Exploring the Feasibility and Effects of a High-Fruit and -Vegetable Diet in Healthy Women[1]

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Abstract

Based on reports that fruits and vegetables may protect against breast cancer, this randomized intervention study tested the feasibility of increasing fruit and vegetable intake among healthy women to 9 daily servings through individual dietary counseling and group activities. Adherence to the dietary recommendations was monitored by 24-h food recalls, log sheets, and plasma carotenoid assessments. To explore possible cancer protective mechanisms of fruits and vegetables, we investigated the treatment effect on plasma phenol levels and on thiobarbituric acid-reactive substances measured as malondialdehyde equivalents, a possible marker of oxidative damage. At baseline, women in the intervention ($n = 13$) and control ($n = 16$) group reported an average daily consumption of 3.3 and 3.2 fruit and vegetable servings, respectively. After 3 and 6 months of intervention, intake in the intervention group had increased to 8.3 and 7.4 servings, whereas the control group reported an average of 4.2 and 4.1 daily servings. An increase of plasma carotenoid levels from 1249 μg/liter at baseline to 1854 and 1827 μg/liter after 3 and 6 months confirmed compliance with the dietary recommendations in the intervention group. Plasma carotenoid levels among controls changed slightly from 1165 to 1231 and 1291 μg/liter Whereas total phenol levels did not respond according to our hypothesis, malondialdehyde levels decreased slightly in the intervention group. These results suggest that motivated women can substantially increase their fruit and vegetable intake, which leads to a notable increase in plasma carotenoid levels.

Introduction

Ethnic differences in breast cancer incidence and the fact that breast cancer risk in Asian women who migrate to the United States approaches the risk among United States whites after a few generations (1) suggest that environmental factors, in particular diet, may be a major determinant of breast cancer risk. A number of case-control studies (2–7) and reviews of epidemiological studies (8–10) have reported a probable protective effect (11) of fruits and vegetables against breast cancer. Possible mechanisms proposed to explain these findings include the antioxidant effect of carotenoids; cancer protection through other micronutrients, such as flavonoids, isoflavones, folic acid, and vitamins E and C (9); and fecal trapping of estrogens by fiber (12–14), leading to a decrease in endogenous estrogen levels, a risk factor for breast cancer (15).

As a preparation for a breast cancer prevention trial, we conducted a pilot study to test the feasibility of increasing the dietary intake of fruits and vegetables to 9 servings per day among healthy women. As a biomarker of fruit and vegetable intake (16), we measured plasma carotenoids. To explore mechanisms that may be responsible for the cancer protective effect of fruits and vegetables, we investigated the effects of a high-fruit and -vegetable diet on total phenols and on TBA-RSs[3] as measured by MDA equivalents, a possible marker of oxidative damage to lipids (17). Phenols are a common constituent of most plants (18) and are hypothesized to reduce cancer risk (19). Total systemic phenols are thought to reflect the total intake of plant products. Lipid peroxides are produced in membranes from unsaturated fatty acids as a result of exposure to oxidants and free radicals (17) and may be involved in mutagenesis or tumor promotion. Antioxidants, such as carotenoids, preferentially react with a variety of oxidants, thereby protecting the cell against oxidative damage (17, 20, 21). The measurement of serum lipid peroxide levels might provide a useful marker of endogenous oxidation or of susceptibility to oxidation.

Patients and Methods

Study Design and Population. We conducted a randomized dietary modification trial among healthy women who were recruited from an ongoing observational study. The study protocol was approved by the Committee on Human Subjects of the University of Hawaii. Eligible women had to be at least 35 years of age, report less than 5 daily servings of fruits and vegetables as assessed by a food frequency questionnaire and a 3-day food record, not take a high-dose vitamin supplement, be free from chronic conditions that may be affected by a change of diet, and have at least 50% mammographic densities as assessed by a computerized method (22). We received 40 positive responses after mailing 55 letters, scheduled 36 initial visits, and randomized 33 women, 16 into the intervention group and 17 into the control group. Smokers were not specifically excluded, but only 1 woman reported current smoking, 7 women were former smokers, and 21 women had never smoked. Approximately one-third of the subjects were postmenopausal, and the others were pre- and peri-

