

## No Effect of 600 Grams Fruit and Vegetables Per Day on Oxidative DNA Damage and Repair in Healthy Nonsmokers<sup>1</sup>

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### Abstract

**In several epidemiological studies, high intakes of fruits and vegetables have been associated with a lower incidence of cancer. Theoretically, intake of antioxidants by consumption of fruits and vegetables should protect against reactive oxygen species and decrease the formation of oxidative DNA damage. We set up a parallel 24-day dietary placebo-controlled intervention study in which 43 subjects were randomized into three groups receiving an antioxidant-free basal diet and 600 g of fruits and vegetables, or a supplement containing the corresponding amounts of vitamins and minerals, or placebo. Blood and urine samples were collected before, once a week, and 4 weeks after the intervention period. The level of strand breaks, endonuclease III sites, formamidopyrimidine sites, and sensitivity to hydrogen peroxide was assessed in mononuclear blood cells by the comet assay. Excretion of 7-hydro-8-oxo-2'-deoxyguanine was measured in urine. The expressions of *oxoguanine glycosylase 1* and *excision repair cross complementing 1* DNA repair genes, determined by real-time reverse transcription-PCR of mRNAs, were investigated in leukocytes. Consumption of fruits and vegetables or vitamins and minerals had no effect on oxidative DNA damage measured in mononuclear cell DNA or urine. Hydrogen peroxide sensitivity, detected by the comet assay, did not differ between the groups. Expression of *excision repair cross complementing 1* and *oxoguanine glycosylase 1* in leukocytes was not related to the diet consumed. Our results show that after 24 days of complete depletion of fruits and vegetables, or daily**

**ingestion of 600 g of fruit and vegetables, or the corresponding amount of vitamins and minerals, the level of oxidative DNA damage was unchanged. This suggests that the inherent antioxidant defense mechanisms are sufficient to protect circulating mononuclear blood cells from reactive oxygen species.**

### Introduction

Results from epidemiological studies indicate that high consumption of fruits and vegetables are associated with healthy lifestyle and low incidence of cancer (1). Probably several mechanisms are involved in the beneficial effects of fruits and vegetables, including vitamins, fibers, and several other bioactive compounds with less well-characterized effects. In addition, vegetables and fruits have high contents of antioxidants that are able to scavenge ROS<sup>4</sup> (2) and probably also inhibit their formation (2, 3).

Oxidative stress occurs when ROS are formed in amounts that exceed the capacity of the antioxidant defense system. The detrimental effects of ROS typically are assessed by the presence of oxidatively altered biomolecules. Oxidative DNA damage formed during oxidative stress probably plays a critical role in carcinogenesis (3). During oxidative stress *in vivo* or when ROS react with DNA *in vitro*, several types of DNA damage are formed, including strand breaks and small base lesions (4). 8-OxodG constitutes one of the most easily formed oxidative DNA lesions and can be detected in both urine and tissues after oxidative stress (5). Alternatively, oxidative DNA lesions can be detected by the enzyme-modified single-cell gel electrophoresis (comet) assay (6). In principle, the comet assay detects DNA strand breaks, and by including DNA glycosylase enzymes, oxidatively damaged nucleobases are excised from the DNA strand and leave strand breaks. Oxidatively altered purines can be detected by the FPG protein and oxidized pyrimidines by ENDOIII. Mixed results of direct DNA strand breaks, enzyme-sensitive sites, and 8-oxodG have been found in dietary antioxidant intervention studies of foods with high antioxidant content, including vegetables and juices (7, 8). In a systematic review, it was shown that many studies apparently showing beneficial effect of such interventions have used designs without proper control of confounding, including period effects (8).

Theoretically, DNA lesions are turned into mutations *in vivo* if they are not removed from the genome by the DNA repair system. In mammalian cells, DNA damage may be excised by two different types of DNA excision repair systems, namely base excision repair and nucleotide excision repair. ERCC1 plays an essential role in the nucleotide excision repair

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<sup>4</sup> The abbreviations used are: ROS, reactive oxygen species; ENDOIII, endonuclease III; ERCC1, excision repair cross complementing 1; FPG, formamidopyrimidine DNA glycosylase; OGG1, oxoguanine glycosylase 1; 8-oxodG, 7-hydro-8-oxo-2'-deoxyguanosine.

