

Greater variety in fruit and vegetable intake is associated with lower inflammation in Puerto Rican adults^{1–3}

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ABSTRACT

Background: Puerto Rican adults have prevalent metabolic abnormalities, but few studies have explored fruit and vegetable (FV) intake and coronary heart disease (CHD) risk in this population.

Objective: We tested the hypothesis that greater FV intake and variety are associated with a lower 10-y risk of CHD and C-reactive protein (CRP) concentrations.

Design: In a cross-sectional study of ≈1200 Puerto Rican adults aged 45–75 y, we assessed FV intake with a food-frequency questionnaire. The 10-y risk of CHD was assessed with the Framingham risk score in participants free of cardiovascular disease. CRP was measured in fasting serum.

Results: Variety, but not quantity, of FV intake was inversely associated with FRS after adjustment for the following: sex; waist circumference; perceived stress; alcohol use; intakes of energy, *trans*, and saturated fatty acids; and use of supplements, cardiovascular medications, and diabetes medications ($P = 0.02$). However, the association was attenuated after adjustment for income ($P = 0.11$). Variety, but not quantity, was associated with a lower serum CRP concentration after adjustment for age, sex, smoking status, alcohol use, servings of FV, white blood cell count, diastolic blood pressure, diabetes, nonsteroidal antiinflammatory medication use, intakes of energy and vitamin B-6, waist circumference, perceived stress, and income. The adjusted odds of a high CRP concentration for those in the highest compared with the lowest tertile of FV variety was 0.68 (95% CI: 0.49, 0.94).

Conclusion: FV variety, but not quantity, appears to be important in reducing inflammation. Although the results are suggestive, larger studies are needed to confirm a possible association with CHD risk score. *Am J Clin Nutr* doi: 10.3945/ajcn.2010.29913.

INTRODUCTION

Heart disease is the leading cause of death in the United States (1). The global total mortality rate attributable to inadequate consumption of fruit and vegetables (FV) has been estimated to be up to 2.64 million deaths per year. It has been projected that by increasing FV consumption to 600 g/d, the worldwide burden of ischemic heart disease and ischemic stroke can be reduced by 31% and 19%, respectively (2). In fact, several epidemiologic studies have consistently shown that greater FV intakes are associated with a lower risk of incident cardiovascular events. Meta-analyses of multiple studies have indicated that each additional portion of FV decreased the risk of coronary heart disease (CHD) by 4% (95% CI: 0.93, 0.99) (3) and the risk of stroke by 5% (95% CI: 0.93, 0.99) (4). In 2 separate meta-analyses, par-

ticipants consuming >5 servings/d had a 26% reduction in risk of stroke (95% CI: 0.83, 0.97) (5) and a 17% reduction in risk of CHD (95% CI: 0.77, 0.89) (6) compared with those consuming <3 servings/d.

Potential mechanisms for the protective effect of FV include their anti-inflammatory properties. A few population-based studies have shown an inverse association between FV intake and C-reactive protein (CRP)—a marker of systemic inflammation (7–9). These inverse associations can be attributed to several nutrients, such as α -carotene, β -carotene, β -cryptoxanthin, lutein, zeaxanthin, lycopene, and vitamin C, which are present in a wide variety of FV. However, supplementation with several of these single nutrients has been shown to have either no effect or adverse effects on heart disease risk in clinical trials (10, 11), which indicates the superior effects of whole foods over isolated nutrients. This concept of food synergy supports the idea of dietary variety and of selecting several foods rich in different nutrients (12). In fact, the 2005 *Dietary Guidelines for Americans* recommend choosing a variety of FV each day (13). In addition, the most recent American Heart Association dietary guidelines for cardiovascular disease (CVD) risk reduction have, for the first time, issued a recommendation to consume a variety of FV (14). However, little is known about how variety in FV intake affects CVD risk. Furthermore, there is a paucity of research on the association between FV intake and heart disease risk in Puerto Ricans—the second largest Hispanic subgroup in the United States. Older Puerto Ricans living in Massachusetts experience a significantly greater prevalence of comorbidities than do non-Hispanic whites residing in the same neighborhood, and these differences remain after adjustment for age, sex, income, and education (15). Few studies have evaluated the relation between FV intake and inflammation in this high-risk group (9). This study was, therefore, undertaken to assess whether FV intake

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was associated with 10-y risk of CHD and inflammation, considering both quantity and variety, among a group of 1222 Puerto Rican adults, aged 45–75 y, living in the greater Boston area.

SUBJECTS AND METHODS

Participants

We used data from the Boston Puerto Rican Health Study (BPRHS)—a prospective cohort study designed to examine the associations between social and psychological stress, vitamin status, allostatic load, and measures of depression, cognitive function, and physical disability in a group of older Puerto Ricans, aged 45–75 y, in Boston, MA. The design of the BPRHS was described in detail elsewhere (16). Briefly, participants were identified from areas of high Hispanic density in the Boston, MA, metropolitan area, as indicated in the year 2000 Census. Blocks were selected for enumeration from the sample frame with ≥ 10 Hispanic persons aged ≥ 45 y from census tracts that contain ≥ 25 Puerto Rican adults in the specified age range (subgroup identity among Hispanics is not available at the block level). After complete block enumeration (blocks were visited up to 5 or 6 times and on weekends and evenings to maximize identification of all relevant households), households with at least one Puerto Rican adult between the ages of 45 and 75 y were identified, and one qualified individual was randomly selected from each and invited to participate. Of a total of 2093 participants who were invited to participate, 282 (13.5%) declined. Primary reasons for declining included not being interested in the study, being too busy, and refusing to have blood drawn. Those who

