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Fruit and vegetable consumption and its relation to markers of inflammation and oxidative stress in adolescents

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Abstract

Background—Fruits and vegetables, foods rich in flavonoids and antioxidants, have been associated with lower risk of stroke, coronary heart disease, and markers of inflammation and oxidative stress in adults. Markers of inflammation and oxidative stress are predictors of coronary heart disease risk; however, it is unknown whether these markers are related to dietary flavonoid and antioxidant intake in youth.

Objective—To determine whether greater intakes of fruit and vegetables, antioxidants, folate, and total flavonoids were inversely associated with markers of inflammation and oxidative stress in 285 adolescent boys and girls aged 13-17 years.

Methods—In this cross-sectional study conducted between February, 1996-January, 2000, diet was assessed by a 127-item food frequency questionnaire. Height and weight measurements were obtained and a fasting blood sample drawn. Spearman partial correlation analyses evaluated the relation of intakes of fruit and vegetables, antioxidants, folate, and flavonoids with markers of inflammation (C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), and 15-keto-dihydro-PGF_{2 α} metabolite and oxidative stress (urinary 8-iso prostaglandin F_{2 α}, a F₂-isoprostane), adjusting for age, sex, race, Tanner stage, energy intake, and body mass index.

Results—Urinary F₂-isoprostane was inversely correlated with intakes of total fruit and vegetables, vitamin C, beta-carotene, and flavonoids. Serum CRP was significantly inversely associated with intakes of fruit, (r = -0.19; p=0.004), vitamin C (r = -0.13, p=0.03); and folate (r=-0.18; p=0.004). Serum IL-6 was inversely associated with intakes of legumes, vegetables, beta-carotene, and vitamin C (r=-0.12, p=0.03). Serum TNF- α was inversely associated with beta-carotene (r=-0.16, p=0.02) and luteolin (r=-0.15, p=0.02).

Conclusion—Study results show that the beneficial effects of fruit and vegetable intake on markers of inflammation and oxidative stress are already present by early adolescence and provide support for the United States Dietary Guideline "to consume five or more servings per day" of fruits and vegetables to promote beneficial cardiovascular health

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INTRODUCTION

Fruits and vegetables, which contain a myriad of vitamins, minerals, and food compounds, have been inversely associated with cardiovascular disease risk factors (1-5), such as inflammation and oxidative stress, in adults (6-9). Folate and antioxidants, such as vitamin C and beta-carotene, are found in a variety of fruits and vegetables and have been related to lower levels of markers of inflammation and oxidative stress in adults (5,10-12). Plant polyphenols, such as flavonoids, also found in many fruits and vegetables may play a role in this relation (13). Flavonoids, the most common subclass of polyphenols, are usually concentrated in the skins and peels and are responsible for the deep color of fruits and vegetables. Polyphenolic compounds provide antioxidant properties and protect the plant from pathogens, ultraviolet light, and other stress. Flavonoids are classified by their structures (anthocyanidins, flavanols (or catechins), flavanones, flavones, flavonols, and isoflavones), which determine their antioxidant properties. The majority of flavonoids consumed by the United States (U.S.) population are found in onions, apples, and tea (14). Although, a dietary recommendation does not exist for flavonoids, daily intake varies from 20 mg/day in the U.S. to more than 70 mg/ day in Holland (15). Data from the National Health and Nutrition Examination Survey 2001-02 showed the average daily intake of vitamin C was 100 mg and 76 mg for boys and girls aged 14-18 years, respectively, which is above their recommended dietary allowance of 75 and 65 mg, respectively (16). In the same survey, which was conducted after initiation of fortification of bread and cereals in 1998, the reported mean intake of folate was 683 and 500 dietary folate equivalents for boys and girls aged 14-18 years, respectively, which is above the current recommended dietary allowance of 400 mcg (16). Currently there is no dietary recommendation for beta-carotene (17).

Although the effects of flavonoid consumption on cardiovascular risk factors among children and adolescents have not been defined, it is known that a heart-healthy diet initiated at a young age reduces the prevalence of obesity, high cholesterol, and high blood pressure (18). This suggests that the inverse relation between flavonoid consumption and markers of inflammation and oxidative stress shown in adults (19-25) may also be operative prior to adulthood, offering a strategy to prevent the early onset of cardiovascular risk, such as low-grade inflammation (26).

