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DNA Damage and Repair: Fruit and Vegetable Effects in a Feeding Trial

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Abstract

Epidemiologic studies have examined the association between fruit and vegetable (F&V) consumption and the risk of cancer. Several cancer-preventive mechanisms have been proposed, such as antioxidant properties and modulation of biotransformation enzyme activities; both may be associated with reducing DNA damage and hence the mutation rate. We investigated, in a randomized, controlled, crossover feeding trial, the effect of 10 servings/day of botanically defined F&V for 2 wk on endogenous DNA damage; resistance to γ-irradiation damage; and DNA repair capacity in lymphocytes, measured by the Comet assay. We also explored the association between the UGT1A1*28 polymorphism and serum bilirubin concentrations and DNA damage and repair measures. Healthy men (n = 11) and women (n = 17), age 20 to 40 yr, provided blood samples at the end of each feeding period. Overall, F&V did not affect DNA damage and repair

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measures in lymphocytes. The number of UGT1A1*28 alleles was inversely associated with sensitivity to γ-irradiation exposure and DNA repair capacity, but a biological mechanism to explain this association is unclear. A larger sample size is needed to investigate the association between bilirubin concentrations and endogenous DNA damage. With inconsistent findings in the literature, additional dietary intervention studies on the effect of F&V on DNA damage and repair are needed.

INTRODUCTION

Epidemiologic studies have examined the association between fruit and vegetable (F&V) consumption and the risk of cancer at various sites, and evidence suggests a protective association with cancers of the mouth and pharynx, esophagus, lung, larynx, stomach, kidney, ovary (vegetables only), bladder (fruit only), colon, and rectum (1). These plant foods contain numerous bioactive compounds, many of which have been shown to have anticarcinogenic properties in experimental systems.

F&V contain antioxidants such as carotenoids, vitamins C and E, flavonoids, and polyphenols that scavenge and neutralize reactive oxygen species or interrupt the free-radical-initiated chain reaction of oxidation (2–5). F&V are also good sources of trace minerals that are required for the synthesis of endogenous antioxidative enzymes such as superoxide dismutase (6). Reactive oxygen species, from both endogenous and exogenous sources, if not quenched by antioxidant defenses, can damage various cellular components including DNA (7). Unrepaired DNA lesions can lead to replication errors and genomic instability during cell division, increasing the prevalence of mutations that may alter the function of protoon cogenes or tumor-suppressor genes and promote carcinogenesis (8,9).

Several phytochemicals found in *Brassica* vegetables, soy foods, and citrus fruit [e.g., glucoraphanin (glucosinolate form of sulforaphane), genistein, quercetin, and tangeretin, respectively] have also been shown to favorably modulate carcinogen metabolism by altering biotransformation enzymes such as UDP-glucuronosyltransferase (UGT) 1A1 (10). UGT1A1 conjugates glucuronic acid to endogenous compounds such as bilirubin (product of heme metabolism that is known to have antioxidant properties) (11), 17β-estradiol, xenobiotic compounds found in foods (12), and cooked-food carcinogens such as 2-amino-1-methyl-6-phenylimidaxo[4,5-b]pyridine (PhIP) (13) and benzo[*a*]pyrene (14), producing soluble excretable metabolites. UGT1A1*28 polymorphism, with an extra dinucleotide (TA) insertion in the TATA box of UGT1A1 promoter (the variant *28 allele contain 7 TA repeats while the wildtype *1 allele contains 6 TA repeats) has been shown to decrease UGT1A1 transcriptional activity (15,16). Studies in humans on the effect of F&V on UGT1A1 induction and the potential effect of UGT1A1*28 polymorphism are very limited. We recently reported that F&V consumption for 2 wk lowered serum bilirubin concentrations (an indicator of UGT1A1 activity) in individuals with the homozygous *28/*28 genotype (17). This observed genotype-by-diet interaction suggested that F&V consumption may also potentially affect bilirubin metabolism and therefore may modify its potential as an endogenous antioxidant.

