1-1-2000

HDL-cholesterol-raising effect of orange juice in subjects with hypercholesterolemia

E. M. Kurowska
Western University

J. D. Spence
Western University, jdspence@uwo.ca

J. Jordan
Western University

S. Wetmore
Western University

D. J. Freeman
Western University

See next page for additional authors

HDL-cholesterol-raising effect of orange juice in subjects with hypercholesterolemia[1–3]

Elzbieta M Kurowska, J David Spence, John Jordan, Stephen Wetmore, David J Freeman, Leonard A Piché, and Paula Serratore

ABSTRACT

Background: Orange juice—a rich source of vitamin C, folate, and flavonoids such as hesperidin—induces hypocholesterolemic responses in animals.

Objective: We determined whether orange juice beneficially altered blood lipids in subjects with moderate hypercholesterolemia.

Design: The sample consisted of 16 healthy men and 9 healthy women with elevated plasma total and LDL-cholesterol and normal plasma triacylglycerol concentrations. Participants incorporated 1, 2, or 3 cups (250 mL each) of orange juice sequentially into their diets, each dose over a period of 4 wk. This was followed by a 5-wk washout period. Plasma lipid, folate, homocyst(e)ine, and vitamin C (a compliance marker) concentrations were measured at baseline, after each treatment, and after the washout period.

Results: Consumption of 750 mL but not of 250 or 500 mL orange juice daily increased HDL-cholesterol concentrations by 21% ($P < 0.001$), triacylglycerol concentrations by 30% (from 1.56 ± 0.72 to 2.03 ± 0.91 mmol/L; $P < 0.02$), and folate concentrations by 18% ($P < 0.01$); decreased the LDL-HDL cholesterol ratio by 16% ($P < 0.005$); and did not affect homocyst(e)ine concentrations. Plasma vitamin C concentrations increased significantly during each dietary period (2.1, 3.1, and 3.8 times, respectively).

Conclusions: Orange juice (750 mL/d) improved blood lipid profiles in hypercholesterolemic subjects, confirming recommendations to consume ≥5–10 servings of fruit and vegetables daily. *Am J Clin Nutr* 2000;72:1095–100.

KEY WORDS Orange juice, hypercholesterolemia, lipoproteins, HDL cholesterol, folate, flavonoids, vitamin C, homocyst(e)ine

INTRODUCTION

Previous epidemiologic studies suggested that a high intake of fruit and vegetables is associated with a reduced risk of coronary heart disease (1). The beneficial effect could be related to minor components, especially flavonoids, which are proposed to exert their action by inhibiting LDL oxidation and platelet aggregation (2), and vitamins C and E and β-carotene, which are thought to act mainly as antioxidants (3). Folic acid and natural folate present in high amounts in citrus fruit and in green vegetables were also reported to reduce plasma total homocyst(e)ine, an intermediate in methionine metabolism, which is implicated as a risk factor in cardiovascular disease (4, 5). Citrus juices, especially orange juice and grapefruit juice, are rich sources of flavonoids, folate, and vitamin C, but their role in cardiovascular health has not been investigated thoroughly. The principal citrus flavonoids, hesperetin from oranges and naringenin from grapefruit, are structurally similar to genistein, a flavonoid from soybean postulated to be hypocholesterolemic (6). Thus, citrus juices could have cholesterol-lowering potential.

The effect of citrus juices and their principal flavonoids on cholesterol metabolism was tested recently in rabbits, rats, and the human liver cell line HepG2. In rabbits with experimental hypercholesterolemia induced by a casein-based, semipurified diet in which drinking water was replaced with either orange juice or grapefruit juice (reconstituted from frozen concentrate at 2 times the normal strength), serum LDL cholesterol decreased by 43% and 32%, respectively (7). In addition, liver cholesterol esters decreased by 42% but fecal excretion of cholesterol or bile acids did not increase, suggesting that juice components, possibly flavonoids, might affect cholesterol metabolism directly in the liver. In support of this hypothesis, serum cholesterol decreased and in vitro activities of hydroxymethylglutaryl-CoA reductase and sterol *O*-acyltransferase—2 key enzymes in cholesterol metabolism—were inhibited in cholesterol-fed rats supplemented with mixtures of principal citrus flavonoids (8). Additionally, treatment of HepG2 cells with both hesperetin and naringenin reduced the net secretion of apolipoprotein B (apo B), the LDL protein, by inhibiting the synthesis of cholesterol esters (9). In addition to flavonoids, citrus juices contain high concentrations of limonoids, mostly bitter triterpene derivatives, which also have shown apo B–lowering potential in HepG2 cells (10).

