

## Relationships between Serum and Colon Concentrations of Carotenoids and Fatty Acids in Randomized Dietary Intervention Trial

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### Abstract

Little is known about the effect of preventive diets on colonic nutrient concentrations. This study randomized 120 persons at increased risk of colon cancer to a Mediterranean versus a Healthy Eating diet for six months. The former targeted increases in whole grains, fruits, vegetables, monounsaturated, and n3 fats. The Healthy Eating diet was based on Healthy People 2010 recommendations. At baseline, dietary fat and carotenoid intakes were poorly associated (Spearman  $\rho < 0.4$ ) with serum and colon concentrations. Strong associations were observed between serum and colon measurements of  $\beta$ -cryptoxanthin ( $\rho = 0.58$ ;  $P < 0.001$ ),  $\alpha$ -carotene ( $\rho = 0.48$ ;  $P < 0.001$ ), and  $\beta$ -carotene ( $\rho = 0.45$ ;  $P < 0.001$ ). After six months, the Healthy Eating intervention increased serum lutein,  $\beta$ -, and  $\alpha$ -carotene significantly ( $P < 0.05$ ). In the Mediterranean arm, the significant increases were in serum lutein,  $\beta$ -cryptoxanthin,  $\beta$ -carotene, monounsaturated, and n3 fats. A significant group-by-time interaction ( $P = 0.03$ ) was obtained for monounsaturated fats. Colonic increases in carotenoids and n3 fats were significant only in Healthy Eating arm, whereas the group-by-time interaction was significant for  $\beta$ -carotene ( $P = 0.02$ ) and  $\alpha$ -carotene ( $P = 0.03$ ). Changes in colon concentrations were not significantly associated with reported dietary changes. Changes in colon and serum concentrations were strongly associated for  $\beta$ -cryptoxanthin ( $\rho = 0.56$ ;  $P < 0.001$ ) and  $\alpha$ -carotene ( $\rho = 0.40$ ;  $P < 0.001$ ). The associations between colonic and serum concentrations suggest the potential use of using serum concentration as a target in dietary interventions aimed at reducing colon cancer risk. *Cancer Prev Res*; 6(6); 558–65. ©2013 AACR.

### Introduction

The relative impact of diet on colorectal cancer prevention has been equivocal in dietary intervention studies. Cohort studies have not found much association between dietary carotenoids and colorectal cancer risk, but in the American Association of Retired Persons study, increased fruit and vegetable intakes during adolescence or 10 years before diagnosis were protective of colorectal cancer (1, 2). In the Polyp Prevention Trial, the intervention diet that targeted decreased fat intakes and increased intakes of fiber, fruits, and vegetables had no effect on polyp recurrence and the increase in plasma carotenoids was modest (3). Subjects with excellent compliance, however, did have decreased polyp recurrence and they also exhibited better dietary intakes at baseline (4). In addition, relatively higher con-

centrations of  $\alpha$ -carotene and vitamin A at baseline or averaged over time were protective in that study (5). Similar findings have been observed in the Women's Health Initiative: women with higher serum  $\beta$ -carotene concentrations averaged over time had lower risk of colon cancer (6). These results indicate that long-term exposures to fruits and vegetables may be necessary for prevention and that blood concentrations are important to measure. Blood concentrations can reflect carotenoid absorption and metabolism in addition to dietary exposures.

A review of the scientific literature completed in 2011 by the American Institute for Cancer Research indicated that there is convincing evidence that colon cancer risk can be affected by fiber-containing foods, red, and processed meat intake (7, 8). The Healthy People 2010 dietary recommendations that were developed by the U.S. Office of Disease Prevention and Health Promotion are consistent with diets that could be useful for colon cancer prevention by encouraging 5 fruits and vegetables/day, whole grains, and decreased saturated fat intakes (9). Mediterranean diets also may be useful for prevention because all the major components of the traditional Greek–Mediterranean diet seem to be protective for colorectal cancer, including olive oil, fish, legumes, whole grains, and fruits and vegetables (10, 11). Epidemiologic data in European populations have found

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Mediterranean diets to be preventive of colorectal cancer (12). Mediterranean diet scores have generally been used in epidemiologic studies, and the scores are calculated on the basis of relative intakes within the population being studied. In the United States, Mediterranean eating scores have not been consistently preventive and may stem from difference in mean intakes in the United States versus Mediterranean countries (12–14).

In this present study, we sought to evaluate the relative effects of counseling for Mediterranean versus Healthy diets on serum and colon concentrations of micronutrients in persons at increased colon cancer risk. This randomized clinical trial provided 6 months of dietary counseling to each study participant. The primary endpoints of the trial were colon carotenoid and fatty acid concentrations. Secondary endpoints were colonic eicosanoids, epithelial proliferation, and nuclear morphology. This present report is focused on the primary endpoints of the trial.

