Plasma C-Reactive Protein and Homocysteine Concentrations Are Related to Frequent Fruit and Vegetable Intake in Hispanic and Non-Hispanic White Elders[1]

Xiang Gao, Odilia I. Bermudez, and Katherine L. Tucker[2]

The Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston, MA

ABSTRACT Elevated C-reactive protein (CRP) and plasma total homocysteine (Hcy) were recently identified as risk factors for cardiovascular disease. However, few studies have related fruit and vegetable consumption to these markers of inflammation and B vitamin deficiency, particularly in the Hispanic population. We examined the relation of fruit and vegetable intake with plasma CRP and Hcy concentrations in a cross-sectional study. Subjects were 445 Hispanic elders and 154 neighborhood-based non-Hispanic white elders living in Massachusetts. Diet was assessed with a FFQ designed for this population. There were significant inverse dose-response associations between fruit and vegetable intake and plasma CRP (P for trend = 0.010) and Hcy (P for trend = 0.033) concentrations, after adjustment for potential confounders. The prevalence of high plasma CRP (> 10 mg/L), and high Hcy (>10.4 μmol/L for women and >11.4 μmol/L for men), was significantly greater among subjects in the lowest quartile of fruit and vegetable consumption relative to those in the highest quartile, 17.9 vs. 9.1% and 58.7 vs. 44.4%, respectively. With each additional serving of fruit and vegetable intake, adjusted odd ratios for high plasma CRP and Hcy were 0.79 (95% CI: 0.65 to 0.97) and 0.83 (95% CI: 0.72 to 0.96), respectively. Greater frequency of fruit and vegetable intake was associated with significantly lower plasma CRP and Hcy concentrations. Because both of these metabolites are known risk factors for CVD, these findings contribute to the evidence that a higher intake of fruit and vegetables may reduce the risk of CVD. J. Nutr. 134: 913–918, 2004.

KEY WORDS: • *C-reactive protein* • *homocysteine* • *fruit* • *vegetable* • *elderly*

Elevated C-reactive protein (CRP)[3] (1–3) and plasma total homocysteine (Hcy) (4–6) were recently identified as risk factors for cardiovascular disease (CVD). CRP is an acute phase reactant secreted by the liver in response to inflammatory cytokines. It was recently identified as a stronger predictor of cardiovascular events than LDL cholesterol (7). Recently, a meta-analysis showed that individuals in the top third of CRP plasma concentrations (>2.4 mg/L) were 2 times as likely to have coronary heart disease (CHD) relative to those in the lowest third of CRP concentrations (<1.0 mg/L) (8). Homocysteine is a sulfur-containing amino acid that is not used for the synthesis of protein. Two recent meta-analyses based on prospective studies demonstrated that every 5 μmol/L increase in Hcy increased the risk for coronary heart disease (CHD) by 20% (9,10).

Increasing fruit and vegetable consumption was shown to protect against CVD (11–14). Results from the Framingham Heart Study showed that the age-adjusted risk ratio for stoke was 0.78 for every additional 3 servings/d of fruits and vegetables (15). In the Nurses' Health Study and the Health Professionals' Follow-Up Study, a protective effect of fruit and vegetables against risk for CHD was also observed (16). One postulated mechanism through which fruit and vegetables protect against CVD is by reduction of plasma Hcy (16). However, few studies have related fruit and vegetable consumption to inflammatory status, and neither inflammation nor Hcy has been studied extensively in Hispanic populations. Therefore, we examined the association of fruit and vegetable intake with plasma CRP and Hcy concentrations in a group of Hispanic elders living in Massachusetts, as well as with a neighborhood-matched group of non-Hispanic white elders.

SUBJECTS AND METHODS

Subjects. The sampling and location for this study were described elsewhere (17,18). Briefly, the Massachusetts Hispanic Elders Study (MAHES), a statewide survey conducted between 1993 and 1997, included a representative sample of elderly Hispanics (≥60 y, $n = 779$) living in Massachusetts, and a neighborhood control group of non-Hispanic whites ($n = 251$). We selected 339 Puerto Ricans, 106 Dominicans, and 154 non-Hispanic whites with dietary intake data and fasting plasma CRP and Hcy measurements. Hispanics of other origin were not included due to small numbers in diverse groups. The Institutional Review Board of Tufts University/New England Medical Center approved the protocol, and subjects gave informed consent before participating.

