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Increasing the Vegetable Intake Dose Is Associated with a Rise in Plasma Carotenoids without Modifying Oxidative Stress or Inflammation in Overweight or Obese Postmenopausal Women[1–3]

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Abstract

The optimal amount of vegetable consumption required to reduce chronic disease risk is widely debated. Intervention trials evaluating biological activity of vegetables at various doses are limited. We conducted a 3-dose, crossover feeding trial to test the hypothesis that vegetable intake is associated in a dose-dependent manner with increased plasma carotenoids and subsequently reduced oxidative stress and inflammation in 49 overweight, postmenopausal women. Participants were assigned in random order to 2 (130 g), 5 (287 g), and 10 (614 g) daily servings of fresh, greenhouse-grown vegetables for 3-wk intervals with a 4-wk washout period between treatments. Plasma total carotenoids significantly increased from 1.63 to 2.07 μmol/L with a dose of 2 vegetable servings, from 1.49 to 2.84 μmol/L with a dose of 5 vegetable servings, and from 1.40 to 4.42 μmol/L with a dose of 10 vegetable servings (pre-post paired *t* tests, all $P <$ 0.001). The change during each feeding period increased with each dose level ($P < 0.001$). Urine concentrations of 8-isoprostane F2α, hexanoyl lysine, and serum high sensitivity C-reactive protein were not affected by any administered vegetable dose. In this variable-dose vegetable study, a dose-response for plasma carotenoids was demonstrated without significant change in oxidative stress and inflammation in overweight, postmenopausal women. J. Nutr. 141: 1827–1833, 2011.

Introduction

Obesity-related chronic disease, including CVD[7], stroke, and cancer, remains the primary cause of death in American adults (1). Increased oxidative stress and/or inflammation have been identified as central pathological processes responsible for the morbidity and mortality of obesity-related disease (2–5). The availability of reliable measures of oxidative stress and inflammation (6–9) makes it possible to test the efficacy of select diet-based interventions in relation to modulation of disease processes. Further, numerous epidemiological studies support the hypothesis that an increase in fruit and vegetable consumption leads to a reduction in chronic disease risk factors (10–12), including through the effect of plant-specific bioactive compounds on oxidative stress and inflammation (13). Several controlled feeding studies have demonstrated efficacy in this regard (14–19). In contrast, other studies have not supported significant improvements in oxidative stress or inflammatory biomarkers with fruit and vegetable interventions, particularly when the population sampled was healthy (20–24).

Despite evidence of a modulating effect of vegetable and fruit intake on oxidative stress and inflammation, few studies have investigated these associations in a dose-specific manner. The question remains: what is the optimal daily dose of fruits and vegetables associated with the greatest reduction in oxidative stress and inflammation, which subsequently could be hypothesized to reduce chronic disease risk? The *Dietary Guidelines for Americans*, based largely on epidemiological evidence, recommends a range in fruit and vegetable intake of 5–9 servings/d (24). In a recent review that used consistent and comparable risk

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[3] Supplemental Figure 1 is available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at jn.nutrition.org.
[7] Abbreviations used: CVD, cardiovascular disease; FW, fruit weight; HEL, hexanyol lysine; hsCRP, high-sensitivity C-reactive protein; 8-Iso-PGF2α, 8-isoprostane-F-2-α.
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assessment of 12 modifiable risk factors, low fruit and vegetable intake was responsible for an estimated 55,000 deaths related to CVD annually in the US (25). Select intervention trials, such as the Dietary Approaches to Stopping Hypertension trial, suggest that optimal intake (in this case for blood pressure health) may be as high as 10 servings/d (26). Additionally, the Nurses' Health Study and the Health Professionals Follow-up Study showed that an intake of 8 servings/d compared with a lower intake demonstrated the greatest reduction in CVD risk, and a recent study among Australian men and women showed that an intake of ≥4 servings/d reduced risk of metabolic syndrome (27,28).

Here, we tested the hypothesis that higher vegetable intake is associated with reduced oxidative stress and inflammation in a sample of overweight and obese adult women. Further, we hypothesized that a dose-response relationship would be demonstrated. This study is among the first to implement a randomized, controlled, crossover design to evaluate the effects of a fresh vegetable intervention on change in oxidative stress and inflammation.