Blood and colon mucosa samples were obtained before and after dietary change. Although serum concentrations of carotenoids have been shown to be useful markers of fruit and vegetables intakes, there is relatively much less information available on colon concentrations of carotenoids. In addition, it was important to evaluate the effects of dietary change on fatty acids concentrations. Increased omega 3 or fish oil fatty acids and conversely decreased omega 6 fatty acids have been associated with decreased colon cancer in experimental models and humans (15, 16). Increased fruits, vegetables, and omega 3 fats and decreased omega 6 fats could work together to suppress colonic inflammation via fatty acid substrate competition for COX enzymes and inhibition of COXs by phytochemicals from plant-based foods (17). It was therefore important to evaluate the impact that Mediterranean and Healthy diets could have on concentrations of colonic fatty acids and carotenoids.

## Materials and Methods

### Subjects and eligibility

The Healthy Eating for Colon Cancer Prevention Study was approved by the University of Michigan (Ann Arbor, MI) Institutional Review Board and was registered at the ClinicalTrials.org (NCT00475722). The study recruited subjects at least 21 years of age who were in good health, had body mass index (BMI) between 18.5 and 35 kg/m<sup>2</sup>, and had a dietary pattern that is noncompliant to the United States Department of Agriculture (USDA) recommended guidelines with respect to fat intake as well as fruit and vegetable servings. Eligible subjects had family history of colon cancer in one first-degree or 2 second-degree relatives. Participants were recruited from Ann Arbor, Michigan and vicinity between July 2007 and November 2010 primarily through advertisements in media and flyers. Details of study eligibility and recruitment have been published (18).

Enrolled subjects were randomized to one of 2 dietary regimes: healthy eating or Mediterranean diet. Subjects in each arm received the same frequency of counseling for 6 months. Each subject had 3 study visits during the study period, at baseline, 3, and 6 months, each of which

consisted of filling out questionnaires, collection of blood samples, and colon biopsies by flexible sigmoidoscopy as described previously (18). Monetary incentives were offered at each visit.

### Assessments

Dietary intake data for each subject at each time point was based on the average of 2 unannounced recalls and 2 written records. The food records and recalls were analyzed using the Nutrition Data System for Research software (version 2010, Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN).

Anthropometric measures of weight, height, blood pressure, waist, and hip circumference were obtained at each study visit. A study questionnaire captured demographic characteristics and health status. Physical activity was assessed using a validated questionnaire from the Women's Health Initiative (19) that records time spent walking at various speeds and carrying out mild, moderate, and strenuous activities leading to a calculation of metabolic equivalents of energy expenditure.

### Dietary intervention

Both diets were designed to be isocaloric with baseline and were delivered by telephone counseling. The consumption goals for the Healthy Eating diet, following the U.S. Healthy People 2010 recommendations (9), included (i) at least 2 servings per day of fruit; (ii) at least 3 servings per day of vegetables; (iii) at least one serving per day of a dark green or dark orange fruit or vegetable; (iv) at least 3 servings per day from whole grains; and (v) less than 10% of calories from saturated fat. The number of goals was more in the Mediterranean arm. The fat goal was to maintain 30% of calories from fat while achieving a PUFA: SFA: MUFA ratio of 1:2:5, which entailed reducing polyunsaturated fatty acids (PUFA) and saturated fatty acids (SFA) intakes by about 50% and 30%, respectively, and increasing monounsaturated fatty acids (MUFA) intake by about 50%. Subjects in this group were asked to consume foods high in omega 3 fatty acids at least twice a week. The whole grain goal was the same as in the Healthy Eating arm. Fruit and vegetable goals were for at least 7 to 9 U.S. Food and Drug Administration servings per day, depending on energy intake, including culinary herbs and allium vegetables.

### Blood and colon sample analyses

Fasting blood sample was obtained from the arm in a coagulation tube for the preparation of serum at baseline and 6 months. Serum was stored at –70°C until analysis. Total serum fatty acids were extracted with Folch reagent and measured as fatty acid methyl ester by gas chromatography with mass spectral detection (20). Carotenoids were extracted with hexane and measured by high-pressure liquid chromatography (20). There was not enough blood for carotenoid analysis from one overweight/obese subject in the Healthy Eating arm at baseline.

Colon mucosal biopsies were obtained circumferentially 15–25 cm above the anal sphincter by flexible sigmoidoscopy

without any prior preparation of the bowels. Six biopsies were frozen in liquid nitrogen within 5 seconds of incision and they were stored at  $-70^{\circ}\text{C}$  until analysis.

Colon samples were analyzed for carotenoids and fatty acids in a similar way as serum except that a colon tissue homogenate was prepared first. A total of 4 frozen biopsies were homogenized together, using pulverization under liquid nitrogen, a technique described previously (21). A portion of the homogenate that was equivalent to one biopsy (150  $\mu\text{L}$ ) was used for carotenoid analysis and an equal portion was used for fatty acid analysis. Samples were diluted with 50  $\mu\text{L}$  PBS before extraction. There was one tissue sample missing at 6 months from an overweight/obese completer in the Mediterranean arm that refused flexible sigmoidoscopy.

