

## Effect of increased vegetable and fruit consumption on markers of oxidative cellular damage

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**The goal of this study was to test the hypothesis that increased consumption of vegetables and fruit would reduce markers of oxidative cellular damage that can be assessed in blood or urine. Twenty-eight women participated in a 14 day dietary intervention. The primary end-points assessed were: 8-hydroxydeoxyguanosine (8-OHdG) in DNA isolated from peripheral lymphocytes, determined by HPLC with electrochemical detection; 8-OHdG excreted in urine, measured by ELISA; malondialdehyde (MDA) in urine, measured by fluorimetric detection following derivatization with thiobarbituric acid and separation via HPLC; urinary 8-isoprostane F-2 $\alpha$  (8-EPG) detected by ELISA. Pre- and post-intervention plasma levels of selected carotenoids were determined by HPLC. Subjects were free living and consumed a completely defined recipe-based diet that increased their average daily consumption of vegetables and fruit from 5.8 servings at baseline to 12.0 servings throughout the intervention. Overall, the level of 8-OHdG in DNA isolated from lymphocytes and in urine and the level of 8-EPG in urine were reduced by the intervention, whereas urine concentrations of MDA were minimally affected. The reduction in lymphocyte 8-OHdG was greater in magnitude (32 versus 5%) in individuals with lower average pre-intervention levels of plasma  $\alpha$ -carotene (56 ng/ml) than in individuals with higher average pre-intervention plasma levels of  $\alpha$ -carotene (148 ng/ml). The results of this study indicate that consumption of a diet that significantly increased vegetable and fruit intake from a diverse number of botanical families resulted in significant reductions in markers of oxidative cellular damage to DNA and lipids.**

### Introduction

Many epidemiological studies have reported an inverse relationship between vegetable and fruit consumption and cancer occurrence (1–3). These data are remarkable in the strength of the inverse associations between vegetable and fruit intake and cancer incidence and the consistency with which a protective effect against cancer has been observed. In animal as well as human studies, the evidence for the effectiveness of whole foods or for mixtures of whole foods in preventing cancer is

stronger than that for plant isolates, suggesting that a number of plant food constituents contribute to the overall effect (4–6). In fact, plant foods are so chemically complex that the list of compounds with possible anticarcinogenic activity is nearly endless. Because of the role of vegetables and fruits in the human diet as a prominent source of essential nutrients, many of which have antioxidant activity, the hypothesis that the antioxidant activity of vegetables and fruits contributes to their cancer preventive activity has been advanced (7–10). However, it is unclear whether the experimentally observed antioxidant effect of vegetables and fruits is achievable in humans under normal dietary conditions. This study was designed to determine whether consumption of a recipe-based diet high in vegetables and fruit from a diverse array of botanical families would reduce indices of oxidative cellular damage.

### Materials and methods

Subjects were recruited from a population of women participating in a clinical program for individuals at risk of breast cancer based on family history. The rationale for recruitment from this population was based on evidence that women at risk of breast cancer may be subject to higher levels of oxidative cellular damage (11,12). Recruitment occurred during a meeting to which all program participants were invited. The purpose of the study was explained and informed consent was obtained from those individuals interested in participating. The study was reviewed and approved by the AMC Cancer Research Center Institutional Review Board.

The intervention comprised a 14 day recipe-defined diet. Subjects were required to prepare all foods in their homes. During the intervention, subjects were asked to refrain from taking dietary supplements. Twenty-four hours a day assistance and the support of three registered dietitians (J.H., C.G. and C.O.) was provided to all participants. A pre-intervention assessment of dietary intake was obtained via a 3 day food record. This assessment provided an estimate of pre-intervention vegetable and fruit consumption. Women's calorie requirements were computed based on the Harris–Benedict formula with adjustments for self-reported levels of physical activity. The energy intake data were used to adjust the portion size of each recipe that was consumed so that subjects would maintain their pre-intervention body weights during the intervention. Pre-intervention and post-intervention blood samples and first void of the morning urine specimens were obtained from each subject. Because of the nature of the study design and the intervention, project staff were not blind to the treatment conditions.

