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Effect of Supplemental Phytonutrients on Impairment of the Flow-Mediated Brachial Artery Vasoactivity After a Single High-Fat Meal

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OBJECTIVES Our objective was to determine if long-term daily administration of phytonutrient supplements can prevent the immediate adverse impact of a high-fat meal and increase the production of nitric oxide.

BACKGROUND Ingestion of a high-fat meal impairs flow-mediated vasodilation of the brachial artery for at least 4 h; however, co-ingestion of vitamin antioxidants or a green salad has been shown to prevent this effect.

METHODS Flow-mediated brachial artery reactivity test (BART) both before and 3 h after a 900 calorie 50 g fat meal was evaluated in 38 healthy volunteers (age 36.4 ± 10.1 years). Subjects were randomized to four weeks of daily supplementation with a powdered fruit vegetable juice concentrate (Juice Plus [JP]) along with a complex supplement providing nutritional antioxidants and various herbal extracts (Vineyard [V]), JP alone, or a matching placebo. At three and four weeks, BART was repeated both before and after the high-fat meal. Serum nitrate/nitrite concentrations were measured at baseline and at four weeks.

RESULTS Four weeks of the JP-V combination blunted the detrimental effect of the high-fat meal (−47.5 ± 23.4% at baseline vs. −1.7 ± 9.7% at four weeks [$p < 0.05$]). Four weeks of JP alone had a similar beneficial effect (−45.1 ± 19.7% at baseline vs. −16.6 ± 10.3% at four weeks [$p < 0.05$]), whereas there was no substantial effect of the placebo. In the subjects treated with supplements, concentrations of serum nitrate/nitrite increased from 78 ± 39 to 114 ± 62 μm/l ($p < 0.02$).

CONCLUSIONS Daily ingestion of modest amounts of a fruit/vegetable juice concentrate with or without adjunctive phytonutrient supplementation can reduce the immediate adverse impact of high-fat meals on flow-mediated vasoactivity and increase nitrate/nitrite blood concentration. (J Am Coll Cardiol 2003;41:1744–9) © 2003 by the American College of Cardiology Foundation

A high intake of fruits and vegetables has been associated with reduced risk for coronary heart disease and ischemic stroke in large prospective case-control studies (1–5). The mechanisms by which fruit and vegetable consumption achieves this benefit remain to be established. They can provide a substantial ration of dietary potassium and soluble fiber and are typically rich in a range of antioxidant phytonutrients. Previous studies show that antioxidant vitamins—notably C and E—can often exert a favorable effect on endothelial function (6–11). In particular, they appear to help preserve the endothelium's capacity to generate bioactive nitric oxide (NO) in various pathogenic circumstances that tend to impair this activity. Nitric oxide acts to stabilize platelets, promote vasodilation, inhibit smooth muscle migration and hyperplasia, and maintain an anti-inflammatory endothelial phenotype that discourages the influx of activated monocytes and other inflammatory cells. To the extent that dietary antioxidants can offset the harmful impact of endothelial oxidants on NO bioactivity, they have the potential to make an important contribution to vascular health (11).

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A sudden increase in the shear stress acting on arterial endothelium induces an endothelium-dependent, NO-mediated vasodilation that is susceptible to non-invasive quantitation by high-frequency ultrasound techniques (12,13). Flow-mediated vasodilation (FMV) of the brachial artery, evoked by the hyperemia that follows relief of pressure-cuff occlusion of arterial blood flow, is now commonly measured to assess the endothelium's capacity for generating bioactive NO in conduit arteries. This FMV is often impaired in patients expressing coronary risk factors associated with endothelial dysfunction, such as hypercholesterolemia, hypertension, hyperhomocysteinemia, insulin resistance, diabetes, and angina (14–24). Our group has previously demonstrated that this vasodilation is transiently impaired for up to 4 h after the ingestion of a fatty meal,

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Abbreviations and Acronyms

BART = brachial artery reactivity test
FMV = flow-mediated vasodilation
JP = Juice Plus
LDL = low-density lipoprotein
NAD(P)H = nicotinamide adenine dinucleotide phosphate (reduced)
NO = nitric oxide
V = Vineyard

presumably owing to the impact of triglyceride-rich lipoproteins and/or high free fatty acid levels (7,25,26); this finding has been confirmed by others (27–32). We have also found that if ample doses of vitamins C and E are administered in conjunction with a fatty meal, the subsequent deterioration of FMV is substantially prevented (7); this suggests that induced endothelial oxidant stress mediates all or most of the adverse impact of the high-fat meal. The objective of this study is to determine whether long- or short-term administration of fruit and vegetable concentrates rich in a range of antioxidant phytochemicals could provide a comparable benefit.

