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Effect of Cruciferous Vegetable Intake on Oxidative Stress Biomarkers: Differences by Breast Cancer Status

Michael D. Wirth[a,b,c], **E. Angela Murphy**[d], **Thomas G. Hurley**[c], and **James R. Hébert**[a,b,c]

[a]Department of Epidemiology and Biostatistics, Arnold School of Public Health, University of South Carolina, Columbia, South Carolina, USA

[b]Cancer Prevention and Control Program, University of South Carolina, Columbia, South Carolina, USA

[c]Connecting Health Innovation, LLC, Columbia, South Carolina, USA

[d]Department of Pathology, Microbiology, and Immunology, School of Medicine, University of South Carolina, Columbia, South Carolina, USA

Abstract

This *post hoc* analysis examined cruciferous vegetable intake on urinary oxidative metabolites in postmenopausal women. Intervention participants (n = 69) received cruciferous vegetables (≥14 cups/week) during a 3-week period. First morning urine measured 8-isoprostane and 8-hydroxy-2′-deoxyguanosine. Dietary intake was estimated using 24-h recalls. When stratified by history of breast cancer, those with breast cancer had significantly lower post-intervention urinary 8-hydroxy-2′-deoxyguanosine values in the intervention arm versus. the control arm (1.1 ng/mL vs. 3.2 ng/mL, p=.01) after adjustment for baseline 8-hydroxy-2′-deoxyguanosine. This was not observed in those without breast cancer. Further work is needed to understand the role of breast cancer in these relationships.

Keywords

Cruciferous vegetables; urine lipoprotein oxidation; breast cancer; diet

CONTACT: Michael D. Wirth, MSPH, PhD, wirthm@mailbox.sc.edu, Cancer Prevention and Control Program, University of South Carolina, 915 Greene Street, Room 233, Columbia, SC 29208, USA.

Declaration of interest

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Introduction

Lifestyle factors, including dietary choices, are known to affect rates of oxidative stress. In particular, evidence shows that dietary antioxidants can significantly lower the incidence of cardiovascular disease (CVD) and, to a smaller extent, cancer (1–4).

Lipid peroxidation-associated deoxyribonucleic acid (DNA) damage has been shown to be elevated in several age-related diseases, including atherosclerosis, diabetes, Alzheimer's disease, and cancer (5–9). In 1990, a frequently used marker of lipid peroxidation belonging to a series of ring-containing prostaglandin-like F-type compounds, 8-isoprostane (8-iso-PGFα) was discovered to be produced in humans (10). Although not well understood, the formation of lipid peroxidation products is catalyzed by free radicals that also inflict DNA damage, suggesting their involvement early in the process of carcinogenesis (11, 12).

A complementary product to assess DNA damage is the tissue and urinary marker of 8-hydroxy- 2′-deoxyguanosine (8oxodG), the nucleoside of 8- hydroxyguanine. Levels of this marker in tissues reflect a dynamic equilibrium between rates of oxidative damage and rates of repair. Excretion of urinary 8oxodG is the preferred indicator of "total body" damage (13–15), even though values for this damage are not consistent across studies, which range from<0.1 to 100 damaged bases per 10^5 unmodified guanines (13, 16– 19), with a conservative estimate for all DNA damage to be approximately $1/10^5$ unmodified DNA bases (20).

Although both oxidative stress markers have been favorably validated using markers of mutagenicity, 8oxodG has been subjected to extensive methodological comparisons (21–23). Case-control studies have shown increased levels of 8oxodG in women with breast cancer (BC) and one study has demonstrated increased levels of 8-iso-PGFα (24–27).

The Nutrition and Breast Health Study of women with a family history of breast cancer (26) demonstrated a significant decline in urinary 8-iso-PGFα with a 12-month low-fat diet in participants who lowered their body mass index (BMI). At baseline, in a large randomized controlled trial (RCT), there were lower levels of 8oxodG with increasing fruit and vegetable servings/day among those consuming 3–4 servings/day (median 8oxodG: 16.7 vs. 20.5, $p = .05$) or ≥5 servings per day (15.6 vs. 20.5, $p = .02$) compared with those consuming ≤2 servings per day. However, this RCT indicated that just supplementing with vitamins C and E will not lower 8oxodG levels (28). Lastly, two large cross-sectional studies of adolescents (29) and healthy older adults (30) demonstrated an inverse correlation of urinary 8-iso-PGFα with higher daily fruit and vegetable intake.