Two studies were conducted. Fifteen women participated in the first study conducted in October 1997 and 13 women participated in a second study conducted in March 1998. The intervention diet consisted of a fully defined 14 day menu of recipes. The major source of recipes for the study was the *5 A Day for Better Health Program*. These recipes and others created by project staff were incorporated into daily diets that instructed participants about everything they were to eat or drink for 2 weeks. Recipes were selected and modified to provide vegetables and fruits from a large number of botanical families (Table I). All recipes were prepared by weight. Recipes were tested for taste, ease of preparation and yield, then converted to gram measurements for precision. Recipes were retested for yield. Menus were then prepared at four calorie levels (range 1400–2200 kcal/day), for ease of tailoring to each woman's calorie intake.

Blood for lymphocyte isolation was processed in CMT tubes (Becton Dickinson, Franklin Lakes, NJ) that contained sodium citrate as the anticoagulant. Lymphocytes were harvested from these tubes and frozen at  $-80^{\circ}\text{C}$  in phosphate-buffered saline containing 10% dimethylsulfoxide for subsequent isolation of DNA from nuclei. Nuclei were isolated from lymphocytes by use of non-ionic detergent (Nonidet P-40) and DNA was isolated from nuclei by a method employing proteinase K and organic extraction. 8-Hydroxydeoxygu-

**Abbreviations:** dG, deoxyguanosine; 8-EPG, 8-isoprostane F-2 $\alpha$ ; MDA, malondialdehyde; 8-OHdG, 8-hydroxydeoxyguanosine.

**Table I.** Botanical families and their frequency of consumption in the intervention diet

Botanical family	Common fruit and vegetable examples	Frequency in the menu (servings per day)	Average consumption (g/day)
Actinidiaceae	Kiwi	0.25	25
Agaricaceae	Mushroom	0.07	6
Chenopodiaceae	Spinach, Swiss chard, beet	0.25	24
Compositae	Artichoke, endive, lettuce	0.21	6
Convolvulaceae	Sweet potato	0.20	20
Cruciferae	Cabbage, broccoli, radish	1.37	96
Cucurbitaceae	Cucumber, zucchini, melon	0.42	36
Ericaceae	Blueberry, cranberry	0.30	22
Gramineae	Corn, bamboo shoots	0.06	5
Leguminosae	Chickpeas, lentils, soybeans	1.02	87
Liliaceae	Chive, garlic, onion, scallion	0.35	27
Musaceae	Banana, plantain	1.20	90
Rosaceae	Apple, peach, strawberry	1.24	124
Rutaceae	Grapefruit, orange, lemon, lime	0.90	167
Solanaceae	Tomato, eggplant, peppers	1.36	163
Umbelliferae	Carrot, celery, parsnip, parsley	0.73	55
Vitaceae	Grape	0.58	23

Average consumption of fruit (g/day): 462.

Average consumption of vegetables (g/day): 514.

anosine (8-OHdG) and deoxyguanosine (dG) in DNA from lymphocytes were measured by use of reverse phase HPLC with electrochemical and spectrophotometric detection for 8-OHdG and dG, respectively. The analysis of 8-OHdG was performed with vigilant attention to conditions that can induce the artificial formation of 8-OHdG, as discussed in a recent communication from our laboratory (13). Analysis was performed on identical samples processed in duplicate. Plasma was separated from non-fasting blood, collected using potassium EDTA as an anticoagulant and stored at  $-80^{\circ}\text{C}$  for subsequent measurement of carotenoids. Urine was collected without preservative in plastic vessels. Upon receipt, urine was aliquoted and stored at  $-20^{\circ}\text{C}$  for analysis of 8-OHdG, 8-isoprostane F-2 $\alpha$  (8-EPG) and malondialdehyde (MDA). 8-OHdG and 8-EPG abundance in urine from first voids was assessed using enzyme-linked immunosorbent assay (ELISA) kits (Genox Corp., Baltimore, MD and Assay Designs, Ann Arbor, MI, respectively). The ELISA assays were performed according to the manufacturer's instructions, with minor modification. Urine samples were diluted 10 $\times$  with assay buffer (Tris-buffered saline) prior to 8-EPG analysis, to standardize sample pH and ionic strength. Urinary 8-OHdG analysis was performed on undiluted urine. The decision to use first voids rather than 24 h collections was based on preliminary data indicating that 24 h averages were not statistically different from values obtained from first voids and on our experience that collecting reliable 24 h urine samples from free-living subjects is problematical. MDA content in urine was assessed by HPLC with fluorimetric detection of the thiobarbituric acid derivative (14). Plasma carotenoid concentrations were determined by reverse phase HPLC with spectrophotometric detection (15).