METHODS

Patient population. Thirty-eight healthy, physically active hospital employees—14 men and 24 women—age 36.4 ± 10.1 years (mean ± SD) were studied. None of the subjects had a history of coronary heart disease, hypertension, diabetes mellitus, or tobacco abuse. Written informed consent was obtained from all subjects, and the protocol was approved by the Institutional Review Board of the University of Maryland at Baltimore.

Baseline evaluation. Studies were begun at 8 AM after a 12-h overnight fast. Fasting blood was drawn for serum total, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein cholesterol, triglycerides, and homocysteine. These assays were performed in the hospital's clinical chemistry laboratory. Combined serum nitrate/nitrite concentrations were determined by a two-step reaction. Each determination required the use of 40 μl of undiluted plasma. The first step consisted of an enzymatic conversion of nitrate to nitrite utilizing nitrate reductase. The second step is the addition of Greiss reagent that converts nitrite to a deep purple azo chromophore, which is quantified at 540 nm and compared to a series of nitrite standards. Homocysteine levels were measured by the high-performance liquid chromatography method. Brachial artery vasodilation was assessed at baseline and after a high-fat meal.

Evaluation of endothelial function. Flow-mediated brachial artery vasodilation was measured using a previously described non-invasive technique (7). Briefly, FMV was assessed in the subject's left arm in the recumbent position in a temperature-controlled room (22°C) after a 10-min equilibration period by a single dedicated ultrasonographer.

Table 1. Assayed Vitamin and Nitrate Content of Juice Plus and Vineyard

	Juice Plus (4 capsules)	Vineyard (4 capsules)
Beta-carotene (i.u.)	9,275	7,188
Vitamin C (mg)	20.4	51
Vitamin E (mg)	24.7	30.4
Thiamin HCL (mg)	0.75	0.17
Riboflavin (mg)	trace	0.03
Niacin (mg)	0.37	1.3
Pyridoxine (mg)	0.07	0.35
Nitrate (mg)	5.7	0.76

With use of 7.5-MHz linear array ultrasound, the brachial artery was longitudinally imaged approximately 5 cm proximal to the antecubital crease, twice at baseline and then 1 min after release of 5 min of upper arm arterial occlusion with a 12.5-cm-wide blood pressure cuff. Photographic images of end-diastolic frames were obtained and were analyzed by two independent investigators blinded to the subject's identity and temporal sequence. Arterial diameter was determined by caliper measurement at the single most equivalently imaged site using side-by-side presentation. Blood pressure and heart rate were also measured.

Flow-mediated vasodilation was quantified as the percent of diameter change of the post-occlusion arterial diameter measurement relative to the mean of the corresponding two baseline measurements. The means of the two measurements by independent observers were calculated. The reproducibility of the two baseline measurements (SD of difference) was 0.87% (coefficient of variation, 4.3%).

After this, the subjects ate a high-fat meal and were re-studied 3 h after consumption of the meal. The high-fat meal (3,766 kJ [50 g of fat, 14 g of saturated fat, 225 mg of cholesterol]) consisted of an Egg McMuffin, Sausage McMuffin, two hash brown patties (McDonald's Corporation), and a non-caffeinated beverage.

Randomization. The subjects were then randomized in a double-blind and 2:2:1 fashion to one of three regimens: Juice Plus (JP), Juice Plus and Vineyard (JP-V), or placebo. Juice Plus is a commercially available capsule supplement providing dried juice concentrates derived from a variety of fruits and vegetables. The fruit concentrate was derived from apples, oranges, pineapples, papaya, cranberries, and peaches. The vegetable concentrate was derived from carrots, parsley, beets, broccoli, kale, cabbage, spinach, and tomatoes. Vineyard is a proprietary capsule supplement incorporating arginine hydrochloride, coenzyme Q10, L-carnitine, mixed tocopherols, ascorbic acid, dried berry juices and extracts, and multiple herbal extracts, including ginkgo biloba, hawthorn berry, grape skin, grape seed, and green tea. Many of these ingredients possess antioxidant activity, and arginine supports effective endothelial production of NO. Table 1 provides the vitamin composition of V and of JP. Two opaque capsules were taken twice a day in a double-blind manner for a four-week period after the

Table 2. Percent Change in Diameter Before and After High-Fat Meal

	Baseline		3 Weeks		4 Weeks	
	Fasting	3 h Postprandial	Fasting	3 h Postprandial	Fasting	3 h Postprandial
Placebo (n = 10)	20.2 ± 4.2	11.7 ± 5.4*	15.7 ± 5.2	9.9 ± 4.9*	15.3 ± 5.3	10.2 ± 4.7*
Juice Plus (n = 14)	13.2 ± 4.2	7.63 ± 3.7*	9.4 ± 4.6	6.4 ± 4.8†	9.7 ± 5.7	7.4 ± 4.0†
Juice Plus and Vineyard (n = 12)	15.8 ± 6.7	8.0 ± 4.5*	15.8 ± 7.1	12.4 ± 4.8†	12.8 ± 6.2	11.2 ± 4.7†

*$p < 0.05$ versus fasting; †not significant versus fasting level.

baseline study. The capsules were withheld on the morning of the four-week study.