Prostaglandins and thromboxanes are bioactive compounds derived from arachidonic acid catalysed by cyclooxygenases and are important mediators of inflammation. $PGF_{2\alpha}$ is a major prostaglandin formed at the site of inflammation, and 15-keto-dihydro-PGF_{2a}, a major metabolite of $PGF_{2\alpha}$ is shown to be a potent indicator of *in vivo* cyclooxygenase-mediated inflammation (27). F2-Isoprostanes are formed during free radical-catalysed peroxidation of arachidonic acid and are reliable markers of oxidative stress *in vivo*, and 8-iso-PGF_{2a} is one of the major F2-isoprostanes (28). The role of C-reactive protein (CRP) and cytokines as inflammatory markers involved in the development of atherosclerosis is well known in adults (29), but only recently in children (26).

The present study was conducted in a cohort of adolescents participating in a longitudinal study of obesity, insulin sensitivity and cardiovascular risk. The study objective was to determine if intake of total and individual flavonoids, antioxidants vitamin C and beta-carotene, folate, and fruits and vegetables was inversely related to markers of inflammation (CRP, interleukin-6 [IL-6], tumor necrosis factor- α [TNF- α], and 15-keto-dihydro-PGF_{2 α} [PGF_{2 α}]) metabolite and oxidative stress (8-iso-F_{2 α}[F₂-isoprostane]).

MATERIALS AND METHODS

Study Population

Approval for this study was obtained from the University of Minnesota Institutional Review Board Human Subjects Committee. Consent for participation was obtained from all participants and their parents or guardians.

Participant recruitment and study methods have been previously described in detail (30). Briefly, after blood pressure screening of 12,043 fifth to eighth grade Minneapolis, Minnesota Public School students, 500 study participants were randomly selected by strata of sex, race (black or white), and blood pressure percentiles (one-half of study participants had blood pressure measurements in the upper 25 percentiles and the other one-half of study participants had blood pressures in the lower 75 percentiles). Of the 400 youth who enrolled in the main study about the influence of insulin sensitivity on blood pressure change, 357 participated at mean age 13 years (range 11 - 15 years) and 304 participated at mean age 15 years (range 13-17 years) between February, 1996-January, 2000. To evaluate the relations between flavonoid intake and biomarkers in the current study, the cohort for this study consisted of 285 participants with complete data for dietary intake collected at two time points, average ages 13 and 15 years, and for clinical measures, fasting blood and overnight urine samples collected at average 15 years.

Measurements

Physical measurements—Height was measured using a wall-mounted stadiometer (Fairgate Rule Co., Cold Springs, NY), weight was measured using a physician balance beam scale (Health O'Meter; Bridgeview, IL), and body mass index (BMI) was computed by study staff as weight in kg divided by height in meters squared (kg/m²). Tanner stage was assessed by a board-certified pediatrician based on pubic hair development in boys and pubic hair and breast development in girls (31).

Laboratory measurements—Fasting blood samples for CRP, IL-6 and TNF- α collected prior to beginning the insulin clamp were centrifuged within 20 minutes of collection and stored at -70°C. Serum CRP was measured using an ultrasensitive colorimetric competitive enzyme-linked immunosorbent assay (ELISA) (32). Serum IL-6 and TNF- α were measured using enzyme-linked immunosorbent assays in the cytokine reference laboratory of the University of Minnesota. The urine samples were analyzed for F2-isoprostane and PGF_{2 α} by radioimmunoassay (RIA), as described by Basu (33,34) and concentrations were adjusted for urine creatinine concentration.

Assessment of dietary intake—At baseline (mean age 13 years) and 2 years later (mean age 15 years), the 127-item Willett Food Frequency Questionnaire (FFQ) (35), including vitamin supplements, was administered by trained and certified staff according to standard procedures, with parental help if needed. Food models were used to facilitate estimation of usual portion size. Fruit and vegetable food groups were created: the fruit group included citrus fruit, berries, melon, apples, pears, peaches, plums, and dried fruit; and the vegetable group included green leafy vegetables, cruciferous vegetables, carotenoid containing vegetables, and miscellaneous vegetables. Fruit juice and French fried potatoes were each examined separately due to the high sugar content in juice and the high fat content in French fried potatoes. Frequency and dose of vitamin supplement intake were queried for multivitamins, vitamins A, C, D, and E, selenium, iron, calcium, and zinc. Servings of fruit, fruit juice, vegetables, French fried potatoes, and legumes, and amount of vitamin C (with and without vitamin supplements), folate (with and without vitamin supplements), beta-carotene, and total and individual flavonoids, including apigenin, kaempferol, luteolin, myricetin, and quercetin, were available

from the Harvard Nutrient Data System. The United States Department of Agriculture Flavonoid Database was used to identify which fruits and vegetables were good sources of the flavonoids of interest (Table 1).

