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Dietary Botanical Diversity Affects the Reduction of Oxidative Biomarkers in Women due to High Vegetable and Fruit Intake[1]

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Abstract

Many health benefits are associated with a high dietary intake of vegetables and fruit (VF); however, little effort has been expended to determine whether the botanical families from which high-VF diets are formulated affect their biological activities. The objective of this study was to determine whether the botanical diversity of high-VF diets alters the response in oxidative biomarkers for lipid peroxidation and DNA oxidation. Two diets were developed that varied in botanical diversity and provided 8–10 servings of VF/d. The high botanical diversity diet (HBD) included foods from the 18 botanical families that induced a reduction in oxidative damage of lipids or DNA. The low botanical diversity diet (LBD) emphasized 5 of these botanical families based on reports that their bioactive components had high antioxidant activity. A total of 106 women completed the study. Participants consumed 9.1 ± 2.6 and 8.3 ± 2.1 servings of VF/d with the LBD and HBD diets. Only the HBD diet induced a significant reduction in DNA oxidation ($P < 0.05$). Both the LBD and the HBD diets were associated with a reduction in lipid peroxidation ($P < 0.01$). These findings indicate that botanical diversity plays a role in determining the bioactivity of high-VF diets and that smaller amounts of many phytochemicals may have greater beneficial effects than larger amounts of fewer phytochemicals. J. Nutr. 136: 2207–2212, 2006.

Introduction

Although many health benefits are associated with consumption of diets having an increased content of vegetables and fruits (VF),[4] relatively little effort has been expended to determine whether the botanical families from which whole food–based diets are formulated have any effect on the biological activities exerted by the bioactive compounds that are available from the diet (1). This situation exists despite intensive investigation of the biological effects of dietary bioactive compounds (DBC) associated with foods from specific botanical families. These include: Brassicaceae (e.g., cabbage), Liliaceae (e.g., garlic), Rutaceae (e.g., orange), and Solanacae (e.g., tomato). (2–5). The classes of chemicals among the DBC that have received particular attention are carotenoids, flavonoids, and sulfur-containing compounds in addition to vitamins C and E.

The DBC in plant foods are likely to modulate the activity of many biological systems in mammalian species (1). However, there is little question that the most widely investigated effect is the role that phytochemical DBC play in protecting biological systems against cellular oxidation. In an effort to extend studies of phytochemical antioxidants from their assessment in test tube assays, cell culture systems, and preclinical models to clinical interventions, our laboratory reported a series of investigations of the effects of carefully formulated diets that varied in their VF content (6–8). In those studies, high (>10 servings of VF/d) vs. low (<3.5 servings of VF/d) intake was associated with a modest reduction in both the level of DNA oxidation in peripheral lymphocytes and the concentration of a marker of lipid peroxidation excreted in urine. Antioxidant effects were also manifest primarily in individuals with elevated levels of oxidation biomarkers at the time they initiated participation in an experiment. These findings provided the foundation for the study reported herein; the primary question asked was whether the botanical diversity of the plant food component of the diet alters the response in oxidative biomarkers to high VF consumption.

Materials and Methods

Subjects

Volunteers for this study were recruited via program advertisement among a group of individuals interested in women's health issues. The eligibility criteria for participation were as follows: female, ≥21 y old, not pregnant or lactating, no known food allergies, not consuming a special diet prescribed by a physician, familiar with cooking, willing to prepare prescribed meals for 2 wk, willing to keep a record of the foods eaten during the study, regularly consumes ≤2 alcoholic beverages/d, does not use tobacco products of any type, does not take multivitamin supplements or willing to stop taking vitamin supplements while participating in the study, not a competitive athlete, maintained approximately the same body weight for the past 6 mo, and does not regularly take medications for things other than birth control or postmenopausal

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[4] Abbreviations used: DBC, dietary bioactive compounds; HBD, high botanical diversity; 8-iso-$PGF_{2\alpha}$, 8-isoprostane $F_{2\alpha}$; LBD, low botanical diversity; 8-oxo-dG,8-hydroxy-2-deoxyguanosine; VF, vegetables and fruit.
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hormone therapy. Failure of interested individuals to meet eligibility criteria formed the basis for exclusion from the study. The clinical protocol was approved by the Institutional Committee for the Protection of Human Subjects at the AMC Cancer Research Center. A total of 111 women enrolled in the study and gave informed consent.