Follow-up studies. Subjects were studied at three weeks (taking the morning dose) and at four weeks (omitting the morning dose). All fasting blood studies performed at baseline were repeated at four weeks. Brachial artery studies were performed similarly to those performed before randomization.

Statistical analysis. Group values are expressed as mean ± SD. Two-tailed paired *t* test was used to compare changes in individual subjects, and two-tailed non-paired *t* tests were used to compare values between groups. A p value <0.05 was considered significant.

RESULTS

Preprandial diameter at baseline (before randomization) was 2.7 ± 0.2, 3.1 ± 0.5 mm, and 3.1 ± 0.6 mm, and blood pressure was 113 ± 6/71 ± 7 mm Hg, 116 ± 6/73 ± 6 mm Hg, and 116 ± 9/73 ± 7 mm Hg in subjects randomized to placebo, JP, or combined JP-V, respectively. These values did not change significantly over the four weeks of the study.

Preprandial and postprandial FMV determinations at baseline (before randomization) and at three and four weeks in the three groups are shown in Table 2. The postprandial percent of FMV compared with the preprandial value (mean ± SD) decreased −40.9 ± 17.9% in the placebo group, −45.1 ± 19.7% in the JP group, and −47.5 ± 23.4% in the combined JP-V group. Differences among the groups at baseline were not statistically significant. There was a trend for the pre-meal FMV to decrease over time, but this did not reach statistical significance. Figure 1 shows the percent of decrease in vasoactivity from preprandial to postprandial for each of the three groups.

In the placebo group, the percent of decrease in vasoactivity with the high-fat meal remained high at three and four weeks (−37.1 ± 19.7% and −37.6 ± 23.4%, respectively). In the JP and JP-V group at both three and four weeks, the percent of decrease in postprandial vasodilation was significantly less than the decrease found before supplementation. The percent of decrease in the JP group after the high-fat meal was −22.3 ± 12.6% at three weeks and −16.6 ± 10.3% at four weeks ($p < 0.05$ compared with the baseline values). The percent of decrease in the JP-V group after the high-fat meal was −13.7 ± 10.2% at three weeks ($p < 0.05$) and only −1.7 ± 9.7% at four weeks ($p < 0.02$ compared with baseline values). Thus, JP and JP-V at three and four weeks significantly decreased the detrimental effect of the high-fat meal on endothelial function.

Lipoprotein, homocysteine, and serum nitrate/nitrite determinations at baseline and at four weeks are shown in Table 3. Total cholesterol and LDL cholesterol decreased significantly ($p < 0.05$) in the group that received JP over the four-week study period, but they did not change in the groups taking JP-V or placebo. There were no changes in other lipoproteins or homocysteine in any group. There was a trend for serum nitrate/nitrite levels to increase in each of the active-treatment groups. When these two groups were combined, serum nitrate/nitrite levels rose from 78 ± 39 μm/l to 114 ± 62 μm/l ($p < 0.02$). There was no significant correlation between the increase in these levels and the change in vasodilation.

DISCUSSION

This study found that the daily use for four weeks of a fruit/vegetable juice concentrate, rich in antioxidant phytochemicals, blunted the detrimental impact of a single high-fat meal on the endothelial function of healthy subjects; similar results were seen with a more complex supple-