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[2] To whom correspondence should be addressed.
E-mail: Katherine.tucker@tufts.edu.

[3] Abbreviations used: CHD, coronary heart disease; CRP, C-reactive protein; CVD, cardiovascular disease; Hcy, homocysteine; NF, nuclear factor; OR, odds ratio.

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Field data collection. Dietary intakes were assessed with a semiquantitative FFQ adapted and validated for use with this population (19). Reported intake frequency of individual fruit and vegetable items was summed to obtain the mean frequency of fruit and vegetables consumed per day for each person. Fruits included apples, pears, bananas, peaches, cantaloupe, watermelon, strawberries, mangoes, oranges, grapefruit, other fruit, organ or grapefruit juice, and other 100% juice. Vegetables included tomatoes, string beans, peas, broccoli, cauliflower, spinach, mustard greens, cole slaw, carrots, green salad, avocado, winter squash, other vegetables, dried beans, beans with rice, chili with beans, peas with rice, vegetable soups, and homemade soups. Median portion sizes of fruit and vegetable items of this population were described elsewhere (19). Outliers ($n = 52$) for energy intake were excluded when values were $<$2.51 MJ/d (600 kcal/d) or $>$16.72 MJ/d (4000 kcal/d). Vitamin supplement use (type, frequency, and dosage) was determined in the home by observing supplement packaging. Supplement information was then entered into the Minnesota Nutrient Data System (NDS) and added into the dietary intake data.

Body weight was measured with a Seca balance scale (Seca) with a capacity of 150 kg. Height was taken with a Harpenden pocket stadiometer (Holtain). BMI was calculated as weight (kg)/height (m)^2.

Duplicate blood pressure measurements were taken by trained field workers. Hypertension was defined as systolic blood pressure $\geq$ 140 mm Hg and/or diastolic blood pressure $\geq$ 90 mm Hg. Subjects were identified as having type 2 diabetes when fasting plasma glucose was $>$7.0 mmol/L, a random plasma glucose was $>$11.1 mmol/L, or they reported use of medications for diabetes (insulin or oral medicines). Information on age (y), household income ($/y), education (y), smoking (current, former, and never), and current alcohol use was calculated from the FFQ. Alcohol intake was categorized as moderate (up to 1 drink/d for women and up to 2 drink/d for men) and heavy (greater then these intakes), based on 13.2 g of alcohol/drink. Use of medication was assessed in the home by obtaining medication packaging. Because medication use has been shown to affect plasma CRP and Hcy concentrations (20–23), we also included the following information in this analysis (yes or no): use of aspirin, nonsteroidal anti-inflammatory drugs, antihypertensives, diuretics or cardiovascular medications, or hormones.

Blood samples were drawn from fasting subjects (12 h), collected in tubes containing 0.15% EDTA, and centrifuged at 2500 × *g* for 20 min at 4°C to separate plasma. CRP was measured with an Immunoturbidimetric assay (SPQ antibody reagent set II, Diasorin) (24). Among the subjects, 71% had CRP values below the lowest measurement limit of 6 mg/L, and these were presented as 3 mg/L (midway between 0 and 6). Homocysteine was measured by HPLC with fluorometric detection (25). A cut-off point of $>$10 mg/L was used to define high CRP concentration. CRP concentration $>$ 10 mg/L is considered clinically elevated, suggesting systemic inflammatory processes (26–28). We used this cut-off because 75.2% of the subjects in our study had one or more of the following cardiovascular conditions: hypertension, and a history of stoke, heart attack, or other heart disease. Two studies showed that CRP $>$ 10 mg/L could predict long-term outcomes after coronary events (29,30). One of these demonstrated that a cut-off point of 10 mg/L had a positive predictive value of 44%, relative to 24% for a cut-off point of 3 mg/L. The negative predictive values of these cut-off points were 92 and 96%, respectively (30). High Hcy was defined as $>$10.4 μmol/L for women