Participants and Methods

Study population

A total of 426 overweight/obese, nonsmoking, postmenopausal women, 50–75 y old, were screened from across southern Arizona over a 10-mo period. Of these, 75 were eligible for study participation: BMI was between 25 and 45 kg/m^2, they reported consumption of alcoholic beverage was ≤12 g ethanol/d, they had stable body weight for the previous 6 mo, they were in general good health with no known allergies to study specific vegetables (peppers, greens, tomatoes, or carrots), and they were willing to complete all study related activities. Fifty women successfully completed the 4-wk run-in period. They were randomized to participate in the diet intervention; eligible women who did not enter the trial were most frequently excluded due to lack of availability throughout the study period or vegetable intolerance, or they reported that the diet was too restrictive. This study was approved by the Institutional Review Board, Human Subjects Protection Committee at the University of Arizona. All participants provided written informed consent prior to participation.

Study design

We conducted a crossover-design study in which the order of diet intervention (3 different vegetable doses) was randomized (**Supplemental Fig. 1**). Prior to randomization, participants completed a 4 wk low-fruit/vegetable washout diet period that also served as the run-in period. During this period, participants were required to consume select low-nutrient/low-phytochemical vegetables at both low and high doses to evaluate the likelihood for longer-term adherence to study protocol during active intervention. Participants also completed study-related activities (diaries, diet questionnaires, etc.) during this period. Participants successful in completing the wash-out/run-in period were randomly assigned to 1 of 6 vegetable intervention groups, each with the same overall vegetable consumption but with a varying order of exposure (2, 5, and 10 servings of vegetables daily).

TABLE 1 Weekly vegetable intakes at doses of 2, 5, and 10 servings/d[1]

Vegetable	2 servings	5 servings	10 servings
		g/wk	
Tomato	400	800	1600
Baby greens mix	90	360	540
Red bell pepper	195	390	780
Baby carrots	230	460	1380
Total vegetables[2]	915	2010	4300

[1] Washout period vegetables included iceberg lettuce, corn, legumes, cucumber, white potatoes

[2] Total vegetable servings for individual vegetable types were adjusted based on qualitative work suggesting acceptable amounts of daily vegetables for consumption and to avoid logistical concerns with participants measuring out portions smaller than one-half portions. Thus, although total dose represents an approximate doubling of amounts between doses, individual portions for each vegetable were not doubled to reach the next dose level.

Intervention vegetables and dosing

Vegetables used in this intervention trial included baby leaf lettuce green mix, tomatoes, red bell peppers, and baby carrots (**Table 1**). These vegetables were strategically selected based on high frequency of intake in the U.S. population, nutrient and bioactive food component composition, and capacity to grow a consistent and reliable yield of crop in a controlled environmental setting. Selection of study vegetables as well as an acceptable maximum daily dose for intake of any individual vegetable was evaluated for participant acceptance using focus group qualitative research prior to finalizing the exposures for study use. Study-provided vegetables were all grown in local greenhouses, except carrots, which were purchased at a local grocery store.

Serving sizes for vegetables were adapted from the USDA My Pyramid guidance such that a serving of baby leaf greens was 1 cup, a serving of tomato was considered one medium tomato, a serving of red bell peppers was one-half of a large pepper, and a serving of carrots was 8–10 baby carrots (as prepackaged for single serving size). Thus, daily vegetable intakes averaged 130 g for 2 servings, 287 g for 5 servings, and 614 g for 10 servings. Participants were instructed to include 5–10 mL of salad dressing or olive oil and to consume the vegetables raw in an effort to somewhat standardize absorption of fat-soluble phytochemicals/nutrients. Throughout the trial, participants were restricted to only study-provided vegetables during the intervention periods and they were instructed to consume no more than 80 g of fruit daily. This restriction in background diet was important, so that any significant changes in the oxidative stress and inflammatory biomarkers could be attributed to the study-specific vegetable intervention.