### Statistical analyses

Subject characteristics such as gender, age, BMI, marital status were compared across the 2 study arms at baseline by means of  $\chi^2$  or Fisher exact test (for categorical characteristics) and 2-sample unpaired  $z$  test (for continuous measures). A similar comparison was carried out between the completers and noncompleters to identify any potential source of bias.

Pairwise correlations between colon micronutrient concentrations, dietary intakes, and serum concentrations of select carotenoids and fatty acids at baseline were obtained. Because of the heavy skewness in the data, Spearman rank correlation was used. Statistical tests to compare the correlations for each of the nutrients were also carried out. Methods are available to compare correlated Pearson correlation coefficients when multivariate data conform to normal distribution, but these methods are not applicable to Spearman correlations. The nonparametric bootstrap method to construct confidence intervals for the pairwise differences in correlation coefficients was therefore used. Briefly, this consisted of resampling with replacement from the pool of 120 subjects and recalculating the correlation coefficients based on the resampled data. The confidence interval for the differences in correlation was calculated using the bias-corrected percentile method (22) based on 2000 bootstrap samples. The difference was deemed statistically significant at 5% level if the 95% bootstrap confidence interval did not include zero. We repeated the procedure with the Spearman correlation coefficient  $\rho$  replaced by  $z_{\rho}$  obtained by Fisher transformation

$$z_{\rho} = \frac{1}{2} \log \left( \frac{1 + \rho}{1 - \rho} \right).$$

Associations between changes in nutrient concentrations in diet, serum, and colon measurements were investigated in a similar manner through correlations of the percent change from baseline in the nutrient concentrations.

To evaluate and compare average changes over time in fatty acids, carotenoids and other nutrients found in serum across 2 groups, linear mixed regression models were used with time, diet group assignment, and the group-by-time interaction as the primary predictors. Mixed linear regres-

sion models provide valid inference in presence of dropouts. Furthermore, because it uses all available data each time point, mixed model analysis is tantamount to an intent-to-treat approach. The models were controlled for baseline age, BMI, and smoking as non-time-dependent covariates. Age was slightly higher in the Mediterranean group than the Healthy group (means of 55 versus 50, respectively). The prevalence of baseline smoking status was slightly different in the 2 study arms (11% in the Healthy arm versus 17% in the Mediterranean arm), although the difference was not statistically significant at 5% level ( $P = 0.06$  based on Fisher exact test). Because smoking status may potentially affect the fatty acid and carotenoid concentrations, it was used as a covariate in the regression models, BMI did not differ appreciably between the 2 groups at baseline, but it is known to affect carotenoid concentrations (23). Furthermore, the samples were analyzed in several analytic batches in the laboratory, which was a potential source of variation, and so batch was used as an additional covariate. Normality of the residuals was assessed through q-q plots and tests for normality, and Box-Cox family of transformations was used to identify a suitably symmetrizing transformation whenever it was necessary (24). SFA was square root transformed. Log transformation was used for all other variables except for MUFA, which required no transformation. Clustering of the pre-post measurements within subjects was accounted for by using a random subject intercept.

Similar models were used for concentrations of fatty acids and carotenoids obtained from colon tissue samples. Apart from baseline age, BMI, and smoking status, the regression models were controlled for variation across laboratory analysis batches. All outcomes required a natural logarithmic transformation with the exception of SFA, which required a square transformation for analysis, and MUFA, which required no transformation.

## Results

### Subject characteristics

A total of 120 subjects were enrolled in the study with 61 assigned to the Healthy arm and the remaining 59 in the Mediterranean arm. A total of 94 subjects had available data at 6 months, with number of completers evenly distributed between the Healthy Eating and the Mediterranean diet arms (47 in each).

There were 43 females at baseline in each study arm. The distribution of the participants' age at study entry exhibited a slight left skew with mean and median age of 52.2 and 53.8, respectively. Among the various subject characteristics compared between the 2 groups at baseline, only age turned out to be significantly different between the 2 groups. The mean (SD) of age in years were 50 (14) and 55 (10) in the Healthy Eating and Mediterranean groups, respectively. The  $P$  value for this comparison based on an unpaired  $z$  test was 0.033. A more complete chart of comparisons between groups was published previously (18). Age also turned out to be the only (borderline) significant demographic characteristics between completers and noncompleters.

Noncompleters were slightly younger with a mean age of 47.7 (SD 14) years versus the completers who had a mean age of 53.6 (SD 11.3) years ( $P = 0.052$ ).