declined were more likely to be older (58.4 compared with 56.7 y; data not shown) and had lived on the US mainland for more years (32.9 compared with 28.8 y; data not shown). No other significant differences in sex, language spoken, or birthplace were observed between those participating and those declining. Nine participants were excluded because of a low Mini-Mental State Examination Score (0.5%), 15 (0.8%) dropped out before completion, 36 (2.0%) were lost before interview, and 251 (13.9%) were not interviewed because of repeated unavailability. Therefore, 1500 of 1802 eligible participants who initially agreed (83.2%) completed the baseline interviews. The first baseline interview was completed in May 2004. At the time of analysis, complete and cleaned data were available on 1222 participants. For the current analyses, we excluded those with implausible energy intakes of <600 or >4800 kcal (<2510 or $>20,083$ kJ) or those with >10 questions left unanswered on the food-frequency questionnaire (FFQ) ($n = 63$) (Figure 1). Compared with participants included in the current analyses, those who were excluded were younger (53.3 compared with 56.9 y), had a lower CRP concentration (2.04 compared with 3.55 mg/L), had higher intakes of energy-adjusted *trans* fatty acids (3.8 compared with 3.1 g/d) and alcohol (19.5 compared with 5.0 g/d), and consumed fewer servings of fruit and vegetables (3.5 compared with 4.2 servings/d). In addition, they were more likely to be male (53.0 compared with 28.2%) and less likely to use cardiovascular medications (44.6% compared with 61.3%). No significant differences in other baseline characteristics were noted. A total of 1159 Puerto Rican adults (324 men and 835 women) aged 45–75 y were included in the following analyses (Figure 1).

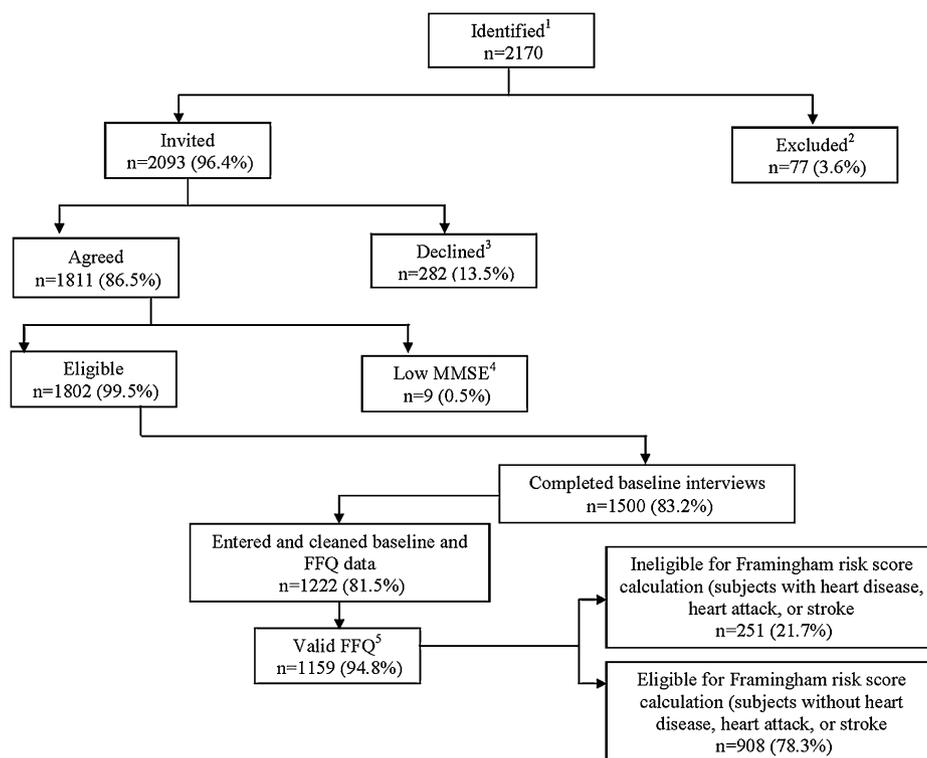


FIGURE 1. Flow diagram of participants included in the current analysis. ¹Identified as of Puerto Rican descent. ²Met the exclusion criteria (eg, serious illness, moved from the study area, homeless, or hostile). ³Reasons for declining participation (eg, not interested, too busy, did not want blood drawn, length of study, or doctor/spouse advised against participation). ⁴Low Mini-Mental State Examination Score (MMSE; <10). ⁵Valid food-frequency questionnaire (FFQ; <10 questions left unanswered and energy intake between 600 and 4800 kcal or between 2510 and 20,083 kJ).

For participants who qualified, additional questionnaires captured information on general background and socioeconomic status, perceived stress, dietary intake, health history, health behaviors, health insurance, and medical conditions. Participants were asked to show containers for medicines and supplements used. Anthropometric and blood pressure measures were also completed. All interviews were administered by trained bilingual interviewers in the participants' homes. Blood samples were drawn in the home by a certified phlebotomist on the day after the home interview or as soon as possible thereafter. All study protocols were approved by the Institutional Review Board at Tufts Medical Center.

Dietary intake assessment

We used a semiquantitative FFQ, specifically developed and validated for this population, to assess dietary intake over the past 12 mo. The food list for this questionnaire was developed by using the format of the National Cancer Institute/Block food frequency, but with data from the Hispanic Health and Nutrition Examination Survey (HHANES) dietary recalls for Puerto Rican adults and tested in Puerto Rican participants aged ≥ 60 y in Massachusetts (17). Because the Puerto Rican population has a typical diet that differs considerably from both the general US population and from Mexican Americans, foods such as plantains, and specific soup and rice-dish recipes were added to the FFQ. Participants reported both frequency of consumption and usual portion sizes for foods. To quantify food group intake, mixed dishes were disaggregated and intake amounts were added to the appropriate food group. Nutrient intakes were obtained by using the Nutrition Data System for Research software version 2007, developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN.