Vegetable production and quality assurance

Study vegetables were grown using hydroponic greenhouses in the Controlled Environment Agriculture Center at the University of Arizona (Tucson, AZ). Briefly, the baby lettuce mix included 2 cultivars of lettuce (Cimmaron Romaine and Red Salad Bowl) and Japanese cruciferous leafy vegetables (komatsuna). Plants were grown in multi-cell trays filled with perlite (for lettuce) or a 1:1 mixture of perlite and vermiculite (for komatsuna) inside the greenhouse where air temperature was maintained at 23–26°C during the day and 16–18°C during the night with a relative humidity of 60%; harvesting occurred on d 23. All cultivars of harvested leaves were mixed together at a 1:1:1 weight ratio in a large plastic tub and kept in the 4°C cooler chamber. After 24 h of cooling, mixtures of lettuce were measured for predetermined servings and packed in Ziploc bags. Mixed greens provided 214.6 mg/kg FW of ascorbic acid as well as ~23 mg/kg FW β-carotene and 25.6 mg/kg FW of anthocyanins.

Red bell peppers (cultivar Triplestar) were grown using a high-wire system inside a greenhouse at The University of Arizona Campus Agriculture Center (Tucson, AZ), with time and nighttime air temperature maintained at 24 and 18°C, respectively. All fruits were harvested when they were at least 90% red and were weighed weekly to assess crop yield. The fruit was then sorted, graded, and packed in Ziploc bags according to predetermined servings. Compositional analysis from Brunswick laboratories showed the mean ascorbic acid concentration was 1.37 g FW and that of β-carotene was 13.0 mg/kg FW.

Cluster-type tomatoes (cultivar Brilliant) were produced and harvested at light-red stage in the local commercial greenhouse (Willcox, AZ) and delivered to Tucson. After postharvest ripening inside a 12°C cooler for 4 d, tomatoes were sorted, graded, and packed in plastic clamshell packs according to the predetermined servings. Baby-type carrots were purchased at local grocery stores (Costco and Safeway). All vegetables were kept at 4°C until distribution to the participants. Tomatoes were the highest source of intervention-related lycopene,

providing 31.4 mg/kg FW. Tomatoes also contained 210 and 8.9 mg/kg FW ascorbic acid and β-carotene, respectively. Carrots, which were purchased at the local supermarket, were also analyzed for nutrient composition and provided 46.4 mg/kg β-carotene and a small amount of phenolic compounds.

Adherence to vegetable intake and diet assessment measures

Study participants kept daily vegetable logs during all 3 vegetable interventions to include serving size, preparation method, time consumed, and foods eaten with study vegetables. In addition, participants were asked to return any uneaten vegetable portions to the study clinic weekly. Returned produce was weighed and a record of total vegetables consumed was estimated from grams provided minus grams returned to establish the net grams consumed per week. Dietary intake also was assessed at baseline and end of study using the validated Arizona FFQ (29). In responding to questionnaire items, participants were asked to report dietary intake over the previous 12 mo for the baseline measure and the study period (or previous 5 mo) for the end-of-study measure. In addition, participants received a supply of multivitamin supplement (One-A-Day Essential, Bayer Healthcare) and were asked to take one daily and discontinue any other dietary supplements throughout the study.

Outcomes and biosample measurements

The primary outcomes for hypothesis testing were changes in urinary and serum biomarkers of inflammation and oxidative stress. Three consecutive, daily, first-morning urine void samples were collected at baseline (end of run-in period) and before and after each vegetable dose using a standard protocol for sample collection. The purpose of collecting a 3-d sample was to establish a pooled sample that would capture intra-individual variability for oxidative stress biomarker analysis. Participants collected urine samples at home in amber-colored urine collection cups; stored samples were placed on ice in the study-provided storage cooler and transported to the study clinic on ice. Samples were pooled, gently vortexed, aliquoted into two 15-mL tubes, centrifuged under refrigeration (4°C) at 1500 × *g* for 10 min, and divided into 2-mL aliquots for immediate storage at −80°C.

To assess cholesterol, carotenoids, and biomarkers of oxidative stress and inflammation, blood samples were collected at baseline and before and after each vegetable dose period from participants after a 12 hr fast. Blood was collected by venipuncture as follows: one 10-mL foil-wrapped plasma tube with heparin additive, one 5-mL whole blood tube with sodium citrate additive, and two 10-mL foil-wrapped serum tubes with no additives. Each tube was centrifuged under refrigeration (4°C) at 1500 × *g* for 10 min, with serum and plasma aliquoted into 2-mL cyrovials and stored at −80°C.