The aim of this study was to investigate whether 10 servings/day of botanically defined F&V, with their antioxidants and UGT1A1-inducing compounds, may affect human endogenous lymphocyte DNA damage, resistance to γ-irradiation damage, and DNA repair capacity as measured by the Comet assay. In addition, we explored the role of UGT1A1*28 polymorphism and serum bilirubin on DNA damage.

SUBJECTS AND METHODS

Subjects

Subjects were a subset of participants who completed a feeding study of diet and glucuronidation (17). In the feeding study, healthy, nonsmoking men and women, aged 20 to 40 yr, were recruited from the greater Seattle area through print advertisements, radio, television, targeted mass mailings, the Fred Hutchinson Cancer Research Center Web site, and flyers sent to university campuses. Potential participants were excluded if any of the following was reported in the eligibility questionnaire: medical history of hepatic, gastrointestinal or renal disorders; current or planned pregnancy or lactation; weight gain or loss greater than 10 lb within the last 2 mo; major changes in eating habits within the past year; antibiotic use within the past 3 mo; body mass index (BMI) greater than 30 kg/m^2 or less than 18 kg/m^2; exercise regimens that require or result in considerable short-term dietary changes; current use of prescription or over-the-counter medications (including oral contraceptives); known allergies to acetaminophen, aspirin, and any foods used in the feeding trial; regular exposure to passive smoke; occupational exposure to smoke or organic solvents; food dislikes that would preclude participation in the feeding trial; alcohol intake of greater than 2 drinks/day (720 ml beer, 240 ml wine, or 90 ml hard liquor); and no interest in participating in a controlled feeding trial. The Center Institutional Review Board approved the study, and informed consent was obtained from all participants prior to the start of the study.

Participants completed a 3-day food record, a self-administered food frequency questionnaire, health and demographic survey, and provided a fasting morning blood sample for genotyping UGT1A1. Individuals with the desired genotypes and normal serum alanine aminotransferase levels (5–42 U/l) were contacted and invited to participate in the feeding study.

Feeding Study Design

The feeding study of diet and glucuronidation was conducted between April 2002 and May 2005. For a detailed description, see Chang et al. (17). Of the 63 individuals who completed the study, 39 provided lymphocyte samples from both diet periods for the Comet assay. The other 24 individuals had finished the parent study before this ancillary study started.

Study Diets

Participants consumed 2 different diets: a basal diet devoid of F&V and the basal diet supplemented with 10 servings/day of cruciferous vegetables, soy foods, and citrus fruits. Details on the diets and dosing are described in Chang et al. (17). Participants were instructed to consume only the food and beverages provided for them during both diet periods, and use of dietary supplements was not allowed. Breakfast, lunch, and snacks were taken home for consumption, and dinner was served at the Human Nutrition Lab dining room under the supervision of the study staff. Dinner contained the major portion of the test F&V. Overall compliance with the study diet was assessed using daily food check-off forms: each checklist covered all foods on the study diets as well as space to record any additional non study foods consumed. Participants were encouraged to report any deviations from the study diets and were asked to bring back study foods that were consumed incompletely so that the staff dietitian could weigh the amount of leftover food.

Female participants were scheduled for the feeding periods according to time in the menstrual cycle, with the goal that sample collection for each feeding period occurred during the same phase. Women were also asked to keep menstrual-cycle diaries.

Specimen Collection

At Day 15 of each feeding period, one 10-h fasting morning blood was collected in a red-top Vacutainer for serum, and one in a yellow-top tube containing acid citrate dextrose Solution A for lymphocyte extraction for the Comet assay. Both samples were sent to Specimen Processing Lab within 1 h of collection for processing. The serum, aliquotted and stored at −80°C, was used to measure total and direct (conjugated) bilirubin concentrations. The lymphocyte samples were cryopreserved at−70°C.

