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Dietary and demographic correlates of serum β -glucuronidase activity

Sonia S. Maruti^{1,2}, Lin Li¹, Jyh-Lurn Chang¹, JoAnn Prunty¹, Yvonne Schwarz¹, Shuying S. Li¹, Irena B. King¹, John D. Potter^{1,2}, and Johanna W. Lampe^{1,2}

¹Fred Hutchinson Cancer Research Center, Seattle, WA

²Department of Epidemiology, University of Washington, Seattle, WA

Abstract

Background— β -glucuronidase, an acid hydrolase that deconjugates glucuronides, may increase cancer risk; however, little is known about factors associated with human β -glucuronidase.

Objective—To examine whether dietary and demographic factors were associated with serum β -glucuronidase activity.

Design—We conducted a cross-sectional study among 279 healthy men and women, aged 20–40 years. Diet, categorized by botanical families and nutrient intakes, was assessed from 3-day food records and a validated semiquantitative food frequency questionnaire. Demographic factors were directly measured or self-reported. Adjusted mean β -glucuronidase activity across categories of exposure variables were calculated by multiple linear regression.

Results—Higher β -glucuronidase activity was significantly associated with being male, older age (≥ 30 years), non-Caucasian, overweight (≥ 25 kg/m²), and higher intakes of gamma-tocopherol. Conversely, lower β -glucuronidase activity was significantly associated with higher intakes of calcium, iron, and magnesium. A suggestive decrease in β -glucuronidase activity was observed for the botanical families, *Cruciferae*, *Rutaceae*, *Compositae*, *Roseaceae*, and *Umbelliferae*, but tests for trend were not statistically significant.

Conclusions—Several dietary and nondietary factors were associated with β -glucuronidase activity, however confirmation of these associations are needed.

Keywords

Biomarkers; Diet; Dietary Recall; Epidemiology; Micronutrients; Vegetables and Fruits

Introduction

β -glucuronidase is an acid hydrolase that deglucuronidates or cleaves glucuronic acid from substrates making such compounds less water-soluble and less able to be excreted. Substrates of β -glucuronidase include drug and non-drug xenobiotics (e.g., dietary factors, toxins), steroid hormones (e.g., estrogen), and other endogenous compounds (e.g., bilirubin) (1–4). This enzyme is present in most tissues particularly the liver, and is dually localized in the endoplasmic reticulum (ER) and lysosomes (1).

Correspondence and request for reprints to J. Lampe, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue N., P.O. Box 19024, M4-B402, Seattle, WA 98109-1024 (jlampe@fhcrc.org).

S. Maruti is presently at Hoffman La Roche, Inc. Nutley NJ.

β -glucuronidase may increase cancer risk (5,6), because potential carcinogens and promoting agents, once deglucuronidated, are converted back to active parent compounds. These can in turn, recirculate and interact with cells. It has been hypothesized that factors inhibiting β -glucuronidase lower cancer risk (7,8).

Kidney β -glucuronidase in rodents is known to be induced by androgens (9), but little is known about what modulates β -glucuronidase in humans. Plants foods are of particular interest because consumption is associated with lower risk for several human cancers (10,11). According to one proposed mechanism, plant intake may raise levels of circulating D-glucaric acid, a plant constituent and potential β -glucuronidase inhibitor. D-glucaric acid has been shown to significantly reduce chemical carcinogen-mediated mammary, liver, and skin tumors in experimental animal models (12-16). D-glucaric acid is converted into D-glucaro-1,4-lactone, a potent inhibitor of β -glucuronidase (17,18), particularly microsomal β -glucuronidase (19), although other chemoprotective mechanisms have been proposed. D-glucaric acid has been detected in several fruits and vegetables, particularly citrus fruits and cruciferous vegetables (19,20) suggesting candidates for β -glucuronidase-lowering activity. In humans, vegetarians have been observed to have higher urinary excretion of D-glucaric acid than omnivores (21).

Few studies have examined the associations between plant food intake and other non-dietary factors on human β -glucuronidase activity. Previously, we conducted a cross-sectional pilot study of 83 men and 120 women (22) and observed weak inverse associations between intakes of plant foods and nutrients and serum β -glucuronidase activity. In a subsequent controlled feeding study, we observed no β -glucuronidase lowering effect from supplementation of 10 servings/d of soy, citrus fruits and cruciferous vegetables (23). With respect to non-dietary factors, β -glucuronidase has been positively associated with males, increased age, and higher BMI; however, results have been based on a few, small-sampled studies (1).