Plasma carotenoids

Plasma samples were shipped express mail on dry ice to the University of California, San Diego, Moores Cancer Center Analytical Laboratory of Dr. Cheryl Rock for carotenoid analysis. Plasma carotenoids were separated and quantified by HPLC methodology (30) using a Varian Star 9010, 9050 system with a variable wavelength UV/visual spectroscopy detector. The analytical method measures 90% of the total plasma carotenoids present and permits quantification of the predominant carotenoids: α-carotene, β-carotene, lycopene, β-cryptoxanthin, and lutein plus zeaxanthin. Accuracy was assessed by periodic analysis of National Institute for Standards and Technology Standard Reference Material, and a pooled plasma reference sample was concurrently analyzed to monitor precision. Values for carotenoid concentrations were within 10% of National Institute for Standards and Technology values, and day-to-day CV during analysis of study samples were <5%. Fasting plasma samples were also used to measure total cholesterol using enzymatic methods (Kodak Ektachem Analyzer System, Johnson & Johnson Clinical Diagnostics).

Biomarkers of oxidative stress

8-Iso-PGF2α. Undiluted, pooled urine samples were analyzed in duplicate for 8-Iso-PGF2α using a competitive ELISA purchased from Oxford Biomedical Research Company (catalogue no. EA85). Analysis followed the protocol provided by the manufacturer and used a kit that was validated using GC-MS methodology. All samples were read using an MRX Revelation micro-plate reader at a wavelength of 450 nm. The mean, SD, and CV were calculated; any sample with a CV > 10% was retested. Measures were normalized to urinary creatinine levels (Cayman Chemical).

HEL. Undiluted, pooled urine samples were shipped express mail on dry ice to Genox Corporation for HEL analysis using a competitive ELISA. Samples were read using a microplate reader at a wavelength of 450 nm. All measurements were performed in duplicate and normalized for urinary creatinine levels. The mean, SD, and CV were calculated; any sample with a CV >10% was retested.

hsCRP biomarker of inflammation. hsCRP was measured in fasting serum using the highly sensitive double sandwich antibody ELISA kit (Diagnostic Systems Laboratories) according to the manufacturer's standard protocol. All measures were completed in triplicate at a wavelength absorbance measurement of 450 nm. Any sample with a CV > 10% was retested.

Anthropometric, demographic, and lifestyle data. Demographic data, including education and race/ethnicity, were self-reported on a standardized questionnaire. Anthropometric measurements were collected using standardized protocols for height, weight, and waist and hip circumferences (31). Body composition was assessed using a hand-held bioelectrical impedance unit (Omron HBF-306) according to manufacturer specifications. Diastolic and systolic blood pressure were measured twice in the sitting position using Omron HEM-711ACN; the mean of 2 measures was recorded for data analysis. All measurements were collected at baseline and before and after each vegetable dose assignment for all study participants.

Statistical analyses. Descriptive statistics were analyzed as means ± SD. Nutrient intakes and body weight before and after the study were compared using paired *t* tests. For each separate dose of vegetables (2, 5, or 10) before and after biomarker values were compared using paired *t* tests. Mixed effects modeling, to produce β-coefficients, was used to assess the effect of vegetable feeding on each biomarker, adjusted for the preintervention value of the biomarker, BMI, and total cholesterol as fixed effects (and individual and intervention regimen order as random effects). In these models, vegetable dose was treated as a categorical variable, with 2 vegetable servings/d as the reference group. To test for significant trends, vegetable dose was treated as an ordinal variable with values of 2, 5, or 10. The α level for all analyses was 5%. All statistical analyses were performed using Stata 11.2 (StataCorp).

Results

The 49 participating women were predominantly white/non-Hispanic (67.4%), received a post-high school education, and ranged in age from 52 to 65 y; most were obese with a BMI >32 kg/m^2 (**Table 2**). Vegetable intervention adherence was excellent with 96% of vegetables consumed and no significant differences across doses (data not shown). The only changes in dietary intake during the study (pre- vs. post-FFQ) were a decrease in carbohydrate intake from 196 ± 98 to 164 ± 85 g/d ($P < 0.05$) and an increase in carotenoid intake from 8 ± 7 to 18 ± 8 mg/d ($P < 0.001$). Body weights did not significantly change during the study (data not shown).