DNA Damage and Repair Evaluated by the Comet Assay

Chemicals and media—Heat-inactivated newborn calf serum (NCS), penicillin-streptomycin (P/S), Dulbecco's phosphate-buffered saline (PBS), and Iscove's Modified Dulbecco's Medium (IMDM) were purchased from Gibco-Invitrogen (Carlsbad, CA). Low-melting DNA-grade agarose was purchased from Fisher Scientific (Fair Lawn, NJ). CometSlides™ were purchased from Trevigen (Gaithersburg, MD). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO).

Isolation and cryopreservation of human lymphocytes—The procedure of isolation and cryopreservation of human lymphocytes has been described previously (18). Briefly, venous blood was mixed gently with an equal volume of RPMI 1640 × P/S in a conical tube, layered onto 10 ml of lymphocyte separation medium, and centrifuged at 400 *g* for 40 min. The supernatant was aspirated, cells resuspended, and centrifuged at 300 *g* for 10 min. This step was repeated once. The supernatant was decanted and the cells resuspended in chilled freezing medium and transferred to 10 1-ml cryovials. Samples were frozen at−1°C/min and stored at −70°C.

Sample thawing and dilution—Samples from the same participant (2 vials, one from each diet period) were assayed together. The lymphocyte-containing cryogenic vial was retrieved and submerged in a 37°C water bath, gently turning the vials until the last trace of ice was melted. The thawed cells were quickly transferred to a 15-ml conical tube previously placed in a 22°C water bath and diluted with 22°C complete medium (RPMI containing 10% heat-inactivated NCS and 1% P/S) to 5 ml. Sample thawing and dilution were completed in 5 min, immediately followed by the Trypan blue dye exclusion test to assess cell viability.

The Comet assay—The Comet assay was conducted to measure 3 endpoints: endogenous (baseline) DNA damage (strand breaks), sensitivity to γ-irradiation challenge, and DNA repair capacity. Samples from the same participants were run side by side, and in each run, an external quality control set of slides made from a batch of aliquoted lymphocytes from a healthy donor were included. Based on cell number during the Trypan blue dye exclusion test, a fraction of the suspension was diluted in a separate microvial with complete medium to produce a 1-ml suspension that contained 4×10^4 cells. One hundred microliters of the suspension were mixed in 1,000 μl of 0.55% (wt/vol) low-melting-point agarose in RPMI at 37°C. 75 μl of the mixture (4×10^3 cells/ml) were immediately pipetted onto each gel spot on the CometSlide. Each measurement consisted of duplicate slides, and each slide contained 2 gel spots. The slides were set on ice in the dark for 15 min to allow the gel to solidify.

The Comet assay was conducted as previously published (18). Briefly, for endogenous DNA damage, cell-embedded slides were immediately immersed in a freshly prepared ice-cold lysis buffer (0.1 M EDTA; 10 mM Tris; 2.5 M NaCl; 1% N-lauroyl sarcosine, pH 10; 1% Triton X-100; and 10% DMSO). After overnight lysis (18–20 h) in the dark at 4°C, the slides were placed in alkaline electrophoresis buffer (0.3 M NaOH, 1 mM EDTA, pH 13) for 1 h to allow DNA unwinding. The slides were then aligned in a horizontal gel

electrophoresis unit from Ellard Instrumentation (Monroe, WA) containing electrophoresis buffer for electrophoresis at 25V (300 mA) for 20 min in at 4°C in the dark. The slides were subsequently rinsed with water and washed with neutralizing buffer (0.4 M Tris-hydrochloride, pH 7.5) at room temperature for 15 min and immersed in pure prechilled ethanol for 5 min. After air drying, the slides were stored at room temperature until analysis. If crystallization occurred on slides (possibly due to the salt-containing residues from the neutralizing buffer that was not completely washed off), we repeated the experiment with another aliquot.

To evaluate sensitivity to γ-irradiation and DNA repair capacity, 2 sets of cell-embedded slides were exposed to 1.23 Gy of γ-irradiation at 4°C. Subsequently, 1 set of the slides was immersed immediately in ice-cold lysis buffer (this set was used to evaluate sensitivity to γ-irradiation) whereas the other set was incubated at 37°C in 95% air and 5% CO_2 in complete medium for 30 min to allow repair before immersing in lysis buffer. The reduced amount of DNA damage in this set in comparison to the previous set was used to determine DNA repair capacity.

