

## Fruits and Vegetables Increase Plasma Carotenoids and Vitamins and Decrease Homocysteine in Humans<sup>1</sup>

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**ABSTRACT** Observational epidemiologic studies have shown that a high consumption of fruits and vegetables is associated with a decreased risk of chronic diseases. Little is known about the bioavailability of constituents from vegetables and fruits and the effect of these constituents on markers for disease risk. Currently, the recommendation is to increase intake of a mix of fruits and vegetables ("five a day"). We investigated the effect of this recommendation on plasma carotenoids, vitamins and homocysteine concentrations in a 4-wk dietary controlled, parallel intervention study. Male and female volunteers ( $n = 47$ ) were allocated randomly to either a daily 500-g fruit and vegetable ("high") diet or a 100-g fruit and vegetable ("low") diet. Analyzed total carotenoid, vitamin C and folate concentrations of the daily high diet were 13.3 mg, 173 mg and 228.1  $\mu\text{g}$ , respectively. The daily low diet contained 2.9 mg carotenoids, 65 mg vitamin C and 131.1  $\mu\text{g}$  folate. Differences in final plasma levels between the high and low group were as follows: lutein, 46% [95% confidence interval (CI) 28–64];  $\beta$ -cryptoxanthin, 128% (98–159); lycopene, 22% (8–37);  $\alpha$ -carotene, 121% (94–149);  $\beta$ -carotene, 45% (28–62); and vitamin C, 64% (51–77) ( $P < 0.05$ ). The high group had an 11% (–18 to –4) lower final plasma homocysteine and a 15% (0.8–30) higher plasma folate concentration compared with the low group ( $P < 0.05$ ). This is the first trial to show that a mix of fruits and vegetables, with a moderate folate content, decreases plasma homocysteine concentrations in humans. *J. Nutr.* 130: 1578–1583, 2000.

**KEY WORDS:** • fruits • vegetables • carotenoids • vitamins • homocysteine • humans

Observational epidemiologic studies have shown that a high consumption of fruits and vegetables is associated with a decreased risk of human cancer at a number of common sites (Block et al. 1992, Miller 1990, Negri et al. 1991, Steinmetz and Potter 1991, Weisburger 1991). A high consumption of fruits and vegetables may also be beneficial with respect to cardiovascular disease risk (Gey et al. 1993, Gramenzi et al. 1990, Hertog et al. 1993, Palgi 1981). The potential health benefits of fruits and vegetables have been attributed to the effects of specific components therein, i.e., vitamins, minerals, dietary fiber and a wide range of secondary metabolites (phytochemicals) responsible for characteristics such as color, flavor and taste. Several of these components have been hypothesized to exert an important influence on human physiologic status (Tomás-Barberán and Robins 1997). One possible defense mechanism of fruits and vegetables is the antioxidant capacity of several components, for example, carotenoids and vitamin C and E. Plasma carotenoids have been associated with a decreased risk of cancer (Astorg 1997) and cardiovascular disease (Palace et al. 1999). Two specific carotenoids,

lutein and zeaxanthin, may be important for protecting the macula lutea in the eye (Eye Disease Case-Control Study Group 1992 and 1993). Another protective mechanism of fruits and vegetables could be the potential influence of folate on homocysteine metabolism. Observational epidemiologic research has shown that a high plasma concentration of homocysteine may be a risk factor for cardiovascular disease. Several intervention studies have shown that supplementation with folic acid is effective in reducing plasma homocysteine concentration (Brouwer et al. 1999a, De Bree et al. 1997, Ubbink et al. 1994). Previous studies on increased fruit and vegetable consumption have been published, but these focused on specific nutrients or products. Zino et al. (1997) and Yeum et al. (1996) showed that plasma carotenoid and vitamin concentrations increased as a result of an increased intake of vegetables and fruits. However, fat intake, an important determinant of bioavailability of carotenoids, especially of lutein esters (unpublished observations), was not controlled in the study of Zino et al. (1997). Yeum et al. (1996) controlled the diet for fat, but selected specifically carotenoid-rich vegetables and fruits. In contrast, De Pee et al. (1995) suggested that  $\beta$ -carotene from vegetables may be poorly bioavailable in tropical diets. Brouwer et al. (1999b) concluded that consumption of foods rich in folate (vegetables and citrus fruits) could

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improve folate and homocysteine status. The current recommendation of health authorities (Steinmetz and Potter 1996) is to increase fruit and vegetable consumption in general (e.g., "five a day"). This is the first study to investigate the effects of this recommendation in a mixed population. The present study investigated whether an increased intake of a mix of fruits and vegetables with a controlled Western diet had effects in human volunteers on plasma carotenoids, vitamin C,  $\alpha$ -tocopherol, plasma homocysteine, vitamin B-12 and folate.