Statistical Analysis

All analyses were conducted using SAS (Version 9.1; SAS Institute, Inc., Cary, North Carolina). The distributions of CRP, IL-6, TNF- α , PGF_{2 α}, and F2-isoprostanes were highly skewed and were log transformed before analysis to achieve normality. To return to the natural scale, means and standard errors (SE) of these analytes were back log-transformed and reported as geometric means. Unadjusted data are expressed as mean \pm standard deviation (SD). To increase precision in the analyses of nutrient and food intake, diet data collected at the two time points were averaged. Spearman partial correlation coefficients were determined to relate intakes of nutrients, flavonoids, and fruit and vegetables with markers of inflammation and oxidative stress, adjusting for age, sex, race, Tanner stage, energy intake (kcal), and BMI. The study was powered at 90% to detect a correlation of 12% or greater between exposure and outcome variables. A significance criterion of p<0.05 was used.

RESULTS

The cohort included 155 boys and 130 girls, age 15 ±1.2 years; 20% were African American. Physical characteristics and laboratory data are shown in Table 2. Tanner stage and BMI were similar for boys and girls and no significant differences were found between genders for CRP, TNF- α , and IL-6. However, PGF_{2 α} metabolite, and F₂-isoprostanes were significantly greater in girls than boys (*p*=0.003 and *p*=0.004, respectively).

Study participants consumed an average of 5.5 ± 0.3 servings (about 2.75 cups) of fruits and vegetables per day. This was reduced to an average of 3.9 ± 1.5 servings (about 2 cups) per day when fruit juice and French fried potatoes were excluded from the total fruit and vegetable group. Of the daily fruit and vegetable consumption, fruit juice contributed nearly 50% of the total fruit servings and French fried potatoes contributed about 10% of the total vegetables. Adolescent boys consumed significantly more kilocalories than girls, but no significant difference was observed for intakes of vitamin C, beta-carotene, folate, legumes, fruit, and vegetables after adjusting for age, race, Tanner staging, and energy intake (Table 3). Total flavonoid intake averaged 14.8 \pm 11.7 mg per day. Consumption of total flavonoids and each of the individual flavonoids was also similar between boys and girls.

Associations of nutrient intake with markers of inflammation and oxidative stress

After adjusting for age, sex, race, Tanner stage, energy intake and BMI, vitamin C intake was inversely related to some markers of inflammation, including CRP and IL-6, and oxidative stress (F2-isoprostane) (Table 4). Intake of beta-carotene was inversely related to IL-6 and TNF- α , while folate intake was inversely correlated with CRP and F2 isoprostanes.

Associations of fruit and vegetable consumption with inflammatory and oxidative stress markers

Fruit and vegetable intakes were correlated with markers of inflammation and oxidative stress after adjusting for age, sex, race, Tanner stage, energy intake and BMI. Fruit intake was inversely related to CRP only. A greater number of legume and vegetable servings were both associated with lower concentrations of IL-6, while the vegetable group was inversely related to TNF- α . Intakes of fruit juice and French fried potatoes were not related to any biomarker. Total fruit and vegetable intake (with or without French fried potatoes and fruit juice) was significantly and inversely related to CRP, TNF- α , IL-6, and F₂-isoprostanes, but not PGF_{2 α}.

Associations of flavonoid consumption with inflammatory and oxidative stress markers

After adjusting for age, sex, race, Tanner stage, energy intake, and BMI, intakes of total flavonoids and individual flavones kaempherol, and quercetin were inversely related to F_{2} -isoprostanes. Only luteolin was inversely correlated with TNF- α . The individual flavonoids myricetin and apigenin were not related to any of the inflammatory or oxidative stress markers.

DISCUSSION

In this study of adolescents, a diet high in fruit and vegetables, and therefore, rich in antioxidants, folate, and flavonoids, was associated with lower levels of markers for inflammation and oxidative stress. These results show that increased consumption of fruit and vegetables, and therefore, antioxidant and flavonoid intake, begin to have an effect on markers of inflammation and oxidative stress early in life and suggest that with an ongoing similar pattern of consumption, these beneficial relations may grow stronger with aging, resulting in lower cardiovascular risk (36).