### Correlation of colon micronutrient concentrations with dietary intakes and serum concentrations at baseline

It was of interest to determine whether dietary intakes or serum concentrations of nutrients would be more closely associated with colon concentrations. The correlations between colon and serum nutrients seemed to be strongest, whereas those between diet and colon were the weakest of all (Table 1). All the nutrients measured in colon, except MUFA, SFA, and n6 fatty acids, were significantly associated with serum concentrations at the 1% level of significance. The strongest associations are exhibited between the serum and colon concentrations of  $\beta$ -cryptoxanthin ( $\rho = 0.58$ ,  $P < 0.001$ ),  $\alpha$ -carotene ( $\rho = 0.48$ ,  $P < 0.001$ ), and  $\beta$ -carotene ( $\rho = 0.45$ ,  $P < 0.001$ ). The only dietary intake to be significantly associated with both serum and colon concentrations was  $\beta$ -cryptoxanthin (Table 1). The corresponding dietary intake was also significantly associated with serum concentrations of  $\alpha$ -carotene ( $\rho = 0.37$ ,  $P < 0.001$ ).

### Changes in serum markers of dietary intakes

Serum fatty acids and carotenoids were measured because they can be useful biomarkers of changes in dietary intakes

**Table 1.** Spearman correlation of dietary, serum, and colon nutrient concentrations at baseline ( $n = 120$ )<sup>a</sup>

Nutrient	Correlation coefficients <sup>b</sup>		
	Diet with serum	Diet with colon	Serum with colon
SFA	0.04	0.20	0.08
MUFA	-0.01	-0.06	0.05
n6 fatty acids	0.06	-0.01	-0.06
n3 fatty acids <sup>c</sup>	0.18	0.01	<b>0.38</b>
total carotenoids	0.18	0.07	<b>0.26</b>
Lutein&zeaxanthin <sup>d,e</sup>	0.04	0.01	<b>0.34</b>
$\beta$ -cryptoxanthin <sup>d,e</sup>	<b>0.37</b>	<b>0.24</b>	<b>0.58</b>
$\alpha$ -carotene <sup>c,e</sup>	<b>0.37</b>	0.18	<b>0.48</b>
$\beta$ -carotene <sup>d,e</sup>	0.17	0.09	<b>0.45</b>
lycopene	0.12	0.10	<b>0.29</b>

<sup>a</sup>Calculations for fatty acids (the first 4 variables) based on available data from 119 subjects.

<sup>b</sup>Significantly ( $P < 0.01$ ) different from zero correlations at baseline are indicated by boldface numbers.

<sup>c</sup>The correlation of diet with colon is significantly different than the correlation of diet with serum.

<sup>d</sup>The correlation of serum with diet is significantly different than the correlation of serum with colon.

<sup>e</sup>The correlation of colon with serum is significantly different than the correlation of colon with diet.

of fat, fruits, and vegetables. With regard to the major classes of fatty acids, there were no significant changes in serum concentrations of saturated fatty acids, but concentrations of MUFA, N3 PUFA, and the ratio of N3/N6 PUFA changed significantly in the expected directions in the Mediterranean arm (Table 2).

For total serum carotenoids, changes were similar in both diet arms, but the increase in total carotenoids was significant only in the Healthy arm. Specific fruit and vegetable goals in the Healthy arm were for 5 servings per day and including at least one carotenoid-rich dark orange or green vegetable. Increases in lutein were significant in both study arms,  $\beta$  and  $\alpha$ -carotene increased significantly only in the Healthy arm, and  $\beta$ -cryptoxanthin increased significantly only in the Mediterranean arm. The fruit and vegetable goals in the Mediterranean arm were to consume at least one serving from each of 7 categories of fruits and vegetables (18). There were no statistically significant changes in lycopene or zeaxanthin concentrations in either arm. Changes that approached statistical significance with  $0.05 < P < 0.10$  were in  $\beta$ -cryptoxanthin in the Healthy arm and in  $\beta$  and  $\alpha$ -carotene in the Mediterranean arm.

### Changes in fatty acids and carotenoids in the colon

Changes in carotenoids and fatty acids in the colon biopsy tissue were in the same direction as in blood, but the changes were smaller and fewer differences were statistically significant (Table 3). Interestingly, concentrations of n3 fatty acids increased in the Healthy arm, but the change was small. Significant increases in several carotenoids were also found in the Healthy arm only (Table 3). Changes in the Mediterranean arm that approached significance with  $0.05 < P < 0.10$  were for N3 PUFA, n3/n6 ratio,  $\beta$ -cryptoxanthin, and  $\alpha$ -carotene.

### Association between the changes in diet with changes in serum and colon measurements

Because the changes in colon concentrations of nutrients were overall smaller than the changes in serum concentrations, it was of interest to evaluate whether the changes in diet, serum, and colon were correlated with each other. Changes in dietary nutrients were more strongly associated with changes in serum concentrations than with changes in colon concentrations. Analogous to the baseline findings, the correlation between changes in serum and colon measures seem to be stronger than for diet with colon measures, with the largest rank correlation of 0.56 obtained for  $\beta$ -cryptoxanthin ( $P < 0.001$ ). This correlation was statistically significantly larger than the corresponding ones between diet and colon or diet and serum. Changes in blood serum concentrations of  $\alpha$ -carotene and  $\beta$ -carotene were significantly correlated with the corresponding changes in either the diet or colon concentrations. However, there was no statistical difference in the strength of association between these pairs. The correlation between changes in  $\alpha$ - and  $\beta$ -carotene levels of colon and diet, was not statistically significant, and was significantly lower than the other 2 correlations in the case of  $\alpha$ -carotene.