Table 1 Subject characteristics and plasma vitamin C and carotenoid concentration in the three groups at baseline (mean and SD)<sup>a</sup>

	Fruit and vegetable	Vitamin supplement	Placebo
No. (males/females)	16 (8/8)	12 (7/5)	15 (7/8)
Age (yr)	29 ± 8	24 ± 4	26 ± 6
Body mass index (kg/m <sup>2</sup> )	23.5 ± 2.3	23.1 ± 1.7	22.6 ± 2.3
Protein (energy percentage) <sup>b</sup>	17 ± 3	17 ± 3	15 ± 3
Fat (energy percentage) <sup>b</sup>	29 ± 6	31 ± 3	29 ± 6
Carbohydrate (energy percentage) <sup>b</sup>	54 ± 8	53 ± 6	55 ± 8
Dietary fiber (g/day) <sup>b</sup>	20 ± 7	16 ± 7	14 ± 7
Alcohol (g/day) <sup>†</sup>	13 ± 16	16 ± 19	17 ± 18
Fruit and vegetable (g/day) <sup>2</sup>	333 ± 178	269 ± 119	260 ± 180
Plasma vitamin C concentration (μM)	75 ± 19	63 ± 14	67 ± 17
Plasma trans-β-carotene concentration (μg/100 ml)	19 ± 10	18 ± 11	20 ± 15

<sup>a</sup> There were not statistically significant differences between the groups at baseline.

<sup>b</sup> Estimated from food frequency questionnaire.

process as a part of the 5'-incision complex (9). The base excision repair enzyme, OGG1, removes mainly oxidized guanine from the DNA (10). To the best of our knowledge, the expressions of the *ERCC1* and *OGG1* genes have not been investigated previously in relation to fruit and vegetable intake.

The aim of this investigation was to compare the level of oxidative DNA damage and repair activity in WBCs between subjects depleted of fruit, vegetables, and antioxidants and subjects supplemented with 600 g of fruits and vegetables/day or a supplement with a corresponding content of vitamins and minerals.

## Materials and Methods

**Study Design and Subjects.** The study was set up as a parallel design, with the subjects randomized to three groups: consuming daily 600 g of fruits and vegetables; vitamin tablets with the same amount of antioxidants and minerals; or no antioxidants (placebo tablet) for 24 days. The study was approved by the Scientific Ethics Committee of the municipalities of Copenhagen and Frederiksberg (01-234/99).

The *a priori* hypothesis was that increased oxidative DNA damage would be observed in the placebo group because of the withdrawal of dietary antioxidants, whereas oxidative DNA damage in the vitamin group and in the fruit and vegetable group would remain unaltered or decreased because of higher intake throughout the study. Expression of DNA repair genes was investigated without any *a priori* hypothesis because this had not been tested before in dietary intervention studies. Before the study, we estimated that the group size needed to be 15 subjects to observe a 50% effect increase in the placebo group at  $\alpha = 5\%$  and  $\beta = 20\%$  and assuming a 50% variation coefficient as observed previously for the comet assay and urinary 8-oxodG excretion (11–14). We considered that a 50% difference was reasonable to expect because several of the studies showing protective effects of antioxidants had reported larger effects in the level of oxidative DNA damage or H<sub>2</sub>O<sub>2</sub> sensitivity after intervention with natural food stuffs or multi-vitamin tablets (15–20).

Healthy normal-weight males and females were recruited for the study by advertisement at universities and other institutions as well as in the local newspaper. Exclusion criteria were smoking, obesity, family history of chronic diseases, hypertension, use of medication, and heavy physical exercise. Forty-eight subjects were recruited to the study after had given informed consent. A total of 43 subjects completed the study (22 men and 21 women, ages 21–56 years). One subject dropped out because he was unable to be present for lunch

within a specific time interval, and 4 subjects dropped out because of events unrelated to the study. The baseline characteristics of subjects that completed the study are provided in Table 1.

Before the start of the controlled diet, the subjects kept a complete weighed diet record for 4 days and filled in a validated food frequency questionnaire (21). According to these data, the habitual intake of fruits and vegetables was ~280 g/day. The subjects were allocated to fixed individual energy intakes based on the body weight, age, and physical activity level (WHO). Throughout the study, body weights were measured every second day, and if the weight varied >1 kg, the subjects were allocated to a different energy level or informed to consume extra energy (provided as energy rolls with identical macronutrient composition as the total diet). The mean energy intake during the study was 13 MJ (range, 9–17 MJ).

