

Hypertension

Dietary Intake of Fruits and Vegetables Improves Microvascular Function in Hypertensive Subjects in a Dose-Dependent Manner

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Background—Observational evidence has consistently linked increased fruit and vegetable consumption with reduced cardiovascular morbidity; however, there is little direct trial evidence to support the concept that fruit and vegetable consumption improves vascular function. This study assessed the dose-dependent effects of a fruit and vegetable intervention on arterial health in subjects with hypertension.

Methods and Results—After a 4-week run-in period during which fruit and vegetable intake was limited to 1 portion per day, participants were randomized to consume either 1, 3, or 6 portions daily for the next 8 weeks. Endothelium-dependent and -independent arterial vasodilator responses were assessed by venous occlusion plethysmography in the brachial circulation before and after intervention. Compliance was monitored with serial contemporaneous 4-day food records and by measuring concentrations of circulating dietary biomarkers. A total of 117 volunteers completed the 12-week study. Participants in the 1-, 3-, and 6-portion/d groups reported consuming on average 1.1, 3.2, and 5.6 portions of fruit and vegetables, respectively, and serum concentrations of lutein and β -cryptoxanthin increased across the groups in a dose-dependent manner. For each 1-portion increase in reported fruit and vegetable consumption, there was a 6.2% improvement in forearm blood flow responses to intra-arterial administration of the endothelium-dependent vasodilator acetylcholine ($P=0.03$). There was no association between increased fruit and vegetable consumption and vasodilator responses to sodium nitroprusside, an endothelium-independent vasodilator.

Conclusions—The present study illustrates that among hypertensive volunteers, increased fruit and vegetable consumption produces significant improvements in an established marker of endothelial function and cardiovascular prognosis. (*Circulation*. 2009;119:2153-2160.)

Key Words: acetylcholine ■ endothelium ■ hypertension ■ nutrition

Meta-analyses of prospective cohort studies have suggested an association between increased fruit and vegetable consumption and a reduced risk of coronary heart disease¹ and stroke.² Such observational evidence has prompted the specific recommendation of increased fruit and vegetable consumption by both the British Hypertension Society³ and the American Heart Association.⁴

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Epidemiological evidence remains open to strong challenge on grounds of residual confounding,⁵ and few intervention studies have examined the conventional wisdom that eating more fruit and vegetables alone improves cardiovascular health. During the 8-week Dietary Approaches to Stop Hypertension (DASH) trial, increased fruit, vegetable, and

fish consumption was combined with reductions in saturated fat and dairy products to achieve significantly lower blood pressures among medication-naïve, hypertensive volunteers.⁶ The Lyon Diet Heart Study compared the effects of specific dietary advice to increase fruit, vegetable, fish, and α -linolenic acid consumption with standard predischarge counseling in patients with myocardial infarction.⁷ This trial was stopped after only 12 months owing to a definite intervention-associated reduction in cardiovascular morbidity, and an extended follow-up confirmed that event-free survival rates were still significantly greater in the intervention group after 5 years (risk ratio for cardiac death was 0.28, $P<0.0001$).⁸

Although the detailed, multifactorial dietary interventions of the DASH and Lyon Diet Heart Studies may indeed reduce morbidity, the 5-a-day fruit and vegetable public health

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message is more readily delivered and remembered. However, there is little clinically applicable randomized, controlled trial evidence to help quantify the vascular effects of any such isolated intervention.

Forearm blood flow responses to intra-arterial acetylcholine, an endothelium-dependent vasodilator, are known to independently predict cardiovascular morbidity among hypertensive patients,⁹ and improved acetylcholine-induced responses after 8 weeks have been significantly correlated with improved coronary event-free survival.¹⁰ With forearm blood flow responses to acetylcholine as a primary end point, the present study aimed to define the dose-dependent effects of fruit and vegetable consumption on arterial health among patients 40 to 65 years of age with grade I (140 to 159/90 to 99 mm Hg) or grade II (160 to 179/100 to 109 mm Hg) hypertension as defined in recent British guidelines.³ Compliance with the intervention was assessed by monitoring vitamin C and a panel of carotenoids, all of which have been proposed as biomarkers of fruit and vegetable consumption.¹¹ These are small molecules present in high concentrations in fruits and vegetables for which plasma levels show a reasonable correlation with fruit and vegetable consumption. They have a range of biological activities, including antioxidant properties, and may themselves contribute to the beneficial effects of fruits and vegetables.

