Rapid Communication

Plant Sterol-Fortified Orange Juice Effectively Lowers Cholesterol Levels in Mildly Hypercholesterolemic Healthy Individuals

Sridevi Devaraj, Ishwarlal Jialal, Sonia Vega-López

Objective—Hypercholesterolemia is a major risk factor for coronary artery disease. Therapeutic lifestyle changes include dietary modifications such as inclusion of phytosterols, which effectively lowers low-density lipoprotein (LDL) cholesterol in margarines and other fats. Their effectiveness in nonfat moieties is not yet established. The aim of this study was to examine if phytosterols alter the plasma lipoprotein profile when incorporated into nonfat orange juice.

Methods and Results—After a 2-week run-in phase with orange juice, 72 mildly hypercholesterolemic healthy subjects were randomized to receive either placebo orange juice (placebo OJ) or plant sterol-fortified orange juice (sterol OJ) (2g/d) for 8 weeks. Fasting blood was obtained at baseline, after 2 weeks of OJ, and after 8 weeks of placebo/sterol-OJ supplementation. Sterol OJ supplementation significantly decreased total (7.2%), LDL (12.4%), and non-high-density lipoprotein (HDL) cholesterol (7.8%) compared with baseline and compared with placebo OJ ($P<0.01$). Apolipoprotein B levels were significantly decreased (9.5%) with sterol OJ. There were no significant changes in HDL cholesterol or triglycerides with the sterol OJ. While folate and B12 levels significantly increased, homocysteine levels were unchanged.

Conclusions—Orange juice fortified with plant sterols are effective in reducing LDL cholesterol and could easily be incorporated into the therapeutic lifestyle changes dietary regimen. **(*Arterioscler Thromb Vasc Biol.* 2004;24:e25-e28.)**

Key Words: phytosterol ■ plant sterol ■ cholesterol ■ lipid profile ■ nonfat ■ diet

Cardiovascular disease (CAD) is the leading cause of morbidity and mortality in the United States. High levels of low-density lipoprotein (LDL) are associated with increased incidence of CAD. The National Cholesterol Education Panel has established dietary therapy as the initial cornerstone of strategies to lower LDL cholesterol (LDL-C) levels and reduce the risk of CAD, and have added phytosterols as part of the therapeutic lifestyle changes dietary guidelines.[1] The US Food and Drug Administration also issued a health claim stating that the foods containing plant stanols and stanol esters may reduce the risk of CAD.[2] Stanols and sterols, found in fat-soluble fractions of plants, chemically resemble cholesterol and exert their cholesterol-lowering action by presumably suppressing intestinal absorption.[3,4] Sterol ester consumption in human subjects reduces plasma total and LDL-C levels. A recent meta-analysis suggests that ingestion of 2 g per day of plant sterols incorporated into dietary fat vehicles such as margarine have yielded a 10% reduction in LDL-C levels in patients with hypercholesterolemia.[3,4] To expand the usefulness of phytosterols, their LDL-lowering potency in nonfat-based moieties should be tested. The data with regard to the effect of phytosterols in nonfat medium, especially in plant sterol-enriched beverages, are scanty and controversial.

The main objective of this study was therefore to examine the effectiveness of a plant sterol-fortified orange juice (a nonfat beverage) supplementation on the lipoprotein profile in healthy human volunteers in a placebo-controlled, double-blind, randomized trial. Furthermore, because orange juice also provides other beneficial antioxidants, such as ascorbate and flavonoids, and is consumed by subjects of all ages, it represents an effective medium to test the effectiveness of plant sterol fortification in a nonfat beverage.

Elevated total homocysteine is emerging as a prevalent and strong risk factor for atherosclerotic vascular disease.[5,6] Hyperhomocystinemia is attributed to commonly occurring genetic and acquired factors including deficiencies of folate and vitamin B12. Supplementation with B vitamins, including folic acid, is an efficient, safe, and inexpensive means to reduce an elevated total homocysteine level. Thus, we also tested the effect of OJ supplementation on levels of homocysteine.