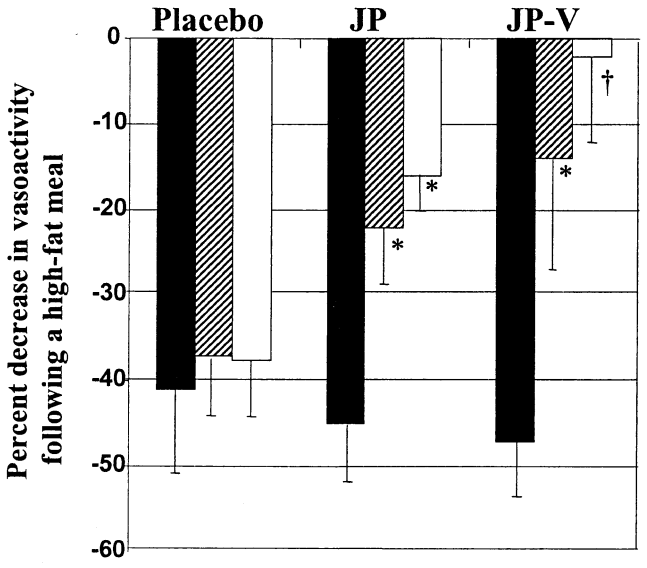

Figure 1. Postprandial decreases on brachial artery vasoactivity at 0, 21, and 28 days after a single high-fat meal in patients randomized to placebo, JP, or JP-V supplementation. *$p < 0.05$, †$p < 0.02$ compared with baseline (day 0). **Solid bars** = day 0; **shaded bars** = day 21; **open bars** = day 28. JP = Juice Plus; V = Vineyard.

Table 3. Lipoprotein, Homocysteine, and Combined Nitrite/Nitrite Levels in 38 Normal Subjects Before and After 28 Days of Placebo or Supplemental Phytonutrient Supplementation

	Placebo n = 10		Juice Plus n = 14		Juice Plus and Vineyard n = 12	
	Baseline	4 Weeks	Baseline	4 Weeks	Baseline	4 Weeks
Total cholesterol (mg/dl)	195 ± 36	191 ± 31	184 ± 30	172 ± 22*	185 ± 31	182 ± 24
LDL cholesterol (mg/dl)	123 ± 36	123 ± 25	110 ± 25	100 ± 26*	113 ± 27	113 ± 22
HDL cholesterol (mg/dl)	56 ± 13	53 ± 14	53 ± 16	51 ± 14	54 ± 16	52 ± 13
Triglycerides (mg/dl)	77 ± 32	77 ± 36	104 ± 45	101 ± 73	85 ± 29	84 ± 34
Homocysteine (μm/l)	6.2 ± 1.1	5.9 ± 0.7	6.8 ± 0.9	6.8 ± 1.3	8.1 ± 2.1	7.8 ± 2.3
NO (μm/l)	63 ± 39	68 ± 31	77 ± 43	110 ± 64†	84 ± 45	115 ± 59†

*$p < 0.05$ versus baseline; †$p < 0.1$ versus baseline. All values are mean ± SD.
HDL = high-density lipoprotein; LDL = low-density lipoprotein; NO = nitrate/nitrite serum level.

mentation regimen incorporating various nutrients and herbal extracts in addition to the fruit/vegetable juice concentrate. This extends our previous observation that pretreatment with a single dose of antioxidant vitamins is effective when taken *immediately before* a high-fat meal (7).

The present design enables us to conclude that prolonged antioxidant phytochemical supplementation can exert a long-term effect such that endothelial function is protected from the adverse impact of a fatty meal even if antioxidants are not administered with that meal. It is interesting to note that, whereas the supplementation provided in this study provided only modest doses of any one antioxidant, the cumulative effect of these antioxidants appeared at least comparable to the protection afforded by large doses of two supplemental antioxidants in our previous study.

Although many previous studies have demonstrated that supplemental intakes of vitamins C and E can benefit impaired endothelium-dependent vasodilation (6–11), relatively few reports are available evaluating the potential of food phytochemicals in this regard. We previously have reported that incorporation of a dark green salad into an olive oil–rich meal offsets the adverse impact of the olive oil on endothelial function (26). Another report indicates that long-term ingestion of a 40% fat diet impairs brachial artery FMV but that daily ingestion of red wine—known to be rich in antioxidant polyphenols—prevents this effect (33). In patients with coronary disease, ingestion of purple grape juice for 14 days likewise has a favorable impact on FMV (34). In these studies with wine and grape juice, endothelium function was assessed in the morning under fasting conditions, without immediate pre-administration of the protective food; thus, their findings are concordant with ours.

Endothelial dysfunction is often associated with, and at least in part mediated by, increased endothelial superoxide generation (11,35). The demonstrable utility of antioxidant vitamins for blunting the adverse impact of fatty meals on endothelial function suggests that enhanced endothelial superoxide production may mediate the concurrent impairment of FMV. The fact that superoxide production by stimulated leukocytes increases sharply after such meals (30,31) is seemingly consistent with this view, inasmuch as the membrane-bound NAD(P)H oxidase system appears to be primarily responsible for superoxide generation in both leukocytes and endothelium (24,35–39). Furthermore, exposure of endothelial cells to free fatty acids has been shown to stimulate superoxide production (40,41). Oxidation of LDL particles renders them more detrimental to endothelial function (42). Thus, there is reason to suspect that an ample dietary intake of antioxidants may promote endothelial health and, more specifically, limit the adverse influence of fatty meals on endothelial function. The results of this study appear consistent with this view.