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[3] The abbreviations used are: TBA, thiobarbituric acid; RS, reactive substance; MDA, malondialdehyde.

menopausal. Shortly after randomization, three women in the intervention group and one woman in the control group dropped out of the study for reasons unrelated to their treatment status. As a result of a stratified randomization approach (Asian *versus* non-Asian), both groups had 11 Asian women. The ethnic distribution for the intervention group was 3 Chinese women, 8 Japanese, 1 Caucasian, and 1 African-American. The control group had 5 Chinese women, 4 Caucasian, 3 Filipino, 3 Japanese, and 1 Vietnamese.

Intervention. During the 6 months intervention period, subjects in the intervention group received an individualized dietary counseling program designed to incorporate at least 9 servings of fruits and vegetables into their daily diet. The emphasis was on achieving the goal of 9 servings with the following recommendations on the type of fruits and vegetables: 3 servings of vitamin C fruits, 1 other fruit, 1 tomato product, 1 dark green vegetable, 1 yellow-orange vegetable, and 2 other vegetables. The definition of a serving was the same as used by the United States Department of Agriculture: 1 cup of raw or 1/2 cup of cooked vegetables or 3/4 cup of juice; for fruits, 1 medium-sized fruit or 1/2 cup of fresh, cooked, or canned fruit or 3/4 cup of juice. The dietitian provided advice on purchasing produce, recipes, and easy-to-prepare dishes. In addition, intervention subjects were invited to attend group meetings with cooking instructions and demonstrations every month. Participants were encouraged to record their daily intake of fruits and vegetables on log sheets. Women in both groups were instructed to consume the same number of calories as before and to avoid weight gain. The control group underwent nutritional counseling based on published guidelines (23) on how to maintain a healthy diet.

Dietary Assessment. An initial diet history using a tested food frequency questionnaire (24, 25) and a 3-day food record established eligibility and baseline fruit and vegetable intake. At 3 months, the dietitian made an unannounced phone call to all subjects and collected a 24-h dietary recall. At the end of the study, all participants except 1 intervention and 2 control subjects completed another 3-day food record. The 3-day food records and 24-h recalls were analyzed using the Food Processor software package, Version 6.0 (ESHA Research).

Blood Collection and Analysis. Participants in both groups donated 10 ml blood samples at baseline and at 3 and 6 months after an overnight fast. The samples were immediately put on ice and centrifuged within 1 h after collection. The plasma was drawn off and aliquoted under yellow light into 2-ml aliquots and stored at −20°C. The samples from all blood draws were analyzed after the entire study was completed, except for the blood lipid analysis. The lab technicians were blinded as to the group status of the study participants.

Levels of 12 carotenoids, retinoids, and tocopherols from plasma were determined by high-pressure liquid chromatography as described elsewhere (16, 26). Analytical accuracy and reliability are verified by participation in the National Institute of Standards and Technology "round robin," from which excellent results were achieved. Plasma triglyceride and total cholesterol levels were determined spectrophotometrically, using enzymatic kits 339-50 and 352-50, respectively, from Sigma Chemical Co. (St. Louis, MO).

A spectrophotometric assay was used to determine total phenol levels in plasma (27). First, 50 μl of plasma were mixed with 50 μl of 95% ethanol followed by vortex mixing for 2 min and centrifugation for 5 min at 1100 $\times$ *g*. Then 25, and 50 μl of clear supernatant were diluted with de-ionized water to 2.0 ml followed by incubation with 0.2 ml of Folin-Ciocalteu phenol reagent (28) for 3 min and incubation with 0.4 ml of saturated aqueous sodium carbonate solution (approximately 35%) and 1.4 ml of de-ionized water for 1 h at room temperature. Thereafter, absorbance was read at 725 nm against a reagent blank (2.0 ml of d.i. water instead of diluted plasma). As reference, a 10 and 20 μM aqueous quercetin solution was measured in the same way. Concentrations of quercetin stock solutions in methanol were determined with absorbance readings at 373 nm using 20,892 as molar absorptivity (29). Final values were expressed as quercetin equivalents in micromolar concentrations.