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TABLE 1. Composition of the Orange Juice

	Phytosterol Orange Juice (per 240 mL)	Control Orange Juice (per 240 mL)
Calories	110	110
Folate (μg)	60	60
Vitamin E (IU)	6	—
Vitamin B6 (mg)	0.4	0.08
Vitamin B12 (μg)	1.2	—
Vitamin C (mg)	72	72
Potassium (mg)	450	450
Thiamin (mg)	0.15	0.15
Free sterol (g)	1.0	—

Methods

Seventy-two subjects, aged 20 to 73 years, participated in this placebo-controlled, double-blind, randomized trial. All subjects gave informed consent, and this study was approved by the Institutional Review Board at the University of California at Davis Medical Center.

Adults with normal complete blood counts, LDL-C >100 mg/dL, normal liver and renal function (normal transaminases, alkaline phosphatase, creatinine), no bleeding diathesis, and normal thyroid function (normal thyroid-stimulating hormone) were included in the study. Secondary causes of hypercholesterolemia such as nephrotic syndrome, cholestasis, and hypothyroidism were ruled out.

The list of exclusion criteria were as follows: pregnancy or lactation, smoking, current use of vitamin supplements, alcohol intake >1 ounce per day, history of cardiovascular disease or chronic inflammatory diseases (eg, Crohns, rheumatoid arthritis, SLE), recent bacterial infection (<2 weeks), antiinflammatory steroidal or nonsteroidal medication use, hypolipidemic or thyroid drug use, oral contraceptive or anticoagulant use, history of sitosterolemia, gastrointestinal problems, and concurrent or recent (within 30 days) intervention study participation.

Study Design

All subjects underwent a 2-week run-in phase in which they received unfortified orange juice. They were then randomized in a blinded fashion to receive plant sterol-fortified orange juice (sterol OJ) or placebo orange juice (placebo OJ) for the next 8 weeks. Placebo OJ and sterol OJ were provided by Minute Maid–The Coca-Cola Company. Plant sterol with the targeted particle size distribution was suspended in orange juice using a process pending patent. Beverage phytosterol was derived from vegetable oils, with the 3 major components distributed approximately as 40% beta-sitosterol, 25% campesterol, and 20% stigmasterol by weight. The suspension was stable throughout the refrigerated shelf-life of the finished beverage (up to 10 weeks). The product was prepared and shipped by the supplier 1 week before disbursement of juice to the subjects. Subjects were given enough juice to last 18 days. They were asked to keep the juice refrigerated and were instructed to shake contents of the container before measuring their 240 mL twice per day. Also, it was packaged in polyethylene containers and, because of the large micron size of the plant sterols used in the study, migration into the polyethylene film is very unlikely. Analytical evaluations of the finished beverage showed that the plant sterol remained in the orange juice throughout shelf-life. Subjects were asked to refrigerate the placebo OJ and sterol OJ. The study investigators were also blinded to protocol assignment until the end of the study. Each subject was asked to consume 240 mL of juice twice per day with meals. This corresponded to 2 g per day of sterol in the sterol OJ. This dose was used because it has been effectively shown to lower cholesterol concentrations in fat matrices and is the dose recommended by the NCEP/ATPIII. Subjects were asked to refrain from consuming any other source of orange juice or citrus fruits or fortified margarines such as Benecol or Take Control 4 weeks before study entry and during the period of the study. Fasting blood was obtained at baseline (average of 2 samples 5 to 7 days apart), after 2 weeks, and after 10 weeks of the study (average of 2 samples 5 to 7 days apart). Subjects were asked to keep a 3-day diet record at the beginning and at the end of the study, which included time of consumption of OJ. The composition of the placebo OJ and sterol OJ are given in Table 1.

TABLE 2. Baseline Subject Characteristics

	Sterol OJ	Placebo OJ
Age (y)	41±13	44±13
BMI (kg/m^2)	25±6	26±4
M/F	13/23	16/20
Total cholesterol (mg/dL)	207±27	209±28
Total triglyceride (mg/dL)	107±45	96±40
LDL-C (mg/dL)	137±21	140±22
HDL-C (mg/dL)	49±15	49±11
Non-HDL-C (mg/dL)	158±25	160±26
ApoB (mg/dL)	105±21	102±19

Data are expressed as mean±SD.
M indicates male; F, female.