#### Statistical analyses

Tests for change (pre-intervention versus post-intervention values) in the outcomes, urinary 8-EPG, urinary and lymphocyte 8-OHdG and urinary MDA, overall and by  $\alpha$ -carotene level ( $>98$  ng/ml, high;  $<98$  ng/ml, low), were performed using a repeated measures, mixed effects model (16). We were concerned with extreme values in urinary 8-OHdG and MDA and therefore used a rank transformation to evaluate the change in pre-intervention versus post-intervention levels of these target analytes; the resulting tests are robust (17). Changes in plasma carotenoids were assessed using a paired *t*-test (18).

## Results

### Subject characteristics

A total of 28 subjects were recruited into two replicate studies and completed the intervention. Since statistical analyses indicated that there were no significant effects attributable to replicate study, data were pooled and are presented as such. The age range of the participants was 27–80 years with an average age of 49.8 years. Based on 3 day pre-intervention

**Table II.** Effect of the dietary intervention on plasma carotenoids

Parameter	Pre-intervention (ng/ml)	Post-intervention (ng/ml)	Change (%)	<i>P</i> value
$\alpha$ -Carotene	95.4 $\pm$ 11.3	169.3 $\pm$ 14.0	77.5	0.001
$\beta$ -Carotene	313.8 $\pm$ 50.1	455.3 $\pm$ 48.3	45.1	0.001
Lutein	156.1 $\pm$ 11.9	229.6 $\pm$ 17.6	47.1	0.001
Lycopene	348.3 $\pm$ 27.6	403.4 $\pm$ 25.0	15.8	0.001
$\beta$ Cryptoxanthin	142.3 $\pm$ 14.7	159.8 $\pm$ 12.1	12.3	0.032

Twenty-eight subjects participated in the intervention. Values are means  $\pm$  SEM. Pre-intervention versus post-intervention differences were evaluated using a paired *t*-test.

food records, average consumption of vegetables and fruits was 5.8 servings/day. The mean pre-intervention energy intake was 1761 kcal/day and the percent of calories from fat was 32.6. The levels of energy intake and percent calories from dietary fat that were maintained throughout the intervention were 1682 kcal and 27.1%, respectively. No significant correlations were found between change in percent kcal from fat, total fat or total calories and the change in the outcomes measures, i.e. 8-OHdG in urine and blood, urinary MDA or urinary 8-EPG. Since none of the parameters correlated univariately with change in the outcome measures, they were not used as covariates in the statistical analyses. Based on food records kept throughout the 14 day intervention, average intake of vegetables and fruits was 12.0 servings/day (2-fold increase) with an overall compliance rate of 95.2% (range 83–101%). Pre-intervention body weights were on average 70 kg (range 47–91 kg) and were not affected by the intervention.