Knowledge of factors that influence β -glucuronidase activity may clarify potential cancer-related mechanisms. We therefore, conducted a cross-sectional study among 279 healthy men and women to investigate whether dietary and demographic factors were associated with serum β -glucuronidase activity. We primarily focused on botanical food groupings and nutrients hypothesized to alter β -glucuronidase activity (8,21). However, because little is known about dietary correlates of β -glucuronidase activity, we also examined other nutrient intakes. As a cross-sectional study, this investigation was mainly exploratory in nature.

Participants and Methods

We recruited 293 healthy, non-smoking men ($n=147$) and women ($n=146$), aged 20-40 years from the Seattle area between 2002 to 2006 through advertisements in college newspapers, flyers in college buildings, and targeted mailings to individuals identified from the Washington State Department of Licensing. Participants completed an eligibility questionnaire and because this sample was the basis of recruitment for a subsequent dietary feeding study (23), individuals were excluded for the following reasons: 1) medical history of gastrointestinal, hepatic or renal disorders; 2) current or planned pregnancy or lactation; 3) major dietary and/or weight change (>4.5 kg) in the past year; 4) antibiotic use within the past 3 months; 5) BMI >30 or <18 ; 6) current use of over-the-counter, recreational and prescription drugs (including oral contraceptives); 7) regular exposure (including occupational) to passive smoke or organic solvents; 8) alcohol intake >2 drinks/day (720 mL beer, 240 mL wine or 90 mL hard liquor); 9) no interest in participating in the subsequent controlled feeding trial of fruits and vegetables; and, 10) exercise regimens that require or result in significant short-term dietary changes. Additionally, participants were asked to

discontinue use of all multivitamins and dietary supplements one week before participating in the study.

In the analyses, we additionally excluded 14 participants because they had either no data on serum β -glucuronidase activity, no dietary data (from dietary assessments described below), or if their calorie intake was implausibly too low or high (< 600 kcal/d and > 4000 kcal/d for women, < 800 kcal/d and > 5000 kcal/d for men, respectively) as reported on a food frequency questionnaire (FFQ). This left 137 men and 142 women for the analyses. This investigation was approved by the Fred Hutchinson Cancer Research Center (FHCRC) Institutional Review Board, Seattle, Washington. Informed written consent was obtained from all participants prior to the start of the study.

3-day food records (DFR)

Recent diet was obtained from three-day food records (3-DFR) where diet was recorded over three consecutive days. A registered dietitian trained participants on how to keep these food records, instructing participants to record all foods/beverages (including portion size and food preparation) at the time of consumption in specially provided booklets. The 3-DFRs were reviewed for completeness and accuracy. Data was analyzed by the trained nutritionists of the Nutrition Assessment Shared Resource (NASAR) using database developed by the Minnesota Nutrient Data System for Research (University of Minnesota's Nutrition Coordinating Center, Minneapolis, MN) (24). Daily intakes of foods were based on standard serving sizes (1 cup raw, $\frac{1}{2}$ cup cooked or canned, $\frac{1}{2}$ cup juice, etc.). Servings/day of fruits and vegetables were calculated as previously described (25,26) and classified into 63 botanical families. We analyzed nine botanical families because they included foods known to contain D-glucaric acid (e.g., *Cruciferae*, *Rutaceae*, *Solanaceae*, *Leguminosae*, *Compositae*, *Roseaceae*, *Umbelliferae*) (19,20) or foods with phytochemicals known to influence other biotransformation enzymes (e.g. *Liliaceae*). Examples of common foods in each family are provided in Table 3. The average daily intake of dietary nutrients was calculated by multiplying the frequency of each food/beverage item by its nutrient content and summing the nutrient contributions of all foods or beverages. Daily intakes of botanicals and nutrients were averaged over all three days.