Few studies have examined the effectiveness of cruciferous vegetables (*Brassicaceae* family) to lower the levels of 8-iso-PGFα or 8oxodG (31–33). In a small cross-sectional study ($n = 71$), Giovannelli et al. found lower adjusted levels of 8oxodG among participants in the highest tertile of cruciferous vegetable consumption compared with participants in the lowest tertile (3.86% vs. 5.47%, respectively, $p = .09$) in healthy Mediterranean adults (32). A small RCT of non-smoking men ($n = 10$) looked at the effect of cruciferous vegetables on 8oxodG (33) and found significantly lower ($p = .04$) levels of 8oxodG after eating 300 g of Brussels sprouts daily over a 3- week period. A small intervention study in healthy Japanese

adults (n = 12) observed lower levels of 8- iso-PGFα after a week of consuming 100-g broccoli sprouts (31).

In light of this noticeable gap in knowledge of the effect of cruciferous vegetable intake on 8oxodG and 8-iso-PGFα, a RCT was conducted to determine the influence of increasing the overall number of cruciferous vegetable servings on these markers of oxidative stress in a population of postmenopausal women. Access to data and samples for this investigation was made available through a study that was originally designed to examine the effect of an intensive cruciferous vegetable-rich dietary intervention on aryl hydrocarbon receptor activation, its protein products, and estrogen metabolites in women undergoing a biopsy following a suspicious mammogram. Study participants were recruited from a breast clinic serving a large African-American population, and included both women who had completed breast cancer treatment and those who were disease-free. For this *post hoc* analysis, we tested whether women in the dietary intervention arm would experience a mean decrease in the two oxidative stress metabolites compared with women in the non-intervention arm. To be consistent with the original design of this intervention study, a secondary analysis stratified the main analyses by breast cancer status.

Material and methods

Subjects

In accordance with the original purpose of this study, participants were recruited between April 2002 and September 2003 in-person at one of two clinical breast centers (Palmetto Health, Columbia, SC, USA) with a local press release. Eligibility requirements included the following: (1) Postmenopausal (>1 year since last menstrual period); (2) not using hormone replacement therapy, or on fixed dose; (3) willing to be randomized; (4) willing to provide post-intervention data, including a needle biopsy from the contra-lateral breast (if history of breast cancer); (5) no health condition that would limit participation; (6) no malignancy in the past 5 years (except non-malignant melanoma of the skin and breast cancer) and having completed all treatments for breast cancer at least 1 year before enrolling in the study; (7) not currently taking thyroid medication, antibiotics, diuretics, or steroids; (8) not following a rigorous diet and no weight change exceeding 5 pounds within the past year; (9) alcohol consumption of <2 drinks/day; and (10) not diabetic. Since it was impossible to ban medications in the study participants in this age group, medication regimens were documented and required to be followed consistently during the intervention. Women were excluded if they did not attend two clinic visits or were unable to provide two urine samples. A total of 69 women were enrolled into the study. The current, *post hoc* analysis is based on these 69 women.

Randomization and informed consent

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by the Department of Defense Institutional Review Board, University of South Carolina Institutional Review Board and Palmetto Health Institutional Review Board. Before recruitment, participants provided informed written consent. Participants were randomly

assigned by the status of breast cancer to the intervention or control group to ensure similarity of participants by study arm and to minimize the influence of background factors.

Study design

The intervention was based on participation in cooking classes. Women randomized to the dietary arm were invited to nine cooking classes conducted by a registered dietitian at one of the two local heart centers (South Carolina Heart Center or Providence Hospital). In addition, the intervention women received an adequate amount of fresh, locally grown cruciferous vegetables to incorporate into their weekly diet outside of cooking class attendance. A 10-point daily system was used to encourage consumption of less commonly consumed foods (e.g., 10 points for a single Brussels sprout compared with two cups of raw cabbage or broccoli). Women in the non-intervention arm were asked to follow usual eating patterns, and they were invited to attend cooking classes after completing their second clinical visit (Figure 1).