### Effect of the dietary intervention on plasma carotenoids

Plasma levels of five commonly reported carotenoids,  $\alpha$ - and  $\beta$ -carotene, lutein, lycopene and cryptoxanthin, were evaluated. Pre-intervention and post-intervention plasma carotenoid data are shown in Table II. Plasma carotenoid levels were elevated, but to different degrees, by the intervention. In all cases the differences in pre- versus post-intervention levels were statistically significant.  $\alpha$ -Carotene was elevated 77.5% above baseline levels. The rank order of effect of the intervention on plasma carotenoids in descending order of magnitude was  $\alpha$ -carotene  $>$  lutein =  $\beta$ -carotene  $>$  lycopene =  $\beta$  cryptoxanthin. Since there was such a wide range of pre-intervention  $\alpha$ -carotene values and  $\alpha$ -carotene has been reported to be the best biomarker of vegetable and fruit consumption (19), a *post hoc* analysis was performed. In this analysis, which should be considered hypothesis-generating rather than hypothesis-testing, we split the 28 individuals into two subgroups based on pre-intervention  $\alpha$ -carotene levels. A natural break in the distribution of data occurred within 5% of the average pre-intervention value of plasma  $\alpha$ -carotene (Table II). When the 28 subjects were divided into groups using this criterion, low ( $n = 16$ ) and high ( $n = 12$ ) pre-intervention  $\alpha$ -carotene subgroups were created. Effects of the intervention on oxidative indices in these two subgroups (Tables III and IV) were then compared and contrasted with the effects observed overall (Table V).

### Effect of the dietary intervention on markers of oxidative damage

During the methods development, we conducted a series of preliminary studies to assess day-to-day variation in urinary

**Table III.** Effect of vegetable and fruit intervention on oxidative indices (pre-intervention  $\alpha$ -carotene <98 ng/ml)

Parameter	Pre-intervention	Post-intervention	Change (%)	<i>P</i> value
Vegetables and fruit (servings/day)	4.7 $\pm$ 0.5	12.0 $\pm$ 0.6	155	
$\alpha$ -Carotene, plasma (ng/l)	56 $\pm$ 6	138 $\pm$ 14	147	0.001 <sup>a</sup>
8-OHdG, lymphocyte (residues/10 <sup>6</sup> dG)	8.6 $\pm$ 1.6	5.8 $\pm$ 1.0	-32	0.038
8-OHdG, urine (ng/mg creatinine)	48.3 $\pm$ 18.1 (27.1)	20.8 $\pm$ 1.9 (18.9)	-57 (-30)	0.173
8-EPG, urine (ng/mg creatinine)	3.6 $\pm$ 0.3	2.3 $\pm$ 0.2	-37	0.004
MDA, urine (nmol/mg creatinine)	10.8 $\pm$ 0.7	10.9 $\pm$ 1.1	1	0.695

Sixteen subjects had pre-intervention plasma  $\alpha$ -carotene concentrations <98 ng/ml. Values are means  $\pm$  SEM. Median values are displayed in parentheses. Primary outcomes were evaluated statistically using a mixed effects, repeated measures analysis of the rank transformation of the outcome measures.

<sup>a</sup>Based on a paired *t*-test.

**Table IV.** Effect of vegetable and fruit intervention on oxidative indices (pre-intervention  $\alpha$ -carotene >98 ng/ml)

Parameter	Pre-intervention	Post-intervention	Change (%)	<i>P</i> value
Vegetables and fruit (servings/day)	7.3 $\pm$ 0.7	12.1 $\pm$ 0.4	66	
$\alpha$ -Carotene, plasma (ng/ml)	148 $\pm$ 15	211 $\pm$ 22	43	0.001 <sup>a</sup>
8-OHdG, lymphocyte (residues/10 <sup>6</sup> dG)	7.0 $\pm$ 1.9	6.7 $\pm$ 1.2	-5	0.938
8-OHdG, urine (ng/mg creatinine)	51.2 $\pm$ 16.9 (21.0)	22.4 $\pm$ 4.7 (20.9)	-56 (-1)	0.375
8-EPG, urine (ng/mg creatinine)	3.8 $\pm$ 0.6	2.5 $\pm$ 0.3	-33	0.039
MDA, urine (nmol/mg creatinine)	13.1 $\pm$ 1.7	13.0 $\pm$ 1.8	-1	0.576

Twelve subjects had pre-intervention plasma  $\alpha$ -carotene concentrations >98 ng/ml. Values are means  $\pm$  SEM. Medians are displayed in parentheses. Primary outcomes were evaluated statistically using a mixed effects, repeated measures analysis of the rank transformation of the outcome measures.