MDA equivalents were determined according to the method of Jentzsch *et al.* (30) Briefly, 200 μl of plasma was combined with 25 μl butylated hydroxy toluene (3 mM in ethanol), 200 μl of orthophosphoric acid (0.2 M), and 25 μl of TBA reagent (0.11 M in 0.1 M NaOH). The sample was vortexed for 10 s with the addition of each reagent and then incubated for 45 min at 90°C. The samples were cooled on ice and extracted with 500 μl of *n*-butyl alcohol along with the addition of 50 μl of a saturated NaCl solution followed by centrifugation at 12,000 rpm for 1 min. The butanol phase was then placed in a microcuvette, and the absorption was determined at 535 and 572 nm on a Shimadzu model UV160U spectrophotometer. MDA equivalents were determined by comparison to a standard curve as described by Jentzsch *et al.* (30)

Statistical Analysis. We used Student's *t* tests (31) to assess the differences in carotenoid levels and fruit and vegetable consumption between groups and paired *t* tests to analyze change over time. Because of the repeated measurements design, the treatment effect on the outcome variables was examined by ANOVA (31). Spearman correlation coefficients were calculated to explore associations between different variables. We applied multiple linear regression models to estimate the relationship between carotenoid levels as a dependent variable and dietary and demographic characteristics as independent variables. If necessary, we used logarithmic transformations for variables that were not normally distributed.

Results

Dietary Intake. The intervention and the control group did not differ significantly in age (47.6 *versus* 50.2 years; $P = 0.73$) and body mass index (22.8 *versus* 22.2; $P = 0.4$). At baseline, the two groups reported similar intakes of total calories, fat, fiber, and servings of fruits and vegetables (Table 1). The mean intake of fruits and vegetables reported at baseline on the 3-day food records was very similar to the results of the food frequency questionnaire, 3.3 *versus* 3.2 servings per day. After 3 months of study participation, the intervention group achieved a mean increase of 5.1 to 8.3 daily servings of fruits and vegetables, whereas the control group reported a mean increase of 0.9 servings. At the end of the study, the 2 groups still differed significantly in fruit and vegetable intake, although the intake in the intervention group had declined to 7.4 servings. An ANOVA model was highly significant ($F = 19.7$; $P = 0.0001$) for group assignment and time but indicated no difference between the 3- and 6-month dietary intakes in fruits and vegetables. According to the 24-h recalls and the 3-day food records, intervention participants added comparable amounts of fruits and vegetables to their diet: 2.1 additional servings of fruits and 3.0 of vegetables after 3 months and 2.0 additional servings of fruits and 2.2 servings of vegetables after 6 months.

The intervention participants returned an average of 22.4 log sheets. Based on information from the log sheets, the average daily fruit and vegetable consumption was 7.8 servings

Table 1 Mean (SD) intake of fruits, vegetables, and selected nutrients

At 6 months, 3-day food records were missing for one intervention subject and two control subjects.

Food item/nutrient	Time of dietary assessment	Control group (n = 16)	Intervention group (n = 13)	P value of difference between groups
Fruits (servings/day)	Baseline[a]	1.3 (0.7)	1.5 (0.8)	0.43
	3 months[b]	2.0 (1.7)	3.6 (1.4)	0.01
	6 months[a]	2.0 (1.3)	3.5 (1.3)	0.01
Vegetables (servings/day)	Baseline[a]	2.0 (0.6)	1.7 (0.6)	0.15
	3 months[b]	2.2 (1.6)	4.7 (1.5)	0.0001
	6 months[a]	2.1 (0.9)	3.9 (1.7)	0.003
Energy (Kcal)	Baseline[a]	1873 (355)	1882 (323)	0.95
	3 months[b]	1872 (606)	1811 (339)	0.75
	6 months[a]	1800 (302)	1854 (177)	0.58
Fat (g)	Baseline[a]	63 (19)	64 (18)	0.88
	3 months[b]	66 (29)	49 (22)	0.10
	6 months[a]	61 (19)	56 (20)	0.52
Calories from fat (%)	Baseline[a]	30 (6)	31 (6)	0.80
	3 months[b]	31 (8)	23 (9)	0.02
	6 months[a]	30 (8)	27 (9)	0.36
Fiber (g)	Baseline[a]	16 (4)	18 (4)	0.10
	3 months[b]	21 (13)	25 (8)	0.30
	6 months[a]	19 (10)	24 (6)	0.12