Servings of FV were obtained by dividing the gram amount of each food by the reference serving amount from the US Department of Agriculture (USDA) Food Guide Pyramid. Fruit included apples, pears, bananas, oranges, grapefruit, peaches, apricots, nectarine, plums, grapes, avocado, kiwi fruit, papaya, mangoes, prunes, cantaloupe, honeydew melon, watermelon, cherries, strawberries, blueberries, raspberries, cranberries, pineapple, olives, and 100% fruit juice. Vegetables included lettuce, spinach, tomato, carrots, string beans, peas, corn, peppers, broccoli, cauliflower, cabbage, beets, asparagus, mushrooms, eggplant, onion, squash, cucumber, radish, celery, cilantro, garlic, parsley, zucchini, basil, and 100% vegetable juice. Starchy vegetables (including potato, plantains, tannier, and cassava), beans, and legumes (including lima beans, pinto beans, white beans, black beans, pink beans, kidney beans, cowpeas, soybeans, split peas, and lentils) were excluded from the analyses. Reported servings of individual FV were summed to obtain the mean servings of FV consumed per day. Variety in FV intake was defined as the total number of unique FV consumed at least once per month over the past 12 mo.

Framingham risk score

We used the Framingham charts by Wilson et al (18) to calculate the estimated 10-y risk of CHD for each subject free of heart attack, heart disease, and stroke (from self report) at baseline ($n = 252$ men and 656 women). Total CHD risk was defined as the risk of angina pectoris, unstable angina, myo-

cardial infarction, or sudden death (18). The risk factors that were considered included age, diabetes, smoking, systolic and diastolic blood pressures, total cholesterol, LDL cholesterol, and HDL cholesterol.

Blood samples

Fasting blood samples (12 h) were drawn from participants by a certified phlebotomist on the morning after the interview or as soon as possible in the participants' homes. To extract plasma, blood was collected into evacuated tubes containing EDTA and immediately centrifuged at $3421 \times g$ at 4°C for 15 min. For serum, blood was allowed to clot at room temperature for ≈ 15 min, after which it was centrifuged at $3421 \times g$ at 4°C for 15 min. All evacuated tubes were shielded from light during specimen collection, processing, and handling. All samples were kept cold and brought back to the Nutrition Evaluation Laboratory at the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University for further processing, portioning, and storage. Samples were stored in cryogenic tubes at -80°C until further analyzed. CRP was measured in serum by using the Immulite 1000 High Sensitive CRP Kit (LKCPR1) on the Immulite 1000 (Seimens Medical Solutions Diagnostics, Los Angeles, CA). This was a solid-phase, 2-site chemiluminescent immunometric assay. Carotenoids were analyzed from serum by using a colorimetric reaction with a Beckman DU640 Spectrophotometer (19). Plasma pyridoxal-5'-phosphate (PLP) was measured by using a radioenzymatic assay in a Beckman LS 6500 Scintillation Counter. Serum folate concentrations were measured with a competitive, liquid-phase, ligand-labeled, protein-binding chemiluminescent assay with a commercially available kit from Diagnostics Products Corporation (IMMULITE 1000; Los Angeles, CA). For processing ascorbic acid, 300 μL perchloric acid was added to 300 μL plasma. The cryogenic tubes were vortex-mixed immediately for 1 min and placed on wet ice. Plasma ascorbate was analyzed by isocratic reversed-phase HPLC (HPLC system with Millennium 32 software; Waters Associates Inc, Milford, MA) coupled with amperometric detection (BAS EC-5 electrochemical detector; Bioanalytical Systems Inc, West Lafayette, IN). The intra- and interassay CVs (%), respectively, for each of these analytes are as follows: CRP (5.2% and 7.3%), carotenoids (6.0% and 7.0%), plasma PLP (5% and 7%), serum folate (6.1% and 6.7%), and plasma ascorbate (4.5% and 6.5%).

Assessment of other covariates

Information on age, sex, smoking status, alcohol use, education, household income, acculturation, psychosocial stress, medical conditions, and medication use was collected by questionnaire. Standing height, weight, and waist circumference were measured in duplicate. Weight was measured with a quality clinical scale (Toledo Weight Plate, model I5S; Bay State and Systems Inc, Burlington, MA), which was calibrated with known weights regularly. Height was measured with a Harpenden pocket stadiometer. Waist circumference was measured with an anthropometric tape on the smallest area of the waist and was recorded to the nearest 0.1 cm. Body mass index was calculated as weight (kg) divided by height squared (m). Blood pressure was measured with an electronic sphygmomanometer (Model HEM-

71; OMRON Healthcare Inc, Vernon Hills, IL) at 3 time points during the interview; an average of the second and third readings was used for each systolic and diastolic blood pressure measurement. Participants were asked to self-report whether they had been diagnosed with heart disease, heart attack, or stroke by a physician. Information on medication use was collected by asking participants to show the bottles of the medications that they were currently taking. Physical activity was assessed with a modified Paffenbarger questionnaire from the Harvard Alumni Activity Survey (20, 21). Smoking and alcohol intake were assessed as current, past, or never. Poverty status was calculated by comparing the subject's total household income with the annual poverty thresholds released by the US Census Bureau, Housing and Household Economic Statistics Division (22). Language acculturation was computed as a score ranging from 0 to 100; greater scores indicated a greater acculturation to the English language. Perceived stress score was measured by using the Spanish version of the Perceived Stress Scale (23). The score was used as a continuous measure; greater scores indicate a greater perceived stress.