**Diet.** All three groups consumed a basic diet that was devoid of flavonoid-containing foods, including fruits, vegetables, juices, teas, alcohol, and chocolate products. The fruit and vegetable group consumed additionally 600 g of vegetables and fruits, containing apples (90 g), pears (90 g), orange juice (120 g), broccoli (100 g), carrots (75 g), onion (25 g), and canned tomato (100 g)/day. The supplemented group received tablets containing the known antioxidant vitamins and minerals calculated to be present in the 600 g of fruits and vegetables, using a computer program (Dankost) that is based on the Danish Veterinary and Food Administrations food composition database. The tablets contained 150 mg of vitamin C, 3.3 mg of  $\alpha$ -tocopherol, 5 mg of  $\beta$ -carotene, 0.3 mg of thiamin, 0.4 mg of riboflavin, 3 mg of niacin, 0.6 mg of vitamin B6, 1.7 mg of pantothenic acid, 3.7 μg of biotin, 125 μg of folic acid, 250 μg of vitamin K (as phylloquinone), 3 mg of Fe (as ferrous fumarate), 0.4 mg of Cu, 1200 mg of K, 180 mg of Ca, 65 mg of Mg, 1.4 mg of Zn (produced by Pharma Vinci, Frederiksværk, Denmark). The content of folic acid and  $\beta$ -carotene in the tablets were 50% lower than the content calculated to be present in 600 g of the fruit and vegetables because of expected higher bioavailability of these antioxidants in the tablet form. The placebo group received a similarly appearing placebo tablet. The subjects and investigators were unaware of the assignment to supplement or placebo groups. The subjects ate one tablet at breakfast, lunch, and dinner (a total of three tablets/day). The supplement and placebo groups consumed an energy drink containing simple sugars, which corresponded to the energy content of the 600 g of fruits and vegetables in the fruit and vegetable group. Most meals were prepared in one batch and frozen until use. The diets consisted of ordinary Danish foods

Table 2 Composition of experimental diet (grams fresh weight/day) at an energy intake of 10 MJ/day

Component	Menu 1	Menu 2	Menu 3	Menu 4
<b>Breakfast</b>				
Bread roll	70	70	70	70
Cheese	25	25	25	25
Sour milk	195	195	195	195
Rye breadcrumbs	33	33	33	33
<b>Lunch</b>				
Rye bread	100	100	100	100
Roast beef, ham, or turkey	28	28	28	28
Ham salad		50		
Egg salad	46			
Tuna salad				50
Mackerel salad			50	
Milk	250	250	250	250
<b>Dinner</b>				
Beef stew	220			
Rice	70			
Cheese sauce		200		
Pasta		70		70
Stew			450	
Rye bread			40	
Meat sauce				190
<b>Snack</b>				
Bread roll		70		70
Cream cheese		20		20
Sponge cake	110		110	
Almond cake		50		50

with four repeated menus (Table 2). On weekdays, lunch was consumed in The Department of Human Nutrition under supervision, whereas beverages, dinner, snack, and breakfast were provided daily as a package with guidelines for its preparation. Food and beverages for the weekend were provided on Fridays. Food that was not eaten was returned to the investigators and registered.

Fasting blood samples for analysis of oxidative DNA damage and mRNA expressions were taken before (day -1), at days 9, 16, and 24 during the intervention, and 4 weeks (day 52) after the end of the intervention when the participants had resumed their habitual diets. Twenty-four-h urine samples were collected before, at four occasions during the intervention period, and on follow-up 4 weeks after the end of intervention.

**Detection of Oxidative DNA Damage in Mononuclear Blood Cells and Urine.** For the comet assay, 7 ml of venous blood were drawn into Vacutainer CPT cell preparation tubes with sodium heparin, and mononuclear blood cells were isolated as described elsewhere (13). The steady-state level of oxidative DNA damage in mononuclear blood cells was detected by the comet assay as previously described with minor modifications (13). Cryopreserved mononuclear cells were thawed and washed in cold PBS and mixed with 0.75% low melting agarose and applied onto Gelbond films (BioWhittaker Molecular Applications, Rockland, ME). The level of DNA damage was scored according to five classes of damage (0-4) in 100 nuclei from each sample (range of score 0-400). The levels of FPG and ENDOIII sites were determined as the difference in strand break between Gelbonds incubated with enzyme and buffer. ENDOIII and FPG enzymes were kind gifts from Dr. Serge Boiteaux (UM217 Center National de la Recherche Scientifique et Commissariat à l'Énergie Atomique, France), and Dr. Andrew Collins (Rowett Research Institute, Scotland). For the detection of hydrogen peroxide sensitivity, mononuclear cells