## MATERIALS AND METHODS

**Subjects.** Subjects aged 40–60 y were recruited from the pool of volunteers of the Institute and through an advertisement in a local newspaper. They were preselected on the basis of a low habitual fruit and vegetable consumption (<250 g/d) and a regular Dutch food intake pattern according to a questionnaire. The protocol was explained to the volunteers before they gave their informed consent. The major inclusion criteria were a body mass index [BMI; body weight (kg)/height<sup>2</sup> (m<sup>2</sup>)] <35 kg/m<sup>2</sup>, alcohol consumption <40 g/d ethanol (men) and 30 g/d ethanol (women), no use of vitamins or other food supplements, no metabolic or endocrine disease, no allergies to fruits and vegetables, not pregnant and/or lactating, no prescribed medication (except paracetamol and oral contraceptives) and a serum cholesterol concentration <8 mmol/L. A total of 48 (24 men and 24 women) eligible volunteers entered the study; 12 were smokers, 6 of each gender.

**Study protocol.** The study was performed according to the guidelines for Good Clinical Practice of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, and was approved by an external Medical Ethical Committee. We performed a 4-wk randomized, single-blind, parallel, diet-controlled intervention study. By design, participants were stratified for gender and smoking habits. Further, several random group (high/low) allocations were generated by a computer program. The random solution on the group level that yielded the most homogeneous age and BMI distribution was then

selected. Volunteers (n = 24) received a diet low in fruits and vegetables (100 g/d; "low" group); another 24 volunteers received a diet high in fruits and vegetables (500 g/d; "high" group) and also drank fruit juices (200 mL/d).

Food intakes were monitored every day by consumption of the evening meal under supervision at the Institute between 1700 and 1900 h. Remaining parts of the dinner were weighed and recorded. The rest of the daily diet (breakfast and lunch) was handed out to the volunteers in a box after the contents were checked. When the subjects returned the next evening, the consumption of breakfast and lunch was controlled and recorded by checking the box. The volunteers had to complete a form with the questions "Did you consume the total supplied amount of food today?" and "Did you consume foodstuffs and drinks other than the provided food and drinks?" The study was executed at the Department of Nutritional Physiology of TNO Nutrition and Food Research, Zeist from September 1997 to October 1997.

**Diets.** All volunteers received the same basic diet with different energy levels according to need (7.7, 8.7, 9.7, 10.7, 11.7 and 12.7 MJ/d). The energy intake of breakfast, lunch and snacks differentiated the energy levels of the diets. The diets were controlled for energy, fat, protein and carbohydrates. During the study period, body weights were measured twice weekly before the evening meal. If the measured body weight deviated  $\geq 1.5$  kg compared with the reference body weight at d 0, the subjects were shifted to a diet one energy level higher or lower. Following the Dutch National Food Consumption Survey-2, fruits and vegetables normally consumed in the age group 40–60 y were selected. Table 1 shows the 7-d menu cycle of fruits and vegetables of the two diets.

**Blood collection.** On d 1 and 29, blood samples were collected from fasting subjects. For the analysis of carotenoids, vitamin C and  $\alpha$ -tocopherol, blood was collected in tubes containing lithium heparin (Vacutainer systems, Becton Dickinson, Leiden, The Netherlands) and further prepared in yellow light. Within 30 min, the tubes were centrifuged (2000  $\times$  g, 10 min, 4°C). Metaphosphoric acid solution (50 g/L; J.T. Baker, Deventer, The Netherlands) was added to the plasma for the analysis of vitamin C. Samples were stored at

TABLE 1

7-d menu cycle of fruits and vegetables of the 'high' and 'low' groups during the dietary intervention period