**Table 2.** Serum concentrations of nutrient biomarkers (mean, SD) at each time-point by diet arm<sup>§</sup>

Serum analyte	Healthy eating		Mediterranean	
	Baseline (n = 61)	6 mo (n = 47)	Baseline (n = 59)	6 mo (n = 47)
SFA (%)	34, 5	34, 5	34, 5	33, 5
MUFA (%) <sup>a</sup>	24, 6	24, 5	24, 5	27, 4 <sup>b</sup>
N6 PUFA (%)	36, 7	36, 7	37, 7	34, 6 <sup>b</sup>
N3 PUFA (%)	3.8, 1.2	4.1, 1.5	3.7, 1.5	4.2, 1.4 <sup>b</sup>
N3/N6 fatty acid ratio	0.11, 0.04	0.12, 0.05	0.11, 0.07	0.13, 0.05 <sup>b</sup>
Total carotenoids (pg/mL)	959, 508	1,240, 873 <sup>b</sup>	1,033, 807	1,154, 746
Lutein (pg/mL)	170, 84	200, 85 <sup>b</sup>	174, 104	219, 152 <sup>b</sup>
Zeaxanthin (pg/mL)	40, 22	46, 21	40, 19	47, 44
β-Cryptoxanthin (pg/mL)	79, 51	115, 106	88, 74	118, 114 <sup>b</sup>
β-Carotene (pg/mL)	229, 227	382, 507 <sup>b</sup>	303, 472	367, 436 <sup>b</sup>
α-Carotene (pg/mL)	78, 70	164, 244 <sup>b</sup>	97, 136	110, 86
Lycopene (pg/mL)	363, 262	333, 236	331, 271	294, 168

<sup>§</sup>Significance testing is based on the model for transformed outcome, with transformations as described in Materials and Methods.

<sup>a</sup>A significant group × time interaction was present at  $P < 0.05$ .

<sup>b</sup>Significantly different than baseline for that diet arm,  $P < 0.05$ . All models had analysis batch, baseline age, BMI, and smoking status as covariates.

## Discussion

The results of this dietary intervention study indicate that concentrations of protective nutrients in the human colonic mucosa can be changed by dietary intervention, but that these changes were small and more closely related to changes in serum than to reported changes in dietary intakes (Table 4). Similarly, at baseline, colon concentrations of nutrients were more closely related to serum concentrations than to dietary intakes (Table 1). This could be due to (i) the short time frame of the intervention because tissue stores

may require more time to reach equilibrium than blood, (ii) errors inherent in dietary assessment, especially when only 4 days are used at each time point, and (iii) to the role of metabolic factors. Most dietary nutrients are absorbed in the small intestine, and colonic exposures to nutrients therefore are likely to occur at the basolateral, not luminal, side via the systemic circulation. This is especially true for the distal colon that was sampled in this study.

The two dietary interventions used in this study differed in several ways, but our unpublished data indicates that

**Table 3.** Colon tissue concentrations (mean, SD) of nutrients at each time-point by diet arm

Nutrient level	Healthy eating		Mediterranean	
	Baseline (n = 61)	6 mo (n = 47)	Baseline (n = 59)	6 mo (n = 47)
SFA (%)	32, 5	32, 4	32, 4	32, 4
MUFA (%)	31, 4	31, 4	32, 5	33, 5
N6 PUFA (%)	32, 7	31, 5	31, 5	31, 5
N3 PUFA (%)	4.8, 2.4	5.1, 2.3 <sup>b</sup>	4.4, 2.3	4.6, 2.2
N3/N6 ratio	0.16, 0.10	0.17, 0.08 <sup>b</sup>	0.15, 0.08	0.16, 0.08
Total carotenoids	17, 15	29, 50 <sup>b</sup>	17, 19	21, 19
Lutein (pg/mL)	8.1, 7.7	14.7, 34.3	8.0, 8.1	10.7, 11.4
Zeaxanthin (pg/mL)	0.79, 0.83	1.03, 1.46	0.74, 0.73	0.83, 0.61
β-Cryptoxanthin (pg/mL)	0.93, 0.68	1.42, 1.48 <sup>b</sup>	1.01, 0.84	1.32, 1.14
β-Carotene (pg/mL) <sup>a</sup>	2.2, 2.9	3.8, 4.7 <sup>b</sup>	2.8, 6.8	3.2, 4.1
α-Carotene (pg/mL) <sup>a</sup>	1.1, 1.7	2.9, 4.2 <sup>b</sup>	1.3, 1.7	1.9, 2.1
Lycopene (pg/mL)	3.5, 5.2	4.9, 11.2	3.3, 3.9	3.0, 3.5

<sup>a</sup>A significant group-by-time interaction was present from mixed linear regression models, after transformation of variables to achieve normality, as described in Methods.