were incubated with 150  $\mu\text{M}$   $\text{H}_2\text{O}_2$  for 5 min on ice. During each electrophoresis, four types of cryopreserved mononuclear cells served as control samples, including nontreated cells, FPG-treated samples, and cryopreserved  $\text{H}_2\text{O}_2$ -treated (150  $\mu\text{M}$ ), as well as freshly  $\text{H}_2\text{O}_2$ -treated cells (150  $\mu\text{M}$ ).

The 24-h urinary excretion of 8-oxodG was determined by high-performance liquid chromatography-electrochemical detection as described previously, and expressed as nmol/24 h (5).

**Quantitation of *OGGI* and *ERCC1* mRNA Expression in Leukocytes.** Total RNA was purified from 1.5 ml of EDTA stabilized full blood using Qiagen blood RNA isolation kit, and the expression of *OGGI* and *ERCC1* mRNA, normalized to 18S RNA, was determined by real-time reverse transcription-PCR as described elsewhere (22). The probes and primers for *OGGI* were: forward primer, 714F, 5'-AAATCCAAGGTGTGCGACTG-3'; reverse primer, 796R, 5'-GCGATGTTGTTGTTGGAGGA-3'; and probe, 5'-FAM-CAAGACCC-ATCGAATGCCTTTTCTCTTT-TAMRA-3'. The probes and primers for *ERCC1* were: forward primer, 512F, 5'-GGC-GACGTAATCCCGACT-3'; reverse primer, 576R, 5'-TAG-CGGAGGCTGAGGAACA-3'; and probe: 5'-FAM-TGTGCT-GGGCCAGAGCACCTGT-TAMRA-3'. Primers and probes were obtained from Applied Biosystems.

**Plasma Levels of Vitamin C and *trans*- $\beta$ -Carotene.** The plasma levels of vitamin C and carotenoids, including *trans*- $\beta$ -carotene, were analyzed as reported previously (23, 24). A complete coverage of alternations in the concentration of plasma antioxidants in the three groups will be published elsewhere.

**Statistics.** In the statistical analysis, the data were analyzed by a multifactorial ANOVA based on repeated measurements and 5% as significance level. The data were tested for homogeneity of variance between groups by Levene's test and for normality by Shapiro-Wilk's W test. The comet assay and urinary 8-oxodG measurements were normally distributed and had homogeneity of variance between groups. There was no homogeneity of variance between groups for the mRNA expressions even after log transformation using the base of 10. Also, the mRNA expression data sets were incomplete because of low quality of mRNA in some samples. Consequently, mRNA expressions were analyzed by Visual GLM General Mancova/Manova with subject identity as random factor and gender, diet assignment, and study day as fixed categorical factors. In an alternative statistical approach, we calculated the mean of samples before (day -1) and after the intervention (day 52) and used the mean value to test for differences in the value of the end of the intervention period. Using this approach, there was no difference in oxidative DNA damage or repair between the three groups, which is in agreement with the statistical analysis by repeated measurements. The *Ps* reported in the text corresponds to the statistical analysis by repeated measurements. Correlation between biomarkers were tested by both Pearson correlation test and Spearman rank correlation test. The statistical analysis was performed in Statistica 5.5 for Windows, Statsoft, Inc. (Tulsa, OK).

