio. Within each treatment group, values with different superscripts are significantly different at $P < 0.01$ off treatment versus on treatment for each variable.

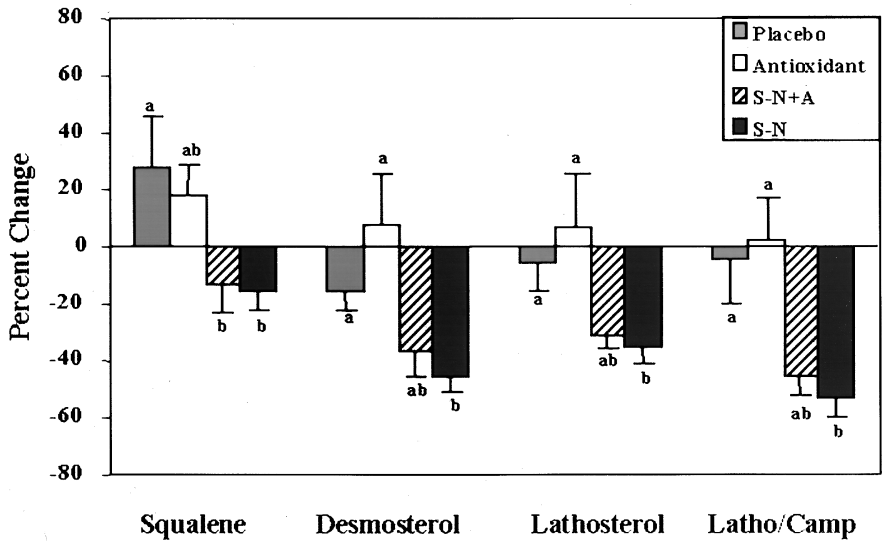

Fig. 1. Mean percent change ± SE (on treatment vs. off treatment) in levels of the cholesterol synthesis markers relative to the concentration of total cholesterol. For each marker, columns with different letters are significantly different ($P < 0.05$).

ward lower lathosterol to campesterol (latho-camp) ratios (potentially a more accurate reflection of cholesterol synthesis rates than the cholesterol synthetic intermediates alone) in the S-N group (53%) versus the S-N+A group (45%) was also observed. The pattern of change in squalene levels (an early intermediate in the cholesterol synthetic pathway) was similar between the S-N and S-N+A groups, although the percent reductions were somewhat less compared with the other synthesis markers.

Among the cholesterol absorption markers, campesterol levels were significantly higher (70% and 54%, respectively) in the S-N groups regardless of the presence of antioxidants relative to the placebo and antioxidant groups (Fig. 2). β-sitosterol levels were increased in the S-N group (50%) relative to the placebo and antioxidant groups, but not the S-N+A group. Despite an observed increase of 46% in β-sitosterol levels in the S-N+A group, the magnitude was diminished so as to be statistically nonsignificant from changes seen in the placebo and antioxidant groups.

Correlation between mean change in percent stenosis, absorption/synthesis markers, and CAD risk factors

Percent change in lathosterol levels ($r = 0.26$, $P = 0.004$) were positively, and β-sitosterol levels ($r = -0.21$, $P = 0.01$) were negatively, correlated with mean change in percent stenosis (**Table 3**). Campesterol levels were not sig-