Classes were participatory, consisting of individualized dietary counseling and “hands-on” cooking classes with menus based on the United States Department of Agriculture (USDA) Food Pyramid. Participants were encouraged to change food intake patterns, advised on how to overcome barriers (i.e., to improve self-efficacy) by substituting food choices, and informed on how to modify serving sizes. Most classes featured vegetables in the form of slaws, salads, and dips to encourage eating raw vegetables. Participants were provided a cruciferous vegetable cookbook and written instructions on how to read labels, incorporate “alternative” foods (e.g., soy products), and use herbs and spices. No advice was given to reduce total food intake or to count calories.

Data collection

Body mass, height, body circumferences (waist, abdomen, and hip), and percentage of body fat were measured by trained study personnel at clinical visits. Waist circumference was taken at the lower rib margin after removing heavy outerwear. Similarly, abdominal circumference was taken midway between the lower rib and iliac crest, and hip circumference was measured at the crotch level. Percentage of body fat was measured with a bioelectrical impedance analyzer (Quantum II Model, RJL Systems, Clinton Twp., MI, USA) with total body resistance (R) and reactance (Xc) recorded to the nearest Ohm. Electrode contact areas were wiped with alcohol before electrodes were placed between the distal prominences of the radius and ulna of the right wrist and between the medial and lateral malleoli of the right ankle. The resistance index (height^2 divided by total body resistance) was calculated.

At baseline, a self-report questionnaire battery was used to collect data on demographics, personal health history, and lifestyle factors, including alcohol intake, reproductive history, breast cancer screening, past week physical activity, dietary knowledge, and personal food preferences.

Dietary data collection and analysis

Trained registered dietitians conducted unannounced telephone-administered 24-h recall interviews (24HR) (two on weekdays and two on weekend days) on randomly selected non-consecutive days during a 3-week sampling window. The Nutrition Data System Version 34 interactive software (Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN, USA), which employs the multi-pass interview protocol, was used to collect all dietary data. Prior to the interviews, participants were provided a two-dimensional food portion poster and briefly trained to use this visual in the estimation of food portion sizes (34). Fruit and vegetable and cruciferous vegetable intakes were calculated based on gram weights of foods consumed using the USDA Pyramid equivalences from the Food and Nutrient Database for Dietary Studies. Data from the 24HRfromeach 3-week measurement period (i.e., pre and during-intervention) were averaged to provide an estimate of current dietary intake.

Urine collection and oxidative biomarker analyses

First-morning urine samples were collected from each woman in the study at the first and second clinical visits (cups contained 125-mg ascorbic acid to 100 mL of urine to prevent oxidation). The volume of urine was recorded as 100 mL of the first catch. Participants were asked to refrain from using dietary supplements or engaging in vigorous physical activity 12 h before urine collection. Urine was kept on ice (4°C) until aliquoted for long-term storage at −80°C.

For all analyses, baseline and follow-up urine samples for each participant were analyzed by a single technician in duplicate and on the same plate, and averaged to control for intra-assay variability. The technician was blinded as to the order of urine collection and identity of participants.

Creatinine was measured using a colorimetric assay kit (catalog No. KGE005; R & D Systems Minneapolis, MN) and according to manufacturer's instructions. All samples were run in duplicate and the coefficient of variation was found to be<2%. In order to standardize urinary metabolite levels, 8oxodG concentration was expressed as ng/mg of creatinine and 8-iso-PGFα concentration was expressed as pg/mg of creatinine.

Urinary metabolites of oxidative stress were measured using commercially available ELISA kits and according to manufacturer's instructions. For 8oxodG, all samples were run in duplicate using a kit (catalog No. KOG-200SE) from Genox Corp (Baltimore, MD, USA) (35). The coefficient of variation between duplicates was found to be <5%. For the 8-iso-PGFα assay, we used a kit (catalog #516351 from Cayman Chemicals, Ann Arbor, MI) that has been validated for measurement of this metabolite in urine (36). All samples were run in duplicate and the coefficient of variation was found to be <2%.