<sup>a</sup>Based on a paired *t*-test.

**Table V.** Overall effect of the vegetable and fruit intervention on oxidative indices

Parameter	Pre-intervention	Post-intervention	Change (%)	<i>P</i> value
8-OHdG, lymphocyte (residues/10 <sup>6</sup> dG)	7.9 $\pm$ 1.2	6.2 $\pm$ 0.8	-21	0.113
8-OHdG, urine (ng/mg creatinine)	49.6 $\pm$ 12.4 (23.9)	21.4 $\pm$ 2.2 (19.2)	-57 (-20)	0.108
8-EPG, urine (ng/mg creatinine)	3.7 $\pm$ 0.3	2.4 $\pm$ 0.1	-36	0.0004
MDA, urine (nmol/mg creatinine)	11.7 $\pm$ 0.8	11.7 $\pm$ 1.0	0	0.516

Twenty-eight subjects were evaluated. Values are means  $\pm$  SEM. Medians are displayed in parentheses. Primary outcome variables were evaluated using a mixed effects, repeated measures analysis of the rank transformation of the outcome measures.

markers. Daily variations in mean values over time were of the order of 13% for urinary 8-OHdG, 11% for urinary 8-EPG and 12% for urinary MDA. None of the differences over time were statistically significant. Table V shows the pre- and post-intervention levels of the markers of oxidative cellular damage that were assayed. The intervention resulted in reductions in lymphocyte 8-OHdG (21.5% reduction) and urinary 8-EPG (35% reduction). There was little effect of the dietary intervention on levels of urinary MDA. Urinary 8-OHdG determined by ELISA was highly variable at baseline (pre-intervention samples) and mean versus median values differed by 48% (Table V). A remarkable reduction in variance was observed in the post intervention samples and the mean and median values differed by only 11%. Considering only mean values, the intervention reduced urinary 8-OHdG by 56.9%, however, the difference in median values was only 19.9%. These results suggest to us that substances in some urine samples may have been interfering with the ELISA assay, which requires the use of undiluted urine. On the other hand, similar effects were not noted in the assessment of 8-EPG. The 8-EPG ELISA kit was designed to use diluted urine, an approach which is likely to reduce interference problems, particularly those associated with differences in urine osmolarity.

Table III shows the effect of the dietary intervention on oxidative indices of individuals in the low pre-intervention plasma  $\alpha$ -carotene subgroup. The average pre-intervention plasma  $\alpha$ -carotene was 56 ng/ml, a value essentially the same as the average concentration found in the US population (20). In these individuals, significant reductions in lymphocyte 8-OHdG and urinary 8-EPG were observed and the reduction in lymphocyte 8-OHdG was greater than the reduction in the value for the entire group (Table V). On the other hand, for individuals in the high pre-intervention plasma  $\alpha$ -carotene group (Table IV), there was no detectable effect of the intervention on lymphocyte levels of 8-OHdG nor was there an effect on urinary 8-EPG. In no case was the magnitude of the effect of the dietary intervention on urinary MDA greater than the level of day-to-day variability observed among individuals in our preliminary studies. The large difference in the mean versus the median pre-intervention values of urinary 8-OHdG were observed in both subgroups (Tables III and IV); variability was lower in post-intervention values. Although there was a 30% difference between the median pre- and post-intervention values of urinary 8-OHdG in the low  $\alpha$ -carotene subgroup, the difference was not statistically significant; there was no similar difference in the high  $\alpha$ -carotene subgroup.

## Discussion

Using indices of oxidative damage to DNA and lipids as surrogate biomarkers for cancer risk and as determinants of *in vivo* antioxidant activity (21), the results of a number of investigations have cast doubt on the hypothesis that nutritional antioxidants present in vegetables and fruits account for the strong association between vegetable and fruit consumption and risk of cancer (22–24). The lack of effect observed raises a critical question, namely does increased consumption of vegetables and fruits in humans under normal dietary conditions have an effect on biomarkers for cancer risk that reflect antioxidant activity? The data shown in Tables III–V indicate that increased consumption of vegetables and fruits does in fact reduce cellular injury measured as the oxidation of DNA and lipids. In the following paragraphs we comment on various aspects of these findings.