Dietary approach

To provide a systematic framework for addressing the question concerning whether the type of plant foods comprising the diet affects antioxidant activity in vivo, plant foods were categorized by botanical family. Using this approach, 2 diets were formulated to provide 8–10 servings of VF/d; one had low botanical diversity (LBD), whereas the second had high botanical diversity (HBD). The HBD diet was formulated from the same 18 botanical families that we investigated previously, families that were shown to induce a modest reduction in oxidative damage of lipids or DNA (8). Both diets were designed to provide 8–10 servings of VF/d depending on the participant's energy intake. The serving size definitions are those adapted by the 5-A-Day for Better Health program (9). Each diet consisted of a fully defined 14-d menu of recipes. Both diets were designed to have the same macronutrient content (% energy from fat, 30%; protein, 18%; carbohydrate, 52%), to meet the Recommended Dietary Allowances for vitamins and minerals, and to be balanced in nutrients from the various food groups.(10)

Study design

Based on our previous work in which we found no evidence that effects of VF observed after 2 wk changed over the next 6 wk of treatment (7), a 2-wk duration for the intervention was chosen for this work. Women who met the eligibility criteria and gave informed consent were randomly assigned to either the HBD or LBD diet groups. Blood samples (from nonfasting subjects) were taken in the morning on which the intervention was initiated, and again on the morning after the last day of the dietary intervention. First, void urine specimens were collected on 3 consecutive mornings preceding each blood draw. The procedure for, and timing of sample collection were based on previous work (6). A total of 111 individuals were randomized, and 53 participants in each diet group completed the study.

Laboratory Measurements

Urinary 8-iso-PGF$_{2\alpha}$. Analysis of 8-isoprostane $F_{2\alpha}$ (8-iso-PGF$_{2\alpha}$) in urine provides a time-averaged index of lipid peroxidation over a relatively prolonged interval that offers greater utility than the more transitory information provided by analysis of blood. Moreover, urine contains very little arachidonic acid, thereby limiting artifactual oxidation and isoprostane production after sample collection. Because we found that intra-individual daily excretion of urinary 8-iso-PGF$_{2\alpha}$ varies considerably in human subjects, we elected to measure 8-iso-PGF$_{2\alpha}$ abundance in pooled urine samples generated from multiple specimens. Urine from the first void of the morning was collected without preservative in plastic vessels on 3 consecutive days. The decision to use first voids rather than 24-h collections was based on our pilot data indicating that 24-h means expressed/mg creatinine did not differ from values obtained from first voids and also on our experience that collecting reliable 24-h urine samples from free-living subjects is problematic. After solid phase extraction of the urine, the analysis was performed using an ELISA assay (Cayman Chemical); our method was described previously in detail (7).

Lymphocyte 8-oxo-dG. To evaluate the effects of the 2 diets on DNA oxidation, the concentration of 8-hydroxy-2-deoxyguanosine (8-oxo-dG) in DNA isolated from peripheral lymphocytes was determined. 8-oxo-dG is a relatively abundant and readily detected product of oxidative DNA damage; as such, it is regarded as a useful and relevant marker for cellular oxidative stress (11). 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 oxidative stress, at the genomic level, to which an organism is subjected (12,13). Our HPLC method using electrochemical detection was described in detail in a recent publication (8).

Plasma carotenoids. Plasma concentrations of α-carotene, β-carotene, lycopene, lutein, and β-cryptoxanthine were determined to provide a biochemical index of compliance with the intervention diets. Our HPLC method using diode array detection was described in detail (7).