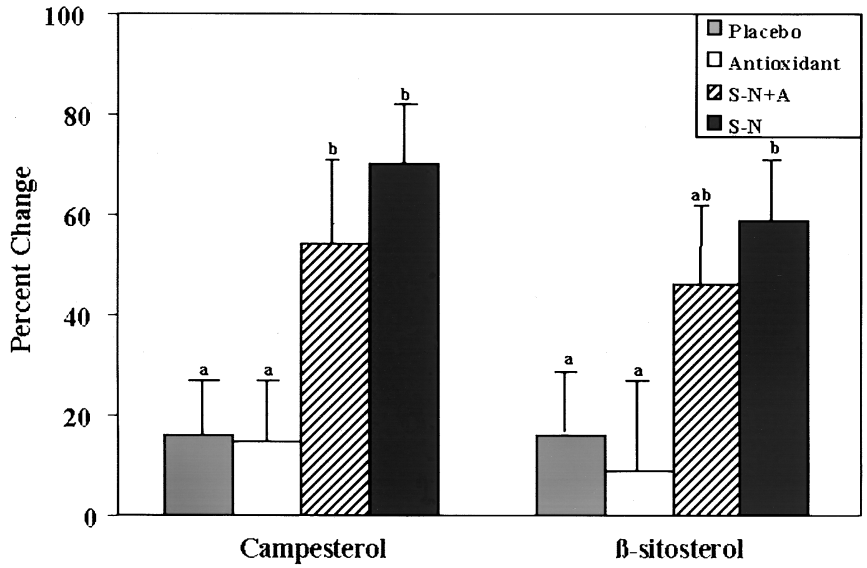

Fig. 2. Mean percent change ± SE (on vs. off treatment) in levels of the cholesterol absorption markers relative to the concentration of total cholesterol. For each marker, columns with different letters are significantly different ($P < 0.05$).

TABLE 3. Correlation between percent change in noncholesterol sterols and stenosis, lipid and lipoprotein data

Variables	Stenosis	Total Cholesterol	LDL-C	HDL-C	Triglyceride
Stenosis	1.00	0.10	0.09	−0.14	−0.02
Squalene	0.12	−0.03	−0.15	−0.21[b]	0.16
Desmosterol	0.06	−0.08	−0.04	0.05	−0.15
Lathosterol	0.26[c]	0.32[b]	0.32[b]	−0.18[c]	−0.13
Campesterol	−0.05	−0.29[a]	−0.28[a]	0.23[b]	0.11
β-Sitosterol	−0.21[b]	−0.33[a]	−0.27[a]	0.27[b]	0.05
Latho/Camp	0.18	0.28[a]	0.23[b]	−0.10	−0.01

n = 123.
[a] $P < 0.001$.
[b] $P < 0.01$.
[c] $P < 0.05$.

nificantly related to mean change in percent stenosis, perhaps due to the greater degree of variability in this measure. A trend toward a positive relation between the latho-camp ratio ($r = 0.18$, $P = 0.06$) and mean change in stenosis was also observed. While no significant associations were observed between mean change in percent stenosis and percent change in lipid levels in this subset of 123 subjects, it should be noted that significant correlations were observed between on-treatment LDL-C levels and mean change in percent stenosis ($r = 0.22$, $P = 0.008$) in the original HATS population (23).

Independent of treatment, the cholesterol synthesis markers were positively associated with total cholesterol ($r = 0.32$, $P = 0.003$ for lathosterol; and $r = 0.28$, $P = 0.001$ for latho-camp ratio) and LDL-C ($r = 0.32$, $P = 0.003$ for lathosterol; and $r = 0.23$, $P = 0.01$ for latho-camp ratio), and negatively associated with HDL-C ($r = -0.18$, $P = 0.04$ for lathosterol; and $r = -0.21$, $P = 0.02$ for squalene) (Table 3). The reverse was observed for cholesterol absorption markers. Campesterol and β-sitosterol were negatively related ($P < 0.02$) to total and LDL-C levels, and positively related to HDL-C levels ($P < 0.05$).

DISCUSSION

As part of a clinical trial that examined the effects of statin and niacin therapy with or without antioxidant supplementation on clinical and angiographical outcomes in CAD patients with low HDL-C, plasma levels of noncholesterol sterols (reflecting cholesterol absorption efficiency and synthesis) were measured off and on therapy to address issues related to mechanism of action. Results of this investigation demonstrate that S-N treatment significantly alters measures of cholesterol metabolism by decreasing cholesterol synthesis and increasing cholesterol absorption, and these findings are consistent with the magnitude of change that would be predicted from the lipid and angiographic data. Antioxidants alone have no significant effect on measures of cholesterol synthesis and absorption. There is a tendency for the supplementation of S-N with antioxidants to diminish these effects. Furthermore, concentrations of these markers appear to more closely parallel changes in stenosis than do changes in plasma lipid levels.