## Results

At baseline, the consumption of energy from carbohydrate, fat, and protein did not differ between the groups. There was no difference in alcohol consumption or intake of fruits and vegetables between the groups (Table 1). Determined from baseline blood samples, the plasma content of vitamin C and  $\beta$ -carotene did not differ between the groups (Table 1). Also, there was no difference in the level of biomarkers of oxidative

Table 3 Plasma concentrations of vitamin C and  $\beta$ -carotene at baseline and on day 24 after 3 weeks of intervention (mean and SD)

Plasma antioxidant	Vegetable	Vitamin	Placebo
Vitamin C (baseline, $\mu\text{M}$ )	74.8 $\pm$ 19.5	62.9 $\pm$ 13.9	67.3 $\pm$ 17.3
Vitamin C (day 24, $\mu\text{M}$ )	78.5 $\pm$ 13.6	71.3 $\pm$ 12.0	21.1 $\pm$ 11.8
$\beta$ -Carotene (baseline, $\mu\text{g}/100\text{ ml}$ )	18.6 $\pm$ 9.5	17.6 $\pm$ 10.9	19.7 $\pm$ 14.5
$\beta$ -Carotene (day 24, $\mu\text{g}/100\text{ ml}$ )	30.9 $\pm$ 15.7	47.5 $\pm$ 18.1	10.4 $\pm$ 8.5

DNA damage and repair between the groups at baseline. Consequently, this indicates that the randomization had been successful.

During the intervention period, plasma concentrations of vitamin C and  $\beta$ -carotene were reduced by 69 and 47% in the placebo group, respectively, whereas high plasma concentrations of vitamin C were maintained in the fruit and vegetable group and the supplemented groups (Table 3). The plasma  $\beta$ -carotene was increased in the fruit and vegetable group and in the vitamin tablet group after 3 weeks of ingestion.

**Steady-State Level of Oxidative DNA Damage in Mononuclear Blood Cells (Comet Assay).** Each sample measurement included strand breaks and  $\text{H}_2\text{O}_2$  sensitivity, as well as oxidative DNA damage detected by ENDOIII and FPG (Table 4). None of the end points were affected by the diet intervention. Various types of standardization were attempted (*i.e.*, subtractions between samples and controls), yet this did not suggest that day-to-day variation masked biological effects of the analysis. The statistical analysis indicated an effect of  $\text{H}_2\text{O}_2$ -induced damage on day 16 in all of the groups ( $P < 0.001$  compared with other days, ANOVA). When stratified by gender, men contributed the most to the period effect (mean and SD for males were  $52.6 \pm 20.1$  and for women  $36.9 \pm 20.2$ ,  $P < 0.001$  posthoc ANOVA on repeated measurements). Overall, there was no effect by gender on any of the comet assay end points.

For each electrophoresis run, we included cryopreserved mononuclear blood cells for detection of strand breaks and  $\text{H}_2\text{O}_2$  treatment (150  $\mu\text{M}$ ). The mean and SD was  $29 \pm 11$  for strand breaks and  $93 \pm 21$  for  $\text{H}_2\text{O}_2$ -treated cells ( $n = 32$ ). FPG-sensitive sites in mononuclear cells were  $62 \pm 24$ , and the mean and SD of freshly  $\text{H}_2\text{O}_2$ -treated (150  $\mu\text{M}$ ) mononuclear cells were  $52 \pm 16$  ( $n = 32$ ). The variation of strand breaks was similar to our previously reported assay variation (14) and to the assay variation reported in other biomonitoring studies (25–27).

**Urinary 8-OxodG Excretion.** There was no difference in 8-oxodG excretion between the three types of diets consumed (Table 5). However, there was a period effect where the samples taken after the intervention period were lower than at the other sampling times ( $P < 0.001$  compared with other days, ANOVA). In accordance with previous observations (28, 29), the men in this study had higher urinary 8-oxodG excretion than women (mean and SD for women  $15.1 \pm 6.9$  nmol/24 h and for men  $21.4 \pm 7.7$  nmol/24 h,  $P < 0.01$ , posthoc ANOVA).

**Expressions of *OGG1* and *ERCC1* mRNA in Leukocytes.** The expressions of *OGG1* and *ERCC1* are outlined in Table 6. The expressions of *OGG1* and *ERCC1* mRNA were determined on 9 different days during the study. A description of the complete data set of the mRNA expressions is reported elsewhere (22). The data presented here only outlines the expressions on the 5 days with comet assay and urinary 8-oxodG excretion measurements. The levels of mRNA expression of the two DNA repair genes were not related to the diet consumed, and there was no effect of gender.

**Correlations.** There were no significant correlations between urinary 8-oxodG and comet assay end points, urinary 8-oxodG and expression of DNA repair genes, and comet assay end points and expression of DNA repair genes.