<sup>b</sup>Significantly different than baseline for that diet arm,  $P < 0.05$ . All models had analysis batch, baseline age, BMI, and smoking status as covariates.

**Table 4.** Spearman correlations of the changes (from baseline to 6 months) in dietary intakes, and serum, and colon nutrient concentrations ( $n = 94$ )

Nutrient	Correlation coefficients		
	Diet with serum	Diet with colon	Serum with colon
SFA	-0.007	0.07	<b>0.27</b>
MUFA	0.14	0.18	0.26
N6 fatty acids	0.10	0.02	-0.12
N3 fatty acids	<b>0.27</b>	0.004	0.14
Total carotenoids <sup>a</sup>	<b>0.27</b>	0.23	0.19
Lutein and zeaxanthin	0.14	0.25	<b>0.34</b>
$\beta$ -Cryptoxanthin <sup>c,d</sup>	<b>0.31</b>	0.26	<b>0.56</b>
$\alpha$ -Carotene <sup>b,d</sup>	<b>0.35</b>	0.09	<b>0.40</b>
$\beta$ -Carotene	<b>0.35</b>	0.26	<b>0.36</b>
Lycopene	-0.004	0.23	0.09

NOTE: Significantly different from zero ( $P < 0.01$ ) correlations at baseline are indicated by boldface numbers.

<sup>a</sup>Calculations based on available data from 93 subjects.

<sup>b</sup>The correlation of diet with colon is significantly different than the correlation of diet with serum.

<sup>c</sup>The correlation of serum with diet is significantly different than the correlation of serum with colon.

<sup>d</sup>The correlation of colon with serum is significantly different than the correlation of colon with diet.

reported fruit and vegetable intakes over 6 months of intervention were unexpectedly similar in the 2 arms. Counseling for fruit and vegetable consumption consistent with Healthy People 2010 goals, namely 2 servings per day of fruit and 3 servings per day of vegetables, with at least one being dark green or orange, was sufficient to increase the concentrations of several serum and colon carotenoids (Table 2). The Mediterranean group with goals for consuming at least 7 servings per day of fruits and vegetables in 7 different categories did not display relatively larger increases in serum carotenoids. One could speculate that subjects in the Healthy arm met their goals with a variety of foods to increase palatability, resulting in a broad spectrum of carotenoid intakes in the Healthy Eating arm. The only serum carotenoid that was increased significantly in the Mediterranean arm, but not the Healthy arm, was  $\beta$ -cryptoxanthin, which is found mainly in fruits. Whether or not other phytochemicals are increased by a Mediterranean diet needs to be investigated. This would include flavonoids such as quercetin, which is high in onions and apples, and phenolic compounds from olives (25, 26).

In experimental models, many individual carotenoids have been shown to be protective of colon cancer including lutein and lycopene (27, 28). A pooled analysis of 11 cohort studies indicated that of the dietary carotenoids, only lutein and zeaxanthin, which were measured together in foods,

displayed weak protective effects for colorectal or colon cancer (2). Colon lutein or lycopene concentrations, however, were not significantly increased after 6 months of either intervention (Table 3). Lycopene in both serum and adipose tissue has been previously found to be poorly related to dietary intakes, and supplementation may be a more feasible method to increase concentrations of this carotenoid (29, 30).

Among the carotenoids, relatively more data is available on  $\beta$ -carotene bioavailability in the colon, and this carotenoid is most widely distributed in fruits and vegetables. Several studies have shown that  $\beta$ -carotene supplementation can increase colonic  $\beta$ -carotene concentrations (31–34). In human trials of boiled vegetables or  $\beta$ -carotene supplements, the lag time in the increase in carotenoids in exfoliated cells in the stool was 5–7 days, suggesting that carotenoids might be obtained from the circulating stores at the crypt base (32, 35). This is consistent with our data in the colonic mucosa showing that blood concentrations correlated with colon concentrations more closely than with diet intakes. The previously published plasma–diet correlations of carotenoids have generally varied with  $r = 0.11$  to 0.52 depending on the carotenoid and on gender (36). In this present study at baseline, concentrations of carotenoids in colonic mucosa were more closely associated with serum concentrations than dietary intakes for all the carotenoids and for n3 fatty acids (Table 1). Change in colon concentrations at 6 months was also more strongly associated with change in serum concentrations than with dietary intakes (Table 4).

Given the similarity between the 2 diet arms in reported fruit and vegetable intakes, the main difference between the 2 interventions was in dietary fat intakes. This is potentially important because fatty acid availability is a key determinant of the types of prostaglandins and other eicosanoids that are produced in cells (37). The Mediterranean diet was unique in increasing serum concentrations of MUFA and n-3 fatty acids, but this may reflect recent diet because phospholipids were not isolated. There were few significant changes in colon fatty acids other than a slight increase in n3 PUFA that was significant in the Healthy arm. This indicates the possible importance of metabolic processes in regulating tissue fatty acids concentrations, and these may be genetically determined.