The antioxidant effect of the dietary intervention on DNA was measured as the abundance of 8-OHdG in DNA isolated from peripheral lymphocytes and excreted in urine. 8-OHdG is a relatively abundant and readily detected product of oxidative DNA damage and as such is regarded as a useful and relevant marker for cellular oxidative stress, particularly with respect to carcinogenesis (21,25). The argument has been made that oxidized adducts of DNA are promutagenic lesions. If they are not repaired, they can result in mutations. Changes in rates of mutation over a lifetime are expected to impact on risk of malignancy. The decision to assess oxidative damage in DNA isolated from lymphocytes was made for several reasons. Lymphocytes are relatively easy to obtain, their half-life in blood is short and they provide a general estimate of the oxidative stress to which an organism is subjected. In addition, levels of DNA oxidation in peripheral lymphocytes have been shown to vary in response to a variety of agents to which individuals have been exposed, including cigarette smoking, exposure to which affects cancer risk (26,27). As shown in Table V, the concentration of 8-OHdG in lymphocyte DNA was reduced by 21.5% by the 2 week intervention. The fact that this response was observed within 2 weeks is consistent with two other reports in which a single food or food extract was fed and shown within 2 weeks to reduce levels of oxidative DNA damage (8,28). The magnitude of the effect was also within the same range. However, in those studies DNA oxidation was estimated by the Comet assay, whereas we measured a specific product of DNA oxidation, 8-OHdG. As shown in Tables III and IV, the intervention reduced lymphocyte levels of 8-OHdG in subjects with low but not high pre-intervention plasma levels of  $\alpha$ -carotene. This observation is particularly important since we did not study the effects of a low vegetable and fruit intervention in this investigation. These data (Tables III and IV) indicate that it is unlikely that the process of following the dietary intervention, rather than the increased level of vegetable and fruit consumption *per se*, accounts for the effects observed on levels of 8-OHdG. This premise is also consistent with the results obtained to date in on-going work in which the effects on the same oxidative indices of the same dietary intervention approach are being studied, but using a recipe-defined menu providing a low intake of vegetables and fruits (3 servings/day). While only 10 subjects have completed the intervention thus far, change in lymphocyte 8-OHdG was <5% over the course of the intervention (data not shown). These results will be reported in detail at a later date. Thus our finding using lymphocyte

8-OHdG as an end-point is important because it indicates that increasing vegetable and fruit intake decreases DNA oxidation, an effect that is not obtained when supplemental antioxidants are administered (22–24).

Urinary excretion of 8-OHdG was also measured. As noted in the Results, we have concerns about the specificity of this assay given the large differences in the mean versus median values that we observed (Tables III–V). Considering the median values for urinary 8-OHdG reported in Tables III–V, it appears that the same pattern of changes detected in lymphocyte 8-OHdG were also detected in urine. This observation is consistent with the expectation that changes in oxidation would be mirrored in these indices (21), however, given our concerns about the specificity of the kit, we are currently addressing this issue using an independent analytical approach which will be discussed in future papers.