Analyses

Plasma was separated from red blood cells after 15 minutes of centrifugation at 600*g*. All analyses were performed in the Clinical Pathology Laboratory at University of California Davis Medical Center in Sacramento. Total cholesterol and total triglyceride were analyzed on the Beckman Access autoanalyzer. LDL-C concentrations were calculated using the Friedewald equation. High-density lipoprotein cholesterol (HDL-C) concentrations were analyzed using the direct HDL-C assay. Apolipoprotein B levels were measured by an immunoturbidimetric assay. Homocysteine levels were measured by high-performance liquid chromatography. Folate and vitamin B12 levels were measured on the Centaur immunoanalyzer after dilution. The inter-assay and intra-assay co-efficient of variation for cholesterol and triglyceride assays was <4%; for homocysteine, it was <5%, and for apo B, folate, and B12, it was <8%. Diet analyses were performed using ESHA Food Processor.

Statistical Analyses

All data are presented either as mean±SD or medians and range (if nonparametric). Plasma lipid concentrations were compared using ANOVA followed by paired *t* tests for parametric data and Wilcoxon signed-rank tests for nonparametric data such as triglycerides. Both between-group and within-group comparisons were made. A probability value of <0.05 was considered statistically significant.

Results

Although 75 subjects entered the study, 3 discontinued participation because of personal reasons (2 in the sterol OJ and 1 in the placebo OJ group), and 72 subjects (n=36/group) completed the study. Thus, compliance was high and body weights were unchanged during the trial. Subjects in both groups (placebo and sterol OJ) were matched for age, gender, and body mass index. Eight subjects in the placebo OJ and 8 subjects in the sterol OJ group were using oral contraceptives. Baseline subject characteristics and baseline lipid profile are reported in Table 2. There were no differences in the baseline lipid profile (total cholesterol, total triglycerides, HDL-C and LDL-C) and the apoB concentrations between the 2 groups. Diet analyses revealed that there were no significant differences in the composition of the diet in the 2 groups before and after OJ supplementation (diet composition: baseline: total fat: 31%, saturated fat: 11%; cholesterol: 235 mg/d; protein: 19%; carbohydrate: 49%; post-supplementation: total fat: 32%, saturated fat: 11%; cholesterol: 239 mg/d; protein: 19%; carbohydrate: 48%).

Sterol OJ supplementation did not result in any changes in complete blood count, liver function tests, blood glucose con-

TABLE 3. Effect of Sterol OJ on the Lipoprotein Profile

	Sterol OJ			Placebo OJ		
	Baseline	Week 2	Week 10	Baseline	Week 2	Week 10
Total cholesterol	207±27	209±32	194±27*†	209±28	213±31	207±29
Total triglyceride	107±45	106±45	104±49	96±40	94±49	102±50
LDL-C	137±21	144±24	125±25*†	140±22	140±24	138±24
HDL-C	49±15	49±11	47±12	49±11	50±11	48±11
Non-HDL-C	158±25	159±28	147±25*†	160±26	158±40	158±30

Data are expressed as mean±SD in mg/dL.
*$P<0.001$ compared to baseline.
†$P<0.05$ compared to placebo.