In summary, this study showed that the intervention using Healthy People 2010 goals resulted in larger changes in colon carotenoids than the intervention using Mediterranean goals. Whether or not other phytochemicals are affected to a relatively greater extent with Mediterranean diets remains to be established, but it is encouraging that the modest goals of the Healthy Eating diet were exceeded and resulted in significant beneficial changes in the colon. These data indicate that a relatively simple intervention may be adequate for increasing carotenoids in colon. Colon concentrations of micronutrients were, however, not well correlated with dietary intakes. Dietary assessment is difficult and has many limitations, but metabolic factors also may

need to be evaluated as determinants of colon nutrient concentrations.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

### Authors' Contributions

**Conception and design:** A. Sen, M.T. Ruffin, D.E. Brenner, Z. Djuric

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### References

- Ruder EH, Thiebaut AC, Thompson FE, Potischman N, Subar AF, Park Y, et al. Adolescent and mid-life diet: risk of colorectal cancer in the NIH-AARP Diet and Health Study. *Am J Clin Nutr* 2011;94:1607–19.
- Mannisto S, Yaun SS, Hunter DJ, Spiegelman D, Adami HO, Albanes D, et al. Dietary carotenoids and risk of colorectal cancer in a pooled analysis of 11 cohort studies. *Am J Epidemiol* 2007;165:246–55.
- Lanza E, Schatzkin A, Daston C, Corle D, Freedman L, Ballard-Barbash R, et al. Implementation of a 4-y, high-fiber, high-fruit-and-vegetable, low-fat dietary intervention: results of dietary changes in the polyp prevention trial. *Am J Clin Nutr* 2001;74:387–401.
- Sansbury LB, Wanke K, Albert PS, Kahle L, Schatzkin A, Lanza E. The effect of strict adherence to a high-fiber, high-fruit and -vegetable, and low-fat eating pattern on adenoma recurrence. *Am J Epidemiol* 2009;170:576–84.
- Steck-Scott S, Forman MR, Sowell A, Borkowf CB, Albert PS, Slattery M, et al. Carotenoids, vitamin A and risk of adenomatous polyp recurrence in the Polyp Prevention Trial. *Int J Cancer* 2004;112:295–305.
- Kabat GC, Kim MY, Sarto GE, Shikany JM, Rohan TE. Repeated measurements of serum carotenoid, retinol and tocopherol levels in relation to colorectal cancer risk in the Women's Health Initiative. *Eur J Clin Nutr* 2012;66:549–54.
- American Institute for Cancer Research. Continuous Update Project: Colorectal Cancer. Washington, D.C.; 2011.
- American Institute for Cancer Research. World Cancer Research Fund/American Institute for Cancer Research, Food, Nutrition and Prevention of Cancer: a global perspective. Washington, D.C.; 2007.
- Office of Disease Prevention and Health Promotion. Healthy People 2010. Available from: <http://www.healthypeople.gov/2010>.
- Gallus S, Bosetti C, La Vecchia C. Mediterranean diet and cancer risk. *Eur J Cancer Prev* 2004;13:447–52.
- Pauwels EK. The protective effect of the Mediterranean diet: focus on cancer and cardiovascular risk. *Med Princ Pract* 2011;20:103–11.
- Agnoli C, Grioni S, Sieri S, Palli D, Masala G, Sacerdote C, et al. Italian Mediterranean index and risk of colorectal cancer in the Italian section of the EPIC cohort. *Int J Cancer* 2013;132:1404–11.
- Fung TT, Hu FB, Wu K, Chiuve SE, Fuchs CS, Giovannucci E. The Mediterranean and dietary approaches to stop hypertension (DASH) diets and colorectal cancer. *Am J Clin Nutr* 2010;92:1429–35.
- Kontou N, Psaltopoulou T, Panagiotakos D, Dimopoulos MA, Linos A. The Mediterranean diet in cancer prevention: a review. *J Med Food* 2011;14:1065–78.
- Cockbain AJ, Toogood GJ, Hull MA. Omega-3 polyunsaturated fatty acids for the treatment and prevention of colorectal cancer. *Gut* 2012;61:135–49.
- Murff HJ, Shu XO, Li H, Dai Q, Kallianpur A, Yang G, et al. A prospective study of dietary polyunsaturated fatty acids and colorectal cancer risk in Chinese women. *Cancer Epidemiol Biomarkers Prev* 2009;18:2283–91.
- Djuric Z. The Mediterranean diet: effects on proteins that mediate fatty acid metabolism in the colon. *Nutr Rev* 2011;69:730–44.
- Djuric Z, Ruffin MT, Rapai ME, Cornellier ML, Ren J, Ferreri TG, et al. A Mediterranean dietary intervention in persons at high risk of colon cancer: recruitment and retention to an intensive study requiring biopsies. *Contemp Clin Trials* 2012;33:881–8.
- Johnson-Kozlow M, Rock CL, Gilpin EA, Hollenbach KA, Pierce JP. Validation of the WHI brief physical activity questionnaire among women diagnosed with breast cancer. *Am J Health Behav* 2007;31:193–202.
- Djuric Z, Ren J, Blythe J, VanLoon G, Sen A. A Mediterranean dietary intervention in healthy American women changes plasma carotenoids and fatty acids in distinct clusters. *Nutr Res* 2009;29:156–63.
- Neilson AP, Djuric Z, Ren J, Hong YH, Sen A, Lager C, et al. Effect of cyclooxygenase genotype and dietary fish oil on colonic eicosanoids in mice. *J Nutr Biochem* 2012;23:966–76.
- Efron B, Tibshirani R. Bootstrap methods for standard errors, confidence intervals, and other measures of statistical accuracy. *Stat Sci* 1986;1:54–75.
- Chai W, Conroy SM, Maskarinec G, Franke AA, Pagano IS, Cooney RV. Associations between obesity and serum lipid-soluble micronutrients among premenopausal women. *Nutr Res* 2010;30:227–32.
- Box G, Cox D. An analysis of transformations. *J R Stat Soc Ser B* 1964;26:211–52.
- Hertog MG, Hollman PC, Katan MB, Kromhout D. Intake of potentially anticarcinogenic flavonoids and their determinants in adults in the Netherlands. *Nutr Cancer* 1993;20:21–9.
- Lucas L, Russell A, Keast R. Molecular mechanisms of inflammation. Anti-inflammatory benefits of virgin olive oil and the phenolic compound oleocanthal. *Curr Pharm Des* 2011;17:754–68.
- Reynoso-Camacho R, Gonzalez-Jasso E, Ferriz-Martinez R, Villalon-Corona B, Loarca-Pina GF, Salgado LM, et al. Dietary supplementation of lutein reduces colon carcinogenesis in DMH-treated rats by modulating K-ras, PKB, and beta-catenin proteins. *Nutr Cancer* 2011;63:39–45.
- Tang FY, Pai MH, Wang XD. Consumption of lycopene inhibits the growth and progression of colon cancer in a mouse xenograft model. *J Agric Food Chem* 2011;59:9011–21.
- Chung HY, Ferreira AL, Epstein S, Paiva SA, Castaneda-Sceppa C, Johnson EJ. Site-specific concentrations of carotenoids in adipose tissue: relations with dietary and serum carotenoid concentrations in healthy adults. *Am J Clin Nutr* 2009;90:533–9.