Other measurements. At baseline, participants completed the NIH 7-item screening questionnaire for fruit and vegetable intake (14); this was the only assessment of preintervention dietary behaviors that was conducted. Participants also completed printed surveys at baseline and postintervention. The baseline surveys provided data on demographics and levels of physical activity. The postintervention surveys provided self-reported compliance and feedback on the intervention.

Statistical analyses

Differences in categorical variables at baseline across randomization groups were evaluated using Fisher's exact test for independence of proportions (15). Continuous data were tested for differences in means using a 2-group, 2-sided t test. Differences with a P-value ≤ 0.05 were considered significant. Distributions for the continuous data were also checked to determine whether transformations were appropriate. None were deemed necessary in this sample.

Maximum likelihood estimates of a repeated-measures model were used for the analysis of the primary outcomes (concentrations of lymphocyte 8-oxodG and urinary 8-iso-PGF$_{2\alpha}$) (16). This approach is conceptually identical to multivariate ANOVA, but avoids the case-wise deletion of subjects with missing assessments. The model provides unbiased estimates under the less restrictive assumption that missing data are missing at random. The maximum likelihood estimates are based on a repeated-measures model of time × group:

$$y_{ij} = \beta_0 + \beta_1 tG,$$

where y_{ij} is the outcome measure for the ith subject in the jth randomization group; j = {1,2}; $t = 11$; $G = A$ if the subject is in the HBD diet group, B otherwise. Hypotheses were tested by setting up appropriate contrasts (linear combinations of model parameters) within the model; we report the P-values associated with the resulting t statistics. A similar model was used to estimate group differences by quartile of the baseline measure; parameterization was modified to facilitate the contrasts. No statistical adjustments for multiple comparisons and multiple end points were made, but the quartile analysis is considered secondary and results should be interpreted with caution. All analyses were done using SAS v 8.2 (SAS Institute).

Results

Characteristics of the study participants. No baseline characteristics differed by randomization groups (**Table 1**). Participants were predominantly white and well-educated (71% had at least 4 y of college). Their mean age was 48.5 y. Nearly 33% were overweight (BMI between 25 and 30 kg/m^2) and 12% were obese (BMI at least 30 kg/m^2); the mean BMI was 25.3 kg/m^2.

Intake of macronutrients and of VF by botanical family. Participants consuming the LBD diet tended to have a greater VF intake than those consuming the HBD diet. Both diets met nutritional guidelines and macronutrient intakes from these diets were similar (**Table 3**). Based on self-report, compliance with the dietary interventions was >85% in both study groups.

Carotenoids. Dietary intake and plasma concentrations of 5 commonly reported carotenoids, α- and β-carotene, lutein, lycopene, and β-cryptoxanthin, were evaluated as markers of

TABLE 1 Baseline characteristics of women by intervention group[1]

Variable	LBD diet	HBD diet
Age, *y*	48.9 ± 8.7	48.1 ± 10.4
Race, *n* (%)		
Black	0 (0)	1 (1.8)
Hispanic	1 (1.9)	3 (5.3)
White (not Hispanic)	51 (96.2)	52 (91.2)
Other	1 (1.9)	1 (1.8)
Weight, *kg*	69.9 ± 13.2	68.4 ± 14.8
Height, *m*	1.65 ± 0.05	1.65 ± 0.05
BMI, kg/m^2	25.5 ± 4.9	25.1 ± 4.6
Education, *n* (%)		
High school graduate	4 (7.5)	1 (1.9)
Some college	11 (20.8)	14 (26.4)
College graduate	18 (34.0)	20 (37.7)
Postgraduate	20 (37.7)	18 (34.0)
Physical activity, *n* (%)		
Sedentary	1 (2.0)	4 (7.6)
Light	18 (36.0)	17 (32.1)
Moderate	27 (54.0)	28 (52.8)
Heavy	4 (8.0)	4 (7.5)
VF, *servings/d*	4.5 ± 2.0	4.3 ± 1.7