[a] Three-day food records were used for dietary assessment.
[b] Twenty-four h recalls were used for dietary assessment.

Table 2 Mean (SD) plasma levels of carotenoids

Micronutrient	Time of dietary assessment	Control group (n = 16)	Intervention group (n = 13)	P value of difference between groups
Total carotenoids (ng/ml)	Baseline	1165 (310)	1249 (488)	0.59
	3 months	1232 (346)	1854 (721)	0.01
	6 months	1291 (475)	1827 (683)	0.02
α-Carotene (ng/ml)	Baseline	50 (20)	61 (45)	0.43
	3 months	84 (49)	132 (72)	0.05
	6 months	63 (49)	87 (57)	0.23
β-Carotene (ng/ml)	Baseline	197 (138)	262 (251)	0.41
	3 months	300 (187)	482 (415)	0.16
	6 months	333 (293)	463 (344)	0.28
β-Cryptoxanthin (ng/ml)	Baseline	210 (107)	174 (84)	0.33
	3 months	169 (54)	296 (119)	0.003
	6 months	194 (104)	296 (112)	0.18
Lutein/zeaxanthin (ng/ml)	Baseline	353 (97)	411 (145)	0.20
	3 months	319 (68)	451 (136)	0.005
	6 months	361 (84)	538 (213)	0.01
Lycopene (ng/ml)	Baseline	174 (77)	154 (51)	0.42
	3 months	179 (57)	242 (60)	0.008
	6 months	170 (87)	184 (70)	0.66

per day with 2.3 servings of vitamin C fruits, 1.5 other fruits, 1.2 dark green vegetables, 0.7 tomato products, 0.7 yellow-orange vegetables, and 1.4 other vegetables. The participants were close to meeting the goal for fruits (3.8 consumed *versus* 4 recommended) but were one serving short in consuming the prescribed 5 servings of vegetables. The participants reported the highest consumption of fruits and vegetables during the first 6 weeks, a slight decrease between week 6 and week 12, a peak in consumption around the time of the second blood draw, and a slight decrease toward the end of the study. However, the weekly averages never dropped below 7 daily servings.

Although the dietary counseling did not recommend any changes in nutrient intake other than fruits and vegetables, intervention participants reported a slightly lower fat intake after 3 and 6 months than at baseline (Table 1). Some women reported that they replaced meat with vegetarian dishes. Whereas intake of energy did not increase during the intervention, fiber intake rose by 25%. Body weight changed very little in either group. The mean in the control group was 127 lbs at baseline and 128 after 3 and 6 months, whereas the mean weight of the intervention group remained approximately 125 lbs. throughout the study.

Plasma Carotenoids, Retinols, Tocopherols, and Blood Lipids. At baseline, the two groups did not differ in their plasma levels of carotenoids (Table 2). Total carotenoids increased by close to 50% in the intervention group, whereas the level of the control group rose by only 10%. In the intervention group, the levels of individual carotenoids were higher after 3 and 6 months than at baseline. The β-carotene levels rose by 52% in the control group and by 84% in the intervention group, and the treatment effect was highly significant (ANOVA, $F = 3.6$; $P = 0.02$).