Methods

Participants in this randomized intervention study were recruited between September 2004 and August 2006. Patients 40 to 65 years of age attending medical outpatient clinics were screened for hypertension. In addition, a press release inviting volunteers was distributed through local media sources. Potential participants had brachial blood pressure measured under standardized conditions (after at least 5 minutes of seated rest, with an Omron M5-I automatic blood pressure monitor [Omron Healthcare, Hoofddorp, Netherlands] in the dominant arm). Three consecutive measurements were recorded, and a summary blood pressure was calculated from the second and third readings. Participants were included if their blood pressure was 140 to 179/90 to 109 mm Hg. The study was approved by the ethics committee of Queen's University Belfast.

Exclusion Criteria

Exclusion criteria were diabetes mellitus, an acute coronary syndrome or transient ischemic attack within the past 3 months, special dietary requirements, food sensitivities or vegetarian/vegan diet by choice, oral anticoagulation therapy, body mass index >35 kg/m², excessive alcohol consumption (defined as >28 U/wk in men and 21 U/wk in women), fasting triglyceride concentration >4 mmol/L, or pregnancy/lactation. Suitable participants gave written informed consent. Baseline demographic information was gathered on smoking status, alcohol consumption, medications, and physical activity. Height and weight were measured according to standardized protocols.

Participants were randomized by use of a block design (block size=9; www.randomization.com) to 1 of 3 groups, consuming 1, 3, or 6 portions of fruit and vegetables daily for 8 weeks. Before this, all participants completed a 4-week run-in phase during which they were asked to consume only 1 portion of fruit and vegetables daily (Figure 1). A portion of fruit and vegetables was quantitatively defined with household measures as outlined by United Kingdom Food Standard Agency guidelines (www.eatwell.gov.uk). Participants were advised on suitable storage and cooking methods and were telephoned weekly to encourage compliance over the 12 weeks. During the 8-week intervention phase, each participant received

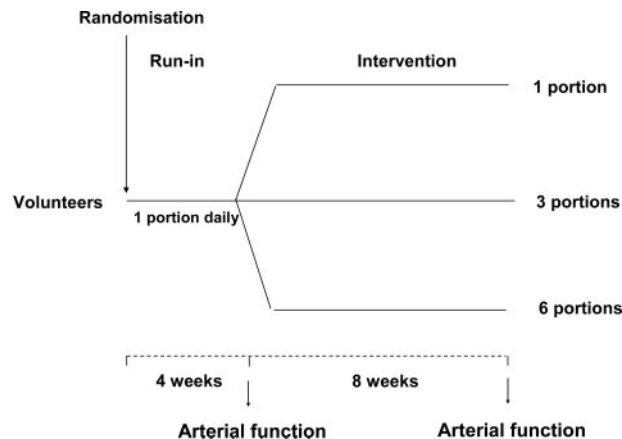


Figure 1. Overview of study design.

self-selected weekly home deliveries of fruit and vegetables from a local supermarket.

Each participant was asked to report newly prescribed or nonprescribed medications and any dose adjustments to existing therapies, and their physicians were encouraged to avoid any changes in medication unless there was a strong clinical indication for a change. Alteration of medical treatment during the 12-week trial was interpreted as an important change in clinical status and prompted exclusion from further participation. Volunteers were also asked to minimize changes in other health and lifestyle behaviors.

Dietary Assessment

Participants kept a series of 4-day (including 1 weekend day) food records: 1 at the start of the study to reflect normal diet, 1 during the run-in period, and 2 during the intervention period (at 4 and 8 weeks). A photographic atlas of portion sizes was included with the food diary.

From these diaries, the actual number of daily portions of fruit and vegetables consumed by the participants was counted. As noted above, a portion of fruit and vegetables was quantitatively defined with household measures as outlined by the United Kingdom's Food Standard Agency guidelines (www.eatwell.gov.uk), ie, 1 apple, 1 orange, half a grapefruit or 1 glass (150 mL) of fruit juice, or 3 tablespoons of vegetables.

Assessment of Vascular Function/Arterial Health

Participants attended for assessments of arterial health before and after the intervention. A dedicated, temperature-controlled vascular laboratory was used for testing. All testing was performed by a single investigator (D. McCall), who remained blind to intervention-phase dietary allocations throughout the collection and analyses of results.

On each occasion, participants attended between 8 and 9 AM having fasted and refrained from caffeine, alcohol, and smoking for at least 12 hours. Those who had been prescribed long-acting nitrate preparations were advised to delay taking them until after the assessment.

Each visit was structured identically. Participants spent 15 minutes of quiet, supine rest before blood pressure determination with the same protocol used at recruitment. A 30-mL blood sample was collected from the dominant arm, and finally, local blood flow responses to intra-arterial vasodilators were determined by venous occlusion plethysmography in the nondominant forearm. All blood samples were processed and frozen at -80°C within 2 hours of collection.