Owing to the fact that NO has an extremely short half-life, its production is most conveniently estimated by measuring serum or urinary levels of its chief metabolic products, namely nitrate and nitrite. It is interesting to note that serum levels of nitrate-plus-nitrite increased significantly in subjects receiving the active supplements in this study. This tells us nothing about the impact of such supplementation on endothelial superoxide production, but it suggests that endothelial NO synthase activity may be enhanced in healthy subjects by an increased intake of phytochemical antioxidants. Because green leafy vegetables contain nitrates, it's conceivable that the JP supplement may have contributed modestly to dietary nitrate intake, but this is unlikely to account for the magnitude of the observed increase in serum levels of NO metabolites. Because protein intake was not specifically controlled for several days before measurement, serum nitrite/nitrate levels have limited reliability. Nitric oxide is known to be the primary mediator of flow-induced brachial vasodilation (43); therefore, the favorable impact of phytochemical supplementation on endothelial function in this study seems likely to reflect preservation of NO bioactivity.

Although antioxidant protection can be provided by high supplemental intakes of specific antioxidant vitamins—vitamins C and E have received the most attention in this regard—there may be limitations to the efficacy of this approach. In prospective cohort studies, high intakes of vitamin E have been linked to a notable reduction in coronary risk (44–46). Nonetheless, except for the small CHAOS trial (47), randomized primary and secondary prevention trials evaluating supplemental vitamin E have demonstrated a lack of beneficial effect of this antioxidant on cardiovascular events (48–50), in contrast to the notably

favorable impact of statin therapy over the same time span. Conceivably, this reflects the fact that vitamin E, as a lipid-soluble antioxidant, can only indirectly influence the oxidation of water-soluble targets such as tetrahydrobiopterin and dimethylarginine dimethylaminohydrolase. Vitamin C, on the other hand, is a versatile water-soluble antioxidant, and a sharp increase in plasma vitamin C levels has often been shown to improve endothelium-dependent vasodilation. Nonetheless, the membrane transporter primarily responsible for carrying ascorbic acid into endothelial cells appears to be saturated by plasma ascorbate concentrations—50 to 100 micromolar (51)—that can be achieved and maintained with a daily intake of about 200 mg (52), this suggests that the protection achievable with vitamin C, while worthwhile, may be limited in scope. These considerations suggest that ingestion of a wide range of both water-soluble and lipid-soluble antioxidants, including but not limited to vitamins C and E, may represent the most effective strategy for achieving comprehensive antioxidant protection of vascular endothelium, and a high intake of fruits and vegetables is a very practical means of attaining this goal. The clear evidence from prospective epidemiological studies linking high fruit and vegetable intake—independent of other dietary factors—with reduced coronary risk may in large measure reflect a beneficial impact of phytochemical antioxidants on endothelial health.

Despite strong clues from epidemiology, the thesis that a high intake of fruits and vegetables diminishes coronary risk still requires validation in prospective controlled trials. The encouraging results of this study suggest that it might be feasible to use encapsulated fruit and vegetable concentrates, analogous to the JP product evaluated here, in such a trial. The merit of this approach is that the study could be conducted in a double-blind fashion without influencing the intake of other foods. Of course, further small studies validating the utility of products such as JP would be required before it would be prudent to use those products in massive long-term prospective trials.

The Vineyard product incorporated in one arm of this study contains a diverse assortment of phytochemicals, herbs, and nutrients that potentially could influence endothelial function, including 1 g daily of arginine hydrochloride. The mechanism by which the administered supplements abrogate the adverse impact of fatty meals on endothelial function has not been determined. We speculate that the active supplements lessened the impact of oxidative stress in vascular endothelium and thereby counteracted the ability of chylomicron remnants and/or free fatty acids to suppress NO synthase activity and NO bioactivity.

CONCLUSIONS

In conclusion, a high-fat meal causes a temporary decrease in flow-mediated brachial artery vasodilatation, likely owing to an induced increase in endothelial oxidative stress. In healthy volunteers, four weeks of daily supplementation with a fruit-and-vegetable juice concentrate, with or without an adjunctive complex phytochemical supplement, blunts the detrimental effect of a high-fat meal on flow-mediated brachial artery vasodilation—even when the supplement is not administered with the meal—and increases the combined serum nitrite/nitrate concentration. However, whether preservation of NO bioactivity mediates the observed benefit is still speculative.

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