[1] From the Departments of Biochemistry, Pharmacology and Toxicology, and Family Medicine, and the Nutrition Program (Brescia College), University of Western Ontario, London, Canada; the Stroke Prevention and Atherosclerosis Research Centre, Robarts Research Institute, London, Canada; and the YMCA, London, Canada.

[2] Supported by Tropicana Products Inc, Bradenton, FL.

[3] Address reprint requests to EM Kurowska, Department of Biochemistry, University of Western Ontario, London, Ontario, Canada N6A 5C1. E-mail: kurowska@julian.uwo.ca.

Received October 1, 1999.

Accepted for publication March 10, 2000.

TABLE 1
Composition of orange juice and its contribution to the daily diet of the study participants[1]

	Dietary period		
	1	2	3
Energy (kJ)	481.3	962.6	1444.0
Protein (g)	2.1	4.2	6.3
Carbohydrates (g)	27.2	54.4	81.6
Sugars (g)	23.0	46.0	69.0
Vitamin C (mg)	74.9	149.8	224.7
Folate (μg)	62.8	125.6	188.4
Calcium (mg)	20.9	41.8	62.7
Potassium (mg)	471.0	942.0	1413.0
Thiamine (mg)	0.16	0.32	0.48
Niacin (mg)	0.84	1.68	2.52
Hesperidin (mg)	11.6	23.2	34.7
Limonoid glucosides (mg)	80.0	160.0	240.0
Limonin glucosides (mg)	45.0	90.0	135.0
Limonin (mg)	0.5	1.0	1.5

[1]Tropicana Pure Premium orange juice (Tropicana Canada, Mississauga, Canada).

The potential cardioprotective effects of orange juice have rarely been investigated in humans. In healthy men, intake of an unspecified dose of fresh orange juice reduced lipoprotein oxidation, presumably because of its high content of vitamin C (500 mg/d), but did not change the plasma lipid profile (11). In another trial, consumption of citrus fruit and green vegetables significantly increased plasma folate and decreased plasma homocyst(e)ine concentrations in healthy subjects (5). The present study was undertaken to determine whether orange juice beneficially alters plasma lipid, folate, and homocyst(e)ine concentrations in subjects with mild-to-moderate hypercholesterolemia.

SUBJECTS AND METHODS

Subjects

Twenty-five subjects (16 men and 9 postmenopausal women) with a mean (±SD) age of 55 ± 11 y and an average body weight of 78 ± 13 kg were recruited from 2 family practice clinics and 1 outpatient clinic in London, Canada. Most of the participants had moderately elevated initial plasma total and LDL-cholesterol concentrations (5.5–8.4 and 3.3–5.1 mmol/L, respectively), whereas 5 subjects were mildly hypercholesterolemic or normocholesterolemic (plasma total and LDL-cholesterol concentrations of 4.4–5.2 and 2.4–3.1 mmol/L, respectively). To be eligible for participation, individuals had to *1*) have initial fasting plasma triacylglycerol concentrations in the normal range (0.8–2.6 mmol/L in 24 subjects and 3.4 mmol/L in 1 subject); *2*) be habitual or occasional orange juice drinkers; *3*) be free of thyroid disorders, kidney disease, and diabetes; *4*) have an alcohol intake of ≤2 drinks/d; and *5*) not be receiving hormone replacement therapy if female. Most of the participants had not been taking cholesterol-lowering medication before the study; those who were (2 women and 4 men) were asked to discontinue the treatment 6 wk before the study began. Participants were also advised to follow the American Heart Association (AHA) Step I lipid-lowering diet for 6 wk before the study began and for the duration of the trial and to avoid taking supplements (eg, vitamins, minerals, or flavonoids) during the study. The experimental protocol was approved by the Human Ethics Committee of the University of Western Ontario and informed consent was obtained from each subject.