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
	g						
High group dinner	french beans (140) red sweet pepper (20) mushrooms (20) onions (20) cucumber (40) tomatoes (35)	carrots (200) chicory (38) apple (20) orange (20)	red cabbage with apples (150) apple-sauce (50) lettuce (45) carrots (30)	cauliflower (200) cucumber (40) tomatoes (35)	sprouts (200) lettuce (50) red sweet pepper (25)	mushrooms (42) onion (30) lettuce (40) white cabbage (38) tomatoes (50) lettuce (63) corn (12)	endive (200) chicory (38) apple (20) orange (20)
High group during the day	orange (200) melon (100) orange juice (200 mL)	orange (200) tangerine (2 $\times$ 90) apple juice (200 mL)	apple (150) apple (75) orange juice (200 mL)	pear (160) kiwi (90) apple juice (200 mL)	apple (150) apple (75) orange juice (200 mL)	orange (200) tangerine (2 $\times$ 90) apple juice (200 mL)	banana (165) grapes (100) orange juice (200 mL)
Low group dinner	french beans (35) red sweet pepper (5) mushrooms (5) onions (5)	carrots (50)	red cabbage with apples (50)	cauliflower (50) cucumber (25) tomatoes (25)	sprouts (50)	mushrooms (15) onion (8) white cabbage (15) red sweet pepper (13) lettuce (42) corn (8)	endive (50) chicory (25) apple (12) orange (13)
Low group during the day	apple (50)	tangerine (50)	orange (50)		orange (50)		

–80°C until analysis. Blood was collected in tubes containing K<sub>3</sub>EDTA (Vacutainer systems, Becton Dickinson) for the analysis of vitamin B-12, folate and homocysteine. Within 15 min, blood was centrifuged (2000 × g, 10 min, 4°C). Plasma samples were immediately frozen in liquid nitrogen and stored at –80°C until analysis. Analyses were carried out after all samples were collected.

**Biochemical analyses.** Carotenoid profiles and  $\alpha$ -tocopherol in plasma were quantified by HPLC using a modified version of a method described previously (Van Vliet et al. 1991). After precipitation of proteins by ethanol (J.T. Baker), carotenoids and tocopherols were extracted from plasma by *n*-hexane (Merck, Darmstadt, Germany) and separated by HPLC (Hyperchrome column; Bisschof, Leonberg, Germany). Two absorbency detectors (ABI Analytical Kratos Division, South Yorkshire, UK) were used in series for the colorimetric determination of carotenoid profiles (450 nm) and  $\alpha$ -tocopherol (286 nm). The CV were as follows: lutein,  $\leq 16.6\%$ ; zeaxanthin,  $\leq 36.8\%$ ;  $\beta$ -cryptoxanthin,  $\leq 7.5\%$ ; lycopene,  $\leq 9.7\%$ ;  $\alpha$ -carotene,  $\leq 23.4\%$ ;  $\beta$ -carotene,  $\leq 6.3\%$ ; and  $\alpha$ -tocopherol,  $\leq 3.2\%$ .

Vitamin C in plasma containing metaphosphoric acid was quantified by HPLC using a modified version of a method previously described (Speek et al. 1984). All ascorbic acid was oxidized to dehydro-L-ascorbic acid using ascorbate oxidase (Boehringer). Quinoxaline derivatate was separated by reversed phase HPLC (Hyperchrome column, Bisschof) after condensation with 1,2-diaminobenzene (Merck). A fluorescence detector (Jasco, Tokyo, Japan) was used for the detection of vitamin C (CV  $\leq 11.6\%$ ).

Vitamin B-12 and folate were quantified by competitive protein-binding assay (Simultrac Radioassay Kit Vitamin B-12 [<sup>57</sup>Co] Folate [<sup>125</sup>I], ICN Pharmaceuticals, ICN Biomedicals, Zoetermeer, the Netherlands), according to Dunn and Foster (1973), Lau et al. (1965) and Kolhouse et al. (1978). In summary, vitamin B-12 and folate in plasma were extracted by heat denaturation of the endogenous binding proteins. In the presence of potassium cyanide (ICN Biomedicals) cobalamins were converted to cyanocobalamin. To prevent oxidation of 5-methyltetrahydrofolic acid, dithiothreitol (ICN Biomedicals) was added. After incubation with <sup>57</sup>CoB-12, <sup>125</sup>I folic acid, Intrinsic Factor (ICN Biomedicals) and  $\alpha$ -lactoglobuline (ICN Biomedicals), the radioactivity was counted. The CV were  $\leq 11.6\%$  and  $\leq 13.0\%$  for vitamin B-12 and folate, respectively.