Plasma total carotenoids decreased between enrollment (pre-run–in period) and the end of the 4-wk run-in period from 1.8 to 1.3 μmol/L ($P < 0.001$) (**Table 3**). Plasma total carotenoids increased during each period and differed among all dose levels ($P < 0.05$) (**Table 4**). Using mixed effect models, we found that doses of 5 and 10 vegetable servings/d increased plasma concentrations of total carotenoids and α and β-carotene, lutein,

TABLE 2 Baseline characteristics of overweight or obese postmenopausal women participating in the vegetable dose study[1]

Baseline characteristic	
Age, *y*	58.6 ± 5.6
Education completed, *n (%)*	
High school or less	13 (26.5)
Some college	17 (34.7)
Completed college	19 (39.8)
Race/ethnicity, *n (%)*	
White	33 (67.4)
Black, African American	5 (10.2)
Hispanic	10 (20.4)
Other	1 (2.0)
Height, *cm*	162.3 ± 6.0
Weight, *kg*	84.7 ± 14.4
BMI, *kg/m*^{2}	32 ± 5
Body fat, *%*	41.5 ± 4.5
Physical activity, *MET*[2] *h/d*	39 ± 6
Waist:hip ratio	0.9 ± 0.1
Plasma total cholesterol, *mmol/L*	5.2 ± 0.9
Systolic blood pressure, *mm Hg*	127 ± 16
Diastolic blood pressure, *mm Hg*	84 ± 11

[1] Values are mean ± SD, or *n* (%); *n* = 49.
[2] MET, metabolic equivalent of task.

and lycopene, but not β-cryptoxanthin compared to 2 servings/d (**Table 5**). However, vegetable dose was not associated with change in 8-Iso-PGF2α, HEL, or hsCRP. There also was no association between change in total plasma carotenoids and 8-Iso-PGF2α, HEL, or hsCRP (data not shown). Stratification by BMI (<30 vs. ≥30 kg/m^2) did not affect the results (data not shown).

Discussion

Recommendations for optimal fruit and vegetable intake have largely been derived from observational studies. This study was designed to define the most effective dose for lowering oxidative stress and inflammation and, subsequently, chronic disease risk in a sample of overweight/obese adult females as has been suggested by earlier work (32–35). Despite a dose-response rise in serum carotenoid concentrations with greater vegetable intake, suggesting good adherence to the study intervention, vegetable dose was not associated with reduced oxidative stress or inflammation. The randomized dose order and crossover design with sufficient washout periods between doses and controlled vegetable growing conditions provided a logistically challenging, but scientifically robust, approach to test the proposed hypotheses. The lack of change in oxidative stress generally replicates the null results of the only other dose-testing study (2, 5, and 8 servings/d) conducted in 64 healthy German males (36). Our findings also are consistent with those of Paterson et al. (21), which demonstrated no effect of a 400-g dose of carotenoid-rich soups and beverages on lipid peroxidation biomarkers. These results do, however, contradict other intervention trials, including a study by Fowke et al. (37) that observed a 15.9% reduction in 8-Iso-PGF2α in 22 adult smokers who consumed 218 g/d of Brassica vegetables for 4 wk. Of relevance, the study by Fowke et al. (37) enrolled smokers, a subgroup of the population known to demonstrate greater oxidative stress; smokers were excluded from participation in the present study.

Studies evaluating HEL, a measure of amide-products of oxidized lipid and protein (8,39), to assess change in oxidative stress are limited. Human interventions with cocoa (38) and black tea polyphenols (40) suggest HEL concentrations are modified by dietary exposures, although the vegetable exposures demonstrated in this study did not affect HEL. Importantly, N-hexanoyl-lysine is elevated in the presence of metabolic syndrome (39). The lack of response in this study may be indicative of competing risks for elevated HEL given the overweight or obese status of the participants.

The increase in plasma carotenoids and, indirectly, exposure to several plant-based bioactive compounds that have the potential to modulate oxidative stress without a change in oxidant status was not unexpected given the body's well-balanced antioxidant system and the relative healthy status of the study population.