Experimental protocol

Standard dosing design procedures were followed, the details of which are described below. Participants consumed the AHA Step I diet during the entire 17-wk study. During that time, subjects were asked to incorporate 1, 2, or 3 cups (250 mL each) of orange juice (Tropicana Pure Premium; Tropicana Canada, Mississauga, Canada) into their diets daily, sequentially, for 3 separate 4-wk periods. This dietary period was followed by a 5-wk washout period. Fasting blood samples were drawn from the antecubital vein of the forearm before the onset of the study (baseline), after each dietary period, and after the washout period. Plasma lipoproteins (VLDL, LDL, and HDL) were separated by discontinuous density-gradient ultracentrifugation, as described by Redgrave et al (12).

Cholesterol and triacylglycerol concentrations were measured in the Clinical Biochemistry Laboratory of the London Health Science Centre (Canada). The evaluations were made with enzymatic timed-endpoint methods by using CHOL Reagent or Triacylglycerol GPO reagent (Beckman Coulter Canada Inc, Mississauga, Canada) on SYNCHRON LX Systems (Beckman Coulter Canada Inc). Plasma concentrations of apo B and apo A-I were analyzed immunonephelometrically at Sick Children Hospital (Toronto) with a BNII System (Dade-Behring Canada Inc, Mississauga, Canada) by using antisera to either human apo A-I (code OUED for apo B) or apo B (code OSAN for apo A-I). A reference curve for each apolipoprotein was generated by using a standard protein serum, and validity controls were run each time the instrument was used. Plasma folate concentrations were evaluated in the clinical laboratory at the London Health Science Centre by using an ACS-180 chemiluminescent automated analyzer (Chiron, Walpole, MA). Plasma homocyst(e)ine determinations were completed at the Robarts Research Institute (London, Canada) by using the HPLC method of Jacobsen et al (13). Plasma vitamin C (a compliance marker) was measured by HPLC (14) in the clinical laboratory of St Joseph's Hospital (London, Canada). Three-day food records were obtained at baseline and during each dietary period, including one weekend day. Practical instructions for completing food records were provided by one of the authors (PS), a registered dietitian. A dietitian maintained contact with the participants at least weekly to ensure comprehension of and compliance with the dietary regimens.

The composition of the orange juice consumed by the participants during each dietary period is presented in **Table 1**. The juice content of all components, except hesperidin (the glucoside of hesperetin, which is abundant in orange juice) and limonoids, was provided by Tropicana Products Inc (Bradenton, FL). The hesperidin concentration in orange juice was measured by reversed-phase HPLC with a method developed by one of the authors (DJF). Briefly, a gradient was developed across the analytic column from 5% acetonitrile in 10 mmol phosphate buffer/L (pH 3.0) to 25% acetonitrile in 10 mmol phosphate buffer/L (pH 3.0) over 15 min at a flow rate of 0.5 mL/min. The column (Nova-Pak C_{18}, 4 μm, 5 cm × 0.39 cm; Canada Waters Ltd, Mississauga, Canada) was maintained at 40 °C and the effluent was monitored at 280 nm. An aliquot of orange juice was centrifuged for 2 min at 145 × *g* at room temperature in an Eppendorf microfuge (VWR Canada, Mississauga) and the supernate diluted 1:10 with the 5% acetonitrile mobile phase. Ten microliters of this mixture

TABLE 2
Energy and nutrient intakes at baseline, after each dietary period, and after a 5-wk washout period[1]