Total plasma homocysteine was determined by HPLC with fluorescence detection using a method previously described (Ubbink et al. 1991). Before reversed-phase HPLC analysis, the plasma thiols were derivatized with ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate (SBD-F), a thiol-specific fluorogenic probe that is commercially available. Retention of SBD-homocysteine was sensitive to pH, and a mobile phase pH of 2.1 ensured baseline separation of plasma thiols within 6 min. The CV was  $\leq 4.5\%$ .

**Chemical analyses of diet.** Total weekly high and low diets were mixed and homogenates were made. Because the meals differed in energy level according to the need of the volunteers, a high and a low diet with a calculated energy level of 10.7 MJ was used for analysis. The homogenates were used for the analysis of vitamin C, carotenoids, vitamin E, vitamin B-6, folate and total energy. Another part of the homogenate was freeze-dried and ground, using a strainer. This freeze-dried homogenate was used for the analysis of protein, fatty acids, carbohydrates, sugars, fiber and glucosinolates. Flavonoid analyses were carried out only in fruits and vegetables with a measurable amount of flavonoids. All of these analyses were performed using standard methods. References are available upon request.

**Statistical analysis.** Data analyses were performed using the statistical software package SAS/STAT (Version 6, SAS Institute, Cary, NC, General Linear Models procedure). First, for description of our data, baseline values were compared between groups (*t* test), and baseline and final values within groups were examined by a paired *t* test. Second, to quantify the unbiased overall effect of the intervention, we used ANOVA to calculate the differences in final values of each variable between the low and high group after adjustment for gender, smoking habits and baseline plasma concentrations of each variable. Analysis of covariance was used for statistical testing. Data are reported as mean  $\pm$  SD unless stated otherwise and *P* < 0.05 indicated significant difference.

## RESULTS

The groups did not differ in age, BMI, total plasma cholesterol, systolic and diastolic blood pressure at baseline and in habitual daily intake of vegetables and fruits before the start of the study (Table 2). At baseline, plasma zeaxanthin concentrations were significantly different between the groups (*P* < 0.05). One participant in the low fruit and vegetable consumption group, a nonsmoking female, did not complete the study and was not replaced. Data for this subject were not used in the statistical analysis. The daily energy intakes of the low and high groups during the trial were 10.2  $\pm$  1.6 and 10.1  $\pm$  1.2 MJ, respectively. The total energy content of both diets was similar. In both diets, the energy percentages (en%) from fat, protein and carbohydrate were near those recommended for protein (12–13 en%), carbohydrate (40–55 en%) and fat (30–35 en%). The amounts of vitamin C, total carotenoids, folate, glucosinolates and flavonoids were 1.7-, 3.6-, 0.7-, 5.3- and 4.0-fold higher, respectively, in the high diet than in the low diet. The amount of  $\alpha$ -tocopherol and vitamin B-6 were comparable in the two diets (Table 3).

The overall compliance in both groups was good. The volunteers consumed 98.9 and 99.7% of the provided energy of the high and low diets, respectively. The compliance of fruit and vegetable intake separately was 99.7% of the energy in the high group and 99.8% of the energy in the low group. The changes in body weight after 25 d in the high and low groups were –1.5 and –1.7%, respectively (*P* < 0.05). These small reductions in body weight did not differ between groups.

In the low group, the concentrations of the carotenoids decreased significantly by 31–67%, depending on the carotenoid (Table 4). Vitamin C concentrations decreased significantly by 13% (*P* < 0.05) and  $\alpha$ -tocopherol concentrations decreased by 8% (*P* < 0.05) in this group. In the high group, there were significant increases in plasma concentrations of  $\alpha$ -carotene (67%), vitamin C (50%) and  $\beta$ -cryptoxanthin (28%), (*P* < 0.05). Plasma lutein and  $\beta$ -carotene concentrations did not change but plasma lycopene decreased by 56% (*P* < 0.05). Plasma  $\alpha$ -tocopherol concentrations decreased by 11% (*P* < 0.05), similar to the change in the low group.