Though our population was generally healthy, our mean baseline urinary 8-Iso-PGF2α concentrations were slightly elevated compared with other healthy study populations (14,41), suggesting some opportunity for modulation. One possible limitation to our approach was that the ELISA used did not include pretreatment of samples with glucuronidase to release isoprostane from its conjugated form. A second possible explanation for a lack of change in the selected biomarkers may be related to the lack of sufficient diversity in the antioxidant and/or antiinflammatory compounds in the vegetables consumed by the study participants compared to diets fed in some of the published trials (17,42). Although there was some variance in

TABLE 3 Post-run–in concentrations of plasma carotenoids and biomarkers of oxidative stress and inflammation in overweight/obese or post-menopausal women[1]

Baseline biomarker	
Total carotenoids, *μmol/L*	1.3 ± 0.5
α-Carotene, *μmol/L*	0.1 ± 0.1
β-Carotene, *μmol/L*	0.4 ± 0.2
Lycopene, *μmol/L*	0.5 ± 0.2
Lutein + zeaxanthin, *μmol/L*	0.3 ± 0.2
β-Cryptoxanthin, *μmol/L*	0.4 ± 0.1
Urine 8-Iso-PGF2α, *ng/mg creatinine*	22.9 ± 8.4
Urine hexanoyl lysine,[2] *nmol/L*	98.3 ± 38.8
Serum hsCRP,[2] *mg/L*	2.5 ± 2.2

[1] Values are mean ± SD, *n* = 49. hsCRP, high-sensitivity C-reactive protein; 8-Iso-PGF2α, 8-isoprostane-F-2-α.
[2] *n* = 48.

TABLE 4 Plasma total carotenoid concentration in overweight or obese postmenopausal women before and after each 3-wk vegetable dose period[1]

Vegetable servings	Pre	Post	Change
		μmol/L	
2	1.63 ± 0.73	2.07 ± 0.73*	0.45 ± 0.42
5	1.49 ± 0.58	2.84 ± 1.12*	1.33 ± 0.94
10	1.40 ± 0.53	4.42 ± 2.08*	3.02 ± 1.98

[1] Values are mean ± SD, *n* = 48 or 49 (5 servings). *Different from Pre, $P < 0.001$ (paired *t* test).

TABLE 5 β-Coefficients of variable dose vegetable intake on plasma carotenoids and urinary biomarkers of oxidative stress and serum biomarkers of inflammation in overweight or obese postmenopausal women[1]

Outcome measure	n[3]	Veg servings	β	(95% CI)	P	P-trend
Total carotenoids[2]	144	2		(Reference)		
		5	0.76	(0.34–1.17)	<0.001	
		10	2.39	(1.97–2.82)	<0.001	<0.001
α-Carotene	145	2		(Reference)		
		5	0.19	(0.06–0.33)	0.005	
		10	0.85	(0.71–0.99)	<0.001	<0.001
β-Carotene	145	2		(Reference)		
		5	0.37	(0.15–0.60)	0.001	
		10	1.23	(1.00–1.46)	<0.001	<0.001
Lycopene	145	2		(Reference)		
		5	0.13	(0.06–0.20)	0.001	
		10	0.15	(0.08–0.22)	<0.001	<0.001
Lutein + zeaxanthin	145	2		(Reference)		
		5	0.08	(0.04–0.12)	<0.001	
		10	0.18	(0.14–0.22)	<0.001	<0.001
β-Cryptoxanthin	145	2		(Reference)		
		5	0.01	(−0.01–0.03)	0.150	
		10	0.05	(0.03–0.07)	<0.001	<0.001
Urine 8-Iso-PGFα	144	2		(Reference)		
		5	−0.50	(−3.61–2.60)	0.751	
		10	−0.16	(−3.27–2.96)	0.921	0.97
Urine hexanoyl lysine	146	2		(Reference)		
		5	−6.67	(−19.8–6.46)	0.319	
		10	−3.62	(− 16.7–9.48)	0.588	0.68
Serum hsCRP	143	2		(Reference)		
		5	−0.17	(−0.60–0.26)	0.442	
		10	−0.08	(−0.51–0.34)	0.699	0.78