Forearm Blood Flow Studies

According to an established protocol,¹² venous occlusion plethysmography was used to determine forearm blood flow during incremental intra-arterial infusions of acetylcholine (50, 100, and 200 nmol/min) and sodium nitroprusside (5, 10, and 20 nmol/min). A

27-gauge sterile needle (Cooper's Needle Works, Birmingham, United Kingdom) was inserted into the nondominant brachial artery by an aseptic technique under local anesthesia. After successful puncture, saline was infused through the needle via an epidural catheter at a rate of 1 mL/min for at least 30 minutes. At baseline and during vasodilator administration, plethysmographic measurements were made with electrically calibrated mercury-in-Silastic strain gauges in both the infused and noninfused limbs, thus accounting for confounding by unexpected systemic or environmental stimuli.¹³ Each strain gauge was attached to a Hokanson EC6 plethysmograph (PMS Instruments, Maidenhead, United Kingdom), which in turn was connected to a dedicated personal computer on which the Hokanson NIVP3 software package (PMS Instruments) had been installed. Vasodilators were administered in random order, with each concentration infused for 5 minutes. After 3 minutes of each infusion step, 5 forearm blood flow readings were made during 7-second periods of venous occlusion separated by 15-second intervals.

NIVP3 software was used to calculate percent change in the infused:control arm blood flow ratio during each infusion step. The maximum vasodilator response observed with acetylcholine/sodium nitroprusside was used as a summary measure for that agent during statistical analysis.⁹

Laboratory Analysis

Plasma ascorbic acid concentrations were determined according to Vuilleumier and Keck.¹⁴ Serum concentrations of lutein, zeaxanthin, β -cryptoxanthin, α -carotene, β -carotene, and lycopene were measured by reverse-phase high-performance liquid chromatography (HPLC) as described by Craft.¹⁵ Assays were standardized against appropriate National Institute of Standards and Technology reference materials. Serum total cholesterol, triglycerides, and HDL cholesterol were measured with standard enzymatic assays (Randox, Crumlin, Northern Ireland) on an automated ILab-600 biochemical analyzer (Instrumentation Laboratories, Warrington, United Kingdom).

Statistical Analysis

Normally distributed continuous variables were summarized as mean and SD. Skewed variables were logarithmically transformed for parametric analysis and were summarized with the geometric mean and interquartile range.

Between-group comparisons of change in each outcome variable were made with 1-way ANOVA. Because the intervention involved increasing numbers of portions of fruit and vegetables, a test for linear trend across groups was used to obtain maximum power in the analysis of study end points. All tests were 2-tailed, and $P < 0.05$ was considered statistically significant. Analyses were performed with SPSS version 12.0.1 (SPSS, Inc, Chicago, Ill).

Power

Endothelium-dependent vasodilatation is an established index of arterial health during short-term intervention studies and was the primary end point of the present study. Because analysis would be based on between-group comparisons of change, data from previous investigators¹² were used to estimate that the SD of percentage change in maximum response to acetylcholine was $\approx 15\%$. With a test for linear trend across all 3 groups, ≈ 35 patients per group would be required to detect a 10% difference in this response variable between the low- and high-intake groups as being statistically significant with 80% power with a 2-tailed test at the 5% significance level.¹⁶

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Summary of Participant Recruitment

The number of participants at each stage of the study is summarized in Figure 2. At recruitment, each participant provided written consent and was assigned a unique study