3-month FFQ and demographic data

Longer-term diet was assessed by FFQs in which participants self-reported their habitual intakes of foods and beverages over the past 3 months. This FFQ was modeled after the previously validated FFQ from the Women's Health Initiative (27) and was designed to reflect U.S. food consumption patterns. Participants reported their usual frequency from "never or less than once per month" to " ≥ 2 per day" for foods and " ≥ 6 per day" for beverages and portion size (small, medium, or large) of approximately 120 foods and beverages; they also answered 12 adjustment questions. Twenty-two of these questions were specific to vegetable intake and 12 to fruit and fruit juices. Nutrient intakes from foods were derived from a nutrient database developed by the Minnesota Nutrient Data System for Research (University of Minnesota's Nutrition Coordinating Center, Minneapolis, MN) (24). The average daily intake of dietary nutrients was calculated by multiplying the adjusted serving frequency (calculated as frequency times portion size) of each food/beverage item by its nutrient content and summing the nutrient contributions of all foods or beverages. Demographic data - age, race, and sex - were obtained from a health and demographics questionnaire. Body weight and height were measured by study personnel.

Serum β -glucuronidase Activity

A 10-hour fasting blood sample was drawn from participants for serum. We used serum β -glucuronidase because it reflects tissue β -glucuronidase resulting from cell turnover,

particularly from the liver (28) which is the main source of this enzyme, and because serum collection requires minimally invasive methods. β -glucuronidase activity from serum was determined using a modification of the Sigma β -glucuronidase procedure (Sigma-Aldrich Company, St. Louis, MO) using methods described in detail previously (23). Briefly, serum was incubated in the presence of phenolphthalein glucuronic acid. The free phenolphthalein was then measured spectrophotometrically. In this manuscript, β -glucuronidase activity is described as μg phenolphthalein released per ml of serum per hr at 37°C . The intra- and inter-assay coefficient of variation (CV) were 2.9% and 5.7%, respectively.

Statistical Analyses

Before all analyses, β -glucuronidase activity was log-transformed to improve normality. To describe dietary intakes and demographic characteristics of participants, raw means and frequencies were calculated (Table 1 and 2). Two-sample t-tests, for continuous data, and chi-square tests, for categorical data, were used to assess whether there was a statistical difference by sex. We used multivariate linear regression to obtain adjusted least-square means of β -glucuronidase activity by categories of dietary intake (Tables 3 and 4). Our dietary exposures were daily servings of botanicals and nutrients. Nutrient intake was categorized into quartiles. Because consumption of some botanicals was low, we categorized data were based on the distribution of intake. Thus, if most participants ($>75\%$) consumed a botanical, intake was divided into quartiles. However, if $<50\%$ participants consumed any of a botanical (e.g., *Compositae*), participants were dichotomized as non-consumer (no intake) versus consumer; otherwise, if $\geq 50\%$ but $<75\%$ consumed a botanical, participants were dichotomized by median intake (e.g., *Cruciferae*, *Rutaceae*, and *Cucurbitaceae*). The adjusted variables included age, sex, race, and BMI which were significantly associated with β -glucuronidase activity in univariate linear regression analyses. We additionally adjusted for total energy intake in analyses of nutrient intakes (Table 4) (29). To facilitate interpretation, the log-back-transformed (exponentiated) least-squared means of β -glucuronidase activity and associated standard deviations are presented. Tests for trend were calculated by modeling the exposures ordinally with the P-value obtained from a F-test. Tests of interaction by sex were performed by entering product terms in a multivariable-adjusted model and using a F-test to obtain a P-value. All statistical tests were 2-sided and $P < 0.05$ considered statistically significant. Analyses were conducted using SAS, version 9.1 (SAS Institute, Cary, NC).

Results

Participants were mostly Caucasian (74%) and, given the study inclusion criteria, had on average, a normal BMI (mean = 23.5 kg/m^2) (Table 1). Compared to women, men had significantly higher BMI ($P = 0.0013$) and as expected, higher energy intake ($P = 0.0001$), but significantly lower carbohydrate intake ($P = 0.06$) and lower consumption of total vegetables ($P = 0.0006$). On average, participants consumed approximately four daily servings of fruits and vegetables. The mean of serum β -glucuronidase activity in the total sample was $5.68\text{ }\mu\text{g/ml/hr}$.

For nondietary factors, higher β -glucuronidase activity was significantly associated with being male, Asian, and overweight ($\text{BMI} \geq 25\text{ kg/m}^2$) in univariate analyses of the total sample (Table 2). In analyses stratified by sex, age was statistically significantly associated with β -glucuronidase activity among men ($P = 0.0002$) but not in women ($P = 0.48$). Moreover, among women, other race was significantly associated with higher β -glucuronidase activity compared with Caucasian or Asian race ($P = 0.009$); however, with only 13 women in the other race group, results may have been due to chance.