Energy and food component	Baseline	Dietary period 1	Dietary period 2	Dietary period 3	Washout
Energy (kJ)	6943 ± 2852	7266 ± 2885	7510 ± 1915	8043 ± 2163	7468 ± 2654
Percentage of energy from orange juice (%)	1.4 ± 2.3	6.9 ± 5.8[2]	12.5 ± 3.4[2]	17.7 ± 5.7[2]	6.8 ± 10.7[2]
Protein (g)	74 ± 25	70 ± 25	80 ± 27	77 ± 25	79 ± 37
Carbohydrates (g)	233 ± 101	242 ± 96	248 ± 72	282 ± 94[2]	235 ± 92
Total fat (g)	50 ± 29	52 ± 25	55 ± 18	53 ± 21	58 ± 26
SFA (g)	17 ± 12	18 ± 11	18 ± 7	17 ± 7	20 ± 10
PUFA (g)	8 ± 5	7 ± 4	7 ± 3	7 ± 4	9 ± 5
P:S	0.5 ± 0.2	0.5 ± 0.2	0.4 ± 0.2	0.5 ± 0.2	0.5 ± 0.3
MUFA (g)	17 ± 10	18 ± 9	17 ± 6	17 ± 8	21 ± 10
Fiber (g)	19.0 ± 9.9	17.7 ± 9.7	15.8 ± 7.7	14.8 ± 7.4[2]	14.0 ± 8.5[2]
Vitamin C (mg)	104 ± 73	128 ± 49	191 ± 50[2]	260 ± 45[2]	133 ± 116
Folate (μg)	209 ± 87	213 ± 73	249 ± 75	350 ± 95[2]	237 ± 111
Vitamin D (mg)	4.8 ± 11.3	3.0 ± 6.6	2.2 ± 2.3	3.4 ± 5.3	2.4 ± 2.1
Sodium (mg)	2555 ± 1228	2830 ± 1142	2842 ± 1365	2798 ± 784	3673 ± 3489
Calcium (mg)	599 ± 312	595 ± 345	586 ± 323	655 ± 307	673 ± 354
Cholesterol (mg)	189 ± 122	161 ± 95	197 ± 96	205 ± 136	196 ± 108

[1] $\bar{x}$ ± SD. Values derived from 3-d dietary food records. SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; P:S, ratio of polyunsaturated to saturated fatty acids.
[2] Significantly different from baseline, $P < 0.05$ (ANOVA followed by Dunnett's t test).

was analyzed and the peak area of hesperidin was compared with that of a standard solution (10 mg/L). Concentrations were calculated electronically with an integrating recorder and correction was made to account for the dilution factor. Limonoid glucoside, limonin, and limonin glucoside concentrations in commercial orange juice were reported previously (15).

Statistics

Changes from baseline after periods 1, 2, and 3 and after the washout period were analyzed by using repeated-measures analysis of variance (ANOVA) followed by Dunnett's t tests. Tukey's honestly significant difference test was used for pairwise comparisons of percentage changes from baseline during the experimental periods. Statistical analyses were performed by using SAS (SAS Institute Inc, Cary, NC).

RESULTS

Energy and nutrient intakes at baseline, after each dietary period, and after the washout period are presented in **Table 2**. These data were derived from the food records. The intakes of total energy, protein, total fat and its constituent fatty acids (saturated, polyunsaturated, and monounsaturated fatty acids), calcium, sodium, and cholesterol were not significantly different between treatment periods. The percentage of energy from orange juice increased significantly from baseline after periods 1, 2, and 3 and after the washout period. (Five subjects, 3 men and 2 women, continued to consume large amounts of orange juice during the washout period.) Fiber intake decreased steadily during the study and was significantly lower than baseline after period 3 and after the washout period. The tendency for the intake of fiber to decrease during periods 1, 2, and 3 may have been due to the sequential increase in orange juice consumption during this time. This was confirmed by the simultaneous increased intake of carbohydrates (largely from orange juice) over the same period, which was significantly higher after period 3 than at baseline. The maintenance of the lower-than-baseline fiber intake during the washout period may have been due in part to the consumption of high-energy foods at the end of the treatment periods because the intakes of total fat, saturated fatty acids, and monounsaturated fatty acids during that period tended to be higher than those at baseline (although the differences were not significant). As expected, vitamin C and folate intakes were significantly affected by the consumption of orange juice. Vitamin C intakes were significantly higher than those at baseline after periods 2 and 3 and folate intakes were significantly higher than those at baseline after period 3. Intakes of both vitamin C and folate returned to baseline values during the washout period.

Changes in the subjects' baseline characteristics during the treatment with increasing doses of orange juice and during the subsequent washout period are presented in **Table 3**. The dietary intervention had no significant effect on body weight (not shown), body mass index (BMI; in kg/m^2), apo B, apo A-I , or most plasma lipid concentrations. However, HDL-cholesterol and total plasma triacylglycerol concentrations increased by 21% ($P < 0.001$) and 30%, ($P < 0.02$), respectively, whereas the LDL-HDL cholesterol ratio decreased by 16% ($P < 0.005$) during the same period. Pairwise comparisons showed that the percentage changes from baseline in HDL cholesterol and in the LDL-HDL cholesterol ratio during period 3 were significantly different from those during periods 1 and 2 but were not significantly different between periods 1 and 2 (data not shown). The increases from baseline in plasma triacylglycerol concentrations observed during period 3 were significantly positively correlated with the changes in VLDL cholesterol ($r^2 = 0.29$, $P = 0.006$).