Table 3 Mean (SD) plasma levels of selected parameters

Measurement	Time of dietary assessment	Control group (n = 16)	Intervention group (n = 13)	P value of difference between groups
Cholesterol (mg/dl)	Baseline	190 (37)	181 (20)	0.43
	3 months	181 (33)	174 (24)	0.51
	6 months	202 (46)	190 (20)	0.36
Triglycerides (mg/dl)	Baseline	92 (49)	95 (41)	0.90
	3 months	91 (50)	83 (54)	0.66
	6 months	98 (40)	116 (104)	0.57
Phenols (μM)	Baseline	1590 (567)	1918 (598)	0.15
	3 months	1690 (560)	1778 (532)	0.67
	6 months	1765 (635)	1744 (584)	0.93
Malondialdehyde (μM)	Baseline	0.327 (0.093)	0.366 (0.093)	0.28
	3 months	0.356 (0.127)	0.300 (0.108)	0.22
	6 months	0.306 (0.108)	0.298 (0.093)	0.84
α-Tocopherol (ng/ml)	Baseline	11798 (4911)	10355 (2230)	0.31
	3 months	11082 (4869)	10360 (3185)	0.65
	6 months	12981 (6323)	13064 (3490)	0.95
γ-Tocopherol (ng/ml)	Baseline	1310 (859)	1072 (615)	0.41
	3 months	1302 (673)	1082 (151)	0.35
	6 months	1094 (760)	1180 (518)	0.73
Retinol (ng/ml)	Baseline	642 (18)	680 (47)	0.45
	3 months	631 (54)	603 (115)	0.59
	6 months	763 (184)	712 (138)	0.42

Retinol and tocopherol levels did not change over time or by group (Table 3). For all participants as a group, the correlation of carotenoid levels with fruit and vegetable intake was greater at the second and third blood draws ($r_s = 0.58$, $P = 0.001$ and $r_s = 0.65$, $P = 0.0003$) than at the first blood draw ($r_s = 0.43$, $P = 0.02$). At no time did the two groups differ significantly in cholesterol or triglyceride levels (Table 3), although cholesterol levels were 10 mg/dl lower at 3 months and 10 mg/dl higher at 6 months than at baseline. Whereas an ANOVA model for total carotenoids was highly significant ($F = 6.9$; $P = 0.003$), the model for cholesterol levels indicated no significant effect of group status ($P = 0.18$) and a borderline effect of time ($P = 0.08$).

The differences in total carotenoids between baseline and 3 months and between baseline and 6 months showed a strong association with the corresponding change in fruit and vegetable intake: 0.53 ($P = 0.003$) and 0.52 ($P = 0.006$), respectively. The average increase per serving fruits and vegetables was 138 ng/ml. For the 11 intervention participants who reported at least 6 daily servings of fruits and vegetables after 3 months, the mean total carotenoid level at that time was 1974 ng/ml (664 ng/ml increase from baseline), whereas it was only 1196 ng/ml (282 ng/ml increase from baseline) for the 2 women who did not comply with the dietary recommendations. The situation after 6 months was similar: a mean of 1966 ng/ml for the nine compliant women *versus* a mean of 1514 ng/ml for the four noncompliant women. A multiple linear regression model with total carotenoids as the dependent variable explained 58% of the variance in carotenoid levels. Fruit intake contributed 34% to the model; percentage of calories from fat, 9%; vegetable intake, 7%; cholesterol level, 5%; and age, 3%. Body mass index, caloric intake, plasma triglycerides, intervention status, and time of blood draw did not contribute significantly to the model.

Total Phenols and MDA Equivalents. We did not observe significant effects of higher fruit and vegetable intake on total phenol levels in plasma (Table 3). At no time did we observe a significant difference between groups. A difference at baseline disappeared toward the end of study. The nonsignificant changes of total phenol levels were in the opposite direction of our hypothesis: the levels increased in the control group and decreased in the intervention group.