[1] Values are means ± SD, $n = 53$, or *n* (%). The groups did not differ in any variable, $P \geq 0.05$.

compliance with the dietary intervention. Based on participants' daily food logs, intake of dietary carotenoids was markedly and significantly different between the LBD and HBD diet groups (Table 3). In both intervention groups, changes in plasma carotenoid concentrations paralleled the intake of dietary carotenoids reported in **Table 4**. The plasma carotenoid data were consistent with the high level of self-reported compliance.

Urinary 8-iso-PGF2α. The urinary concentration of 8-iso-PGF2α was lower in the postintervention than in the preintervention samples of subjects in both intervention groups (**Table 5**, $P < 0.001$). The extent of reduction tended to be greater in the HBD intervention group, although the postintervention concentrations of 8-iso-PGF2α in the HBD and LBD diet groups did not differ significantly ($P = 0.141$).

In other studies (7,8), we observed that the magnitude of the reduction in 8-iso-PGF2α is related to the level of this analyte in the urine at baseline. To examine the effect of botanical diversity on lipid peroxidation, as affected by peroxidation status at baseline, the data were subdivided by baseline quartile of urinary 8-iso-PGF2α and the effects of the dietary interventions evaluated. Neither intervention diet affected the concentration of 8-iso-PGF2α in individuals in the lowest quartile. The HBD intervention significantly reduced the urinary concentration of 8-iso-PGF2α in the 2nd through 4th quartiles, whereas the effect of the LBD diet was significant only for individuals in the 3rd and 4th quartiles.

Lymphocyte 8-oxo-dG. The concentration of 8-oxo-dG in genomic DNA isolated from peripheral lymphocytes (Table 6) was lower in the postintervention than in the preintervention samples from subjects in the HBD diet group ($P = 0.05$), but not the LBD diet group ($P = 0.78$). These data were further evaluated by dividing the participants into quartiles by concentration of lymphocyte 8-oxo-dG at baseline. The LBD diet did not affect the concentration of this analyte irrespective of the baseline concentration of 8-oxo-dG. On the other hand, in the HBD, the concentration of 8-oxo-dG was lower in post- than in preintervention samples of participants in the 3rd ($P = 0.003$) and 4th quartiles ($P = 0.007$).

TABLE 2 Daily intake of vegetables and fruit by botanical family in women consuming LBD or HBD diets[1]

Botanical family	Common foods	LBD diet[2]	HBD diet
		servings/d	
Actinidiaceae	Kiwi		1.08 ± 0.32
Chenopodiaceae[2]	Spinach, Swiss chard, beet	1.25 ± 1.13	0.40 ± 0.24
Compositeae	Artichoke, endive, lettuce	0.93 ± 0.67	0.58 ± 0.25
Convolvulaceae	Sweet potato		0.76 ± 0.42
Cruciferae[2]	Cabbage, broccoli, radish	1.47 ± 0.79	0.80 ± 0.32
Cucurbitaceae	Cucumber, zucchini, melon		0.98 ± 1.01
Ericaceae	Blueberry, cranberry		0.42 ± 0.16
Gramineae	Corn, bamboo shoots		0.96 ± 0.65
Leguminosae	Chickpeas, lentils, soybeans	0.87 ± 0.61	0.98 ± 0.70
Liliaceae[2]	Chive, garlic, onion, scallion	3.11 ± 1.38	1.35 ± 0.82
Musaceae	Banana, plantain		0.89 ± 0.32
Rosaceae	Apple, peach, strawberry		0.91 ± 0.34
Rutaceae[2]	Grapefruit, orange, lemon, lime	1.91 ± 0.83	0.57 ± 0.36
Solanaceae[2]	Tomato, eggplant, peppers	1.88 ± 0.90	1.28 ± 0.69
Umbelliferae	Carrot, celery, parsnip, parsley	0.24 ± 0.10	0.79 ± 0.36
Vitaceae	Grape		1.64 ± 0.60
Agaricaceae	Mushroom		0.56 ± 0.24
Bromeliaceae	Pineapple		0.32 ± 0.06
Total Servings/d[3]		9.10 ± 2.62	8.33 ± 2.13