We next investigated whether botanicals that contain D-glucaric acid were associated with β -glucuronidase activity (Table 3). We used intakes from 3-DFR, in which participants reported individual meals, foods, and beverages, because this data enabled more accurate classification of botanicals than the FFQ, which inquired about commonly eaten foods in a semiquantitative format. The range of daily intakes for *Cruciferae*, *Rutaceae*, *Compositae*, *Cucurbitaceae* were low requiring them to be dichotomized (see Methods). Higher intakes of most botanicals were suggestively associated with lower mean β -glucuronidase activity. The test for trend for total botanicals (i.e., all 63 botanical families) was at near significance (P for trend=0.06; mean β -glucuronidase activity across quartiles of dietary intake: $Q_1=6.60$ $\mu\text{g/ml/hr}$; $Q_2=7.18$; $Q_3=6.17$; $Q_4=6.05$) and suggestive for *Liliaceae* (P for trend=0.07; mean β -glucuronidase activity: $Q_1=6.73$ $\mu\text{g/ml/hr}$; $Q_2=6.63$; $Q_3=6.86$; $Q_4=6.51$) but not statistically significant for other botanicals. Qualitatively, comparing extreme intakes (i.e., highest versus lowest groups), a negative percent difference in β -glucuronidase activity was observed for most botanicals, including *Cruciferae* (-2.3%) and *Rutaceae* (-3.4%); exceptions were for intakes of *Solanaceae* (10.7%) and *Leguminosae* (4.8%) where we observed higher activity.

Because sex-differences have been observed in biotransformation enzymes (30,31), we investigated whether β -glucuronidase response to botanicals differed by women versus men (Table 3). Women were significantly different from men in their response to *Compositae* (P for interaction=0.04), with a suggestive positive association among women but a negative association among men. Tests of interaction by sex were not statistically significant for the other botanicals (Table 3).

We next examined the influence of nutrients found in plant foods and other major nutrients in relation to β -glucuronidase activity (Table 4). We used nutrient data from the 3-DFR (recent diet) and FFQs, which represented diet over a longer period of time (i.e., 3 months). Associations with β -glucuronidase activity using dietary data from the 3-DFR were generally similar to those reported on the FFQ, and to facilitate reporting, results from 3-DFR are generally described here. Lower β -glucuronidase activity was statistically significantly associated with higher intakes of energy (P for trend $_{\text{DFR}}=0.04$), the minerals calcium (P for trend $_{\text{DFR}}=0.03$), iron (P for trend $_{\text{DFR}}=0.005$ and P for trend $_{\text{FFQ}}=0.02$), and magnesium (P for trend $_{\text{FFQ}}=0.01$); there was also a marginally nonsignificant inverse association for phosphorous (P for trend $_{\text{DFR}}=0.06$). Interestingly, higher β -glucuronidase activity was statistically significantly associated with increased intakes of the plant nutrient, gamma-tocopherol in the FFQ (P for trend $_{\text{FFQ}}=0.03$; $Q_1=5.86$ $\mu\text{g/ml/hr}$; $Q_2=6.36$; $Q_3=6.19$; $Q_4=7.40$) and there was a suggestive but not significant positive trend for data reported from the DFR (P for trend =0.13). Qualitatively, comparing extreme intakes (i.e., fourth versus first quartiles), a negative percent difference in β -glucuronidase activity was also observed for many of the other nutrients, including nutrients in plants such as alpha-carotene (-9%, from 3-DFR), beta-carotene (-8.3% from 3-DFR), beta-cryptoxanthin (-4.6% from 3-DFR), and alpha-tocopherol (-6.3% from 3-DFR). In contrast, total fat, saturated fat, gamma-tocopherol, and caffeine appeared to be positively associated with β -glucuronidase activity (9.8%, 16.5%, 13.1%, 5.3% respectively from 3-DFR). Interaction by sex were not statistically significant for any of the nutrients except for beta-cryptoxanthin (P for interaction $_{\text{FFQ}}=0.01$); for this nutrient, there was a suggestive positive association for women (for FFQ: $Q_1=6.02$ $\mu\text{g/ml/hr}$; $Q_2=5.76$; $Q_3=5.32$; $Q_4=6.61$) but a possible negative association for men (for FFQ: $Q_1=7.08$ $\mu\text{g/ml/hr}$; $Q_2=7.13$; $Q_3=8.17$; $Q_4=6.52$).