In addition to influencing blood lipids, consumption of orange juice had a significant effect on plasma concentrations of vitamin C and folate (Table 3). Plasma vitamin C concentrations were substantially elevated during all treatment periods ($P < 0.001$). The responses progressed in a sequential manner with increasing doses of orange juice (2.3-, 3.1-, and 3.8-fold increases from baseline during periods 1, 2, and 3, respectively). Plasma folate concentrations increased by 18% ($P < 0.01$) during the treatment with 750 mL orange juice/d but remained unchanged from

TABLE 3
BMI and vitamin C, blood lipid, folate, and homocyst(e)ine concentrations at baseline, after each dietary period, and after a 5-wk washout period

Variable	Baseline	Dietary period			Washout
		1	2	3	
BMI (kg/m^2)	27.9 ± 4.4	28.0 ± 4.5	27.9 ± 4.4	27.7 ± 4.5	27.6 ± 4.6
Plasma cholesterol level (mmol/L)					
Total	6.3 ± 1.0	6.4 ± 0.9	6.4 ± 1.0	6.5 ± 0.9	6.3 ± 1.0
VLDL	0.8 ± 0.4	1.0 ± 0.6	0.8 ± 0.4	0.9 ± 0.4	0.8 ± 0.4
LDL	3.6 ± 0.7	3.8 ± 0.6	3.7 ± 0.7	3.6 ± 0.7	3.5 ± 0.7
HDL	1.0 ± 0.3	1.0 ± 0.3	1.1 ± 0.3	1.2 ± 0.3[2]	1.3 ± 0.4[2]
Change in HDL cholesterol (%)	—	5	7	21	27
LDL:HDL cholesterol	3.8 ± 0.9	3.8 ± 0.9	3.6 ± 1.0	3.1 ± 0.9[2]	3.0 ± 1.0[2]
Change in LDL:HDL cholesterol (%)	—	0	−4	−16	−20
Total triacylglycerol (mmol/L)	1.6 ± 0.7	1.9 ± 1.0	1.8 ± 0.8	2.0 ± 0.9[2]	1.7 ± 1.0
Apolipoprotein B (g/L)	1.4 ± 0.2	1.4 ± 0.2	1.4 ± 0.2	1.4 ± 0.2	1.4 ± 0.2
Apolipoprotein A-I (g/L)	1.4 ± 0.2	1.4 ± 0.2	1.4 ± 0.2	1.4 ± 0.2	1.4 ± 0.2
Vitamin C (μmol/L)	8.5 ± 3.3	19.1 ± 4.8[2]	26.8 ± 11.6[2]	32.5 ± 16.3[2]	15.7 ± 7.3[2]
Folate (nmol/L)	37.5 ± 10.3	38.9 ± 12.9	40.7 ± 13.2	44.1 ± 15.4[2]	41.0 ± 10.6
Homocyst(e)ine (μmol/L)	10.8 ± 2.2	10.9 ± 3.4	10.1 ± 2.3	10.6 ± 2.6	10.3 ± 2.2

[1] $\bar{x}$ ± SD.
[2] Significantly different from baseline, $P < 0.05$ (ANOVA followed by Dunnett's t test).

baseline during the treatment with 250 or 500 mL orange juice/d. Plasma homocyst(e)ine concentrations were not significantly affected by the dietary intervention (Table 3).

By the end of the washout period, the significantly lower LDL-HDL cholesterol ratio and the elevated HDL-cholesterol and plasma vitamin C concentrations had not returned to baseline values (Table 3). In fact, the increase in HDL cholesterol and the decrease in the LDL-HDL cholesterol ratio observed during the washout period tended to be greater than those observed during period 3 (a 27% increase and a 20% decrease, respectively). The 1.8-fold increase in plasma vitamin C produced during the washout period was less pronounced than that produced during period 3. The rise in plasma folate concentration observed during period 3 was reversed during the washout period. However, folate concentrations during the washout tended to remain higher than those at baseline, although the difference was not significant. HDL-cholesterol concentrations during the washout and the changes from baseline in HDL cholesterol after the washout were not positively correlated with plasma vitamin C concentrations during that period. Additionally, HDL-cholesterol concentrations did not tend to be elevated in subjects who consumed large amounts of orange juice during the washout period. Plasma triacylglycerols and other indexes [eg, BMI and plasma apo B, apo A-I, homocyst(e)ine, and total, VLDL, and LDL cholesterol], which were not influenced by the intake of orange juice, were not significantly different from baseline during the washout period.