The relation of flavonoid intake to inflammation has not been previously characterized in an adolescent population. However, several in vivo experiments and observational studies conducted in middle age and elderly populations have examined this association and noted inverse relationships (37-41), consistent with our study outcomes in adolescents. Grape flavonoids decreased TNF- α and IL-6 concentrations in both pre- and postmenopausal women (42), and IL-6 was shown to be inversely related to quercetin (43) and tea flavonoids in vivo (44). In the present study total flavonoids and the individual flavonoids, kaempferol and quercetin, were significantly inversely associated with oxidative stress. However, with the exception of the inverse relation of luteolin to TNF- α , none of the flavonoids was significantly associated with levels of the other inflammatory factors. This may suggest that the earliest effect of flavonoids is on oxidative stress, with a significant effect on inflammation not noted until adult life, as previously described (36-44). It is important, however, to study the development of subclinical inflammation in youth without chronic disease to determine its early mechanisms.

One study of 79 children aged 6-14 years observed non-significant associations between antioxidant intake (vitamins C and E and beta-carotene) and inflammatory markers CRP, IL-6, and TNF-a; however, the study had insufficient power to detect weak associations (45). The present study of 285 adolescents mean age 15 years demonstrated significant and inverse associations of antioxidant intake with markers of inflammation and oxidative stress.

The proposed beneficial effects of antioxidants, folate, and flavonoids, such as lowering blood cholesterol and homocysteine, reducing blood pressure, decreasing platelet aggregation, and scavenging free radicals and reactive oxygen species (ROS), are associated with a reduction in the inflammatory markers and prostaglandins evaluated in this study, thus supporting the presumption that they are intermediaries in the pathway to development of cardiovascular disease (46-51). Decreases in cytokines, such as IL-6, are directly associated with a decrease in inflammation (52,53). Decreases in oxidative stress are associated with lower levels of ROS, free radicals, and reactive nitrogen species, resulting in a reduction in lipid oxidation and formation of atherosclerotic plaques (54-56). A 4-week randomized intervention trial in healthy, non-smoking men showed that a diet rich in carotenoids (8 servings per day of fruits and vegetables) reduced serum CRP (57). In a cross-sectional analysis of data from the Massachusetts Hispanic Elder Study a significant inverse dose-response relation was found for fruit and vegetable intake with CRP and plasma total homocysteine (3). A recent prospective study of 40-59 year old English males found that fruit intake had a significant inverse association with CRP, blood viscosity, and tissue plasminogen activator antigen (11). Results from the present study have shown for the first time that consumption of antioxidants, total

flavonoids, and total fruit and vegetables was associated with lower levels of oxidative stress (F2-isoprostanes) in healthy adolescents.

While male sex has been strongly associated with risk factors for atherosclerosis and cardiovascular disease (58), and previous research has shown increased oxidative stress in young adult, healthy, non-smoking men in comparison to a similar group of women (59), the present study found that girls at mean age 15 had a significantly higher concentration of F2-isoprostanes. It is possible that this may be related to the age of the boys and girls, with effects of recent puberty influencing levels of oxidative stress and antioxidant activity (60,61). Alternatively, it may be related to changes in percentages of body fat versus lean body mass at this age; however, regression models were adjusted for Tanner staging and body mass (62). Previous research has demonstrated a significant reduction in oxidative stress, represented by F2-isoprostane, in obese men and women undergoing a nutritional and behavioral intervention (63); and decreased oxidative cellular damage to DNA and lipids was observed following a 14 day dietary intervention that included 12 servings of fruits and vegetables (64).

Although antioxidant and flavonoid analyses have been conducted successfully in prior studies (65,66), there are potential errors associated with assessing the antioxidant and flavonoid content of foods (67). Determination of flavonoid content is difficult due to the complex manner in which polyphenols are synthesized in food products and lack of a comprehensive food composition database for flavonoids. Quantification of antioxidant and flavonoid consumption may be further complicated by food storage, handling, processing, and preparation (68-70). Water-soluble antioxidants and flavonoids are released into high temperature cooking water and discarded.