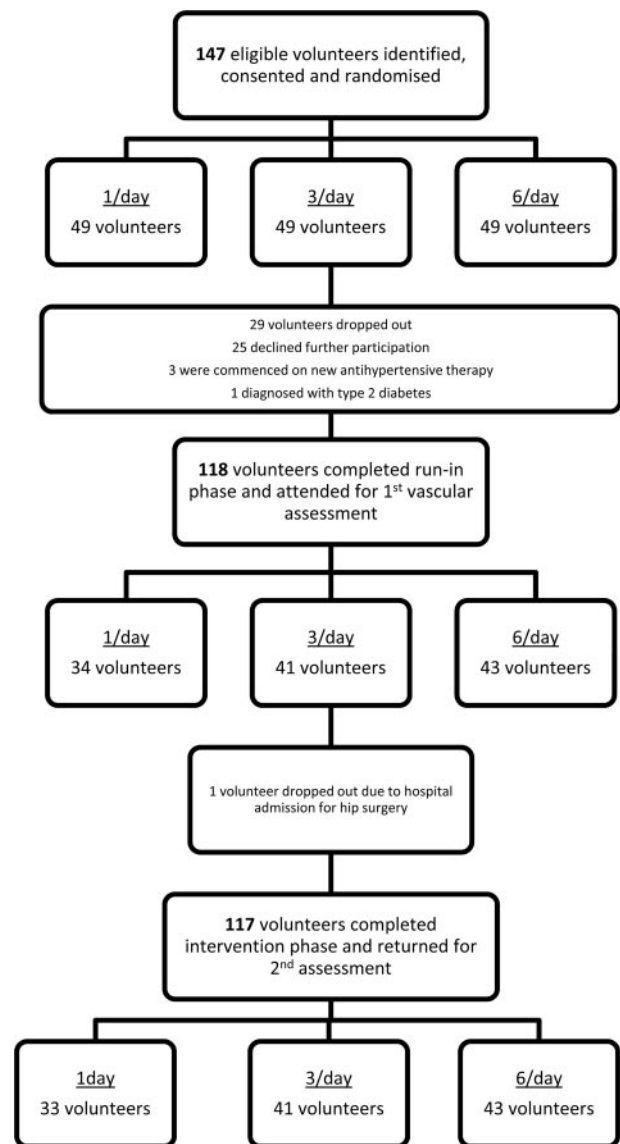


Figure 2. Progress of volunteers through study.

code. The latter corresponded to an intervention-phase fruit-and-vegetable-group allocation. A total of 147 eligible individuals agreed to participate and commenced the common run-in phase of 1 portion per day. There were 29 dropouts during this period, the majority among participants randomized to the 1-portion/d group for the intervention phase, because of a perceived difficulty in adhering to this allocation for a 12-week period. Once the intervention period had begun, only 1 participant failed to attend postintervention testing, because a cancellation had expedited this participant's elective hip surgery.

A total of 117 participants completed the 8-week intervention study. Five individuals had either preintervention or postintervention serum C-reactive protein values > 10 mg/L. In line with American Heart Association guidelines,¹⁷ this was taken as evidence of active infection or inflammation, and these participants were excluded from further analyses. Of the remaining 112 volunteers for whom data are presented, there were 3 missing blood samples, and brachial artery cannulation was unsuccessful on 3 occasions.

Table 1. Preintervention Phase Population Characteristics of 112 Participants According to Daily Fruit and Vegetable Allocation

	1/day (n _{max} =33)	3/day (n _{max} =39)	6/day (n _{max} =40)
Age, y	52.4±7.9	56.1±8.4	53.7±7.1
Men, %	70	62	63
Body mass index, kg/m ²	29.7±4.4	28.2±3.2	28.8±3.3
Systolic blood pressure, mm Hg	139.4±15.0	144.6±18.1	145.3±15.7
Diastolic blood pressure, mm Hg	82.0±11.9	81.1±11.1	86.3±11.0
Current smokers, %	39	3	15
Former smokers, %	36	44	35
Antihypertensive therapy, %	36	46	63
Lipid-lowering therapy, %	52	67	55
Triglycerides, mmol/L*	2.10 (1.54–3.07)	1.75 (1.14–2.68)	1.56 (1.09–2.52)
Total cholesterol:HDL ratio	4.37 (1.35)	4.18 (1.45)	4.31 (1.31)
C-reactive protein, mg/L*	1.98 (1.11–4.46)	1.59 (0.82–2.98)	1.40 (0.81–1.95)
Maximal response to acetylcholine, %*	276 (172–398)	389 (268–663)	348 (227–497)
Maximal response to sodium nitroprusside, %*	215 (144–313)	231 (166–347)	223 (139–345)

For all variables, n=33, 39, and 40 for the 1-, 3-, and 6-portions/d groups, respectively, except for triglycerides, cholesterol:HDL ratio, and C-reactive protein, for which n=32, 39, and 38, respectively, and maximal response to acetylcholine and to sodium nitroprusside, for which n=31, 38, and 39, respectively.

Continuous variables are summarized as mean±SD, except where data are skewed (*), and a geometric mean with interquartile range is quoted.

Baseline Characteristics

The habitual fruit and vegetable intake of the participants as reported during the first pre-run-in food record was a mean of 2.3 portions (184 g) per day. Preintervention characteristics after the 4-week run-in period are summarized in Tables 1 and 2. There were no significant imbalances between the groups at baseline.

Dietary Changes During the Intervention Phase

Analysis of preintervention and postintervention 4-day food records confirmed that reported fruit and vegetable intake increased significantly across the allocated groups ($P<0.001$ for linear trend; Table 3).

Changes in Circulating Micronutrient Concentrations

Preintervention and postintervention variations in micronutrient status according to group are shown in Table 4. Increases in lutein ($P=0.002$) and β -cryptoxanthin (<0.001) concentration were significant across the 3 intervention groups, whereas changes in vitamin C ($P=0.060$) and zeaxanthin ($P=0.089$) approached significance.