Statistical analyses

All statistical analyses were performed by using SAS (version 9.2; SAS Institute, Cary, NC). A P value < 0.05 was considered significant. Participants were divided into tertile categories of servings of FV intake and FV variety. We calculated the age- and sex-adjusted means for lifestyle and dietary characteristics across increasing tertile categories of FV intake and FV variety by using SAS PROC GLM. We assessed the significance across categories of FV intake and FV variety using linear (for continuous variables) or logistic (for categorical outcome variables) regression. Similarly, nutrient intakes and circulating concentrations of nutrients were examined across tertiles by using analysis of variance [general linear models procedure (PROC GLM)] and were adjusted for age, sex, and energy intake. Tests for linear trend were conducted by assigning each participant the median servings or the median variety of FV intake for the corresponding tertile and treating this value as a continuous measure. We used PROC GLM to test the associations of FV intake and FV variety with the Framingham risk score (FRS) with the following adjustments: In our first model, we adjusted for traditional risk factors for CHD that were not part of the Framingham risk algorithm. These include sex, waist circumference, and intakes of total energy, alcohol, *trans* fatty acids, and saturated fatty acids. Furthermore, to separate the effect of FV variety from FV quantity, they were adjusted for each other in model 1. Because stress is associated with FV intake and is an independent risk factor for CHD, we further adjusted for perceived stress score in model 2. We also controlled for confounding by indication by including vitamin supplement use, cardiovascular medication use, and diabetes medication use in model 2. Because household income may also influence FV intake, FV variety, and CHD risk, we adjusted for this in our final model. Models with log CRP as the outcome variable were constructed as follows:

- 1) Model 1 was adjusted for traditional risk factors, including, age, sex, energy intake, smoking, and alcohol use. To control for elevations in CRP from acute infections, white blood cell count was also included in model 1.
- 2) Because waist circumference, diastolic blood pressure, diabetes status, vitamin B-6 intake, and nonsteroidal antiinflammatory medication use are known to affect CRP concentrations, we adjusted for these variables in model 2. In addition, because stress is known to increase CRP concentrations and affect FV intake, we also adjusted for perceived stress in model 2.
- 3) In our final model, we adjusted for total household income to control for potential confounding due to socioeconomic status.

For all linear models, we checked assumptions of linearity and homogeneity by examining the residuals of our outcome variables (FRS and CRP) compared with our primary exposure (FV intake and FV variety) variables. For models not meeting this assumption, we applied a logarithmic transformation to our outcome variable. Log-transformed values were back-transformed, and the results were expressed as geometric means. We used ordinal logistic regression (PROC LOGISTIC) to calculate the proportional odds ratios (ORs) and 95% CIs for the association between CRP categories [normal (<1 mg/L), intermediate (1–3 mg/L), high (>3 mg/L)] and FV intake and FV variety. A test for proportional odds was conducted. We also tested for potential effect modification by sex by including an interaction term with the exposure variable in the regression model. All analyses were adjusted for multiple comparisons by using Tukey's honestly significant difference test. Sensitivity analyses included repeating models replacing income with education, replacing categorical smoking and drinking variables with quantified variables, and rerunning models with individuals with a CRP concentration >10 mg/L excluded.

RESULTS

A nearly 3-fold difference in the intake of FV was observed between participants in the highest and lowest tertiles. The median daily serving size for each tertile was 2.3, 3.6, and 5.9, respectively (**Table 1**). Those in the highest compared with the lowest tertile of FV intake were more likely to be female, older, to engage in light physical activity, to be moderate alcohol drinkers, to have greater educational status and household income, to be more acculturated, and to report lower perceived stress. Similarly, these participants were less likely to be sedentary, current smokers, nondrinkers, or to have diabetes. Participants in the highest variety tertile consumed twice the variety of FV compared with participants in the lowest tertile. The median variety score for tertiles 1, 2, and 3 were 17.8, 26.2, and 34.0, respectively (**Table 2**). Compared with participants with the least FV variety, those with the highest variety in FV intake were more likely to be female, to be former smokers, to be moderate drinkers, to engage in light physical activity, to have greater educational status and household income, to be more acculturated, to report lower perceived stress, and to use vitamin supplements and nonsteroidal antiinflammatory drugs. These participants were also less likely to be sedentary, current smokers, and nondrinkers.

Those in the highest tertiles of FV intake had significantly higher intakes of dietary fiber and higher concentrations of serum carotenoids, serum folate, and plasma ascorbic acid compared with those in the lowest tertile (**Table 3**). Likewise, relative to

TABLE 1
Characteristics of Puerto Rican men and women by tertile of fruit and vegetable intake¹

	Tertile of fruit and vegetable intake			<i>P</i> for trend
	1	2	3	
Fruit and vegetable intake (servings/d)	2.3 (0.08–2.9) ²	3.6 (3.0–4.4)	5.9 (4.5–15.1)	
No. of subjects	383	383	383	
Age (y) ³	55.9 ± 0.4 ⁴	57.8 ± 0.4 ⁵	58.2 ± 0.4 ⁶	<0.0001
Female (%) ⁷	61.4	74.9 ⁶	80.9 ⁶	<0.0001
BMI (kg/m ²) ⁸	31.0 ± 0.3	30.9 ± 0.3	31.4 ± 0.4	0.35
Abdominal obesity (%) ⁸	62.6	63.0	63.3	0.96
Physical activity (%) ⁸				
Sedentary	55.7	42.0 ⁹	35.3 ⁶	<0.0001
Light	38.1	54.5 ⁶	59.1 ⁶	<0.0001
Moderate	5.1	2.8	4.7	0.94
Heavy	1.1	0.7	0.8	0.69
Smoking (%) ⁸				
Never	40.7	43.3	40.3	0.80
Former	30.1	30.0	35.4	0.06
Current	29.2	26.7	23.8	0.08
Alcohol use (%) ⁸				
Nondrinker	65.2	53.5 ⁵	53.0 ⁹	0.002
Moderate	24.0	38.5 ⁶	38.1 ⁶	0.0003
Heavy	8.5	6.6	6.8	0.38
Education (%) ⁸				
<8th grade	50.8	47.9	44.5	0.08
9th–12th grade/GED	39.0	37.9	34.1	0.14
College/some graduate school	9.9	14.2	21.3 ⁶	<0.0001
Household income (US\$/y) ⁸	16,110 ± 1823	18,664 ± 1870	23,653 ± 1913 ⁵	0.003
Below poverty (%) ⁸	59.4	55.6	52.9	0.09
Acculturation score (%) ⁸	22.6 ± 1.1	25.9 ± 1.1	28.8 ± 1.2 ⁹	0.0001
Perceived stress score ⁸	24.5 ± 0.5	23.0 ± 0.5	21.5 ± 0.5 ⁶	<0.0001
Diabetes (%) ⁸	40.1	45.8	34.6	0.05
Hypertension (%) ⁸	67.2	69.6	70.2	0.41
Vitamin supplement use (%) ⁸	54.5	56.4	61.1	0.06
Medication use (%) ⁸				
Cardiovascular	59.2	63.6	61.4	0.66
Nonsteroidal antiinflammatory	28.3	32.8	31.8	0.39
Diabetes	33.6	37.9	27.2	0.03