[1] β-Coefficients from a mixed-effects model compared with 2 vegetable servings/d, adjusted for preintervention values of the biomarker, BMI, and total cholesterol (random effects: individual and regimen order). hsCRP, high-sensitivity C-reactive protein; 8-Iso-PGF2α, 8-isoprostane-F-2-α; veg, vegetable.
[2] n = 3 missing values for total carotenoids, 1 for cholesterol.
[3] These n are not independent, as each person is represented 3 times.

the percentage of total vegetables from the individual vegetables consumed at each dose, this was necessary based on qualitative research suggesting participants would not adhere to an equivalent escalation in doses that would have, resulted in unacceptable amounts and logistically challenging portions of prescribed intake (e.g. tomato at doses of 0.5, 1.25, and 2.5/d). Further, our goal was to evaluate the effect of each vegetable does on circulating carotenoid concentrations rather than the effect of individual vegetables.

Differential bioavailability of carotenoids also could explain inconsistent findings from fruit/vegetable intervention trials. For example, lycopene (processed tomato products) and bioactives in cruciferae have been shown to modulate oxidant stress, suggesting that food matrix and dose are relevant to bioavailability and biological effects demonstrated (43–45). Tomatoes for this study were consumed raw and cruciferous vegetables comprised only a small portion of the mixed green product. We speculate that the limited bioavailability and/or amount of these bioactives contributed to the lack of change in oxidative stress.

Although reduction in chronic systemic inflammation is one proposed mechanism whereby obesity-related chronic risk is modified (6,32,46–48), the intervention did not significantly reduce hsCRP despite it being an independent predictor of chronic disease risk (49–51). Contradictory results were reported by Watzl et al. (15) in adults who consumed 8 servings/d of carotenoid-rich fruits and vegetables compared to 2 or 5 daily servings and also by Valtueña et al. (52), who compared a high- and low-antioxidant diet (provided by fruits and vegetables) in healthy postmenopausal women wherein hsCRP was significantly reduced with intervention. Of note, both studies (15,52) provided a greater diversity in plant foods (vegetables and fruits) than what was provided in this study. Here again, in our sample of overweight, postmenopausal women, baseline inflammation (hsCRP) was only mildly elevated (53) and baseline inflammation (and oxidative stress) is known to be predictive of intervention response in previous diet intervention trials (41,54).

A longer dietary intervention also may be necessary to observe a significant decrease in markers of inflammation or oxidative stress. Participants in the current study received treatment for 3 wk for each of the 3 vegetable doses (2, 5, and 10 servings/d). Whole-food dietary interventions testing changes in biomarkers of systemic inflammation generally range from 2 to 12 wk in length (15,17,52,54–56). In our study, the repeated washout periods between vegetable doses likely reduced the long-term exposure to plant-based bioactive compounds necessary to modify oxidative stress and/or inflammatory biomarkers (6,10,32,46).

Although duration of vegetable exposure, bioavailability, and vegetable botanical diversity may have contributed to study limitations, adherence to the study vegetable protocol was not a limiting factor. Evaluation of plasma carotenoid concentrations as biomarkers of vegetable intake (57–59) suggested adherence was high. In fact, the adherence rate was similar to that reported by Thompson et al. (7) in an intervention comparing high and low doses of fruits and vegetables. Further, we demonstrated sufficient washout periods between vegetable doses. This was demonstrated by the fact that the circulating total plasma carotenoid concentrations prior to each dose were not significantly different from the post-run–in value for the population, another important factor for interpreting results of crossover, multiple-dose studies.

By 2020 chronic diseases will account for an estimated 75% of all deaths worldwide (60). Dietary modification is a relatively inexpensive, noninvasive means for reducing chronic disease risk in both men and women and oxidative stress and inflammation remain important targets for obesity-related risk reduction. Based on the current findings, future investigations to identify the optimal vegetable dose should include diets high in biologically diverse plant foods delivered over a prolonged intervention period and should target at-risk subgroups that demonstrate elevated levels of systemic oxidative stress and inflammation to reduce chronic disease risk. Even with improvements in study design, acknowledgment of the body's capacity to tightly regulate oxidative balance may preclude any substantial modulation of oxidant stress pathology in the context of short-term diet interventions.

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