Discussion

In this cross-sectional study, we investigated dietary and non-dietary correlates of serum β -glucuronidase activity. We observed suggestive inverse associations between several D-

glucaric acid-containing botanical families and plant-related nutrients and β -glucuronidase activity but these did not reach statistical significance. The results were consistent with our earlier pilot study (22), in which we observed inverse correlations for serum β -glucuronidase activity with intakes of plant protein, dietary fiber, and the botanical groupings containing foods with D-glucaric acid. These results tentatively support our original hypothesis that natural dietary sources of D-glucaric acid are associated with lower β -glucuronidase activity.

Several non-dietary factors were associated with β -glucuronidase activity. Our finding of a positive association in men is consistent with reports in humans (22,32) and other data. For instance, in rodents, androgens are known to increase rates of β -glucuronidase gene transcription in the kidney (9,33). In a human cell line, 17 β -estradiol treatment decreased secreted β -glucuronidase in a dose-dependent manner, potentially explaining lower β -glucuronidase activity in females (34). Our discrepant finding that higher energy intake was associated with lower β -glucuronidase activity but being overweight ($\text{BMI} \geq 25 \text{ kg/m}^2$) was associated with higher enzyme activity is not clear. Our results for a positive association between BMI and β -glucuronidase activity is supported by at least one other report (32). Inflammation may be one mechanism potentially underlying our positive finding for being overweight; immune cells (e.g., macrophages, neutrophils, etc.) release lysosomal β -glucuronidase at sites of inflammation (1,35,36), and obesity is associated with higher concentrations of inflammatory markers (37). The positive association between age and β -glucuronidase activity, despite the narrow age range of our study population, is consistent with some but not all studies in humans (1,32); in rodents, increased leakage of lysosomal enzymes has been observed with older age (38). This is the first study, to our knowledge, reporting on race and β -glucuronidase activity; differences in enzymatic activity by race may point to underlying genetic polymorphisms.

Although we observed a positive association between gamma-tocopherol and β -glucuronidase activity, we did not observe increased β -glucuronidase activity with intakes of alpha-tocopherol, which like gamma-tocopherol is a form of vitamin E. No other study, to our knowledge, has examined gamma-tocopherol in relation to this enzyme and the mechanisms are unclear. Additionally, we did not observe a significant association between ascorbic acid intake and serum β -glucuronidase activity. This is in contrast with the results of Young et al (39) who reported that oral supplementation of ascorbic acid (1500 mg/d) lowered urinary β -glucuronidase levels by 24% in a double-blind crossover study of 17 healthy male volunteers. The divergence with our results may be due to differences in β -glucuronidase activity in urine versus serum or due to different intakes of ascorbic acid.

For minerals, intakes of calcium, iron, magnesium and potentially phosphorous were associated with lower serum β -glucuronidase activity. Typical food sources of these nutrients are meats, and we observed a nonsignificant inverse association for animal protein. Consistent with our results, in human cell lines, compounds that raise intracellular Ca^{2+} concentrations (the calcium ionopore A23187 and separately, the calcium ATPase inhibitor, thapsigargin) reduced β -glucuronidase activity (40); one proposed mechanism is the decrease of β -glucuronidase transcription. However, contrary to our results, two studies showed that administration of ionized calcium Ca^{2+} in the physiological range (0.1- 5 μM) increased β -glucuronidase activity in rat livers (8,41). We do not know of other investigations examining iron, magnesium, and phosphorous in relation to β -glucuronidase activity. A complicating factor is that, in humans, calcium and other minerals are highly regulated. Nonetheless, the association of several divalent cations with β -glucuronidase activity warrants further investigation.

This study has several strengths. To our knowledge, this is the first study examining a broad range of botanical and nutrient intakes, including mineral intakes, in relation to serum β -

glucuronidase activity in humans. In addition, the 3-DFR gave us the ability to examine recent diet and the FFQ data gave us an ability to assess 3-month habitual diet.