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30. Campbell DR, Gross MD, Martini MC, Grandits GA, Slavin JL, Potter JD. Plasma carotenoids as biomarkers of vegetable and fruit intake. *Cancer Epidemiol Biomarkers Prev* 1994;3:493-500.
31. Mobarhan S, Shiau A, Grande A, Kolli S, Stacewicz-Sapuntzakis M, Oldham T, et al. beta-Carotene supplementation results in an increased serum and colonic mucosal concentration of beta-carotene and a decrease in alpha-tocopherol concentration in patients with colonic neoplasia. *Cancer Epidemiol Biomarkers Prev* 1994;3:501-5.
32. Gireesh T, Nair PP, Sudhakaran PR. Studies on the bioavailability of the provitamin A carotenoid, beta-carotene, using human exfoliated colonic epithelial cells. *Br J Nutr* 2004;92:241-5.
33. Simone F, Pappalardo G, Maiani G, Guadalaxara A, Bugjanesi R, Conte AM, et al. Accumulation and interactions of beta-carotene and alpha-tocopherol in patients with adenomatous polyps. *Eur J Clin Nutr* 2002;56:546-50.
34. Pappalardo G, Maiani G, Mobarhan S, Guadalaxara A, Azzini E, Raguzzini A, et al. Plasma (carotenoids, retinol, alpha-tocopherol) and tissue (carotenoids) levels after supplementation with beta-carotene in subjects with precancerous and cancerous lesions of sigmoid colon. *Eur J Clin Nutr* 1997;51:661-6.
35. Nair PP, Lohani A, Norkus EP, Feagins H, Bhagavan HN. Uptake and distribution of carotenoids, retinol, and tocopherols in human colonic epithelial cells *in vivo*. *Cancer Epidemiol Biomarkers Prev* 1996;5:913-6.
36. Ascherio A, Stampfer MJ, Colditz GA, Rimm EB, Litin L, Willett WC. Correlations of vitamin A and E intakes with the plasma concentrations of carotenoids and tocopherols among American men and women. *J Nutr* 1992;122:1792-801.
37. Rao CV, Hirose Y, Indranie C, Reddy BS. Modulation of experimental colon tumorigenesis by types and amounts of dietary fatty acids. *Cancer Res* 2001;61:1927-33.