Intervention-Associated Changes in Functional Vascular Assessments

Preintervention and postintervention values for forearm blood flow responses, body mass index, blood pressure, and lipid parameters are summarized according to fruit and vegetable

Table 2. Preintervention Phase Characteristics of 108 Participants According to Daily Fruit and Vegetable Allocation: Dietary Intake of Fruit and Vegetables and Micronutrient Status

	1/day (n _{max} =31)	3/day (n _{max} =39)	6/day (n _{max} =38)
Fruit and vegetable intake, g/d	71 (60–90)	79 (60–102)	86 (60–115)
Fruit and vegetable intake, portions/d	0.9 (0.8–1.1)	1.0 (0.8–1.3)	1.1 (0.8–1.4)
Vitamin C, μ mol/L	23.6 (15.6–33.4)	26.2 (20.9–37.7)	27.9 (19.2–45.0)
Lutein, μ mol/L	0.13 (0.10–0.17)	0.15 (0.11–0.19)	0.16 (0.13–0.21)
Zeaxanthin, μ mol/L	0.03 (0.02–0.05)	0.03 (0.02–0.05)	0.04 (0.02–0.06)
β -Cryptoxanthin, μ mol/L	0.05 (0.03–0.08)	0.06 (0.04–0.09)	0.07 (0.04–0.11)
α -Carotene, μ mol/L	0.11 (0.08–0.18)	0.14 (0.10–0.22)	0.18 (0.12–0.24)
β -Carotene, μ mol/L	0.22 (0.14–0.33)	0.27 (0.15–0.52)	0.36 (0.20–0.51)
Lycopene, μ mol/L	0.58 (0.29–0.81)	0.59 (0.33–0.94)	0.67 (0.35–0.92)

For all variables n=31, 37, and 36 for the 1-, 3-, and 6-portions/d groups respectively, except for vitamin C, for which n=31, 39, and 38, respectively, and fruit and vegetable intake in g/day or portions per day, for which n=28, 34, and 36, respectively.

Continuous variables are summarized as geometric mean with interquartile range because data were skewed.

Table 3. Fruit and Vegetable Intake During the Run-In and Intervention Phases According to Daily Fruit and Vegetable Allocation

	Fruit and Vegetables (Portions)*			<i>P</i> (for Linear Trend)
	Pre	Post	Change From Baseline (95% CI)	
1/day (n=27)	0.9 (0.4)	1.1 (0.5)	0.2 (0.0–0.3)	
3/day (n=33)	1.1 (0.5)	3.2 (0.8)	2.2 (1.8–2.5)	<0.001
6/day (n=34)	1.1 (0.5)	5.6 (0.9)	4.5 (4.2–4.7)	

Pre indicates preintervention run-in phase; Post, intervention phase.

Preintervention and postintervention values are mean (standard deviation). The changes in each variable are expressed as percentage of baseline (95% CI). Changes were compared between groups by 1-way ANOVA, with linear trend fitted.

allocation in Table 5. Between-group comparison of change in maximum response to acetylcholine suggested a significant linear relationship between increasing fruit and vegetable consumption and improved endothelium-dependent forearm blood flow response ($P=0.02$ for linear trend). No such relationship was seen for sodium nitroprusside ($P=0.51$ for linear trend).

Reductions in systolic blood pressure were greater with increased daily fruit and vegetable allowance, but the test for linear trend across the groups failed to reach statistical significance ($P=0.19$). There were no significant between-group changes for any other variable.

Reanalysis With Fruit and Vegetable Consumption as a Continuous Variable

Statistical analysis was initially performed according to intervention allocation; however, food records completed before and after the intervention phase also allowed a more direct estimate of actual change in fruit and vegetable consumption for each participant who completed the study. The relationships between change in fruit and vegetable consumption and forearm blood flow responses to acetylcholine and sodium nitroprusside were examined by univariate linear regression analysis. With this approach, there appeared to be a significant positive relationship between increased fruit and vegetable consumption and endothelium-dependent forearm blood flow responses (shown in Figure 3; $P=0.032$).

No such relationship was seen for sodium nitroprusside ($P=0.170$). An extra portion of fruit and vegetables daily was estimated to increase the maximum response to acetylcholine by 6.2% (95% CI 0.5% to 12.2%).

Discussion

Although widely promoted through public health messages (for instance, www.eatwell.gov.uk) and advocated by clinical practice guidelines,³ the potential cardiovascular benefits of increased fruit and vegetable consumption have rarely been assessed within randomized, controlled trials. The present study attempted to establish whether a dose-response relationship exists between fruit and vegetable consumption and microvascular health in hypertensive volunteers. We have shown that each additional portion of fruit and vegetable consumed daily led to a 6.2% improvement in endothelium-dependent forearm blood flow responses.