TABLE 1

Characteristics of elderly Hispanic and non-Hispanic men and women by quartile of fruit and vegetable intake

	Quartile			
	1	2	3	4
	1.4 (0.2–2.2)[1]	2.7 (2.3–3.2)	3.8 (3.3–4.3)	5.5 (4.4–14.8)
n	149	150	151	149
Age,[2] *y*	69.0 ± 0.6	70.5 ± 0.6	68.8 ± 0.6	69.8 ± 0.6
Female, %	52.4	66.0	58.3	61.1
BMI,[3] *kg/m*^{2}	27.2 ± 0.5	27.7 ± 0.5	28.4 ± 0.5	28.4 ± 0.5
Ethnicity				
Puerto Rican, %	23.6	25.4	26.6	24.4
Dominican, %	21.7	23.6	22.6	32.1
Non-Hispanic white, %	29.9	25.3	24.0	20.8
Smoking, %				
Never	38.0	44.3	47.3	46.7
Former	35.3	37.6	39.3	37.3
Current	26.7	18.1	13.4	16.0
Alcohol use,[4] %				
Nondrinker	75.0	77.3	70.3	72.4
Moderate	18.7	18.6	26.9	22.8
Heavy	6.3	4.1	2.8	4.8
Education, *y*	6.3 ± 0.4	6.0 ± 0.4	6.8 ± 0.4	6.8 ± 0.4
Household income, *$/y*	12733 ± 1744	16074 ± 1732	12741 ± 1736	13318 ± 1736
Diabetes, %	30.9	32.0	36.4	32.9
Hypertension, %	71.8	68.7	71.5	69.8
Vitamin supplement use, %	20.0	27.5	29.3	34.6**
Regular use of medication, %				
Aspirin	14.7	6.6*	7.1	7.0
Nonsteroidal anti-inflammatories	21.7	27.7	26.4	17.6
Antihypertensives	59.7	57.7	57.9	55.6
Diuretics or cardiovascular	62.8	59.9	65.7	59.9
Hormones	29.5	32.9	33.6	31.0

[1] Median (range), times/d.

[2] Mean ± SEM, adjusted for sex.

[3] Mean ± SEM, adjusted for age (y) and sex.

[4] Heavy drinker $>$1 drink/d for women and $>$2 drink/d for men; moderate drinker = 0.1–1 drink/d for women and 0.1–2 drink/d for men; and nondrinker. Based on 13.2 g alcohol/drink.

* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, relative to the lowest quartile, adjusted for age (y) and sex.

TABLE 2

Energy and nutrient intake of elderly Hispanic and non-Hispanic men and women by quartile of fruit and vegetable intake[1]

	Quartile			
	1	2	3	4
n	149	150	151	149
Energy intake, *MJ/d*	6.2 ± 0.2	6.7 ± 0.2	7.4 ± 0.2***	8.5 ± 0.2***
Vitamin C, *mg/d*				
Food intake	70.2 ± 6.7	105 ± 6.7**	146 ± 6.7***	193 ± 6.7***
Total intake	130 ± 18.7	161 ± 18.5	199 ± 18.6**	261 ± 18.6***
α-Tocopherol, *mg/d*				
Food intake	5.5 ± 0.4	5.4 ± 0.4	6.5 ± 0.4	7.7 ± 0.4***
Total intake	19.5 ± 6.6	20.3 ± 6.6	27.6 ± 6.6	35.0 ± 6.6
Folate, *μg/d*				
Food intake	243 ± 12.6	274 ± 12.6	340 ± 12.6***	404 ± 12.5***
Total intake	306 ± 20.0	349 ± 20.0	411 ± 20.0***	509 ± 20.0***
Vitamin B-6, *mg/d*				
Food intake	1.6 ± 0.1	1.7 ± 0.1*	2.0 ± 0.1***	2.4 ± 0.1***
Total intake	2.0 ± 0.6	2.6 ± 0.6	2.4 ± 0.6	4.4 ± 0.6*

[1] Values are means ± SEM, adjusted for age (y) and sex. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, relative to the lowest quartile, adjusted for age (y) and sex.

and >11.4 μmol/L for men, based on the 95th percentile for young adults in the third National Health and Nutrition Examination Survey (31).