[1] Values are means ± SD, $n = 53$; these values were computed on the basis of actual intake as recorded in the daily food records kept by each participant.
[2] Botanical families emphasized in the LBD diet group.
[3] Column sums will not equal total servings/d because not all foods were eaten on all days.

TABLE 3 Intake of macronutrients and carotenoids in women consuming LBD or HBD diets

	Diet[1]	
	LBD	HBD
Energy,[2] *kJ/d*	6669.6 ± 966.0	6825.0 ± 793.8
Fat, *% energy*	105.0 ± 7.1	100.8 ± 7.1
Saturated fatty acid, *% energy*	37.4 ± 3.4	32.8 ± 2.1
Monounsaturated fatty acid, *% energy*	39.9 ± 7.6	46.6 ± 3.8
PUFA, *% energy*	27.7 ± 5.0	21.4 ± 1.7
Carbohydrate, *% energy*	238.1 ± 6.3	239.8 ± 8.0
Protein, *% energy*	76.9 ± 2.9	79.4 ± 3.4
α-Carotene, $\mu g/d$	108 ± 44	1744 ± 251*
β-Carotene, $\mu g/d$	7178 ± 1227	9069 ± 1808*
β-Cryptoxanthine, $\mu g/d$	895 ± 169	184 ± 34*
Lycopene, $\mu g/d$	8885 ± 2076	7319 ± 1323*
Lutein, $\mu g/d$	12114 ± 2125	5818 ± 814*

[1] Values are means ± SD, $n = 53$; values were computed on the basis of actual intake as recorded in the food records kept by each participant. *Different from LBD, $P < 0.001$.
[2] The 2 diets were designed to have the same macronutrient content (% energy from fat, 30%; protein, 18%; carbohydrate, 52%), to meet the Recommended Dietary Allowances for vitamins and minerals, and to be balanced in nutrients from the various food groups. Nutritional analyses were performed using the NDS nutrient analysis system.

TABLE 4 Effect of consuming LBD or HBD diets on plasma carotenoids in women[1]

Carotenoid	Diet group	Preintervention	Postintervention	Mean difference
		pmol/L		
α-Carotene	LBD[2]	205.3 ± 130.1	145.2 ± 87.3	−60.1 ± 63.4***
	HBD	177.6 ± 205.0	277.1 ± 180.5**	99.5 ± 132.6***
β-Carotene	LBD	803.0 ± 634.4	777.4 ± 456.2	−25.6 ± 271.2
	HBD	936.9 ± 1447.8	969.8 ± 928.4	32.9 ± 651.7
Lutein	LBD	255.5 ± 122.1	433.5 ± 183.9	178.0 ± 115.6***
	HBD	217.8 ± 120.5	322.4 ± 149.6**	104.7 ± 106.0***
Lycopene	LBD	765.8 ± 285.3	657.4 ± 199.1	−108.4 ± 248.1***
	HBD	657.4 ± 268.8*	636.6 ± 209.0	−20.8 ± 181.4
β-Cryptoxanthine	LBD	183.9 ± 84.6	244.5 ± 81.6	60.6 ± 62.9***
	HBD	176.6 ± 133.4	163.0 ± 104.6**	−13.6 ± 57.4

[1] Values are means ± SD; $n = 53$. *Preintervention mean differs from LBD, $P < 0.05$; **postintervention mean differs from LBD, $P < 0.05$; ***significant mean difference (post- minus preintervention) for that group, $P < 0.01$.