Conclusions drawn from nutritional epidemiology are usually based on the prolonged follow-up of healthy professionals,¹⁸ and dietary intervention trials often selectively recruit medication-naïve participants in a controlled feeding design.⁶ By studying free-living individuals, many of whom were already taking antihypertensive and/or lipid-lowering therapy, we have attempted to maximize the clinical and public health application of the findings.

The use of venous occlusion plethysmography in quantifying forearm blood flow responses to endothelium-dependent agonists such as acetylcholine is well established in cardiovascular research.¹⁹ Evidence to support the role of this technique as an independent prognostic indicator in selected patient groups, however, has only emerged relatively recently.^{9,10} Although there is considerable variability in how the findings of forearm blood flow studies are expressed, we followed the example of Perticone and colleagues⁹ in adopting the maximum response to endothelium-dependent/independent vasodilator agonists as our primary outcome measure. These authors divided their cohort of 262 hypertensive patients into tertiles according to maximum acetylcholine-induced change in forearm blood flow, which demonstrated significantly more cardiovascular morbidity among the poorest responders (ie, the first tertile). The present study illustrated an absolute increase of $\approx 6\%$ in maximum endothelium-dependent forearm blood flow response for each

Table 4. Micronutrient Status During Run-In and Intervention Phases According to Daily Fruit and Vegetable Allocation

	1/day (n _{max} =31)		3/day (n _{max} =38)		6/day (n _{max} =38)		<i>P</i> for Linear Trend
	Pre	Post	Pre	Post	Pre	Post	
Vitamin C, $\mu\text{mol/L}$	23.7 (15.5–34.3)	25.8 (18.7–37.6)	25.7 (20.9–36.7)	38.9 (29.5–53.8)	27.9 (19.2–45.0)	42.3 (32.7–60.8)	0.06
Lutein, $\mu\text{mol/L}$	0.13 (0.10–0.17)	0.14 (0.11–0.18)	0.15 (0.11–0.19)	0.17 (0.13–0.24)	0.16 (0.13–0.21)	0.20 (0.15–0.27)	0.002
Zeaxanthin, $\mu\text{mol/L}$	0.03 (0.02–0.05)	0.03 (0.02–0.06)	0.03 (0.02–0.05)	0.04 (0.03–0.05)	0.04 (0.02–0.06)	0.04 (0.03–0.07)	0.089
β -Cryptoxanthin, $\mu\text{mol/L}$	0.05 (0.03–0.08)	0.05 (0.04–0.08)	0.06 (0.04–0.09)	0.07 (0.04–0.13)	0.07 (0.04–0.11)	0.11 (0.07–0.17)	<0.001
α -Carotene, $\mu\text{mol/L}$	0.11 (0.08–0.18)	0.12 (0.09–0.18)	0.14 (0.10–0.22)	0.14 (0.10–0.22)	0.18 (0.12–0.24)	0.20 (0.13–0.30)	0.82
β -Carotene, $\mu\text{mol/L}$	0.22 (0.14–0.33)	0.24 (0.17–0.34)	0.28 (0.16–0.52)	0.28 (0.16–0.44)	0.36 (0.20–0.51)	0.39 (0.23–0.75)	0.98
Lycopene, $\mu\text{mol/L}$	0.58 (0.29–0.81)	0.66 (0.36–1.02)	0.59 (0.33–0.94)	0.52 (0.20–0.88)	0.67 (0.35–0.92)	0.60 (0.27–0.94)	0.091

Pre indicates preintervention run-in phase; Post, intervention phase.

For all variables n=31, 37, and 36 for the 1-, 3-, and 6-portion/d groups, respectively, except for vitamin C, for which n=29, 38, and 38, respectively. All values are geometric mean (interquartile range). Changes were compared between groups with 1-way ANOVA, with linear trend fitted.

Table 5. Patient Descriptors During the Run-In and Intervention Phase According to Fruit and Vegetable Intervention Group