To determine whether selected baseline indexes (HDL cholesterol and the LDL-HDL cholesterol ratio) were important in producing beneficial plasma lipid responses in subjects treated with orange juice, regression analysis was carried out between these indexes and changes from baseline in plasma lipid and folate concentrations during period 3. The results showed that changes in the LDL-HDL cholesterol ratio induced by the intake of 750 mL orange juice/d were significantly inversely related to the initial LDL-HDL cholesterol ratio ($r^2 = 0.23$, $P = 0.016$). Similarly, changes in HDL-cholesterol concentrations tended to be inversely correlated with baseline HDL cholesterol but the association was not significant. Changes in plasma triacylglycerol and plasma folate concentrations induced by the highest dose of orange juice were not significantly correlated with the initial LDL-HDL cholesterol ratio or with the initial HDL-cholesterol concentration (data not shown).

Because many previous studies showed that diet-induced increases in serum folate are associated with decreases in plasma homocyst(e)ine concentrations, regression analysis was also carried out between serum folate and plasma homocyst(e)ine concentrations during treatment with the highest dose of orange juice. The results showed no significant relation between the 2 indexes when all data were included. A slight significant inverse correlation was shown after exclusion from the analysis of 2 subjects with unusually high plasma homocyst(e)ine concentrations ($r^2 = 0.20$, $P = 0.03$); however, values after the exclusion were still nonsignificant by ANOVA.

DISCUSSION

This study showed, in a group of subjects consisting mainly of individuals with mild-to-moderate hypercholesterolemia, that consumption of 750 mL (3 cups) but not of 250 or 500 mL orange juice/d for 4 wk improved the plasma lipoprotein profile by significantly increasing HDL-cholesterol concentrations and by reducing the LDL-HDL cholesterol ratio. The reduction in the LDL-HDL cholesterol ratio observed during treatment with the highest dose of orange juice was entirely due to changes in HDL-cholesterol concentrations. This observation contrasts with a substantial reduction in LDL cholesterol induced in orange juice–fed, hypercholesterolemic rabbits (7), with a cholesterol-lowering effect of citrus flavonoids observed in rats (8) and with the lack of changes in plasma lipids in normocholesterolemic, young men consuming unspecified doses of fresh orange juice for 2 mo (11). The disagreement between our data in humans and the results in animals could be due to the lower amount of the juice or its minor components consumed by participants of the study than by rabbits or rats. Differences in responses between human trials could be due to the fact that some of the participants had hypercholesterolemia initially and some did not. The

increases in HDL cholesterol but not in apo A-I concentrations observed during the treatment with 750 mL orange juice/d suggest that the beneficial alterations occurred mostly in HDL_2, a subclass of HDL containing greater proportions of cholesterol but lower proportions of apo A-I than another major HDL subclass, HDL_3 (16). This response could provide an additional cardioprotective effect because previous studies reported a reduction in HDL_2 but no changes in HDL_3 in individuals with coronary heart disease (17).

Significant elevations in plasma triacylglycerol produced in response to treatment with 750 mL orange juice/d did not exceed the normal range (Table 3) and may not be clinically significant or result in increased cardiovascular risk. Similar changes were also found in hypercholesterolemic rabbits (7) but not in normocholesterolemic men given orange juice (11). The effect was most likely not due to fructose and sucrose, which are abundant in orange juice, because increases in plasma triacylglycerol induced by these sugars in human trials (18, 19) were associated with a decline rather than an increase in HDL cholesterol (19) and because plasma triacylglycerols were not elevated in rabbits fed grapefruit juice, which consumed only 23% less of both sugars than animals given orange juice (7). Likewise, previous studies do not suggest that increases in plasma triacylglycerols are due to intake of hesperidin. In rats, serum triacylglycerols were not altered by consumption of a 0.1%-hesperidin diet (8) and actually decreased after consumption of a 10%-hesperidin diet, presumably because of the inhibition of lipase (20).