¹ GED, General Education Development.² Median; range in parentheses (all such values).³ Adjusted for sex.⁴ Mean ± SEM (all such values).^{5,6,9} Significantly different from tertile 1, adjusted for age and sex by ANOVA (PROC GLM; SAS Institute, Cary, NC): ⁵*P* < 0.01, ⁶*P* < 0.0001, ⁹*P* < 0.001.⁷ Adjusted for age.⁸ Adjusted for age and sex.

those with the least variety in FV intake, participants with the greatest variety in FV intake had significantly higher intakes of dietary fiber and higher circulating concentrations of carotenoids, folate, PLP, and ascorbic acid (*P* for trend <0.0001) (Table 4).

The FRS was not normally distributed, but residuals of FRS on FV intake and FV variety were normally distributed. When treated as a continuous variable, analysis of covariance showed a significant inverse association between FV variety and FRS (*P* < 0.05). However, this association was no longer significant after adjustment for total household income (Table 5). For serum CRP, normality of the residuals improved after a logarithmic transformation of CRP. A strong inverse association between FV variety and log CRP (*P* < 0.01) was found, which was attenuated, but remained significant, after adjustment for

total household income (*P* < 0.05). No significant associations were found between total FV intake, FRS, and log CRP (Table 5). The proportional odds assumption, which tests whether the ordered logit coefficients are equal across the levels (normal, intermediate, or high) of CRP, was met. The multiple-adjusted proportional odds ratio (OR) for high CRP compared with the combined effect of intermediate and low CRP (or the combined effect of high and intermediate CRP compared with low CRP) was 0.88 (95% CI: 0.64, 1.21) for those in the third tertile of FV intake compared with those in the lowest tertile (Figure 2). For those in the third tertile of FV variety, the proportional odds of high CRP compared with the combined effect of intermediate and low CRP (or the combined effect of high and intermediate CRP compared with low CRP) were 0.68 times lower than those in the first tertile of FV variety (95% CI: 0.49, 0.94). Similarly,

TABLE 2
Characteristics of Puerto Rican men and women by tertile of fruit and vegetable variety¹

	Tertile of fruit and vegetable variety			P for trend
	1	2	3	
Fruit and vegetable intake (no.) ²	17.8 (2.0–22.4)	26.2 (22.4–29.7)	34.0 (29.7–44.7)	
No. of subjects	386	387	386	
Age (y) ³	56.7 ± 0.4 ⁴	57.5 ± 0.4	57.4 ± 0.4	0.18
Female (%) ⁵	66.8	76.0 ⁶	73.5 ⁷	<0.0001
BMI (kg/m ²) ⁸	30.7 ± 0.3	31.6 ± 0.3	31.0 ± 0.3	0.58
Abdominal obesity (%) ⁸	67.3	76.0	68.6	0.89
Physical activity (%) ⁸				
Sedentary	52.1	48.3	39.5 ⁹	<0.0001
Light	44.0	49.9 ⁷	54.0 ⁶	0.002
Moderate	3.1	0.8	6.0	0.15
Heavy	0.8	1.0	0.5	0.80
Smoking (%) ⁸				
Never	44.0	45.5	49.4	0.39
Former	22.8	33.6 ⁹	32.2 ⁶	0.005
Current	33.2	20.9 ⁶	18.4 ¹⁰	<0.0001
Alcohol use (%) ⁸				
Nondrinker	67.0	62.0 ⁷	54.6 ¹⁰	<0.0001
Moderate	24.5	34.6 ⁶	40.1 ¹⁰	<0.0001
Heavy	8.4	3.4 ⁷	5.3	0.13
Education (%) ⁸				
<8th grade	53.4	48.6	42.9 ⁶	0.002
9th–12th grade/GED	38.7	40.1	30.4 ⁷	0.04
College/some graduate school	7.5	11.4 ⁷	26.8 ¹⁰	<0.0001
Household income (US\$/y) ⁸	15,320 ± 1824	16,626 ± 1839	25,964 ± 1839 ¹⁰	<0.0001
Below poverty (%) ⁸	65.3	58.7 ⁷	51.4 ¹⁰	<0.0001
Acculturation score (%) ⁸	20.7 ± 1.1	26.2 ± 1.1 ⁹	31.1 ± 1.1 ¹⁰	<0.0001
Perceived stress score ⁸	24.6 ± 0.5	23.0 ± 0.5 ⁷	21.7 ± 0.5 ¹⁰	<0.0001
Diabetes (%) ⁸	39.4	42.7	36.9	0.24
Hypertension (%) ⁸	67.1	69.3	68.3	0.88
Vitamin supplement use (%) ⁸	52.6	58.7	67.3 ⁹	0.0001
Medication use (%) ⁸				
Cardiovascular	60.9	64.3	60.0	0.48
Nonsteroidal antiinflammatory	27.7	35.8 ⁷	36.1 ⁷	0.02
Diabetes	31.1	36.0	31.2	0.64

¹ GED, General Education Development.

² Median number of unique fruit and vegetables consumed at least once per month; ranges in parentheses.

³ Adjusted for sex.

⁴ Mean ± SEM (all such values).

⁵ Adjusted for age.