Statistical analyses. Statistical analyses were completed with SAS version 8.1 (SAS Institute). Logarithmic transformations were performed for plasma CRP and Hcy concentrations to normalize the distribution of data. We created an overall variable for frequency of total fruit and vegetable intake by summing the intake of all fruit and vegetables reported on the FFQ. Subjects were divided into quartile categories of frequency of total fruit and vegetable consumption. Means were compared using the General Linear Model procedure in SAS, with Dunnett adjustment for multiple comparisons (the lowest quartile as reference). Logistic regression was used to test differences in prevalence across quartiles and to calculate odds ratios (ORs) and 95% CIs. Analyses were adjusted for age (y), sex, ethnicity (Hispanic vs. non-Hispanic white), BMI (kg/m^2), diabetes status (yes or no), hypertension status (yes or no), smoking (current, former, and never), and current alcohol use (heavy drinker, moderate drinker, and nondrinker), vitamin supplement use (yes or no), total energy intake (MJ/d), meat intake (times/d), cereal intake (times/d), dairy product intake (times/d), aspirin use, nonsteroidal anti-inflammatory drug use, antihypertensive use, diuretic or cardiovascular medication use, and hormone use. Linear trends were tested for significance by assigning each subject the median frequency of total fruit and vegetable intake (times/d) for the quartile and treating this value as a continuous variable. Interactions between fruit and vegetable intake and ethnicity were tested in all analyses. An α of 0.05 was used in all of our analyses.

RESULTS

There was a fourfold difference in the median frequency of total fruit and vegetable intake between the highest and lowest quartiles of the population (**Table 1**), with values of 1.4, 2.7, 3.8, and 5.5 for each quartile, respectively. Those with the highest fruit and vegetable intake were less likely to use aspirin, more likely to use vitamin supplements, and had higher intakes of total energy, folate, vitamin B-6, vitamin C, and vitamin E relative to those in the lowest intake quartile (**Table 2**).

There were significant inverse dose-response associations between fruit and vegetable intake and plasma CRP (P for trend = 0.017) and Hcy (P for trend = 0.049) concentrations, after adjustment for potential confounders (**Table 3**). To evaluate the independent effects of Hcy and CRP with the consumption of total fruit and vegetables, we adjusted for the

TABLE 3

Plasma CRP and Hcy concentrations of elderly Hispanic and non-Hispanic men and women by quartile of fruit and vegetable intake

	Quartile				*P* for trend	
	1	2	3	4	Model 1[1]	Model 2[2]
n	149	150	151	149		
CRP,[3] *mg/L*	4.8 ± 1.1	4.8 ± 1.0	4.5 ± 1.1	3.9 ± 1.1*	0.017	0.010
Hcy,[3] *μmol/L*	11.6 ± 1.0	10.8 ± 1.0	11.0 ± 1.0	10.5 ± 1.0*	0.049	0.033

[1] Adjusted for age (y), sex, ethnicity (Hispanic vs. non-Hispanic white), BMI (kg/m^2); presence of diabetes or hypertension; smoking (current, former, and never); current alcohol use (heavy drinker >1 drink/d for women and 2 drink/d for men, moderate drinker = 0.1–1 drink/d for women and 0.1–2 drink/d for men, and nondrinker based on 13.2 g alcohol/drink); vitamin supplement use (yes/no); total energy intake (MJ/d), frequency of meat, cereal, and dairy product intake (times/d); and use of aspirin, nonsteroidal anti-inflammatories, antihypertensives, diuretics or cardiovascular medications, and hormones (each yes/no).

[2] Hcy concentration was also adjusted for CRP, and CRP for Hcy.