Statistical analysis

Differences at baseline in categorical variables (i.e., age, race, marital and employment status, education, participation in sports, and no history of breast cancer) were assessed using the Chi-square or Fisher's Exact test for independence of proportions. Mean values for

age, BMI, percentage of body fat, total cruciferous vegetable and fruit and vegetable intake, 8oxodG, and 8-iso-PGFα were compared between intervention and control arms at baseline using t-tests. In addition, using paired t-tests, differences in baseline and post-intervention 8oxodG and 8-iso-PGFα by treatment group were examined. All of the following were considered as potential confounders during analysis: physical activity status, cruciferous vegetable intake, fruit intake, vegetable intake, and fruit and vegetable intake. Through a series of multivariable analyses (i.e., treatment group, baseline outcome measure, and plus confounder) each potential confounder was individually substituted into this model. Variables with a p value of ≤.20 were added to a "full" model. A backward confounder selection procedure was then used to develop final models that included all variables that were statistically significant ($\alpha \leq 0.05$) or, when removed from the model, changed the beta coefficient of the primary independent variable (i.e., treatment group) by at least 10%.

To test for normality, models were run using the linear regression options to output normality statistics, including the model's residuals. Using the Shapiro–Wilk normality test, both measures of oxidative stress were found to have non-normal distributions and therefore were log-transformed to obtain normally distributed model residuals that adhered to the normality assumption of linear regression. Mean values and confidence intervals were back-transformed for presentation. General linear models were used to compute least square (LS) mean values of each dependent variable (i.e., 8oxodG and 8-iso-PGFα) to test the difference in post-intervention 8oxodG and 8-iso-PGFα values between control and intervention arms after adjustment for baseline 8oxodG and 8-iso-PGFα values. Differences between the control and intervention arms were evaluated using the least significant difference statistics with the control arm as the referent. Secondary analyses stratified by breast cancer status. Statistical analyses were conducted using SAS® statistical software version 9.3 (Cary, NC, USA) and all statistical tests were 2-tailed with a critical $\alpha = 0.05$.

Results

Characteristics of the study population

At baseline, women randomized to the treatment arm did not significantly differ from those in the control arm by relevant anthropometric, demographic, or fruit and/or vegetable categories. In addition, no differences in 8oxodG and 8-iso-PGFα were observed at baseline (Table 1). Overall, women were 61 ± 8.6 years of age (range 46–83); were, on average, overweight (BMI (kg/m^2): 28.9±5.64; had moderate percentage of body fat: 39.6%±5.6%; and consumed an average of 5.6±2.4 fruit and vegetable servings per day. A high percentage of participants (78.3%) had at least completed high school. A majority of women were European-American (71%). An equal number of women (29%) with prior breast cancer (i.e., completed all treatment >1 year before entering study) were in the treatment and control arms.

Differences between 8oxodG and 8-iso-PGFα

Consumption of cruciferous vegetables is significantly increased by 2.6 ± 1.5 srv/d in the intervention group but decreased slightly by −0.15 ± 0.6 srv/d ($p < .01$) in controls. Paired t-tests indicated an increase of 117 pg/mL and a decrease of 193 pg/mL in control and

intervention groups, respectively, for 8-iso-PGFα at post-intervention compared with baseline. However, neither of these differences was statistically significant. As for 8oxodG, at post-intervention compared with the baseline, there was a 0.52 increase in controls but a 0.74 decrease in the intervention group; neither change was statistically significant (data not tabulated). Table 2 displays post-intervention 8-iso- PGFα and 8oxodG after adjustment for selected confounders and the baseline levels of those outcomes. There was no statistically significant difference in 8- iso-PGFα in the treatment group at post-intervention after adjusting for baseline levels of 8-iso-PGFα. There was a suggestion of a difference between intervention and control arms in post-intervention 8oxodG after adjustment for baseline levels (2.0 ng/mL vs. 2.8 ng/mL, respectively, $p = .11$), although not different statistically (Table 2).

To be consistent with the design and original intention of the study, we performed a secondary analysis by stratifying the main results by breast cancer status. The breast cancer status-by-treatment group interaction was not statistically significant for 8-iso-PGFα ($p = .63$), but it was for 8oxodG ($p = .03$). When comparing the intervention with the control arm at follow-up after adjustment for baseline values, there were no statistically significant differences in 8oxodG or 8-iso-PGFα in women without a history of breast cancer, or in 8-iso-PGFα in women with a history of breast cancer. However, women with a history of breast cancer in the intervention arm showed statistically significantly lower 8oxodG levels than women with a history of breast cancer in the control arm (1.1 ng/mL vs. 3.2 ng/mL, respectively, $p = 0.01$, Table 3). In an attempt to determine whether there was a differential increase in cruciferous vegetable intake, we found that women with a history of breast cancer (0.1 to 3.2 servings/day, $p < .01$) and women without history of breast cancer (0.5 to 3.1 servings/day, $p < .01$) undergoing the intervention had similar increases in cruciferous vegetable consumption.