	1/day $n_{\max}=32$		3/day $n_{\max}=39$		6/day $n_{\max}=40$		<i>P</i> for Linear Trend
	Pre	Post	Pre	Post	Pre	Post	
Maximal response to acetylcholine, %*	285 (181–398)	274 (189–397)	389 (268–663)	409 (301–599)	348 (227–497)	436 (330–766)	0.02
Maximal response to sodium nitroprusside, %*	219 (153–316)	256 (183–375)	231 (166–347)	236 (174–318)	223 (139–345)	236 (177–367)	0.51
Body mass index, kg/m ²	29.9 (4.5)	29.0 (4.1)	28.2 (3.2)	28.5 (4.3)	28.8 (3.3)	29.5 (5.4)	0.14
Systolic blood pressure, mm Hg	138.9 (14.9)	137.5 (15.9)	144.6 (18.1)	140.6 (14.3)	145.3 (15.7)	140.1 (14.9)	0.19
Diastolic blood pressure, mm Hg	82.2 (12.1)	81.9 (10.2)	81.1 (11.1)	81.3 (8.4)	86.3 (11.0)	83.8 (11.4)	0.29
Cholesterol:HDL	4.4 (1.4)	4.5 (1.4)	4.1 (1.4)	4.1 (1.6)	4.3 (1.3)	4.3 (1.3)	0.64
Triglycerides, mmol/L*	2.1 (1.5–3.1)	2.1 (1.5–3.4)	1.7 (1.1–2.7)	1.7 (1.1–2.3)	1.6 (1.1–2.5)	1.8 (1.2–2.6)	0.11

Pre indicates preintervention run-in phase; Post, intervention phase.

For all variables, $n=32$, 39, and 40 for the 1-, 3-, and 6-portions/d groups, respectively, except for body mass index, for which $n=30$, 39, and 40, respectively, and maximum response to acetylcholine and maximum response to sodium nitroprusside, for which $n=31$, 38, and 39, respectively. Variables are summarized as mean (SD) or geometric mean (interquartile range) where data were skewed (*). Changes were compared between groups with 1-way ANOVA, with linear trend fitted.

additional portion of fruit and vegetables consumed daily. At baseline, the (geometric) mean maximum response to acetylcholine for all participants in the present study was 330%, which put them just between Perticone's second and third tertiles (185% to 333% and 339% to 760%, respectively). Because food records suggested that mean daily habitual fruit and vegetable intake was ≈ 2.5 portions, the present findings suggest that increasing this to a target of 5 portions per day would translate into significantly improved endothelium-dependent responses, which may in turn reduce cardiovascular morbidity, although this would have to be tested in a trial with hard clinical end points.

Such a conclusion must be qualified, however, to account for the experimental conditions under which it was drawn, as well as a consideration of the strengths and weaknesses of the

study design. A 4-week washout period of low fruit and vegetable consumption was used before randomization, and thus, assessments of arterial health made at this point are unlikely to reflect true baseline status. Although the inclusion of volunteers taking steady doses of antihypertensive and/or lipid-lowering medication widens the clinical relevance of the present study, it should also be acknowledged that these therapies influence microvascular reactivity. However, the fact that these medications were maintained at constant doses throughout the study minimizes the likelihood that inclusion of these participants would have influenced the study findings. Whether or not the dose-response relationship between fruit and vegetable consumption and endothelium-dependent vasodilatation proposed here holds true for individuals who usually consume >1 portion each day cannot be commented on definitively. Equally, a threshold effect beyond the maximum portion allocation used here cannot be excluded. The fruit and vegetable-associated improvements in endothelium-dependent forearm blood flow responses observed here can be contrasted with several negative ascorbic acid supplementation studies that have used a similar vascular assessment technique.^{20–22} In this context, it can be concluded that the positive findings of the present study are not a result of altered plasma ascorbate concentrations. Rather than searching for the single magic bullet micronutrient, a more practical approach is likely to consider whole foods and associated dietary patterns. Thus, increasing fruit and vegetable consumption is likely to have numerous beneficial effects due to synergistic effects of bioactive compounds that improve the vascular phenotype but may not be readily detected by routine clinical or biochemical examination.

Although the association between the change in forearm blood flow and change in fruit and vegetable intake was statistically significant, the value of $r=0.23$ is not particularly strong, and this suggests that the change in fruit and vegetable intake only explained 5% of the variation in forearm blood flow, with other factors also influencing it. This weak correlation is typical of nonpharmacological

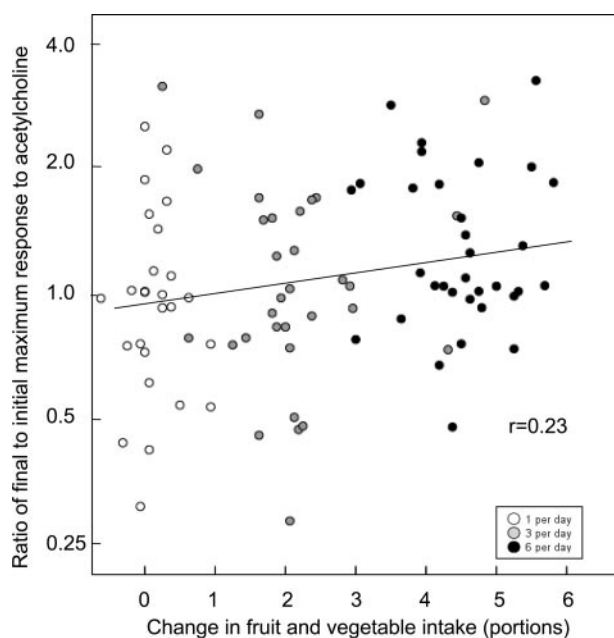


Figure 3. Change in maximum response to acetylcholine plotted against change in daily fruit and vegetable intake.

dietary interventions and also reflects the natural variability in forearm blood flow.