## Discussion

In this study, we have found that depletion of fruit and vegetables, or consumption of 600 g of fruit and vegetables, or the corresponding amount of vitamins and minerals given as a supplement for 24 days did not alter the level of strand breaks, oxidative DNA base damage, or  $\text{H}_2\text{O}_2$  sensitivity in mononuclear blood cells. The expressions of DNA repair genes involved in base excision repair (*OGG1*) and nucleotide excision repair (*ERCC1*) were unaltered by the diet consumed, suggesting that the unaltered levels of oxidative DNA damage were not because of change in DNA repair activity. This notion is additionally strengthened by the unchanged 8-oxodG excretions in urine, which represent the sum of 8-oxodG repair, sanitation of the nucleotide pool, cell turnover, and mitochondrial turnover in the whole body (7). Collectively, our data strongly suggest that excess of oxidative DNA damage were not produced in subjects during the period of low antioxidant intake.

The amount of fruit and vegetable was chosen to represent antioxidant consumption in a range from virtually zero intake to the consumption of 600 g/day as recommended by the health authorities in many countries and the Danish Veterinary and Food Administration. Eating 600 g of fruit and vegetable is within the range showing decreased relative risk of cancer in epidemiological studies (1). Our results are in agreement with the results reported by van den Berg *et al.* (30) showing that subjects having relatively low fruit and vegetable intake had unaltered biomarker levels of comet assay end points, lipid peroxidation, oxidized/reduced glutathione ratio, and activity of glutathione *S*-transferases and nuclear transcription factor- $\kappa\text{B}$  after 3 weeks consumption of vegetable burgers and fruit drinks (equivalent to 500 g of mixed fresh vegetable and fruit drinks), although the concentration of vitamin C, carotenoids, and total plasma antioxidant capacity increased in plasma. In our study, the plasma levels of relevant antioxidants were clearly decreased in the placebo group. This may indicate that more severe depletion of antioxidants is required to elevate the levels of oxidative DNA damage. Alternatively, we cannot exclude that the intake of vegetable and fruit must be higher than 600 g/day to show protective effect on biomarkers related to oxidative DNA damage. However, unaltered levels of 8-oxodG in lymphocytes and urine were seen in subjects that doubled their intake of fruits and vegetables (baseline servings 5.8/day; Ref. 31).

Although our data are supported by two studies of fruit and vegetable consumption (30, 31), investigations of other sources of antioxidants have found beneficial effects. Consumption of carotenoid-rich food products (tomato juice, carrot juice, and spinach powder) decreased the level of strand breaks and ENDOIII sites (16). Also subjects consuming tomato puree (25 mg/day for 2 weeks) in addition to the normal diet had decreased  $\text{H}_2\text{O}_2$  sensitivity in lymphocytes (20). However, none of these studies were placebo controlled. A sequential 1-week supplementation study of spaghetti sauce (126 g/day), tomato juice (450 ml/day), or oleoresin capsules (2.5 g/day) as sources of lycopene did not alter the level of 8-oxodG (32). Two better-controlled studies revealed that drinking green tea or red wine decreased the steady-state level of 8-oxodG in leukocytes (18, 19). It is possible that large consumption of one type of antioxidant (*e.g.*, carotenoids) will decrease the steady-state

Table 4 Level of DNA strand breaks, ENDOIII- and FPG-sensitive sites, and hydrogen peroxide sensitivity in mononuclear blood cells detected by the comet assay (mean and SD)<sup>a,b</sup>