One potential limitation of this study is the lack of smoking information for the study participants. It is likely that smoking would have attenuated the relation between dietary intake and the outcome factors (71). The weak correlations between dietary intake and markers of inflammation and oxidative stress ranging from -0.13 to -0.19 (all p<0.5) may be considered a potential limitation of the study. However, associations of BMI with markers of inflammation and oxidative stress are known to be moderately strong in adults ($r \ge 0.35$; p < 0.001) (72,73). In the present study with 90% power to detect small associations and after adjusting for BMI and other covariates, dietary antioxidant, flavonoid, and fruit and vegetable intake remained significantly and inversely related to markers of inflammation and oxidative stress in adolescent boys and girls. Furthermore, it is likely that the duration of low-grade inflammation and oxidative stress concentrations in this sample of adolescents are not as great as in adults (26,62); and therefore, the strength of the associations would not be as great in adolescents compared to those in adults (12,36-44). Despite these limitations, this study provides encouraging results with regard to the adolescent diet and its relation to inflammatory markers and oxidative stress.

On a negative note, dietary intake of adolescents enrolled in this study showed that they did not achieve the Food Guide Pyramid recommendations (74) for fruit and vegetable consumption. Adolescents enrolled in this study consumed about ½ of the recommended amounts for fruit (1.5-2 cups/day) and vegetables (2.5-3.5 cups) (74). This is consistent with recent reports on the diet of adolescents in the U.S. (75). In addition, most adolescents consumed lower amounts of total flavonoids than the U.S. national average estimate for adult flavonoid consumption (~20 mg/day) (15); however, vitamin C intakes were greater than the recommended amount (16,75). Thus, strategies designed to increase fruit and vegetable intake, and therefore antioxidant and flavonoid intakes, in adolescents may result in an overall reduction in cardiovascular risk indicators. This is consistent with studies on the pathophysiology of cardiovascular disease showing that arterial plaque formation and other

negative cardiovascular sequale begin early in life and that 5-6 or more daily servings of fruit and vegetables are associated with fewer adverse events in adulthood (36,76,77).

Conclusion

Evidence is accumulating that suggests low-grade inflammation and oxidative stress predicts an increased risk for chronic disease in adults (29,46-54) and adolescence (26,78). As shown in the present study, fruit and vegetable intake was associated with lower concentrations of systemic oxidative stress and inflammation in adolescents, which is similar to studies in adults (1-5). Anti-inflammatory mechanisms, such as dietary antioxidants, may inhibit the development of atherosclerosis (29,55), and therefore, following the recommendations of the U.S. Dietary Guidelines for Americans (79), including a diet rich in fruit and vegetables, may be an important strategy for disease prevention (36,40,76-78).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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TABLE 1

Common food sources rich in flavonoids

Individual Flavonoid	Flavonoid Subclass	Common Food Sources
Apigenin	Flavone	Parsley, celery, rutabagas, spinach, peppers, cauliflower, beets, brussels sprouts
Kaempferol	Flavonol	Onions, peppers, rutabagas, brussels sprouts, broccoli, grapefruit, spinach, watercress
Luteolin	Flavone	Parsley, celery, rutabagas, lemons, olives, spinach, peppers, cauliflower, beets, brussels sprouts
Myricetin	Flavonol	Broccoli, beans, carrots, kale, onion, peppers, turnips, watercress
Quercitin	Flavonol	Onions, apples, berries, black grapes, tea, broccoli, kale, peppers, turnips, citrus fruits

Source: United States Department of Agriculture Flavonoid Database (14).

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CL. and defined in	Boys	(n=155)	Girls (i	1=130)	<i>q</i>
Characteristic	Mean	SD ^a	Mean	SD a	<i>p</i> -value
Age, years	15.1	1.22	14.9	1.23	0.13
Physical characteristics					
Tanner stage	4.46	0.83	4.56	0.59	0.22
Body mass index (kg/m ²)	23.3	5.02	24.0	5.31	0.21
Laboratory characteristics					
CRP (ng/mL) cd	0.77	1.89	0.85	1.87	0.12
TNF-α (pg/mL) ^{ce}	4.01	1.96	4.33	2.15	0.33
IL-6 (pg/mL) ¢f	2.16	2.09	2.41	1.95	0.17
F2-iso (nmol/mmol creatinine) ^{cg}	0.30	1.65	0.36	1.51	0.004
PGF_{2a} (nmol/mmol creatinine) ch	0.17	1.40	0.20	1.40	0.003
^a SD, standard deviation					
b p-values represent significance level of the difference	e between boys and girls				
c Geometric mean					
dCRP = c-reactive protein					
e TNF-a = Tumor necrosis factor- α					
f_{IL} -6 = Interleukin-6					
g F2-iso = F2-iso prostane					
$h_{PGF2a} = Prostaglandin F2a$					