Evaluation of DNA damage and repair—Before scoring, Rthe slides were stained with 50 μl fluorescent dye 10X SYBR® Green. Comet images were recorded using a Nikon Eclipse E400 fluorescent microscope (Melville, NY) equipped with a digital camera from QImaging (Burnaby, British Columbia, Canada). Images were taken and stored by an image analysis system (LAI Comet analysis system version 2.2.2; Loats Associates, West-minister, MD). Tail moment (the product of comet tail length and percent DNA in the tail) and tail moment arm (tail moment divided by the normalized integrated tail intensity to measure the average distance of DNA migration within the tail) were determined from 150 randomly selected cells. To acquire images, the slide was first searched based on upward movement. When the edge of the gel was in proximity, we positioned the view to the right and searched for images on downward movement until we were near the edge of the gel again. The same scoring pattern was repeated until we scored 75 cells for each gel. For each sample, we used the median DNA damage level of the 150 cells (75 cells × 2 samples) for statistical analysis.

The rationale for choosing 1.23 Gy as the irradiation intensity was based on a dose study (0–5 Gy) in which at this intensity, it maintained cell viability, induced sufficient damage to study repair capacity, and at the same time did not generate a substantial number of "hedgehog" or "ghost" cells [discussed in (18)]. Meanwhile, the potential effect of the presence of these hedgehog cells was further taken into account by scoring more cells per slide and then using median instead of mean values for statistical analysis.

Determination of UGT1A1 Genotypes

Genotyping of the UGT1A1 polymorphism was done as described previously in Chang et al. (17). Briefly, a fluorescently labeled forward primer (5′6FAM-GTCACGTGACACAGTCAAAC-3′) and a tailed reverse primer (5′-GTTTCTTTTTGCTCCTGCCAGAGGTT-3′) were used to amplify the promoter region that contained the TA repeat. Amplified fragments of 103 bp, 105 bp, 107 bp, and 109 bp were analyzed using an ABI PRISM 3100 Genetic Analyzer and Genotyper 2.5 software (Applied Biosystems, Foster City, CA).

Determination of Serum Bilirubin

Serum total and direct (conjugated) bilirubin were measured using a Cobas MIRA Plus centrifugal analyzer as described previously in Chang et al. (17). Indirect (unconjugated) bilirubin was calculated by subtracting direct bilirubin from total bilirubin.

Statistical Analysis

Prior to analysis, logarithmic transformations were done on total, direct, and indirect bilirubin concentrations to normalize distributions. We examined the DNA damage variables and found that they were reasonably normally distributed, and the logarithmic transformation did not improve normality; therefore, these variables were not transformed. To test for the effect of the dietary intervention on endogenous DNA damage, γ-irradiation sensitivity, and DNA repair capacity, we used a linear mixed model including diet, UGT1A1 genotype, feeding periods, diet order, age, and sex as fixed effects and participant as a random effect. To assess the correlation between endogenous DNA damage and bilirubin concentration, we used the same statistical model, adding bilirubin and diet-by-bilirubin interaction terms as fixed effects. All the tests were 2-sided. All statistical analyses were performed using Stata version 9.0 (College Station, TX).

RESULTS

Among the 39 pairs of samples, 11 did not yield useable data due to experimental errors (samples did not survive cryopreservation and thawing process, loss of gel during experiment, and undetected DNA repair activity due to unknown factors); therefore, data from only 28 participants (17 women and 11 men) were included in the statistical analysis. Baseline characteristics of the 28 individuals (Table 1) did not differ by diet order, although those who were assigned to basal then F&V had lower BMI and a higher proportion of women than those with periods in the reverse order.