The 18% increase in plasma folate concentration induced by treatment with the highest dose of orange juice (providing 188.4 μg folate/d) was relatively moderate. After similar 4–5-wk periods, more pronounced increases in plasma folate were reported (30%, 50%, and 100%, respectively) for individuals with coronary heart disease supplemented with 127 μg folic acid/d (21), for healthy subjects who consumed a vegetable- and citrus-rich diet enriched with 350 μg folate/d (5), and for women who consumed 250 μg folic acid/d (22). However, other studies showed that in young women, a dose of 400 μg folic acid/d increased plasma folate concentrations only after 8 wk of consumption and not after 4 wk (23). Treatment with 750 mL orange juice/d did not decrease plasma homocyst(e)ine, although its lowest concentrations were generally found in subjects with the highest plasma folate concentrations. The lack of association may have been due to insufficient increases in plasma folate or to a relatively low dose of folate in the juice. As suggested, folate intakes need to be ≥200 μg/d to produce decreases in plasma homocyst(e)ine (24).

The residual effect of the orange juice intervention on plasma HDL cholesterol and on the LDL-HDL cholesterol ratio observed during the washout period was unlikely due to continued consumption of large amounts of juice by some subjects, as confirmed by a lack of correlation between plasma vitamin C and HDL-cholesterol concentrations during the washout. The tendency toward more pronounced responses observed for plasma HDL cholesterol and for the LDL-HDL cholesterol ratio during the washout period (27% and −20%, respectively) than during period 3 (21% and −16%, respectively) suggests a long-term effect of the juice components, possibly flavonoids, on hepatic lipoprotein metabolism. This could not be verified in vivo because after oral administration of orange juice, plasma hesperetin concentrations are below accurate detection limits (25). However, in our previous study in HepG2 cells, the apo B–lowering effect of citrus flavonoids was partly reversible, implying a long-term effect of these compounds or their metabolites on lipoprotein metabolism in the liver (9).

A significant negative correlation between the baseline LDL-HDL cholesterol ratio and the reduction in this ratio caused by the intake of 750 mL orange juice/d indicates that individuals with the highest initial LDL-HDL cholesterol ratio are most likely to experience a reduction in this ratio by consuming orange juice. A similar association was found in hypercholesterolemic subjects after consumption of a diet enriched with soybean products (26).

Our results do not imply that consumption of large quantities of orange juice (≈20% of daily energy) should be recommended to hypercholesterolemic individuals. According to the US food guide pyramid, a healthy diet includes ≥5–10 servings of fruit and vegetables daily as well as adequate quantities of fiber. Thus, cardioprotective nutrients in amounts equivalent to those in 750 mL orange juice should be provided from a combination of different foods.

We thank Carina Banh, Nicki Mitek, and Megan Smith for their expert technical assistance.