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 Table 3

 Average daily nutrient and food intake of 285 adolescent boys and girls
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	Boys (n=155)		Girls (n=130)		<i>c</i>
Dany Nucrem Intake	Mean	SE^{b}	Mean	$\operatorname{SE} b$	<i>p</i> -value
Energy (kcal)	2562	83.2	2234	93.9	0.01
Protein (g)	91.4	2.5	90.2	2.8	0.76
Carbohydrate (g)	347	4.1	341	4.6	0.36
Fiber (g)	21.4	0.4	22.9	0.5	0.03
Total fat (g)	78.8	1.4	80.9	1.6	0.34
Vitamin C, food+suppl (mg) ^d	250.0	15.2	251.3	17.1	0.96
Vitamin C, food only (mg) e	204.6	9.0	175.9	10.2	0.04
Beta-carotene, food only(IU) ^e	9047	565	9047	589	0.31
Folate, food+suppl(mcg) ^d	471.2	15.2	462.6	17.3	0.71
Folate, food only (mcg) e	429.9	10.8	392.8	12.2	0.03
Daily Food Intake, serving ^a					
Fruit, 1 piece or $1/2$ cup f	1.4	0.09	1.4	0.09	0.90
Fruit juice, ½ cup	1.7	0.10	1.2	0.12	0.02
Vegetables, $1/2 \operatorname{cup} ^{\mathcal{B}}$	2.3	0.11	2.6	0.12	0.14
French fried potatoes, ½ cup	0.5	0.20	0.5	0.25	0.43
Legumes, ½ cup	0.2	0.03	0.2	0.03	0.89
Fruits and vegetables, ½ cup h	5.6	0.27	5.5	0.31	0.79
Daily Flavonoid Intake a					
Total flavonoids (mg)	14.2	0.58	15.4	0.66	0.16
Myricetin (mg)	0.92	0.07	1.03	0.08	0.27
Kaempferol (mg)	2.51	0.28	3.06	0.32	0.21
Quercetin (mg)	10.2	0.33	10.8	0.38	0.23
Luteolin (mg)	0.08	0.04	0.04	0.04	0.43
Apigenin (mg)	0.52	0.03	0.55	0.04	0.53

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 a Daily nutrient and food intakes were adjusted for age, race, Tanner staging, and total energy intake

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 $^{b}\mathrm{SE,}$ standard error

 $^{c}_{}$ p-value represents significance level of the difference between boys and girls

 $d_{\rm Values}$ represent nutrient from food + vitamin supplements

^eValues represent nutrient from food only

 $f_{\rm Fruit\ excludes\ fruit\ juice}$

^gVegetables exclude French fried potatoes

 $h_{\rm T}$ Total fruit and vegetables include fruit juice and French fried potatoes

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Table 4 Correlation of flavonoidsand fruit and vegetable intake with markers of inflammation and oxidative stress among adolescents, N= 285 Z NIH-P/

	(ng/mL)	(pg/mL)	(pg/mL)	(nmol/mmol creatinine)	(nmol/mmol creatinine)
Nutrient Intake ^a					
/itamin C, mg ^b	-0.13 *	NS	-0.14 *	NS	-0.16
seta-carotene, IU	NS	-0.14 *	-0.15 *	NS	NS
dolate, mcg b	-0.18	NS	NS	NS	-0.21 **
7000 Intake, servings a					
'ruit(w/o juice)	-0.19	NS	-0.13 *	NS	NS
ruit juice	NS	NS	NS	NS	NS
$^{\prime}$ egetables c	NS	-0.13 *	-0.15 *	NS	NS
rench fried potatoes	NS	NS	NS	NS	NS
egumes	NS	NS	-0.16 *	NS	NS
ruit and vegetables d	-0.15 *	-0.13 *	-0.16 ⁸	NS	-0.13 *
lavonoid Intake ^a					
otal flavonoids (mg)	NS	NS	NS	NS	-0.14
Myricetin (mg)	NS	NS	NS	NS	NS
Kaempferol (mg	NS	NS	NS	NS	-0.15 *
Quercetin (mg)	NS	NS	NS	NS	-0.15 *
Luteolin (mg)	NS	-0.15 *	NS	NS	NS
Apigenin (mg)	NS	NS	NS	NS	NS
F2-iso = F2-isoprostane					
PGF2a = Prostaglandin F2a					
><0.05					
* p<0.01					

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 $\boldsymbol{b}_{\text{Nutrients}}$ include food and vitamin supplement values

 $^{\mathcal{C}}$ Vegetables exclude French fried potatoes

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