Discussion

In this study, we observed no significant differences in either marker of oxidative stress as a result of a 3- week cruciferous vegetable dietary intervention among all women. However, we did find significantly lower levels of 8oxodG (but not for 8-iso-PGFα) in women with a history of breast cancer. It is not clear exactly why we observed this finding, given that all women had a significant increase in cruciferous vegetable consumption. It is possible that another underlying mechanism is driving the differences among women with breast cancer. Those in the intervention arm previously diagnosed with breast cancer (and who completed treatment>1 year before entering the study) had significantly lower ($p = .03$) mean values of 8oxodG than those without a prior diagnosis of breast cancer after the 3-week dietary intervention, which was not observed in women without a history of breast cancer. Regardless of breast cancer history, cruciferous vegetable consumption increased similarly in all women undergoing the intervention; this limits the possibility of differential increases in cruciferous vegetable intake between women with a history of breast cancer and women without a history of breast cancer undergoing the intervention as an explanation for the observed findings. On average, cruciferous vegetable consumption increased by 2.6 ± 1.5 srv/d among women in the intervention arm and decreased among those in the control arm by −0.15 ± 0.6 srv/d ($p < .01$).

Recent meta-analyses based on case-control studies have concluded that cruciferous vegetable consumption is associated with a lower risk of several cancer types (37–40). It also related to lower levels of markers of inflammation and oxidative stress (41, 42). However, few studies have examined the specific effect of cruciferous vegetables on oxidative damage in cancer patients. Of the larger intervention studies that have used fruit and vegetable intake to decrease levels of 8oxodG and 8-iso-PGFα in cancer patients, the Women's Healthy Eating and Living (WHEL) RCT of women previously treated for breast cancer (43) found statistically significant decline in the levels of 8oxodG and 8-iso-PGFα after a 12-month low fat, high-fruit and vegetable dietary intervention. However, this analysis was based on a sub-sample of participants; no women without breast cancer were included as a control group, and the design used measured serum cholesterol rather than cruciferous vegetables to assess dietary adherence. In contrast to our results, two studies have shown decrease in 8-iso- PGF2α, including a small (n = 12) 1-week feeding study using broccoli sprouts (100 g/d) (31), as well as a more recent randomized cross-over intervention (n = 20) that asked participants to eat at least two cups (>160 g) of these vegetables daily (44). Lowering levels of oxidative damage as a result of cruciferous vegetable intake have been confirmed in small intervention trials, regardless of participants' smoking status (45–47).

There is a wide range of effects observed as a result of using different markers of oxidative damage in epidemiological studies. Djuric and colleagues found significant differences by gender using a marker of oxidative damage (5-hydroxymethyl-2′-deoxyuridine [5-OHmdU] in DNA from nucleated blood cells) in a small isoflavone supplementation study (n = 12) (25). It took longer to observe a decrease inoxidative damage in men (>3 weeks) than in women (~1 week) possibly due to including premenopausal women who may exhibit lower levels of reactive oxygen species due to significant variability by phase of the menstrual cycle (48, 49) and because the small level of supplementation (50 mg) was not enough to impact the typically higher weight of men. However, no difference was observed using isoprostane biomarkers (25).

Isoprostane concentrations vary widely in healthy adults due to dietary intake differences and endogenous antioxidant defenses. Some healthy humans appear to have higher rates of lipid peroxidation, even when consuming comparable diets (50–53). These individuals could be at greater risk of diseases involving lipid peroxidation, such as atherosclerosis and cancer. The evidence suggests that variations in oxidative damage, as measured by isoprostanes, may be modified by random individual variation in lipid peroxidation as well as the type of dietary intervention (i.e., use of supplements rather than whole foods).