^{6,7,9,10} Significantly different from tertile 1, adjusted for age and sex by ANOVA (PROC GLM; SAS Institute, Cary, NC): ⁶*P* < 0.01, ⁷*P* < 0.05, ⁹*P* < 0.001, ¹⁰*P* < 0.0001.

⁸ Adjusted for age and sex.

for those in the second tertile of FV variety, the multivariate-adjusted proportional odds of high CRP compared with the combined effect of intermediate and low CRP (or the combined effect of high and intermediate CRP compared with low CRP) were 0.72 times lower (95% CI: 0.52, 0.98) compared with participants with the least variety (**Figure 3**). We repeated our analyses for models with CRP as the dependent variable by excluding participants with CRP concentrations >10 mg/L (*n* = 194). Exclusion of these participants did not change the results, and associations between FV variety and CRP remained significant in a comparison of tertile 2 with tertile 1 (OR: 0.70; 95% CI: 0.50, 0.97) and of tertile 3 with tertile 1 (OR: 0.67; 95% CI: 0.48, 0.95). Models replacing income with education or categorical smoking and alcohol use variables with pack-years and intake quantity did not change any of the results. We found no

significant interactions between sex and FV intake and FV variety.

DISCUSSION

In this population of older Puerto Rican adults living in the greater Boston area, we found that greater FV variety, but not intake, was associated with lower inflammation. This association persisted after adjustment for a variety of potential confounding factors. In addition, the association between FV variety and FRS was significant before the inclusion of income and approached significance (*P* = 0.11) after adjustment for income. Although this limits our conclusions about this association, it may also be argued that income is, at least partially, in the causal pathway (as purchasing a greater variety of FV is directly affected by

TABLE 3Energy and fiber intakes and circulating concentrations of select nutrients of Puerto Rican men and women by tertile of fruit and vegetable intake¹

	Tertile of fruit and vegetable intake			<i>P</i> for trend
	1	2	3	
Fruit and vegetable intake (servings/d)	2.3 (0.08–2.9) ²	3.6 (3.0–4.4)	5.9 (4.5–15.1)	
No. of subjects	383	383	383	
Energy intake (kcal/d)	2270 ± 44 ³	2083 ± 46 ⁴	2345 ± 47	0.07
Dietary fiber intake (g/d)	17.5 ± 0.3	18.6 ± 0.3 ⁵	21.2 ± 0.3 ⁶	<0.0001
Serum carotenoids (μmol/L)	1.63 ± 0.04	1.71 ± 0.04	1.92 ± 0.04 ⁶	<0.0001
Serum folate (nmol/L)	41.6 ± 1.1	41.7 ± 1.1	45.6 ± 1.1 ⁵	0.004
Plasma pyridoxal-5'-phosphate (nmol/L)	14.3 ± 0.8	14.4 ± 0.9	15.9 ± 0.9	0.16
Plasma ascorbic acid (μmol/L)	44.2 ± 1.1	46.7 ± 1.1	50.0 ± 1.2 ⁷	0.0002

¹ The models were adjusted for age, sex, and energy intake by using ANCOVA (PROC GLM; SAS Institute, Cary, NC).² Median; range in parentheses (all such values).³ Least-squares mean ± SEM (all such values).^{4–7} Significantly different from the lowest tertile, adjusted for age, sex, and energy intake: ⁴*P* < 0.01, ⁵*P* < 0.05, ⁶*P* < 0.0001, ⁷*P* < 0.001.

income); therefore, its inclusion contains some overadjustment (24). Contrary to these findings, total FV intake was not associated with either 10-y risk of CHD or inflammation.

Most epidemiologic studies have shown that increased consumption of FV is associated with a lower risk of acute coronary syndrome (25), a lower progression of carotid atherosclerosis (26), a lower incidence of venous thromboembolism (27), and favorable changes in cardiac autonomic function (28). The most widely studied mechanism for the protective effect of FV on CHD risk has been inflammation (29–34). Inflammatory markers—such as CRP, interleukin-6, and tumor necrosis factor-α—have emerged as strong independent risk indicators for CVD (35, 36). In fact, CRP is known to exert pro-atherogenic effects on vascular endothelial cells (37, 38), induce matrix metalloproteinase production (39, 40), and modulate associated signaling pathways (38). Despite strong epidemiologic evidence of a potential role of FV in inflammation (32–34, 41), results from clinical trials have largely been inconsistent. In an 8-wk randomized controlled trial in hypertensive volunteers, daily consumption of 6 portions per day did not significantly reduce CRP concentrations compared with daily consumption of 1 portion per day (42). Likewise, Freese et al (43), in a 6-wk randomized controlled trial, found no differences in CRP concentrations be-

tween those with a high and low intake of vegetables, berries, and apple. On the other hand, daily consumption for 4 wk of 8 servings of vegetables and fruit significantly reduced CRP concentrations compared with consumption of 2 servings per day (44). Similarly, consumption of a vegetable soup (containing ≈80% raw vegetables) decreased biomarkers of both oxidative stress and inflammation (45).