Diet	Before	Day 9	Day 16	Day 24	After <sup>c</sup>
Strand breaks					
Placebo	26.6 ± 13.3	25.4 ± 18.7	26.8 ± 15.0	23.2 ± 12.8	33.0 ± 13.5
Vitamin	29.8 ± 12.8	23.4 ± 12.0	32.3 ± 15.7	27.8 ± 14.6	33.3 ± 12.3
Vegetable	25.1 ± 13.4	30.1 ± 13.0	34.1 ± 25.6	25.6 ± 14.1	28.1 ± 16.9
ENDOIII sensitive sites					
Placebo	27.0 ± 24.5	32.1 ± 20.1	21.6 ± 22.0	27.8 ± 23.4	20.3 ± 17.3
Vitamin	33.2 ± 15.7	32.3 ± 24.9	23.5 ± 14.8	20.3 ± 26.5	32.2 ± 10.0
Vegetable	36.5 ± 14.4	29.5 ± 15.4	31.4 ± 26.5	27.3 ± 18.8	22.0 ± 26.2
FPG-sensitive sites					
Placebo	42.2 ± 25.3	39.9 ± 29.4	35.9 ± 15.7	44.9 ± 25.3	35.7 ± 19.6
Vitamin	28.9 ± 28.1	37.6 ± 29.1	31.2 ± 18.8	33.9 ± 18.4	36.5 ± 20.1
Vegetable	38.3 ± 19.6	36.2 ± 22.2	39.5 ± 26.3	39.7 ± 23.1	35.3 ± 26.2
Hydrogen peroxide-sensitive sites					
Placebo	33.6 ± 10.6	28.4 ± 15.9	45.6 ± 18.1	31.4 ± 12.3	35.0 ± 14.0
Vitamin	39.3 ± 12.5	37.7 ± 13.1	42.3 ± 15.6	34.3 ± 10.6	37.3 ± 16.4
Vegetable	36.7 ± 16.4	41.1 ± 16.5	45.6 ± 26.7	37.7 ± 10.8	40.9 ± 19.7

<sup>a</sup> The data are expressed as arbitrary units.

<sup>b</sup> There were no interaction between the diets consumed and time-point in the study. For strand breaks, ENDOIII, and FPG-sensitive sites there were no statistically significant effects of the diet consumed or the time point in the study.

<sup>c</sup> The "after" samples were obtained 4 weeks after cessation of the intervention period (day 52).

Table 5 Level of 24 h urinary 8-oxodG excretion (mean and SD)<sup>a</sup>

Diet	Before	Day 2	Day 9	Day 16	Day 24	After <sup>b</sup>
Vegetable	19.5 ± 8.6	17.5 ± 7.3	17.0 ± 8.4	18.4 ± 9.0	17.7 ± 10.0	13.8 ± 7.9 <sup>c</sup>
Placebo	19.5 ± 8.6	18.6 ± 7.4	21.0 ± 10.6	21.0 ± 9.8	18.8 ± 8.9	17.0 ± 9.8
Vitamin	18.8 ± 3.9	19.5 ± 5.4	19.8 ± 5.1	18.4 ± 5.8	17.9 ± 5.0	15.0 ± 3.4

<sup>a</sup> The data are expressed as nmol/24 h.

<sup>b</sup> The "after" samples were obtained 4 weeks after cessation of the intervention period (day 52).

<sup>c</sup> n = 15 (other days n = 16).

Table 6 Expression of *OGG1* and *ERCC1* mRNA relative to 18S in leukocytes isolated from whole blood (mean and SD with number of subjects in parenthesis)<sup>a,b</sup>

Diet	Before	Day 9	Day 16	Day 24	After <sup>c</sup>
Expression of <i>OGG1</i> mRNA					
Placebo	10.0 ± 5.3 (15)	9.5 ± 5.9 (15)	7.6 ± 4.6 (9)	10.5 ± 6.7 (16)	11.0 ± 6.8 (13)
Vitamin	7.2 ± 2.9 (11)	6.9 ± 3.5 (12)	6.7 ± 2.7 (9)	7.3 ± 3.0 (12)	10.9 ± 7.3 (12)
Vegetable	8.9 ± 4.1 (15)	11.1 ± 6.6 (15)	6.3 ± 1.6 (11)	9.0 ± 6.5 (15)	9.8 ± 9.0 (13)
Expression of <i>ERCC1</i> mRNA					
Placebo	29.0 ± 14.6 (14)	28.3 ± 12.9 (14)	24.8 ± 13.6 (9)	33.6 ± 18.5 (15)	41.6 ± 28.0 (12)
Vitamin	22.9 ± 6.5 (11)	23.7 ± 7.9 (12)	20.6 ± 6.5 (9)	27.1 ± 11.5 (12)	36.5 ± 17.9 (12)
Vegetable	29.0 ± 13.1 (15)	31.4 ± 17.1 (15)	19.9 ± 6.9 (11)	28.8 ± 18.4 (15)	30.9 ± 15.9 (13)

<sup>a</sup> The data are expressed as mRNA relative to the expression of 18S (per million).

<sup>b</sup> There were no interaction between the diets consumed and time point in the study, nor were there any single factor effects of the diet consumed and time point in the study.