After adjustment for gender, smoking habits and baseline plasma concentrations, the final plasma concentrations of the high in comparison with the low group were significantly higher for lutein, +46% [95% confidence interval (CI) +28 to +64];  $\beta$ -cryptoxanthin, +128% (+98 to +159); lycopene,

TABLE 2

Characteristics of participants at baseline in the 'low' and 'high' groups<sup>1</sup>

	Intervention	
	Low group	High group
Age, y	50 $\pm$ 4.38	48.6 $\pm$ 5.63
Men/women, n	12/11	12/12
Smokers, %	35	33
Intake of vegetables and fruits, g/d	158 $\pm$ 54	162 $\pm$ 46
Body weight, kg	77.5 $\pm$ 11.8	79.5 $\pm$ 11.4
Body mass index, kg/m <sup>2</sup>	25.2 $\pm$ 0.6	26.3 $\pm$ 0.7
Height, cm	175.2 $\pm$ 9.9	173.9 $\pm$ 7.5
Total plasma cholesterol, mmol/L	6.0 $\pm$ 0.9	5.9 $\pm$ 0.9
Systolic blood pressure, mm Hg	128 $\pm$ 19	126 $\pm$ 20
Diastolic blood pressure, mm Hg	78 $\pm$ 11	79 $\pm$ 14

<sup>1</sup> Values are means  $\pm$  SD unless stated otherwise.

TABLE 3

Daily intake of nutrients and energy during the dietary intervention period<sup>1</sup>

Energy/Nutrient	Intervention	
	'Low' diet	'High' diet
Total energy, MJ	11.2	11.4
Protein, en%	14.6	14.9
Total fat, en%	32.7	29.9
Carbohydrate, en%	53.5	53.3
Saturated fatty acids, en%	14.4	13.1
Monounsaturated fatty acids, en%	7.5	6.8
Polyunsaturated fatty acids, en%	6.5	6.0
Fiber, g	47.9	56.2
Vitamin C, mg	65.0	172.5
Total carotenoids, mg	2.9	13.3
Lutein, mg	0.5	2.0
Zeaxanthin, mg	0.04	0.13
$\beta$ -Cryptoxanthin, mg	0.04	0.27
Lycopene, mg	0.06	0.66
$\alpha$ -Carotene, mg	0.27	1.33
$\beta$ -Carotene, mg	0.63	2.98
$\alpha$ -Tocopherol, mg	17.6	19.9
Vitamin B-6, mg	1.61	1.96
Folate, $\mu$ g	131.1	228.1
Total glucosinolates, $\mu$ mol	2.3	14.6
Total flavonoids, mg	0.75	3.74

<sup>1</sup> Values are based on the chemical analyses of weekly diets (see Materials and Methods).

+22% (+8 to +37);  $\alpha$ -carotene, +121% (+94 to +149);  $\beta$ -carotene, +45% (+28 to +62); and vitamin C, +64% (+51 to +77).

No significant changes were found in either group for folate and vitamin B-12 after 4 wk of dietary intervention. Plasma homocysteine concentrations decreased significantly by 10% ( $P < 0.05$ ) in the high group (Table 5). Differences in final plasma levels between the high group and the low group (high-low) were as follows: folate, +15% (+0.8 to +30;  $P < 0.05$ ) and homocysteine, -11% (-18 to -4;  $P < 0.05$ ). Plasma vitamin B-12 concentrations did not differ between the two groups.

## DISCUSSION

The main findings of this study are that the consumption for 4 wk of 500 g fruits and vegetables in comparison with 100 g fruits and vegetables resulted in significantly higher plasma carotenoids concentrations, including lutein (46%),  $\beta$ -cryptoxanthin (128%), lycopene (22%),  $\alpha$ -carotene (121%),  $\beta$ -carotene (45%) and vitamin C (64%). Other important findings are an 11% lower plasma homocysteine concentration in the high group than in the low group. Furthermore, we found a small but significant plasma effect on folate (15%). Plasma concentrations of zeaxanthin,  $\alpha$ -tocopherol and vitamin B-12 were not affected by the intervention, as could be expected.

In this single blind trial, volunteers were carefully prestratified and were administered a habitual Dutch diet, which was completely controlled for energy, fat, protein and carbohydrate content. Reported compliance was very good because the major part of the diet containing fruits and vegetables was consumed under supervision. After the consumption for 4 wk of 100 g fruits and vegetables, plasma concentrations of carotenoids and vitamins decreased. The selected volunteers had an initial consumption of ~150 g/d fruits and vegetables. The observed decrease of plasma concentrations is not surprising. Also, our brief questionnaire may have underestimated fruit and vegetable consumption somewhat.