Analysis of moment arm data showed that after adjusting for genotype, age, sex, diet order, and period, there was no effect of F&V diet on the endogenous DNA damage level, γ-irradiation sensitivity, and DNA repair capacity (Table 2). Examining the effect of genotypes using data from both feeding arms combined, however, we found that sensitivity to γ-irradiation and DNA repair capacity were inversely associated with the number of UGT1A1*28 allele (Table 3): individuals with *28/*28 genotype exhibited the lowest sensitivity to γ-irradiation challenge ($P = 0.02$) and the lowest DNA repair capacity ($P = 0.02$). Results for the tail moment analysis showed similar findings except that the inverse association with γ-irradiation sensitivity was not statistically significant.

The potential antioxidant property of bilirubin (19) prompted us to examine its association with lymphocyte endogenous DNA damage. A statistically significant diet-by-bilirubin interaction was detected for total and direct bilirubin ($P = 0.045$ and 0.003, respectively), with the association being inverse (higher bilirubin concentration, lower endogenous DNA damage) during the basal diet period and positive (higher bilirubin concentration, higher DNA damage) during the F&V diet. Examination of the scatter plots of total bilirubin and DNA damage by diet revealed that 2 potential outliers might be driving the correlations (1 outlier in each feeding period). After removing both data points (5.6 SD and 2.7 SD from the mean of endogenous DNA damage), the diet-by-bilirubin interaction was much attenuated for both total and direct bilirubin ($P = 0.82$ and 0.89, respectively). Total bilirubin and DNA damage (without outliers) in both diets suggested a weak inverse association.

DISCUSSION

This randomized, crossover, dietary intervention showed that approximately 10 servings/day of cruciferous vegetables, soy foods, and citrus fruit for 2 wk had no effect on endogenous DNA damage, γ-irradiation sensitivity, and DNA repair capacity measured in peripheral lymphocytes. Several dietary antioxidant intervention studies on DNA damage conducted in the past decade have been summarized in reviews (20,21); overall, the available data suggest that single-dose antioxidant interventions tend to reduce DNA damage in the immediately

subsequent hours, but continuous ingestion of antioxidants produces mixed results. The number of intervention studies that have used food-based approaches in humans is even smaller (20,21). Study designs vary (from sequential treatments to crossover designs to multiple-arm trials) as do the intervention duration and sample size. Results of these studies have also varied, even when similar foods have been used. The only controlled feeding study prior to this randomized 43 participants to 3 different dietary treatment groups (antioxidant-free basal diet, basal diet plus 600 g of F&V, and basal diet plus a supplement containing the corresponding amounts of vitamins and minerals) and reported that 24 days of F&V intervention had no effect on oxidative DNA damage and the expression of DNA-repair genes (22). On the other hand, in the study with the largest sample size and longest treatment period—a randomized, crossover design supplementing the habitual diets of 60 participants with 85 g/day raw watercress for 8 wk—the investigators observed a statistically significant reduction in basal DNA damage and oxidative DNA damage and increased H_2O_2 resistance with the watercress (23). In their review, Moller and Loft (21) suggested that the protective effects of F&V were more observable in oxidatively stressed subjects who exhibited higher baseline DNA damage levels such as diabetics. Thus, the impact of F&V on DNA damage and repair is probably influenced by the type of F&V, the study design, the duration of exposure, and the population being studied, including preexisting illnesses.

Increasing evidence suggests that bilirubin, the end product of heme catabolism and the endobiotic substrate of UGT1A1, contains antioxidant properties in vitro and in vivo (24–26). It has also been suggested that bilirubin may be beneficial in preventing atherosclerosis, cancer, as well as other inflammatory and degenerative diseases (19,27). Environmental factors (e.g., F&V consumption) that modify UGT1A1 activity are likely to affect serum bilirubin concentration and therefore its antioxidative potential. In examining the association between bilirubin concentration and endogenous DNA damage, we observed a diet-by-bilirubin interaction. After omitting 2 potential outliers in our data set, the interaction was attenuated. However, we did not have any a priori explanation for the outliers; therefore, we described both results. A study with a larger sample size that displays a wider range of endogenous DNA-damage levels will be needed to determine the relationship.