This dietary intervention successfully increased total vegetable servings as well as cruciferous vegetables regardless of race or breast cancer status. Our results are in agreement with the WHEL study on women with a history of breast cancer (43) as well as another large clinical trial (28), both of which demonstrated decrease in 8oxodG levels with increasing intake of fruits and vegetables. It is possible that the decreases observed in previous studies may be partly due to larger effects in men than in women rather than generally higher intake of vegetables (44, 54).

Many factors could explain why a reduction in oxidative stress is not observed due to dietary interventions. These include inherent biological variations in absorbing essential phytochemicals and rapid degradation, inactivity, or clearance of these chemicals, as well as differences in study methodology. Study design may further contribute by not accounting for individual genotypic differences in processing cruciferous vegetable metabolites (55–57) and requiring a larger quantity of cruciferous vegetables to observe metabolic changes.

Our study had several limitations that could be addressed in the future trials. It was limited by a small sample size ($n = 69$) compared to much larger sample sizes (i.e., $n > 200$) in more recent studies examining circulating levels of markers of inflammation and oxidative stress in women (41, 58). We know that self-reports of dietary intake are subject to biases of various types, including social desirability bias (59). So, it is conceivable that measurement error could explain some of these null results. For analysis of 8-iso-PGFα and 8oxodG in urine samples, although we used immunoassays, mass spectrometry analysis generally provides better results (60, 61). The exact amount of cruciferous vegetables consumed and its bioavailable functional components could not be determined during the class sessions or from foods consumed outside class sessions. The lack of information on the nutritional composition of cruciferous vegetables introduced (especially for bioactive constituents such as isothiocyanates) or a circulating marker of intake make it difficult to evaluate the strict relationship between the cruciferous treatment and the oxidative stress markers analyzed. Providing specific meal plans in addition to providing vegetables has been previously suggested to improve consumption of cruciferous vegetables and enhances the probability of differences during the study in the dietary behavior between participants (62–64). The future analyses may consider excluding women exposed to tobacco smoke (either active or passive) because of its well-known role in promoting oxidative damage (65–67). In addition, it may be useful to examine the role of caffeine, alcohol, and supplements in explaining the racial differences in oxidative damage over time.

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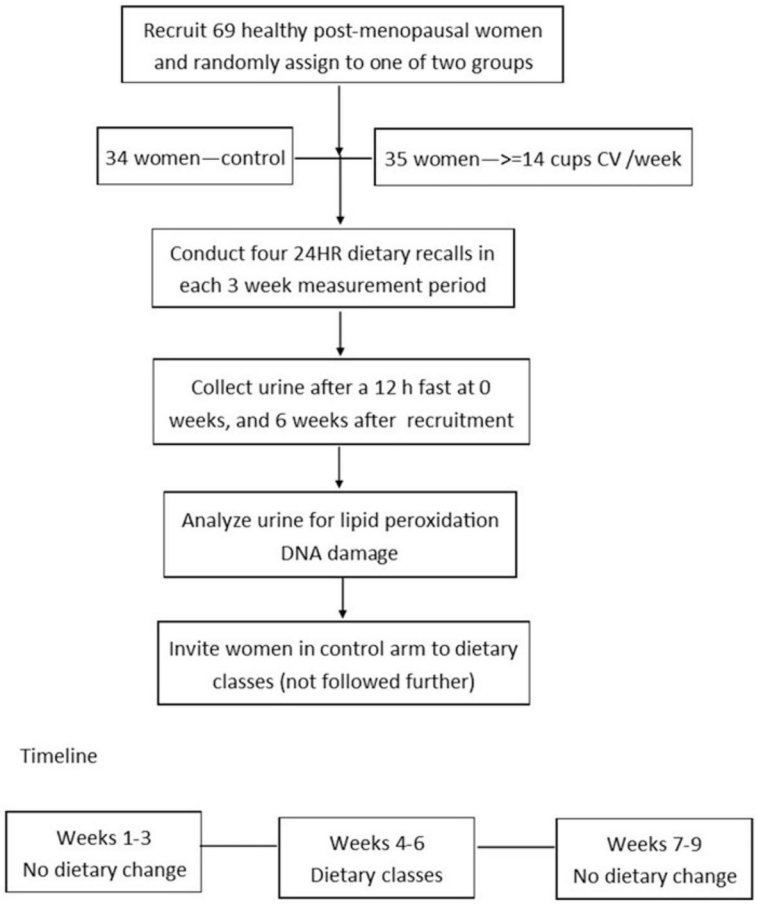

Figure 1.
Graphical representation of the study timeline and design.