This study has several limitations. While this study is the largest study to date, results were based on limited sample size and may be one reason for our lack of significant results. The number of participants in this study was based on findings of our earlier pilot study. However, post-hoc calculations showed that we needed 110 participants per quartile (or 440 people total) to observe a statistically significant effect comparing the quartiles of total botanicals with the highest and lowest mean β -glucuronidase activity with 80% power. Second, few participants were in the extreme categories of fruit and vegetable intake; potentially higher intakes of fruit and vegetables eaten habitually would have provided greater biologically relevant concentrations of D-glucaric acid. Moreover, diet from food records may not reflect bioavailability of some nutrients. Future studies may want to examine plant nutrient levels from serum as a more accurate measure of some dietary exposures. Third, some statistically significant results may be due to chance because of the high number of statistical tests we conducted. Still, the p-values for BMI and sex were very low and still may be considered significant considering a false discovery rate. Potentially, non-dietary factors may be more important or stronger than dietary effects. Fourth, the strict selection criteria may have limited the generalizability of results; however, this also enabled us to assess associations in a well-characterized population. Lastly, we assessed β -glucuronidase from serum, because blood draws are minimally invasive compared to liver biopsies, the gold standard, and activity is correlated. However, serum β -glucuronidase activity is much lower than in the liver (32) and may underestimate hepatic enzyme activity.

Further study of the human β -glucuronidase gene and genetic polymorphisms may provide insight into the mechanisms of xenobiotic regulation of the enzyme. The human β -glucuronidase gene is on chromosome 7 with 12 exons (42). Analysis of the 5'-flanking region suggests binding sites for 3 ubiquitous transcription factors which may have possible roles in regulating β -glucuronidase: nuclear factor κ B (NF κ B), activating protein-2 (AP-2), and specificity protein 1 (Sp1) (cited in (43)). Over 20 polymorphisms leading to a total or near total lack of β -glucuronidase activity have been found among patients with mucopolysaccharidosis type VII (MPS VII), a lysosomal storage disorder resulting from the accumulation of undegraded glycosaminoglycans (44). Among healthy people without MPSVII, a 193-person study (32) examined β -glucuronidase from plasma and reported 6 genetic variants among those participants in the top and bottom 10% of β -glucuronidase activity; three of these variants were associated with altered activity or expression of β -glucuronidase. Whether these polymorphisms are common and how they may influence β -glucuronidase activity in the general population remains to be determined.

There are many gaps in our understanding of β -glucuronidase activity in humans. While data on β -glucuronidase activity and cancer risk in animal models have accumulated, there is no study, to our knowledge, that has examined β -glucuronidase activity and cancer risk among humans. A case-control or cohort study would be useful in examining the association between enzyme activity and cancer risk in humans. Additional long-term human intervention studies investigating effects of plant food intake on β -glucuronidase activity and circulating D-glucaric acid concentrations concurrently are warranted.

In conclusion, these results tentatively support the idea that nondietary and dietary factors may be associated β -glucuronidase activity and suggest the possibility of lifestyle modification of β -glucuronidase activity. However, there are many gaps in our understanding of β -glucuronidase activity in humans. Confirmation of these associations and an understanding of underlying mechanisms are important.