Plasma lycopene concentrations of subjects that consumed 500 g of fruits and vegetables were 22% greater after 4 wk compared with subjects that consumed 100 g fruits and vegetables. However, lycopene concentrations decreased in both groups. In a study of Yeum et al. (1996), lycopene concentrations increased significantly after consumption of a diet with a high carotenoid content (of the 16 mg/d, 3.3 mg/d was lycopene). Possible explanations for the decrease of plasma lycopene during our study include a lower lycopene intake during the study in comparison with the prestudy period and a limited bioavailability of lycopene from the nonprocessed products (Van het Hof et al. 1998). The preselection of volunteers was based on fruit and vegetable intake as a whole. The lycopene intake cannot be estimated from this questionnaire. In the prospective cohort study on diet and cancer in the Netherlands, the mean daily intake of lycopene was  $1.05 \pm 1.56$  and  $1.33 \pm 1.88$  mg/d for men and women, respectively (Gold-

TABLE 4

Effect of 100 g/d fruits and vegetables ('low group') and 500 g/d fruits and vegetables ('high group') on plasma carotenoids (all-trans isomers), vitamin C (ascorbic acid) and  $\alpha$ -tocopherol concentrations in fasting humans during a 4-wk dietary intervention period<sup>1</sup>

	Low group	Change	High group	Change	High-Low After study <sup>3</sup>
	(n = 23) <sup>2</sup>		(n = 24) <sup>2</sup>		
	Baseline		Baseline		
	$\mu$ mol/L				
Lutein	0.35 $\pm$ 0.16	-0.12 $\pm$ 0.12	0.32 $\pm$ 0.14	0.02 $\pm$ 0.11	0.11 (0.07-0.14)*
Zeaxanthin	0.05 $\pm$ 0.04	-0.02 $\pm$ 0.02	0.04 $\pm$ 0.02	-0.004 $\pm$ 0.02	0.005 (-0.004-0.01)
$\beta$ -Cryptoxanthin	0.13 $\pm$ 0.07	-0.04 $\pm$ 0.04	0.18 $\pm$ 0.20	0.05 $\pm$ 0.14	0.13 (0.11-0.16)*
Lycopene	0.52 $\pm$ 0.28	-0.35 $\pm$ 0.20	0.39 $\pm$ 0.26	-0.22 $\pm$ 0.18	0.04 (0.01-0.06)*
$\alpha$ -Carotene	0.06 $\pm$ 0.04	-0.02 $\pm$ 0.04	0.06 $\pm$ 0.02	0.04 $\pm$ 0.02	0.06 (0.04-0.06)*
$\beta$ -Carotene	0.43 $\pm$ 0.22	-0.17 $\pm$ 0.13	0.37 $\pm$ 0.19	-0.02 $\pm$ 0.11	0.11 (0.07-0.15)*
$\alpha$ -Tocopherol	33.2 $\pm$ 7.2	-2.7 $\pm$ 4.9	33.2 $\pm$ 7.7	-3.7 $\pm$ 4.2	-1.0 (-3.4-1.3)
Vitamin C	51.0 $\pm$ 20.5	-6.7 $\pm$ 15.4	47.9 $\pm$ 16.9	24.0 $\pm$ 18.0	28.1 (22.3-34.0)*

<sup>1</sup> Values are means  $\pm$  SD unless stated otherwise.

<sup>2</sup> For some individual measurements, data are missing.

<sup>3</sup> Difference in final values (95% confidence interval), corrected for gender, smoking habit and baseline values in the 'high' vs. 'low' group, covariance analyses; \* $P < 0.05$ .

TABLE 5

Effect of 100 g/d fruits and vegetables ('low' group) and 500 g/d fruits and vegetables ('high' group) on plasma vitamin B-12, folate and homocysteine concentrations in fasting humans during a 4-wk dietary intervention period<sup>1</sup>

	Low group (n = 23) <sup>2</sup>		High group (n = 24) <sup>2</sup>		High-Low After study <sup>3</sup>
	Baseline	Change	Baseline	Change	
Vitamin B-12, pmol/L	251.5 ± 121.8	-0.14 ± 40.7	270.8 ± 85.2	-2.5 ± 29.7	0.02 (-19.5 to 19.6)
Folate, nmol/L	13.2 ± 6.0	-0.96 ± 4.18	15.5 ± 10.2	0.14 ± 4.64	1.99 (0.11 to 3.87)*
Homocysteine, μmol/L	13.8 ± 6.0	-0.56 ± 3.03	12.1 ± 3.6	-1.2 ± 2.1	-1.36 (-2.25 to -0.47)*

<sup>1</sup> Values are means ± SD unless stated otherwise.