<sup>c</sup> The "after" samples were obtained 4 weeks after cessation of the intervention period (day 52).

level of oxidative DNA damage in leukocytes, whereas ingestion of a mixture of antioxidants in fresh fruits and vegetables provide less protective effect. One reason could be that the bioavailability of antioxidants in fluid products (juices) and food products (puree) is higher than in fresh fruit and vegetables. Another possible limitation in this study is that the study was designed to detect a 50% difference, which may be more than can be achieved by dietary changes in placebo-controlled intervention studies with well-nourished subjects, although marked differences were observed in plasma antioxidant concentrations. Changes of 20–25% may be more realistic to expect in placebo-controlled intervention studies, although the power in large-scale placebo-controlled intervention studies usually is insufficient to detect 20–25% differences. However,

the study design is likely to be equally important. It is worth considering that the study design used in our study is very strong because parallel groups were investigated simultaneously. This type of design is less prone to period effects than sequential studies or crossover studies. Period effects may be caused by external exposures unrelated to the study or introduced into the study if people who enter a study change dietary habits. For example, we observed increased H<sub>2</sub>O<sub>2</sub> sensitivity 2 weeks (day 16) into the intervention period. On the same day, the lowest level of mRNA expressions were observed for the two DNA repair enzymes, although it was not significantly different from other days. Had this been a sequential fruit and vegetable withdrawal study, we could have erroneously assumed that low antioxidant intake was associated with low

DNA repair activity and increased H<sub>2</sub>O<sub>2</sub> sensitivity. Similarly, the 8-oxodG excretion decreased significantly in all groups after the diet intervention period. In a recent 10-week crossover study of flavonoids, we also observed an overall decrease in 8-oxo-dG excretion and in several other markers of oxidative damage that were not related to the antioxidants consumed (33). In a critical review of studies, including 8-oxodG and the comet assay, we suggested that antioxidant intervention studies with strong designs tended to produce null outcomes (8). Also, for urinary 8-oxodG excretion, most of the intervention trials have found no difference (7).

In the scientific community and the public, there is strong belief that consumption of fruits and vegetables is healthy because of an antioxidant effect and a majority of case-control studies support a protective effect against many cancers (1). Thought-provokingly, it appears that in recent years, several biomarker studies have produced null effects in antioxidant intervention studies, indicating that the antioxidant property may not be the major contributor of the beneficial effect of fruits and vegetables and other antioxidant-rich foodstuffs. However, it should be recognized that the data from biomarker studies are based on detection of oxidative DNA damage in leukocytes as a surrogate tissue or in urine as an overall estimate of the oxidative DNA damage produced in the body. The distribution and tissue concentration of relevant dietary antioxidants are virtually unresolved. Another limitation in the biomarker-based studies concerns the possibility that beneficial effects of dietary antioxidants only are observed when given in an oxidative stress situation. Moreover, some data indicate that the effect of antioxidants only are observed within a short time after ingestion (34–37). This indicates that fasting blood samples may not be suitable for the detection of antioxidant effects of fruits and vegetables and other antioxidant-rich products. Despite the mentioned limitations, we suggest that the antioxidant effect of fruits and vegetables is less important than previously expected. Still, it is pertinent to stress that fruit and vegetable intake in biomarker-based studies show plenty of other putative disease-preventive effects (38). Thus, in relation to risk communication, it is still important to inform the public that eating fruit and vegetables is healthy.

In conclusion, this 24-day fruit and vegetable intervention trial showed no beneficial effects on oxidative DNA damage or expression of DNA repair genes. This may suggest that the antioxidant properties or effects on oxidative DNA damage play little or no role in the cancer preventive effect of fruit and vegetables. Alternatively, it is possible that the subjects enrolled in the study were not exposed to any type of oxidative stress that would have produced oxidative damage. Also, it needs to be taken into consideration that the subjects selected for the study had a relatively high habitual intake of fruits and vegetables and were nonsmokers. Additional studies on the effect of fruit and vegetables on oxidative DNA damage in oxidative stress situations are needed to support a role for fruit and vegetables in the prevention of cancer.

**Note added in proof.** After submission of this paper, Collins *et al.* (Carcinogenesis (Lond.) 24: 511–515, 2003) have as the first researchers published data on expression of OGG1 mRNA in relation to kiwi fruit intake.

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## No Effect of 600 Grams Fruit and Vegetables Per Day on Oxidative DNA Damage and Repair in Healthy Nonsmokers

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