Our results do not agree with available epidemiologic and clinical trial evidence for a protective role of FV in reducing CHD risk (46, 47) and inflammation (32–34, 41). The lack of a significant negative association between servings of FV intake, FRS, and CRP could be attributed to the limited distribution of FV in this population. The median intake of FV (3.2 servings/d) was lower than that reported in other large-scale cohorts, such as the Health Professionals Follow-Up Study (5.1 servings/d) (48), Nurses' Health Study (5.8 servings/d) (48), Women's Health Study (5.5 servings/d) (49), Atherosclerosis Risk in Communities Study (3.5 servings/d) (47), and the National Health and Examination Survey follow-up study (3.3 servings/d) (46). Studies that found a significant inverse association between FV intake and incident CVD events had a greater contrast in FV intake between extreme quantiles. For example, in the Health Professionals Follow-Up Study and the Nurses' Health Study (48), participants

TABLE 4Energy and fiber intakes and circulating concentrations of select nutrients of Puerto Rican men and women by tertile of fruit and vegetable variety¹

	Tertile of fruit and vegetable variety			<i>P</i> for trend
	1	2	3	
Fruit and vegetable intake (no.) ²	17.8 (2.0–22.4)	26.2 (22.4–29.7)	34.0 (29.7–44.7)	
No. of subjects	386	387	386	
Energy intake (kcal/d)	2224 ± 45 ³	2274 ± 46	2245 ± 46	0.74
Dietary fiber intake (g/d)	18.1 ± 0.3	19.2 ± 0.3 ⁴	19.9 ± 0.3 ⁵	<0.0001
Serum carotenoids (μmol/L)	1.59 ± 0.04	1.70 ± 0.04	1.94 ± 0.04 ⁵	<0.0001
Serum folate (nmol/L)	39.4 ± 1.1	44.3 ± 1.1 ⁶	45.1 ± 1.1 ⁷	<0.0001
Plasma pyridoxal-5'-phosphate (nmol/L)	12.4 ± 0.8	14.6 ± 0.8	17.5 ± 0.8 ⁵	<0.0001
Plasma ascorbic acid (μmol/L)	43.3 ± 1.1	45.4 ± 1.1	51.4 ± 1.1 ⁵	<0.0001

¹ Models were adjusted for age, sex, and energy intake by using ANCOVA (PROC GLM; SAS Institute, Cary, NC).² Median number of unique fruit and vegetables consumed at least once per month; ranges in parentheses.³ Least-squares mean ± SEM (all such values).^{4–7} Significantly different from the lowest tertile, adjusted for age, sex, and energy intake: ⁴*P* < 0.05, ⁵*P* < 0.0001, ⁶*P* < 0.01, ⁷*P* < 0.001.

TABLE 5

Multivariate associations between quantity and variety of fruit and vegetable intake, Framingham risk score, and C-reactive protein

	β (95% CI) ¹	
	Fruit and vegetable servings ²	Fruit and vegetable variety ³
Framingham risk score ⁴		
No. of subjects ⁵	870	870
Model 1	0.08 (−0.21, 0.36)	−0.06 (−0.13, 0.00)
Model 2	0.03 (−0.24, 0.29)	−0.08 (−0.14, −0.02)
Model 3	−0.01 (−0.28, 0.26)	−0.06 (−0.13, 0.01)
Log C-reactive protein ⁶		
No. of subjects ⁵	1145	1127
Model 1	0.005 (−0.010, 0.019)	−0.006 (−0.010, −0.002)
Model 2	0.007 (−0.007, 0.021)	−0.004 (−0.008, −0.001)
Model 3	0.006 (−0.009, 0.020)	−0.004 (−0.008, −0.001)

¹ β -Coefficients (95% CIs) were calculated by using ANCOVA (PROC GLM; SAS Institute, Cary, NC).

² Servings per day.

³ Number of unique fruit and vegetables consumed at least once per month.

⁴ Model 1: adjusted for sex, energy intake (kcal/d), alcohol use (current, former, or never), waist circumference (cm), *trans* fatty acid intake (g/d), saturated fatty acid intake (g/d). Model 2: adjusted as for model 1 plus vitamin supplement use (yes or no), cardiovascular medication use (yes or no), diabetes medication use (yes or no), and perceived stress score. Model 3: adjusted as for model 2 plus household income (US\$/y). Fruit and vegetable servings and variety models were adjusted for each other.

⁵ Note that the sample sizes for each analysis fluctuate around the reported value because of missing data for some covariates.

⁶ Model 1: adjusted for age (y), sex, energy intake (kcal/d), smoking (current, former, or never), alcohol use (current, former, or never), white blood cell count (mm³). Model 2: adjusted as for model 1 plus additional adjustment for waist circumference (cm), diastolic blood pressure (mm Hg), diabetes status (yes or no), vitamin B-6 intake (mg/d), nonsteroidal antiinflammatory medication use (yes or no), and perceived stress score. Model 3: adjusted as for model 2 plus additional adjustment for household income (US\$/y). Fruit and vegetable servings and variety models were adjusted for each other.

in the highest quintile of FV intake consumed nearly twice the median servings of those in the highest tertile in our population (10.2 and 9.2 compared with 5.9 servings/d). In the Women's Health Study, median intake in the third quintile approximated intakes in our highest tertile (5.5 compared with 5.9 servings/d), whereas those in the highest quintile had a median intake of 10.2 servings/d (49). The limited distribution in FV intake in this Puerto Rican population may be inadequate to detect associations with CHD risk. Our results are consistent with those of Genkinger et al (50), who also observed limited variation in FV intake (0.87 and 4.89 servings/d in extreme quintiles) and found no association with cardiovascular mortality.

Whereas several epidemiologic studies have consistently shown an association between increased FV intake and CHD, none that we are aware of have explored associations between FV variety, CHD risk, and inflammation. The biological mechanisms for the protective effect of greater variety in FV intake are not entirely clear, but may be attributable to the singular or synergistic action of several bioactive compounds. Mounting evidence shows that bioactive compounds such as carotenoids, flavonoids, phytoestrogens, dietary fiber, and resveratrol, which are present in a wide variety of FV, influence the risk of CHD by preventing