[3] Adjusted geometric mean ± SEM (model 1), * $P < 0.1$, relative to the lowest quartile.

respective measure. The coefficients changed only slightly for both CRP (from −0.053 to −0.057) and Hcy (from −0.021 to −0.023), with no change in significance. *P*-values for trend were 0.010 for CRP and 0.033 for Hcy. We also examined whether ethnicity was an effect modifier in the relation between fruit and vegetable intake and plasma CRP and Hcy. Interactions of ethnicity with fruit and vegetable intake were not significant for either CRP ($P = 0.18$) or Hcy ($P = 0.87$). Therefore, results are presented for Hispanics and non-Hispanic whites combined, with adjustment for ethnicity in the model.

The prevalence of high plasma CRP was significantly greater among subjects in the lowest quartile of fruit and vegetable consumption, relative to the highest quartiles (17.9 vs. 9.1%, $P = 0.014$). The prevalence of high Hcy was also significantly greater in subjects in the lowest relative to the highest quartile of fruit and vegetable intake (58.7 vs. 44.4%, $P = 0.012$). Those in the highest quartile of total fruit and vegetable intake were 65% less likely to have high plasma CRP and 54% less likely to have high Hcy, relative to those in the lowest quartile, after adjustment for other potential confounders and plasma Hcy or CRP concentrations, respectively (**Fig. 1**). For each serving of fruit and vegetable intake, the multiple-adjusted ORs for high plasma CRP and Hcy were 0.79 (95% CI: 0.65 to 0.97) and 0.83 (95% CI: 0.72 to 0.96), respectively.

DISCUSSION

We observed clear and significant dose-response relations for both plasma CRP and Hcy concentrations with frequency of fruit and vegetable intake after adjusting for the respective measure and for other covariates. The likelihood of having high CRP or Hcy was reduced by 21 and 17% per serving of fruit and vegetable intake, respectively. CRP and Hcy have been well documented as risk factors for cardiovascular disease. CRP is a marker as well as an amplifier of inflammation (32). It is directly involved in atherothrombogenesis by binding to damaged tissue and to LDL, and in stimulating tissue factor biosynthesis by macrophages (22,33,34). Homocysteine contributes to arteriosclerosis and thrombosis by several potential mechanisms, including endothelial cell damage, platelet activation, and deleterious effects on thrombomodulin expression (35). Effects on these two, therefore, may be mechanisms through which fruit and vegetable intake reduces the risk of CVD.

Average CRP concentrations were higher in our study population than was seen in other studies. The Women's Health Study reported values of 1.5 and 6.6 mg/L as 50th and 90th percentiles (7). Similar ranges were shown elsewhere (36). In this mostly Hispanic population, >70% of subjects had hypertension and >30% were obese and diabetic (17,18). These chronic conditions are related to high plasma CRP concentration (22). Because we measured CRP using an immunoturbidimetric assay, we could not quantify CRP values < 6 mg/L. This limits our ability to extend the association of fruit and vegetable intake with CRP concentration at the lower end of the CRP distribution. However, despite this limitation, we saw a clear dose-response relation between fruit and vegetable intake and CRP concentration. This finding suggests that fruit and vegetable intake may modulate the inflammatory response. We expect that stronger associations may be observed in future studies using high-sensitivity CRP measurement.

The antioxidant components of fruit and vegetables, i.e., carotenoids, vitamin E, vitamin C, and flavonoids, may contribute to this anti-inflammatory effect. Several studies, in both humans and rats, showed an inverse relation between inflammation and dietary antioxidant intake. Consumption of a diet low in antioxidants was shown to result in inflammation (37), whereas antioxidant supplementation decreases inflammation (38). Flavonoids were shown to inhibit the synthesis and gene expression of cytokines (39). Nuclear factor (NF)-κB, an oxidant-sensitive upstream regulator of proinflammatory mediator synthesis, plays a key role in this process (38). Oxidative stress leads to NF-κB activation and DNA binding (40,41). As a result, the inflammatory cascade is triggered and CRP is subsequently produced (42). Antioxidants were shown to block NF-κB activation and DNA binding (41,43–45). A recent intervention study showed that after drinking 500 mL/d of high-pressurized orange juice for 14 d, plasma CRP was reduced by 40% (from 0.25 to 0.15 mg/L) and 56% (from 0.23 to 0.10 mg/L) in men and women, respectively (46). In that study, decreases in plasma prostaglandin E_2 were also observed in both men and women (46).