REFERENCES

1. Bors W, Heller W, Michel C, Saran M. Flavonoids as antioxidants: determination of radical scavenging efficiencies. Methods Enzymol 1990;186:343–55.
2. Cook NC, Samman S. Flavonoids—chemistry, metabolism, cardioprotective effects, and dietary sources. J Nutr Biochem 1996;7:66–76.
3. Charleux JL. Beta-carotene, vitamin C, and vitamin E: the protective micronutrients. Nutr Rev 1996;54:S109–14.
4. Jacques PF, Selhub J, Bostom AG, Wilson PWF, Rosenberg IH. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. N Engl J Med 1999;340:1449–54.
5. Brouwer IA, Dusseldorp MV, West CE, et al. Dietary folate from vegetables and citrus fruit decreases plasma homocysteine concentrations in humans in a dietary controlled trial. J Nutr 1999;129:1135–9.
6. Anthony MS, Clarkson TB, Hughes CL Jr, Morgan TM, Burke GL. Soybean isoflavones improve cardiovascular risk factors without affecting the reproductive system of peripubertal rhesus monkeys. J Nutr 1996;126:43–50.
7. Kurowska EM, Borradaile NM, Spence JD, Carroll KK. Hypocholesterolemic effects of dietary citrus juices in rabbits. Nutr Res 2000;20:121–9.
8. Bok SH, Lee SH, Park YB, et al. Plasma and hepatic cholesterol and hepatic activities of 3-hydroxy-3-methyl-glutaryl-CoA reductase and acyl CoA:cholesterol transferase are lower in rats fed citrus peel extract or a mixture of citrus bioflavonoids. J Nutr 1999;129:1182–5.
9. Borradaile NM, Carroll KK, Kurowska EM. Regulation of HepG2 cell apolipoprotein B metabolism by the citrus flavanones hesperetin and naringenin. Lipids 1999;34:591–8.
10. Kurowska EM, Hasegawa S, Manners GD. Regulation of apo B production in HepG2 cells by citrus limonoids. In: Berhow EM, Hasegawa S, Manners GD, eds. Citrus limonoids: functional chemicals in agriculture and foods. Washington, DC: American Chemical Society, 2000:175–84. (ACS Symposium Series 758.)
11. Harats D, Chevion S, Nahir M, Norman Y, Sagee O, Berry EM. Citrus fruit supplementation reduces lipoprotein oxidation in young men ingesting a diet high in saturated fat: presumptive evidence for an interaction between vitamins C and E in vivo. Am J Clin Nutr 1998;67:240–5.
12. Redgrave TG, Roberts DCK, West CE. Separation of plasma lipoproteins by density gradient ultracentrifugation. Anal Biochem 1975;63:42–9.
13. Jacobsen DW, Gatautis VJ, Green R, et al. Rapid HPLC determination of total homocysteine and other thiols in serum and plasma: sex differences and correlation with cobalamin and folate concentrations in healthy subjects. Clin Chem 1994;40:873–81.

14. Wagner E, Lindley B, Coffin R. High performance liquid chromatography determination of ascorbic acid in urine. J Chromatogr 1979;163:225–9.
15. Fong CH, Hasegawa S, Herman Z, Ou P. Limonoid glucosides in commercial citrus juices. J Food Sci 1990;54:1505–6.
16. Gidez LI, Miller GJ, Burstein M, Slagle S, Eder HA. Separation and quantitation of subclasses of human plasma high density lipoproteins by a simple precipitation procedure. J Lipid Res 1982;23: 1206–23.
17. Gofman JW, Young W, Tandy R. Ischemic heart disease, atherosclerosis, and longevity. Circulation 1966;34:679–97.
18. Hollenbeck CB. Dietary fructose effects on lipoprotein metabolism and risk for coronary artery disease. Am J Clin Nutr 1993;58(suppl): 800S–9S.
19. Truswell AS. Food carbohydrates and plasma lipids—an update. Am J Clin Nutr 1994;59(suppl):710S–8S.
20. Kawaguchi K, Mizuno T, Aida K, Uchino K. Hesperidin as an inhibitor of lipases from porcine pancreas and *Pseudomonas*. Biosci Biotechnol Biochem 1997;61:102–4.
21. Malinow MR, Duell PB, Hess DL, et al. Reduction of plasma homocyst(e)ine levels by breakfast cereal fortified with folic acid in patients with coronary heart disease. N Engl J Med 1998;338:1009–15.
22. Brouwer IA, van Dusseldorp M, Thomas CMG, et al. Low-dose folic acid supplementation decreases plasma homocysteine concentrations: a randomized trial. Am J Clin Nutr 1999;69:99–104.
23. Bronstrup A, Hages M, Prinz-Langenohl R, Pietrzik K. Effects of folic acid and combinations of folic acid and vitamin B-12 on plasma homocysteine concentrations in healthy, young women. Am J Clin Nutr 1998;68:1104–10.
24. Ward M, McNulty H, McPartlin J, Strain JJ, Weir DG, Scott JM. Plasma homocysteine, a risk factor for cardiovascular disease, is lowered by physiological doses of folic acid. Q J Med 1997;90:519–24.
25. Ameer B, Weintraub RA, Johnson JV, Yost RA, Rouseff RL. Flavanone absorption after naringin, hesperidin, and citrus administration. Clin Pharmacol Ther 1996;60:34–40.
26. Kurowska EM, Jordan J, Spence JD, et al. Effects of substituting dietary soybean protein and oil for milk protein and fat in subjects with hypercholesterolemia. Clin Invest Med 1997;20:162–70.