Table 1

Demographic, anthropometric, and fruit and vegetable consumption by treatment group at baseline.

	Group		
	Intervention (*n* = 35)	**Control (*n* = 34)**	***p* value**
Race[a]			
European-American	24 (69%)	25 (74%)	.65
African-American	11 (31%)	9 (26%)	
Married/living with partner[a]			
Yes	25 (71%)	18 (56%)	.20
No	10 (29%)	14 (44%)	
Education[a]			
≤High school	7 (20%)	8 (24%)	.72
>High school	28 (80%)	26 (76%)	
Employment status[a]			
Full time	13 (37%)	14 (45%)	.78
Part-time	6 (17%)	4 (13%)	
Unemployed	16 (46%)	13 (42%)	
Participation in sports[a]			
Yes	31 (89%)	29 (91%)	.99
No	4 (11%)	3 (9%)	
Breast cancer status[a]			
History of breast cancer	10 (29%)	10 (29%)	.94
No history of breast cancer	25 (71%)	24 (71%)	
Age[b]	60.3 ± 8.7	61.9 ± 8.7	.44
Body Mass Index[b]	29.3 ± 5.0	28.8 ± 6.3	.69
Percentage of body fat[b]	40.1 ± 4.9	38.9 ± 6.2	.41
Total cruciferous vegetable intake (srv/d)[b]	0.42 ± 0.5	0.64 ± 0.7	.24
Total vegetables intake (srv/d)[b]	2.79 ± 1.0	3.32 ± 1.6	.10
Total fruit intake (srv/d)[b]	1.67 ± 1.1	1.61 ± 1.3	.83
Total vegetable and fruit intake (srv/d)[b]	4.47 ± 1.6	4.94 ± 2.4	.33
8-iso-PGFα (pg/mL)[c]	1394 ± 1491	1006 ± 799	.66
8oxodG (ng/mL)[c]	4.78 ± 4.56	4.96 ± 4.84	.86

[a]Frequency (%), *p* value based on Chi-square or Fisher's Exact test.

[b]Mean ± standard deviation, *p* value based on t-tests.

[c]Mean ± standard deviation, *p* value based on the Wilcoxon rank sums test.

Table 2

Mean post-intervention adjusted 8-iso-PGFα and 8oxodG by treatment groups.

Variable/treatment	LS Mean	LS Mean CI	*p* value
8-iso-PGFα (pg/mL)[a]			
Intervention (*n* = 35)	696	524–923	.39
Control (*n* = 34)	826	626–1091	Referent
8oxodG (ng/mL)[a]			
Intervention (*n* = 35)	2.0	1.4–2.8	.11
Control (*n* = 34)	2.8	2.0–4.0	Referent

[a]From analysis, using log values, mean values were back-transformed for presentation.

Adjustments: 8-iso-PGFα = baseline 8-iso-PGFα, age, and body mass index.

Table 3

Mean post-intervention-adjusted 8-iso-PGFα and 8oxodG by treatment groups stratified by breast cancer status.

Variable/treatment	LS Mean	LS mean CI	*p* value
BC history			
8-iso-PGFα (pg/mL)[a]			
Intervention (*n* = 10)	729	445–1197	.37
Control (*n* = 25)	997	608–1637	Referent
8oxodG (ng/mL)[a]			
Intervention (*n* = 10)	1.1	1.0–1.9	.01
Control (*n* = 25)	3.2	1.8–5.7	Referent
No BC history			
8-iso-PGFα (pg/mL)[a]			
Intervention (*n* = 10)	670	478–966	.67
Control (*n* = 24)	755	536–1064	Referent
8oxodG (ng/mL)[a]			
Intervention (*n* = 10)	2.5	1.7–3.7	.81
Control (*n* = 24)	2.6	1.8–4.0	Referent

[a]From analysis using log values, mean values were back-transformed for presentation.

Adjustments: 8-iso-PGFα = baseline 8-iso-PGFα, age, and body mass index; 8oxodG = baseline 8oxodG and education status.