In the present study, we used plasma biomarkers of fruit and vegetable consumption to assess compliance with the intervention. The biomarkers that we measured may themselves have physiological effects, and increased levels could be mediators of the beneficial effects of fruit and vegetables. However, if this is the case, levels of mediators in the arterial endothelium may be more significant than plasma levels, and the relationship between plasma levels and tissue levels of these biomarkers is largely unknown.

Additional fruit and vegetable consumption may be associated with other potentially favorable dietary alterations, including reduced salt and fat consumption. Because the present work was conducted among free-living volunteers, these factors were not specifically controlled. Although food record analyses revealed that between-group comparisons of change in fat and salt intake did not reach statistical significance, trends toward reduced consumption of both of these were associated with increasing daily fruit and vegetable allowance. Thus, it remains plausible that the favorable vascular effects observed here are linked to reductions in potentially deleterious dietary elements. Equally, although volunteers were asked to minimize changes in other health and lifestyle behaviors, and they reported no changes in smoking or physical activity behaviors, subtle alterations of potentially relevant factors such as activity levels cannot be discounted.

There was a nonsignificant trend for systolic blood pressure reduction with increasing fruit and vegetable consumption. The magnitude of this decrease among those consuming 6 portions per day was comparable to that previously described in a larger study performed among medication-naïve volunteers.⁶ As expected, a small, nonsignificant fall in systolic blood pressure was noted among those participants randomized to 1 portion per day. This “regression to the mean” phenomenon is well described for blood pressure end points²³ and often necessitates large study sizes, particularly when relatively small between-group differences are anticipated, as would be expected with dietary rather than pharmacological interventions.

With variability data from the 1-portion/d group, a retrospective power calculation was undertaken, and it was estimated that to detect a 5-mm Hg difference in blood pressure change as being statistically significant (2-tailed test, $\alpha=0.05$) with 90% power, 115 participants per group would have been required. Although the present study was therefore undoubtedly underpowered to detect between-group differences in blood pressure reduction as being statistically significant, the trend observed, as well as the fact that we found significant within-group reductions in systolic blood pressure in both the 3- and 6-portions/d groups, would suggest that a larger study among similar participants may have positive findings. This hypothesis has important clinical and public health implications, because it suggests that a clinically relevant reduction in systolic blood pressure can be achieved through relatively simple dietary interventions among free-living patients, some of whom have already been established on antihypertensive medication.

In conclusion, among hypertensive participants, there is a significant dose-response relationship between fruit and vegetable consumption and endothelium-dependent vasodilation, with an extra daily portion improving the maximum forearm blood flow response to acetylcholine by $\approx 6\%$. This finding links an achievable dietary goal with improvement in a vascular measure of known prognostic value. Although this is an encouraging endorsement of the 5-a-day message, it also provides evidence that just eating 1 extra portion a day has potential benefits. This could be important for those who perceive 5-a-day as unattainable, and therefore, it has important public health implications.

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CLINICAL PERSPECTIVE

Observational evidence has consistently linked increased fruit and vegetable consumption with reduced rates of cardiovascular morbidity. Although health promotion literature and clinical guidelines suggest that eating 5 or more portions of fruit and vegetables daily may have beneficial vascular effects, this specific hypothesis has rarely been addressed in intervention studies in free-living participants. We conducted a randomized, controlled trial among 117 volunteers with mild hypertension to examine the dose-dependent effects of altered fruit and vegetable consumption on microvascular function. A significant relationship between increased fruit and vegetable consumption and improvements in forearm blood flow responses to intra-arterial acetylcholine was observed. Such findings link a potentially achievable dietary goal with favorable changes in an established predictor of cardiovascular morbidity. This study was conducted among free-living volunteers rather than under controlled feeding conditions, and many of the participants were taking antihypertensive and/or lipid-lowering therapies. These details allow application of our findings within a broader patient population and should encourage further trials to assess the impact of nutrition and lifestyle intervention on cardiovascular health. Such work is vital both in providing physicians with a sound evidence base for nonpharmacological prescription and in scientifically informing future public health advice.