Baseline MDA levels were highest for Japanese women (0.39 μM), followed by Chinese (0.35 μM), Caucasian (0.31 μM), Filipino (0.25 μM), and other (0.28 μM). We observed no significant correlations of baseline MDA levels with the dietary intake of meat, dietary fat, saturated fat, or plasma triglycerides. Although the differences in MDA levels between intervention and control group (Table 3) were small, the decrease in MDA levels for the intervention group compared to the control group was significant at 3 months ($T = 2.59$; $P = 0.02$) but not at 6 months ($T = 1.22$; $P = 0.23$). MDA levels showed a weak correlation with carotenoid levels which increased from the first ($r_s = 0.2$; $P = 0.31$) to the second ($r_s = 0.28$; $P = 0.14$) and third ($r_s = 0.38$; $P = 0.04$) blood draws, as well as a moderate correlation between change in carotenoid and MDA levels from baseline to 3 months ($r_s = -0.49$; $P = 0.04$). The association between MDA measurements for individual study participants was $r_s = 0.58$ ($P = 0.001$) between first and second blood draws and $r_s = 0.64$ ($P = 0.0002$) between second and third blood draws.

Discussion

This randomized trial achieved a successful change in dietary intake of fruits and vegetables among healthy women. Compliance was confirmed by a large increase in plasma carotenoid levels. These results agree with several dietary intervention studies reporting increases in fruit and vegetable intake of 3.8 (32), 4.7 (33), and 5.3 (16) servings per day. Whereas the correlation coefficient between the change in fruit and vegetable intake and plasma carotenoids was slightly lower than in a previous report (Ref. 16; 0.52 *versus* 0.69), the mean increase in carotenoids per servings was higher in this study (160 *versus* 77.7 μg/liter). The β-carotene baseline levels in our study were higher than in the Finnish and the United States carotene intervention studies and similar to the 221 ng/ml reported from the Physicians' Health Study (34). The findings from the intervention studies are consistent with results from cross-sectional studies reporting a correlation of 0.59 for total carotenoids (35) and 0.36 for β-carotene (36, 37) with fruit and vegetable intake. As in earlier reports (16), retinol and tocopherol levels were not affected by dietary change. With the

exception of a report from New Zealand (33), previous interventions randomized patients with cancer (16, 32, 33) or cardiovascular disease (38) who are presumed to be highly motivated to make lifestyle changes. Therefore, this report adds to the limited evidence that healthy women can succeed in substantially increasing fruit and vegetable intake.

To our knowledge, this is the first report presenting changes in TBA-RSs following a dietary intervention using the method described by Jentzsch *et al.* (30). The results of this study indicate that plasma levels of MDA, a product of lipid peroxidation, may be more likely to decrease in subjects whose carotenoid levels increase, but the results are suggestive only. Neither an increase in fruits and vegetables nor a decrease in fat intake affected MDA levels in a dietary intervention (38) assessing MDA levels with the method of Yagi (39). Based on a cross-sectional study (17) using the Yagi assay (39) to estimate lipid peroxidation, we expected a decrease by as much as 30% in MDA levels. The advantage of the method presented here was that the assay results were independent of triglyceride levels. Although the decrease in MDA was not as large as expected, subjects in the intervention group did experience a decrease in MDA levels that was related to plasma carotenoid levels. The decrease in MDA levels between the 3- and 6-month blood draws among the control group cannot be explained easily. Carotenoids are not the only micronutrients that affect MDA levels. The control group participants may have made dietary changes, such as replacing polyunsaturated fats with monounsaturated oils high in tocopherols. Other antioxidants that were not measured may have contributed to the change in MDA levels. The ethnic diversity may have also affected the MDA levels, despite the stratified randomization approach to balance the ethnic composition of the two groups. We observed slightly higher MDA baseline levels in the Japanese and Chinese women than in the other groups. Our findings agree with a report that TBA-RSs were significantly lower in vegetarians when oxidative modification was assessed in native and oxidatively modified low-density lipoprotein among 19 subjects (40). That MDA levels may be relevant to breast cancer risk was suggested by a Canadian study (41), in which the urinary excretion of MDA was associated with mammographic dysplasia. Autoantibodies recognizing 5-hydroxymethyl-2′-deoxyuridine, a possible biomarker for oxidative DNA damage, were found to be higher among healthy women who were diagnosed with breast cancer during the following 6 years than among age-matched controls (42).