In addition to bilirubin, UGT1A1 also conjugates a wide range of substrates with various, sometimes contradictory, physiological functions: Some cause damage to DNA [e.g., N-hydroxy-PhIP (13)], and some have complex functions (e.g., estradiol) with downstream effects possibly associated with oxidative stress. We therefore explored the potential role of UGT1A1*28 polymorphism on DNA damage and repair outcomes. The inverse association of radiation sensitivity and DNA-repair capacity by the number of UGT1A1*28 alleles was unexpected because UGT1A1 is related to phase II biotransformation and has no known physiological function in DNA repair. We speculate that a possible UGT1A1 substrate may have temporary genotoxic effect, causing DNA damage and inhibiting DNA repair. The reduced enzyme activity in UGT1A1*28/*28 would result in higher concentrations of such a genotoxic substrate in the circulation, leading to higher genotoxicity in lymphocyte samples. Further investigation, such as using microarrays to examine gene expression in response to dietary treatments, may shed light on the observed correlation.

A primary strength of this study was the design, as few food-based human dietary interventions investigating DNA damage and repair have used such a controlled crossover. Participant compliance was very good, as described in Chang et al. (17). In addition, with the crossover design, each participant served as his or her own control, hence minimizing the within-subject variation. With the parent study recruiting participants based on UGT1A1 genotypes, we could explore the role of UGT1A1*28 polymorphism on DNA damage and repair measures. The 2-wk wash-out period was chosen based on the outcomes of the parent

study (a study of glucuronidation in humans); the duration is also commonly used in dietary intervention studies using the Comet assay to measure DNA damage endpoints (23,28–31). Another strength is that all slides in this study were scored by one technician to minimize interscorer variation. We scored more cells per slide than most similar studies (see **Subjects and Methods**). As automated slide reading becomes increasing available (32,33), it should help reduce sample variability further.

Our study also had limitations: The use of lymphocytes as the surrogate tissue for DNA damage and repair measures may be less than optimal; it is possible that F&V and bilirubin have more physiological effects in tissues other than the circulating lymphocytes. In addition, although the parent study addressed the potential order effect by randomizing half the participants to a basal-then-F&V order and half to the reserve order with balanced numbers for sex and genotype, this was not the case for this substudy. By the time funding and blood collection for this study began, one-third of the participants had completed the study without providing blood samples for the Comet assay. The baseline characteristics indicated uneven numbers of men and women and in genotype distribution (Table 1) by diet order, although the numbers are only slightly different by diet order. We also adjusted for order in all analyses. As crystallization occurred on some slides, we repeated the experiment with another aliquot. However, at that point, we encountered low cell viability, probably due to long-term storage. Our decision was to not perform the experiment on samples that exhibited cell viability less than 70%. In addition, we did not detect DNA repair in some samples from 5 participants; therefore, their baseline DNA damage, induced damage, and DNA repair capacity were treated as missing values. Concerned that these 5 participants might share certain characteristics, we examined their sex and UGT1A1 genotypes and found that among them were 2 men and 3 women and 1 *1/*1, 2 *1/*28 and 2 *28/*28 in UGT1A1 genotype. This suggests that the undetected repair is unlikely to be attributable to certain sex or genotype differences. This ancillary study has a small sample size; however, it was sufficient to test for differences of a physiologically relevant magnitude if they existed. Post hoc power estimates suggested that we had 80% power to detect a 10% difference with the sample size in our study. Further, we had 78% power to detect the magnitude in DNA repair capacity between UGT1A1 *1/*1 and *28/*28 genotypes (Table 3).

In summary, supplementation of a F&V-free basal diet with cruciferous vegetables, soy foods, and citrus fruits for 2 wk did not affect endogenous DNA damage, γ-irradiation sensitivity, and DNA repair capacity in lymphocytes. The variant *28/*28 genotype of UGT1A1 was associated with reduced γ-irradiation sensitivity and DNA repair capacity compared to the wildtype genotypes; however, the mechanism behind this association is not established. Our study design was rigorous, but larger studies are needed to investigate the association between bilirubin concentrations and endogenous DNA damage. The study participants were relatively young and healthy and were not exposed to oxidative stress and were thus a group in which any protective effect may be less observable.