The lowering of plasma total and LDL-C levels observed in patients treated with S-N can be ascribed to the reduction in cholesterol synthesis. This finding is consistent with the known mechanism of action of statins in inhibiting HMG-CoA reductase activity, the rate-limiting enzyme in cholesterol biosynthesis (31, 32). The current findings also confirm previous observations of increased ratios of phytosterols during statin treatment (32–34). This response appears to reflect a compensatory increase in absorption efficiency in order to restore cholesterol homeostasis altered by the suppression of cholesterol synthesis. In turn, the decreased synthesis leads to diminished cholesterol turnover, presumably resulting in decreased biliary elimination of sterols (the primary route of elimination of phytosterols) and thus further increases circulating phytosterol levels. Since phytosterols are obtained solely from diet, different intake levels could be responsible for the changes observed. While dietary phytosterol levels were not directly measured in the HATS, it has been previously shown by others that the polyunsaturated to saturated fatty acid ratio (P-S) of the diet is directly related to plasma phytosterol levels (35). Analysis of the total caloric, cholesterol, and P-S ratio intake data from HATS subjects demonstrated that there were no significant differences in these parameters on and off treatment within each group as well as among groups. Furthermore, it has been demonstrated that a several 100-fold increase in the dietary phytosterol load is required to double the plasma β-sitosterol levels in humans (36). Consequently, variation in dietary phytosterol intakes is unlikely to explain the alterations in plasma levels.

The interesting yet contradictory finding of this study was the response of cholesterol synthesis and absorption in patients taking antioxidant supplements alone or in combination with S-N treatment. While the pattern of change in levels of the cholesterol synthesis and absorption markers in the S-N+A group were similar to those observed in the S-N group, the extent of change was diminished, and consequently the differences observed were rendered nonsignificant relative to the placebo and antioxidant treatment. Additionally, in the group that received antioxidants alone, a nonsignificant trend toward increased levels of all the cholesterol synthesis markers was observed. The reason for this effect is not immediately clear, but animal and in vitro studies have shown that vitamin E supplementation at doses >20 mg/day stimulates cholesterol synthesis, possibly via posttranscriptional upregulation of HMG-CoA reductase (37, 38). The presence of vitamin E in the antioxidant cocktail may have partially counteracted the statin-induced inhibition of HMG-CoA reductase, thereby reducing the extent of cholesterol synthesis downregulation with S-N+A treatment. Furthermore, it has also been reported that statins undergo oxidation catalyzed by cytochrome P-450 in the liver and intestine to form mono- and dihydric phenols, potent antioxidants shown to reduce cholesterol accumulation in macrophages by blocking cholesterol esterification and

endocytosis of modified lipoproteins (39–41). Supplementation of S-N with antioxidants could theoretically prevent formation of these metabolites, and thereby diminish the efficacy of statin treatment on stenosis development. This could explain why S-N alone promoted regression of coronary disease, but supplementation of S-N with antioxidants mitigated this effect. It has also been shown that phytosterols preferentially accumulate in HDL (phytosterol to cholesterol ratios are almost 40% higher than in VLDL or LDL), so any factor that interferes with HDL metabolism would subsequently affect plasma phytosterol levels. In the entire HATS population, the increase of HDL-C and apoA-I levels was significantly diminished by the addition of antioxidants to S-N treatment (24). It has been postulated that fat soluble vitamins entering the cell on internalized HDL might decrease expression of apoA-1 and/or genes encoding for specific transporters (ABCA1, ABCG5, and ABCG8) or enzymes (LCAT, CETP, and LPL) involved in cholesterol metabolism (42), resulting in increased uptake and degradation of HDL-C, and consequently lower phytosterol levels. While HDL-C and apoA-I kinetics were not measured in this study, our findings of significant associations between phytosterol levels and HDL-C partly support this hypothesis.

Statin therapy increased markers of cholesterol absorption efficiency and decreased markers of cholesterol synthesis, and resulted in CAD regression. Addition of antioxidants to statin therapy potentially diminishes these responses, which could explain the lack of CAD regression in the S-N+A relative to the S-N group. Markers of cholesterol metabolism were stronger predictors of disease progression than plasma lipid levels. Measurement of cholesterol absorption and synthesis markers may potentially serve as new markers of CAD progression.

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