The lack of correlation between total plasma phenols and fruit and vegetable intake may be a result of interferences with plasma proteins, which may not have been completely eliminated with this assay. This problem does not usually occur when urine that contains only traces of protein is analyzed for phenols. Also, other dietary sources of phenols (18), such as coffee, tea, red wine, and isoflavones from soy may have concealed a possible "9-A-Day" intervention effect. A recent breast cancer case-control study (27) described a protective effect of high urinary phenol levels in combination with a high excretion of isoflavones, which is in agreement with the anticarcinogenic properties of phenolic compounds (19).

To minimize the interassay variability of the laboratory assays, all samples from different blood draws were analyzed in one batch. The only exception were the blood lipids, for which the first and the second batch were measured together and the third batch at a different time. The variation in cholesterol and triglyceride levels observed among all study subjects may be a result of seasonal variations that have been described previously (43). The highest levels of cholesterol were observed in March and April and the lowest levels in September and October. Baseline blood draws in our study occurred in March, and the study ended in September.

As in all dietary research studies, there is a question of the validity of the self-reported dietary intake. The fact that the control group did not complete log sheets to report their daily fruit and vegetable intake has to be considered a limitation. However, the strong association of fruit and vegetable intake with plasma carotenoid levels suggests fairly accurate reporting of daily servings. The control group made some dietary changes; however, they were small in comparison to the intervention group. Several factors are responsible for the individual differences in plasma carotenoid response to fruit and vegetable intake. Carotenoid content of different fruits and vegetables varies considerably between a high of 400 ppm in carrots and 0–0.1 ppm in bananas (44). Second, dietary fat intake influences the absorption of lipid soluble nutrients. Also, the bioavailability of carotenoids differs for raw and cooked vegetables. Finally, plasma carotenoid levels can be affected by season (43), even in a tropical climate. Although women taking high doses of vitamins were ineligible for the study and participants were discouraged from taking any vitamin preparation, we suspect that a few subjects were taking vitamin supplements, because some α-tocopherol levels were higher than what can normally be achieved through dietary means. We consider it a strength of this study that we examined fruit and vegetable consumption rather than the intake of micronutrients. Because the cancer protective effect of fruits and vegetables may be multifaceted, looking at individual nutrients or micronutrients may overlook the total effect of a diet rich in fruits and vegetables, as demonstrated in a lung cancer case control study (45, 46), in which the relative risk for the quartile with the highest vegetable consumption as compared to the group with the lowest consumption was 0.31, and the relative risk for β-carotene was 0.62.

The small sample size is a severe limitation of the study. Because the well-educated and highly motivated women in this study, who had participated in a mammography study, were not representative of women in their age group, other populations may not achieve the same success in dietary change using the same type of intervention. There is a need to repeat the intervention in a larger, less selected group of women. Given the relatively short duration of this intervention, we also do not know whether the dietary changes will be maintained over time. During the exit interview, several women expressed their intention to continue the high fruit and vegetable intake, but probably at a level around 7 servings per day. The question of long-term change needs to be investigated by a larger scale trial that may incorporate less intensive intervention strategies and more behavioral techniques than were applied in this study. Whereas some women in this study expressed concerns about the time and effort to buy and prepare the produce, the required number of servings and financial aspects were not identified as major barriers. From the self-reports, tomato and yellow-orange products were the most difficult to include into the regular diet. Beneficial effects, such as greater regularity and softer breasts, were reported spontaneously by several intervention subjects.

In conclusion, this study confirmed our hypothesis that healthy women can be motivated to increase dietary intake of fruits and vegetables. As many women shared with us, they are concerned about breast cancer risk and are willing to make dietary changes despite the lack of conclusive evidence that this intervention strategy will be effective to prevent breast cancer. As in previous studies, we found total plasma carotenoids to be a good measure of compliance to the dietary recommendations. Future studies need to investigate whether MDA, a product of lipid peroxidation, may serve as a useful marker of oxidative damage or anitoxidant status.

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