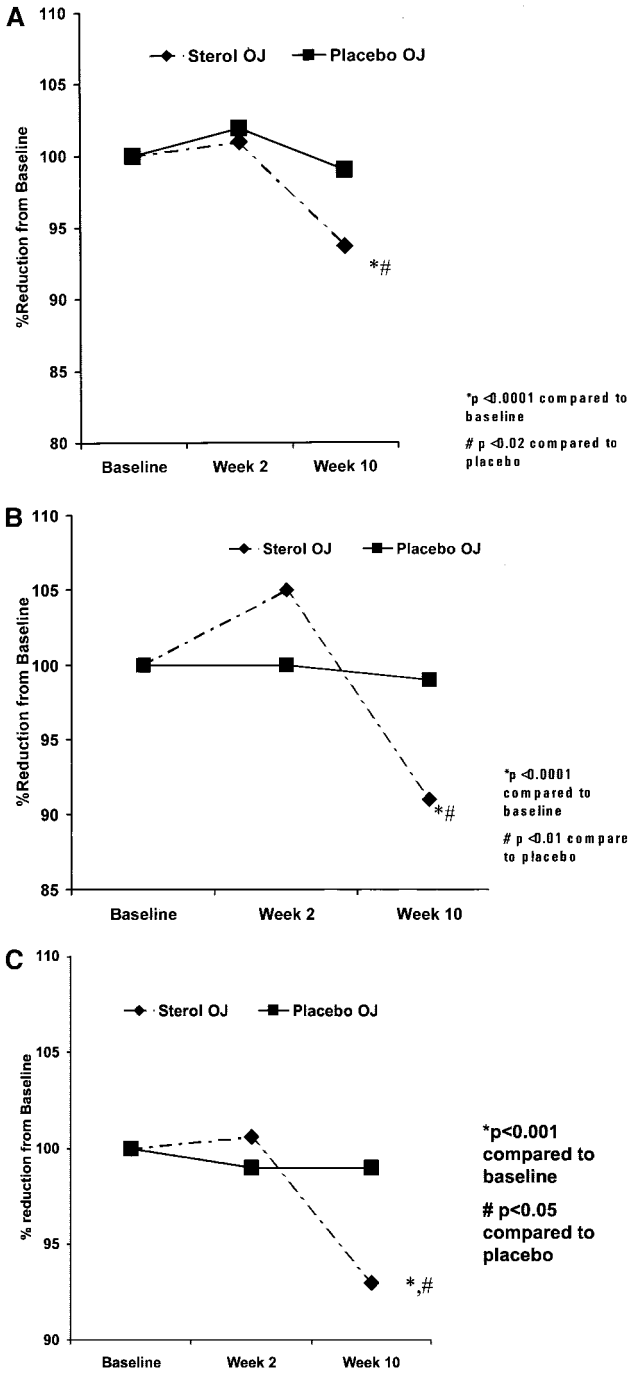
centrations, or renal function. Mean baseline, week 2 (run-in phase), and endpoint concentration of total cholesterol, LDL-C, non-HDL-C, HDL-C, and total triglycerides are provided in Table 3. Overall, there were no significant differences in any of these parameters between week 0 and week 2 or the run-in phase with control OJ. No significant changes in the lipid profile were observed with the placebo OJ. With regard to total cholesterol and LDL-C, there was a significant reduction in concentrations between groups as well as compared with baseline within the sterol OJ group (7.2% decrease in total cholesterol and 12.4% decrease in LDL-C; $P<0.001$) (Figure 1a and 1b). There were no significant changes in triglyceride levels or HDL-C concentrations compared with baseline or between the 2 groups. We also examined the effects of OJ supplementation on non-HDL-C. Whereas non-HDL concentrations were essentially unchanged in the placebo OJ group, there was a significant (7.8% reduction, $P<0.01$) reduction in non-HDL-C concentrations in sterol OJ group compared with baseline and compared with the placebo OJ group (Figure 1c). ApoB levels were monitored at baseline and after 8-weeks of supplementation with placebo and sterol OJ. There was a significant (9.5%, $P<0.01$) reduction in Apo-B concentrations only in the sterol group but not in the placebo OJ group.

Because the OJ contained folate and was fortified with B6 and B12, we examined the effect of OJ supplementation on homocysteine concentrations. Although there were significant increases in plasma concentrations of folate (baseline: 23±17 ng/mL; week 10: 34±20 ng/mL; $P<0.05$) and vitamin B12 (baseline: 595±219 pg/mL; week 10: 677±289 pg/mL; $P<0.05$), there was no significant reduction in the plasma concentrations of homocysteine (baseline: 7.3±1.7 μmol/L; week 10: 7.2±1.3 μmol/L) after OJ supplementation.

Discussion

This is the first placebo-controlled, double-blind trial to our knowledge to report a significant cholesterol-lowering effect of plant sterols incorporated in a nonfat beverage in normal healthy subjects with mild hypercholesterolemia. In this study, we show significant lowering of total cholesterol (7.2%), LDL-C (12.4%), and non-HDL-C (7.8%) with sterol OJ compared with baseline and compared with placebo OJ. ApoB levels were significantly decreased with sterol OJ compared with baseline and compared with placebo OJ.