<sup>2</sup> For some individual measurements, data are missing.

<sup>3</sup> Difference in final values (95% confidence interval), corrected for gender, smoking habit and baseline values in the 'high' vs. 'low' group, covariance analyses; \*P < 0.05.

bohm et al. 1998). These daily intakes are ~0.4–0.7 mg/d higher than that of the high diet. In a study of Van het Hof et al. (1999), plasma concentrations of lycopene decreased by ~38% after consumption of 490 g/d vegetables with a lycopene content even higher (1.1 mg/d) than that of our high diet.

Concentrations of β-carotene showed a difference of +45% in the high group in comparison with the low group. However, the concentrations remained more or less stable in the high group and decreased significantly in the low group. Zino et al. (1997) performed a trial in which the intervention group was asked to increase their fruit and vegetable consumption to eight servings (~1000 g) per day. The plasma concentration of β-carotene was higher after 4 wk compared with the present trial, but the β-carotene content of the intervention diet in the trial of Zino et al. (1997) was ~1.5 mg/d higher than in our trial. Also, subjects in the Zino study continued to consume their freely chosen habitual diet, including processed foods.

No difference between the changes in plasma zeaxanthin and α-tocopherol concentrations were observed between the low and high group. The CV for zeaxanthin measurements could be the reason for the lack of a significant difference. The daily dietary intake of α-tocopherol was comparable between the groups because fruits and vegetables do not contain a high amount of vitamin E. Therefore, no major differences between the diet treatments would be expected.

The strength of this study in comparison with previously described trials is the completely controlled intervention period for energy, fat, protein and carbohydrate intake and the good compliance. Therefore, effects on plasma (fat-soluble) vitamins and carotenoids can be attributed to the differences in fruit and vegetable consumption alone. We examined the effects of increased fruit and vegetable consumption as a whole and not an increase of a specific micronutrient and/or specific fruit or vegetable as is to be expected in a population.

In this study, the daily low diet contained 1.6 mg vitamin B-6 and 131 μg folate, whereas the daily high diet contained 2.0 mg vitamin B-6 and 228 μg folate. These differences in intake could theoretically not be expected to have a major influence on plasma homocysteine. Minimal doses of supplemented folic acid of 250 μg/d with a high bioavailability have been reported to lower homocysteine concentrations (Brouwer et al. 1999a). Food contains folate polyglutamate derivatives, which are hydrolyzed to monoglutamate forms in the gut before absorption. Therefore the bioavailability of food folate is lower than the bioavailability of folic acid supplements. In the intervention trial of Brouwer et al. (1999b), folate status was improved by 7 nmol/L and homocysteine concentrations were decreased by 2 μmol/L after consumption of vegetables

and citrus fruits high in folate (560 μg/d). In comparison, the high diet in our trial contained 228 μg/d. During the study, the volunteers consumed a normal Dutch diet, not a diet with vegetables and fruits high in folate concentration. Although we can only speculate here, our results suggest that other components in fruits and vegetables may influence plasma homocysteine.

The biological relevance of an 11% lower plasma homocysteine concentration, ~1.4 μmol/L, is not clear, but observational epidemiologic studies have found an inverse relationship between plasma homocysteine and cardiovascular disease. Thus, it is advisable to increase fruit and vegetable consumption, which could lower homocysteine concentrations.

In conclusion, our study suggests that a 4-wk period of 500 g/d fruit and vegetable consumption in comparison with 100 g/d of fruits and vegetables with a diet controlled for energy, fat, protein and carbohydrates significantly increases plasma carotenoids, vitamin C, folate and decreases homocysteine concentrations, but has no effect on zeaxanthin, vitamin B-12 and α-tocopherol plasma concentrations. Extra consumption of fruits and vegetables (400 g) and 200 mL/d fruit juices with an equal fat consumption could increase plasma carotenoid and vitamin concentrations by 22–128% and influence plasma folate and homocysteine. Plasma homocysteine (in addition to carotenoids) can be an important indicator of fruit and vegetable intake and is responsive to changes in the intakes of these foods.

Whether changes in plasma concentrations of vitamins and carotenoids have an effect on disease risk remains to be established. High plasma homocysteine concentrations are considered to be a risk factor for cardiovascular disease (Hankey and Eikelboom 1999). This is the first trial that shows that a mix of fruits and vegetables, with a moderate folate content, will decrease plasma homocysteine levels.

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