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TABLE 1

Characteristics of study subjects by diet order[a]

Subjects	All	Basal Then F&V	F&V Then Basal
n	28	15	13
Age (yr)	29.1 ± 5.5	29.6 ± 5.7	28.5 ± 5.5
BMI (kg/m^2)	23.5 ± 2.8	22.5 ± 2.9	24.6 ± 2.3
Sex			
Women	17	10	7
Men	11	5	6
UGT1A1 genotype			
*1/*1	9	5	4
*1/*28	12	6	6
*28/*28	7	4	3

[a]Abbreviations are as follows: F&V, fruit and vegetable; BMI, body mass index; UGT, uridine diphosphate glucuronosyl transferase. Values for continuous variables are means ± SD and for categorical variables are count.

TABLE 2

DNA damage and repair measures by diet[a]

Outcome	Basal Diet	F&V Diet
Moment arm[b]		
Endogenous DNA damage	2.31 ± 0.21	2.30 ± 0.37
After 1.23 Gy γ-irradiation	9.28 ± 2.37	9.41 ± 2.19
After 30-min repair	5.01 ± 1.85	5.12 ± 1.55
DNA repair capacity[c]	46% ± 15%	44% ± 16%
Tail moment[d]		
Endogenous DNA damage	0.053 ± 0.005	0.053 ± 0.007
After 1.23 Gy γ-irradiation	0.306 ± 0.173	0.306 ± 0.161
After 30-min repair	0.112 ± 0.050	0.112 ± 0.042
DNA repair capacity	58% ± 16%	57% ± 19%

[a] Abbreviation is as follows: F&V, fruit and vegetable. Values for baseline damage and γ-irradiation challenge are least-squares means ± SE and for DNA repair capacity are percentages adjusted for diet, sex, age, diet order, and diet period.

[b] Moment arm = tail moment divided by the normalized integrated tail intensity to measure the average distance of DNA migration within the tail).

[c] DNA repair capacity = [(median value of induced damage level−median value of damage level after 30 min of repair)/(median value of induced damage level)] × 100%.

[d] Tail moment = the product of comet tail length and percent DNA in the tail.

TABLE 3

DNA damage and repair measures stratified by UGT1A1 genotype[a]

Endpoints	UGT1A1 *1/*1 (*n* = 9)	UGT1A1 *1/*28 (*n* = 12)	UGT1A1 *28/*28 (*n* = 7)
Moment arm[b]			
Endogenous DNA damage	2.31 ± 0.04	2.30 ± 0.03	2.33 ± 0.03
1.23 Gy γ-irradiation challenge	10.16 ± 0.28	9.43 ± 0.25	8.05 ± 0.33[e]
DNA repair capacity[c]	51% ± 1%	43% ± 1%	36% ± 2%[e]
Tail moment[d]			
Endogenous DNA damage	0.053 ± 0.001	0.053 ± 0.001	0.053 ± 0.001
1.23 Gy γ-irradiation challenge	0.328 ± 0.018	0.326 ± 0.017	0.244 ± 0.023
DNA repair capacity	65% ± 2%	56% ± 2%	47% ± 2%[e]

[a] Abbreviation is as follows: UGT, uridine diphosphate glucuronosyl transferase. Values for baseline damage and γ-irradiation challenge are least-squares means ± SE and for DNA repair capacity are percentages adjusted for diet, sex, age, diet order, and diet period.

[b] Moment arm = tail moment divided by the normalized integrated tail intensity to measure the average distance of DNA migration within the tail.

[c] DNA repair capacity = [(median value of induced damage level − median value of damage level after 30 min of repair)/(median value of induced damage level)] × 100%.

[d] Tail moment = the product of comet tail length and percent DNA in the tail.

[e] Significantly different from UGT1A1 *1/*1 at $P < 0.05$.