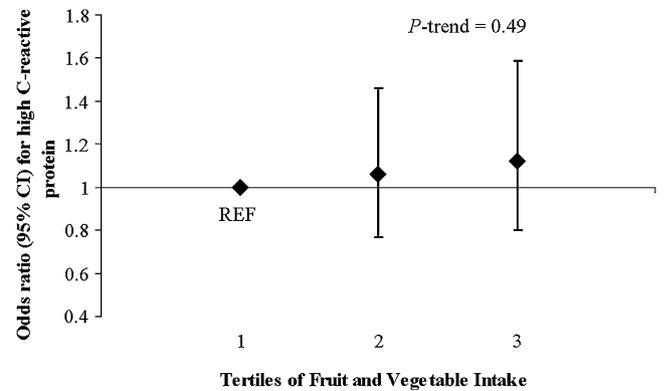


FIGURE 2. Odds ratios (95% CIs) of high C-reactive protein category across tertiles of fruit and vegetable intake (servings/d). Data were adjusted for age (y), sex, energy intake (kcal/d), smoking (current, former, or never), alcohol use (current, former, or never), white blood cell count (mm³), variety in fruit and vegetable intake (number of unique fruit and vegetables consumed at least once per month), waist circumference (cm), diastolic blood pressure (mm Hg), diabetes status (yes or no), perceived stress score, vitamin B-6 intake (mg/d), nonsteroidal antiinflammatory medication use (yes or no), and total household income (US\$/y) by cumulative logistic regression (PROC LOGISTIC; SAS Institute, Cary, NC). Tertile 1, *n* = 383; tertile 2, *n* = 383; tertile 3, *n* = 383. REF, reference.

oxidation of cholesterol in the arteries and by decreasing oxidative stress (51–53). Although trials have not specifically evaluated the effect of FV variety on CHD, supplementation trials using a mixture of FV juices have been shown to lower plasma homocysteine (54) and improve antioxidant (54–56) status. However, like FV intake, we noted inverse, albeit borderline nonsignificant, associations between FV variety and the FRS. Because the FRS is calculated only for participants without a history of CVD, we did not have enough power to detect associations in this smaller subset. It may also be that inflammation may be the primary operational pathway through which FV variety decreases CHD risk. Low dietary intakes of FV and low serum concentrations of flavonoids, carotenoids, and

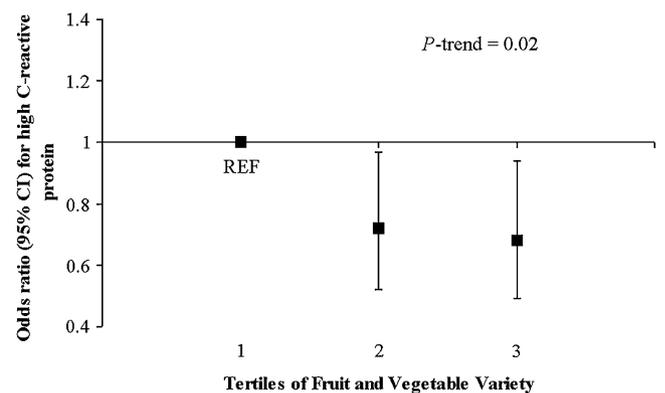


FIGURE 3. Odds ratios (95% CIs) of high C-reactive protein category across tertiles of fruit and vegetable variety (number of unique fruit and vegetables consumed at least once per month). Data were adjusted for age (y), sex, energy intake (kcal/d), smoking (current, former, or never), alcohol use (current, former, or never), white blood cell count (mm³), fruit and vegetable intake (servings/d), waist circumference (cm), diastolic blood pressure (mm Hg), diabetes status (yes or no), perceived stress score, vitamin B-6 intake (mg/d), nonsteroidal antiinflammatory medication use (yes or no), and total household income (US\$/y) by using cumulative logistic regression (PROC LOGISTIC; SAS Institute, Cary, NC). Tertile 1, *n* = 386; tertile 2, *n* = 387; tertile 3, *n* = 386. REF, reference.

vitamin C, which may indirectly represent low FV variety, have been associated with increased inflammatory status in several populations (7, 29, 30, 57). Greater variety may ensure exposure to a wide spectrum of antioxidants and phytonutrients that are needed to reduce both CHD risk and cytokine-mediated inflammation. The totality of this evidence supports dietary recommendations for consuming a variety of FV.

Our findings need to be interpreted in the context of a few limitations. First, because of the cross-sectional nature of our study, we used the Framingham risk equations to predict the 10-y risk of CHD. Because the FRS was derived from the experience of the Framingham Heart Study, a predominantly non-Hispanic white population in Massachusetts, concern exists as to whether the FRS can be generalized to other populations. Using much earlier data from Puerto Rico, D'Agostino et al (58) showed that the FRS systematically overestimated 5-y CHD events in Puerto Ricans. However, these data were derived from men participating in the Puerto Rican Heart Health Program in the mid 1960s, and the prevalence of CVD risk factors in Puerto Ricans has increased considerably since then (59, 60). Whereas absolute individual risk estimates may be more accurately obtained with recalibration, participants in our study should be ranked appropriately according to their risk estimates. Furthermore, using the FRS as a continuous measure as opposed to a categorical variable (low, medium, or high risk), we avoided potential misclassification of our participants. Thus, observed associations between FV intake and the FRS should be valid. Second, we used cross-sectional data. Prospective cohort studies are ideal to study the role of FV in the development and progression of CHD, because dietary data are assessed before the outcome, thus avoiding potential bias. Nonetheless, the current study showed, for the first time, the importance of variety in FV intake in the Puerto Rican population. Third, even after adjustment for several important covariates, residual confounding is still a possibility. Finally, it may be possible that selection bias may have occurred in our study. Although it may not be possible to accurately assess how this may affect our outcomes, it is possible that those who declined participation may have a higher FRS because they were more likely to be older.

In summary, the present study supports the hypothesis that variety in FV intake is associated with lower serum CRP. The results of this study indicate that one potential mechanism underlying the relation between FV intake and CHD may involve influencing the process of systemic inflammation. Our findings underscore recommendations by several national agencies to increase variety in FV intake, particularly in this high-risk population.

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The authors' responsibilities were as follows—SNB and KLT: study design and analytic plan; SNB: analysis, data interpretation, and writing of the manuscript; and KLT: supervision of all phases of the project. All authors critically reviewed the manuscript. None of the authors had a conflict of interest.

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