A secondary focus of our work was to determine if diets high in vegetables and fruit would decrease lipid peroxidation. Lipid peroxidation chain reactions can produce an exponential increase in reactive compounds that can oxidize cellular macromolecules, including DNA, and certain antioxidants are known to inhibit such events (29). MDA was chosen as one marker of lipid peroxidation for this study since it is a widely used and easily assessed biochemical end-point that has been shown to change in response to changes in exposure to oxidative stress (15). The data shown in Table V are not consistent with our prediction that increasing fruit and vegetable intake would decrease urinary MDA. These observations may indicate that urinary MDA is a relatively insensitive marker for detecting differences in peroxidation and that both the pre-intervention diets of the participants and the intervention diet were adequate with respect to protecting against the peroxidation of lipid reflected in urinary levels of MDA. Alternatively, these results may reflect changes in dietary MDA consumption that could obscure changes in endogenous levels of lipid peroxidation. In animal models, ingested MDA from diets high in polyunsaturated fat have been shown both in our laboratory (unpublished data) and by others (reviewed in ref. 21) to contribute substantially to urinary MDA excretion. Thus, MDA absorbed from dietary sources and excreted in the urine can complicate the ability to discriminate between MDA endogenously produced due to cellular oxidative events and that resulting from dietary ingestion.

In an effort to further assess the effect of the dietary intervention on lipid peroxidation, we also measured urinary excretion of 8-EPG. The 8-epimer of prostaglandin  $F_{2\alpha}$  is an isomer of a class of prostanoids, referred to collectively as F2 isoprostanones, that are produced *in vivo* by free radical-induced lipid peroxidation (21,30). Evidence indicates that they are generated primarily by cyclooxygenase-independent pathways, as their production is not modulated by cyclooxygenase inhibitors such as ibuprofen, aspirin and indomethacin. They have been shown to arise *in situ*, be esterified to phospholipids and to be excreted in urine (31). Some work indicates that urinary F2 isoprostanones serve as a time-integrated marker for *in vivo* free radical-induced lipid peroxidation (21,32). As shown in Table V, the vegetable and fruit intervention resulted in a 35% reduction in urinary levels of 8-EPG. Interestingly, this effect was observed in both the low (Table III) and high (Table IV) pre-intervention plasma  $\alpha$ -carotene subgroups, although the effect was numerically greater in the low  $\alpha$ -carotene subgroup. The fact that in the on-going study referred to above we have

not observed changes in urinary excretion of 8-EPG in response to a dietary intervention providing three servings of vegetables and fruits per day indicates that the effect reported in Table V is likely to be due to the consumption of increased levels of vegetables and fruits rather than the dietary intervention *per se*. These data also imply that urinary MDA is not a sensitive indicator of the overall level of *in vivo* lipid peroxidation.

One innovative aspect of this work was the development of a structured, recipe-defined, nutritionally balanced diet that provided a botanically diverse array of plant foods on a daily basis rather than over-emphasizing a single botanical family (illustrated in Table I). This approach was implemented for several reasons. First, we hypothesize that botanical diversity is a key to maximizing exposure to an array of beneficial phytochemicals, many still unidentified, present in edible plants, while minimizing the potential for deleterious effects that could arise from over-emphasizing a particular botanical family(s) (33). Second, we judge that there is a need for a dietary approach that can be modified systematically to evaluate the contributions of specific classes of vegetables and fruits in cancer risk reduction. Table II shows pre- and post-intervention mean plasma levels of five typically measured plasma carotenoids. As expected, the diet outlined in Table I caused a significant increase in the levels of all the carotenoids measured, but the magnitude of increase varied. The percent change in pre- versus post- intervention mean carotenoid levels ranged from as low as 12% to as high as 78%. Whether this pattern of change is optimal for cancer prevention or requires adjustment remains to be determined by additional experimentation. However, we judge that the use of plasma carotenoid patterns could guide future work in this field by serving as a biomarker in the design of botanical patterns that maximize the cancer preventive activity of the diet. The third reason underlying the development of our dietary approach is the importance of offering a diet, which we prefer to call a cuisine, that has the potential for high consumer acceptability by virtue of its palatability.

In summary, the fact that DNA oxidation and lipid peroxidation were reduced by adherence to a diet that increased vegetable and fruit intake is consistent with these foods having an antioxidant effect that could be causally related to carcinogenesis and its inhibition. The design of the experiment reported in this study provides a basis for systematically evaluating the many existing questions about vegetables and fruits in cancer prevention. Among those questions, determining how many servings of vegetables and fruits per day afford maximal protection and which particular botanical families of edible plants are critical to include in the diet have particular merit.

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