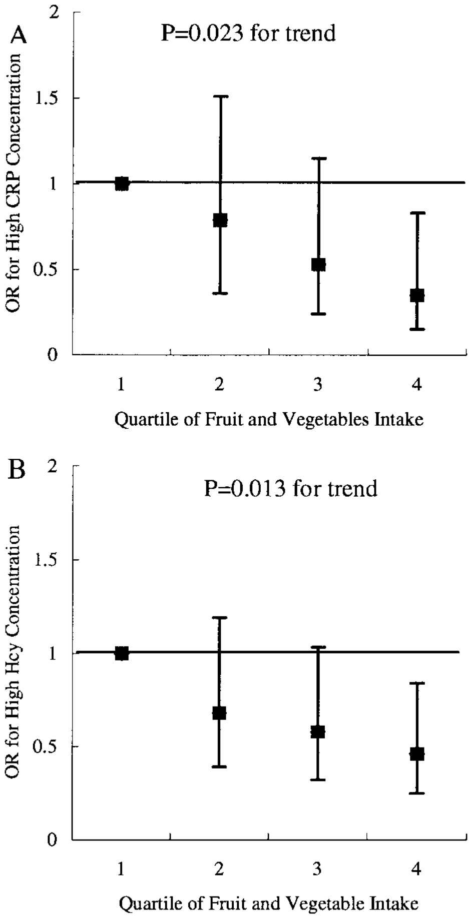

FIGURE 1 ORs for high plasma CRP (>10 mg/L; *Panel A*), and high plasma total Hcy (>10.4 μmol/L for women and >11.4 μmol/L for men; *Panel B*) by fruit and vegetable intake. Adjusted for age (y), sex, ethnicity (Hispanic vs. non-Hispanic white), BMI (kg/m^2); presence of diabetes or hypertension; smoking (current, former, and never); current alcohol use (heavy drinker > 1 drink/d for women and 2 drink/d for men, moderate drinker, and nondrinker, based on 13.2 g alcohol/drink); vitamin supplement use (yes/no); total energy intake (MJ/d), frequency of meat, cereal, and dairy product intake (times/d); and use of aspirin, nonsteroidal anti-inflammatories, antihypertensives, diuretics or cardiovascular medications, and hormones (each yes/no). CRP and Hcy concentration were also adjusted for each other.

Our observation of an inverse association between fruit and vegetable intake and plasma Hcy concentration is consistent with results from the Framingham Heart Study in which a clear dose-response relation was identified for plasma Hcy with greater frequency of fruit and vegetable consumption (47). Several trials have shown that a high intake of fruit and vegetables reduces plasma Hcy (48–51). This may be explained by the folate and vitamin B-6 content of fruit and vegetables (52–54). Folate is required in the remethylation of Hcy to methionine, and vitamin B-6 is involved in the transsulfuration of Hcy to cystathionine (55). The relations of dietary folate and vitamin B-6 intake and plasma Hcy have been well established (31,52,56).

Kuller et al. (57) proposed that inflammatory processes may result in an increased demand for folate, thereby leading to secondary elevation of Hcy. Friso et al. (58) found that vitamin B-6 was associated with an elevation of CRP. These observations suggest that CRP and Hcy may share common pathways. However, we found that the significant associations of fruit and vegetable intake with plasma CRP and Hcy were independent of each other. Furthermore, we found no relation between CRP and Hcy after adjustment for age, sex, ethnicity, BMI, smoking, and alcohol use (data not shown), which is consistent with other studies (59–61).

In summary, greater frequency of fruit and vegetable intake was associated with lower plasma CRP and Hcy concentrations. Because both of these metabolites are known risk factors for CVD, these findings contribute to the evidence that higher intake of fruit and vegetables may reduce the risk of CVD.

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