Discussion

We reported previously a modest but significant reduction in markers of lipid peroxidation and DNA oxidation in response to consumption of diets with a high vs. low VF content when these diets were consumed for periods ranging from 2 to 8 wk (7,8). The modest nature of those effects is not consistent with expectations of much higher antioxidant activity based on test tube assays of plant foods for total antioxidant capacity (17–19). One possible explanation for these modest effects is that the type of plant foods selected to formulate those high VF diets was not optimal for antioxidant effects to be manifest in vivo. The botanical families emphasized in the LBD diet were selected on the basis of reports that their dietary bioactive components had high antioxidant activity in test assays (17–19). Nonetheless, only the HBD diet induced a significant reduction in DNA oxidation. Both the LBD and the HBD diets were associated with a significant reduction in lipid peroxidation, although concentrations of 8-iso-PGF2α were reduced to a greater extent by the HBD diet than by the LBD diet. Detailed consideration of these findings is presented below.

There was a significant decrease in the urinary concentration of 8-iso-PGF2α in response to either the LBD or HBD diet (Table 5). Although the effect was greater in women that consumed the HBD diet, that difference was not significant (LBD vs. HBD, $P = 0.141$). This finding was contrary to our initial prediction that the reduction in oxidation would be greater in the LBD group because that diet was formulated primarily from botanical families considered to be particularly rich sources of foods with high potential for antioxidants effects. The fact that a reduction in oxidative damage to DNA (Table 6) was observed only in the HBD diet group provides additional evidence that the LBD and HBD diets differed in their in vivo activity in reducing cellular oxidation.

Our laboratory recently published evidence supporting the hypothesis that the response to dietary antioxidants is not uniform, but rather is conditional, based on a participant's oxidation status at the time the participant enters a study (7,8). Hence, null effects of a potential antioxidant intervention could be due to the oxidation status of the study population rather than the lack of antioxidant activity of the intervention. To

TABLE 5 Effect of LBD or HBD diets on urinary 8-iso-PGF2α in women[1]

Baseline quartile 8-iso-PGF2α, $\mu mol/mol$ creatinine	Dietary intervention	*n*	Preintervention 8-iso-PGF2α, $\mu mol/mol$ creatinine	Postintervention 8-iso-PGF2α, $\mu mol/mol$ creatinine	Mean difference
Overall	LBD	53	0.30 ± 0.01	0.25 ± 0.02	−0.05 ± 0.02*
	HBD	53	0.34 ± 0.02	0.21 ± 0.02	−0.13 ± 0.02*
1st (0.12, 0.19)[2]	LBD	13	0.15 ± 0.01	0.13 ± 0.01	−0.02 ± 0.01
	HBD	13	0.16 ± 0.01	0.13 ± 0.01	−0.03 ± 0.01
2nd (0.20, 0.29)	LBD	13	0.25 ± 0.01	0.24 ± 0.02	−0.01 ± 0.02
	HBD	13	0.25 ± 0.01	0.20 ± 0.01	−0.05 ± 0.01*
3rd (0.30, 0.38)	LBD	13	0.33 ± 0.01	0.23 ± 0.02	−0.10 ± 0.02*
	HBD	13	0.34 ± 0.01	0.23 ± 0.01	−0.11 ± 0.02*
4th (0.39, 0.85)	LBD	14	0.48 ± 0.02	0.33 ± 0.03	−0.15 ± 0.04*
	HBD	14	0.60 ± 0.05	0.35 ± 0.03	−0.25 ± 0.04*

[1] Values are means ± SEM. *Significant mean difference (post- minus preintervention) for that group, $P < 0.01$.
[2] Values for baseline quartiles of 8-iso-PGF2α are in parentheses.