While several trials in different populations have shown that phytosterol consumption in fat matrices (margarine, butter, or salad dressing) results in decreased total cholesterol and LDL-C concentrations (3.4% to 11.6% and 5.4% to 15.5%, respectively),[3,4] the incorporation of phytosterols in reduced-fat matrices have yielded variable results. Maki et al[7] reported a 7.6% and 8.1% LDL-C-lowering with a 50% fat spread providing 1.1 and 2.2 grams phytosterols per day, respectively. However, no difference in cholesterol concentrations was observed in another trial comparing the effects of phytosterols at 3 g per day in reduced-fat spread versus 6 grams in a 28% fat dressing versus 9 grams in reduced-fat spread and dressing.[8] Recently, consumption of low-fat (1%) yogurt containing 1 gram per day of phytosterols significantly lowered total cholesterol and LDL-C concentrations, however, the weakness of this study is that the placebo, albeit nonsignificant, decreased total cholesterol and LDL-C concentrations, and comparisons versus placebo were not made.[9] Mensinck et al[10] have also shown a 13.7% LDL-C-lowering using esterified stanols (3 g per day) in low-fat yogurt. The only study of phytosterols in low-fat and nonfat beverages so far has been conducted by Jones et al,[11] and they did not observe any significant differences in total cholesterol and LDL-C between placebo and the 2 groups. In that study, the subjects were on precisely controlled diets, which resulted in a 5% to 12% decrease in total cholesterol and LDL-C concentrations; however, there was no difference between placebo, low-fat, and nonfat beverages containing phytosterols. It is possible that their decreased sample size (n=15/group), the preparation of the sterol mixture, beverage consumption for 3 weeks, and the use of controlled diets that already produced significant cholesterol-lowering prevented them from observing an additional significant effect with the phytosterols. In our study, subjects were asked to eat their normal American diet and drink 240 mL of juice along with breakfast and dinner. It is possible that because the orange juice was consumed with meals and their daily diet was composed of ≈31% total fat, 11% saturated fat, and ≈235 mg per day of cholesterol (similar to AHA Step 1 diet), this resulted in effectively lowering total cholesterol and LDL-C concentrations. The fat in the meal may have helped to emulsify the sterols; alternatively, the bile salt and phospholipid in the bile, whose contraction is produced by a meal, may have aided the plant sterols in competing more effectively with cholesterol for absorption and, finally, contraction of the gall bladder allows presentation of biliary cholesterol to the intestine, which is a target of reduced absorption. Previously, agents such as AOMA, cytellin, and sucrose polyester at doses of 5 to 50 g per day have also exhibited cholesterol-lowering, as reviewed by



Effect of sterol OJ supplementation on total cholesterol (a), LDL-C (b), and non-HDL-C (c). Fasting blood was obtained at baseline, after 2 weeks of supplementation with orange juice, and after 8 weeks of supplementation with placebo OJ or sterol OJ. All analyses were performed as described in Methods. Data are presented as percent reduction from baseline.

Crouse et al.[12] However, because we did not test the sterol OJ without meals, it is difficult to speculate on the exact mechanism for LDL-C-lowering observed with the sterol OJ, and this should be tested in future studies.

With regard to plasma homocysteine concentrations, although folate and B12 levels were increased after sterol OJ supplementation, there was no difference in homocysteine concentrations, most likely because of the low levels of folate and B vitamins in the sterol OJ and because of the fact that the subjects already had normal homocysteine concentrations at baseline (median 7.3 μmol/L).

In conclusion, this study shows a novel LDL-C-lowering effect of plant sterols (2 g per day) in a nonfat beverage (orange juice). Nonpharmacological treatment is considered to be an important strategy for patients with moderate hypercholesterolemia. Incorporating phytosterols in such nonfat beverages is an important step in keeping with a heart-healthy diet and an important component of the therapeutic lifestyle dietary regimen recommended by the NCEP/ATP III guidelines. It is also important to note that this study was conducted in free-living ambulatory subjects to examine its relevance to the population at large. The strategy of supplementing orange juice with plant sterols is very attractive, especially because it is also an excellent source of other micronutrients/antioxidants, such as ascorbate (provides the recommended daily allowance), folate, and other flavonoids, in addition to being often consumed at breakfast, and it does not provide an additional source of fat as do other phytosterol products. Such beverages could be used to lower cholesterol in subjects of all ages, from teenage to old age, and has broad appeal.

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