TABLE 6 Effect of LBD or HBD diets on lymphocyte 8-oxo-dG in women[1]

Baseline quartile 8-oxo-dG/10^6dG	Dietary intervention	*n*	Preintervention 8-oxo-dG/10^6dG	Postintervention 8-oxo-dG/10^6dG	Mean difference
Overall	LBD	53	6.88 ± 0.40	6.85 ± 0.44	−0.03 ± 0.34
	HBD	53	6.90 ± 0.38	6.09 ± 0.44	−0.81 ± 0.33*
1st (3.15, 4.39)[2]	LBD	13	3.87 ± 0.12	4.35 ± 0.26	0.48 ± 0.24
	HBD	13	3.91 ± 0.11	5.15 ± 0.51	1.24 ± 0.48
2nd (4.40, 5.44)	LBD	13	4.79 ± 0.09	5.60 ± 0.62	0.78 ± 0.63
	HBD	13	4.90 ± 0.09	5.37 ± 0.42	0.48 ± 0.45
3rd (5.45, 7.40)	LBD	13	6.29 ± 0.20	6.17 ± 0.84	0.13 ± 0.81
	HBD	13	6.57 ± 0.16	5.21 ± 0.25	−1.40 ± 0.24**
4th (7.41, 20.16)	LBD	14	12.55 ± 1,22	11.67 ± 1.43	−0.88 ± 0.25
	HBD	14	12.21 ± 1.23	9.71 ± 1.39	−2.67 ± 0.84**

[1] Values are means ± SEM. Asterisks indicate a significant mean difference (pre- minus postintervention) for that group: *$P < 0.05$; **$P < 0.01$.

[2] Values for baseline quartiles of 8-oxo-dG /10^6dG are in parentheses.

determine whether this might account for our observations, the 8-iso-PGF2α and 8-oxo-dG data were subjected to subgroup analysis based on preintervention concentrations of each analyte. Those analyses indicate that the reduction of lipid peroxidation was observed primarily in the participants in the highest 2 quartiles of baseline 8-iso-PGF2α, an observation consistent with our previous reports. Interestingly, both the quartile in which the HBD diet was observed to reduce lipid peroxidation significantly, and the magnitude of the reduction observed in each quartile relative to the effects of the LBD diet, are consistent with the HBD diet having greater activity in reducing lipid peroxidation than the LBD diet. The results of a quartile-based analysis of the effects of the 2 diets on concentrations of 8-oxo-dG in genomic DNA isolated from peripheral lymphocytes (Table 6) also support the hypothesis that only individuals with high baseline levels (3rd and 4th quartiles) of 8-oxo-dG experienced a reduction in the concentration of this analyte, and this effect was significant only in individuals consuming the HBD diet. Hence, these results are also consistent with the existence of a differential response in oxidative damage to the LBD vs. HBD diet.

It is likely that the LBD and HBD diets provided different amounts and types of phytochemicals, an observation supported by the carotenoid intake profiles for each diet (Table 3). Although differences in the chemical composition of these diets undoubtedly contributed to the effects on the peroxidation of lipids and oxidation of DNA, speculation about what the key chemical factors might be is considered unwarranted because so many other issues relating to absorption and metabolism of ingested phytochemicals could also contribute to the observed effects. On the other hand, what can be learned from these data is that the botanical diversity of the diet is likely to play a role in determining the bioactivity of dietary chemicals and that smaller amounts of many phytochemicals may have greater potential to exert beneficial effects than larger amounts of fewer phytochemicals. If this observation is correct, it gives added meaning to one of the most widely pronounced dietary recommendations, i.e., that variety and moderation are the key to a healthy diet.

A growing number of studies of the effects of plant foods, their DBC, and of known antioxidants on oxidative cellular damage in vivo are being reported, and the results are mixed. The significant number of null findings has spawned the antioxidant conundrum as outlined by Seifried et al. (20). The contribution made by the botanical diversity of the diets studied in explaining the mixed results was not investigated previously. The findings presented here represent an initial inquiry concerning the potential importance of plant food selection in a high VF background in determining biological activity; they demonstrate an approach that makes it feasible to investigate the effects of botanical diversity on disease risk biomarkers in a systematic manner.

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