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References

1. Costas, I. *Enzyme Systems that Metabolise Drugs and Other Xenobiotics*. Chichester, UK: John Wiley and Sons; 2002.
2. Zhu BT, Evaristus EN, Antoniak SK, Sarabia SF, Ricci MJ, et al. Metabolic deglucuronidation and demethylation of estrogen conjugates as a source of parent estrogens and catecholestrogen metabolites in Syrian hamster kidney, a target organ of estrogen-induced tumorigenesis. *Toxicol Appl Pharmacol* 1996;136:186–93. [PubMed: 8560473]
3. O'Leary KA, Day AJ, Needs PW, Sly WS, O'Brien NM, et al. Flavonoid glucuronides are substrates for human liver beta-glucuronidase. *FEBS Lett* 2001;503:103–6. [PubMed: 11513863]
4. Whiting JF, Narciso JP, Chapman V, Ransil BJ, Swank RT, et al. Deconjugation of bilirubin-IX alpha glucuronides: a physiologic role of hepatic microsomal beta-glucuronidase. *J Biol Chem* 1993;268:23197–201. [PubMed: 8226839]
5. Dwivedi C, Downie AA, Webb TE. Net glucuronidation in different rat strains: importance of microsomal beta-glucuronidase. *Faseb J* 1987;1:303–7. [PubMed: 3115856]
6. Hanausek M, Walaszek Z, Slaga TJ. Detoxifying cancer causing agents to prevent cancer. *Integr Cancer Ther* 2003;2:139–44. [PubMed: 15035900]
7. Walaszek Z. Potential use of D-glucaric acid derivatives in cancer prevention. *Cancer Lett* 1990;54:1–8. [PubMed: 2208084]
8. Whittaker M, Sokolove PM, Thurman RG, Kauffman FC. Stimulation of 3-benzo[a]pyrenyl glucuronide hydrolysis by calcium activation of microsomal beta-glucuronidase. *Cancer Lett* 1985;26:145–52. [PubMed: 3978604]
9. Paigen K. Mammalian beta-glucuronidase: genetics, molecular biology, and cell biology. *Prog Nucleic Acid Res Mol Biol* 1989;37:155–205. [PubMed: 2672109]
10. Vainio H, Weiderpass E. Fruit and vegetables in cancer prevention. *Nutr Cancer* 2006;54:111–42. [PubMed: 16800779]
11. Riboli E, Norat T. Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk. *Am J Clin Nutr* 2003;78:559S–569S. [PubMed: 12936950]
12. Dwivedi C, Downie AA, Webb TE. Modulation of chemically initiated and promoted skin tumorigenesis in CD-1 mice by dietary glucarate. *J Environ Pathol Toxicol Oncol* 1989;9:253–9. [PubMed: 2509679]
13. Dwivedi C, Oredipe OA, Barth RF, Downie AA, Webb TE. Effects of the experimental chemopreventative agent, glucarate, on intestinal carcinogenesis in rats. *Carcinogenesis* 1989;10:1539–41. [PubMed: 2752528]
14. Singh J, Gupta KP. Calcium glucarate prevents tumor formation in mouse skin. *Biomed Environ Sci* 2003;16:9–16. [PubMed: 12747003]
15. Abou-Issa H, Moeschberger M, el-Masry W, Tejwani S, Curley RW Jr, et al. Relative efficacy of glucarate on the initiation and promotion phases of rat mammary carcinogenesis. *Anticancer Res* 1995;15:805–10. [PubMed: 7645962]
16. Walaszek Z, Hanausek-Walaszek M, Webb TE. Dietary glucarate-mediated reduction of sensitivity of murine strains to chemical carcinogenesis. *Cancer Lett* 1986;33:25–32. [PubMed: 3768860]
17. Horton D, Walaszek Z. Conformations of the D-glucarolactones and D-glucaric acid in solution. *Carbohydr Res* 1982;105:95–109.
18. Levvy GA. The preparation and properties of beta-glucuronidase. IV. Inhibition by sugar acids and their lactones. *Biochem J* 1952;52:464–72. [PubMed: 13018259]
19. Dwivedi C, Heck WJ, Downie AA, Larroya S, Webb TE. Effect of calcium glucarate on beta-glucuronidase activity and glucarate content of certain vegetables and fruits. *Biochem Med Metab Biol* 1990;43:83–92. [PubMed: 2346674]

20. Walaszek ZS J, Hanausak M, Adams AK, Sherman U. D-Glucaric acid content of various fruits and vegetables and cholesterol-lowering effects of dietary D-glucarate in the rat. *Nutrition Research* 1996;16:673–682.
21. Kyle E, Carper A, Bowen P. Caffeine consumption and vegetarian diets affect D-glucaric acid excretion in man. *Nutrition Research* 1987;7:461–470.
22. Lampe JW, Li SS, Potter JD, King IB. Serum beta-glucuronidase activity is inversely associated with plant-food intakes in humans. *J Nutr* 2002;132:1341–4. [PubMed: 12042456]
23. Maruti SS, Chang JL, Prunty JA, Bigler J, Schwarz Y, et al. Serum beta-glucuronidase activity in response to fruit and vegetable supplementation: a controlled feeding study. *Cancer Epidemiol Biomarkers Prev* 2008;17:1808–12. [PubMed: 18628435]
24. Schakel SF, Sievert YA, Buzzard IM. Sources of data for developing and maintaining a nutrient database. *J Am Diet Assoc* 1988;88:1268–71. [PubMed: 3171020]
25. Horner NK, Kristal AR, Prunty J, Skor HE, Potter JD, et al. Dietary determinants of plasma enterolactone. *Cancer Epidemiol Biomarkers Prev* 2002;11:121–6. [PubMed: 11815409]
26. Kristal AR, Vizenor NC, Patterson RE, Neuhouser ML, Shattuck AL, et al. Precision and bias of food frequency-based measures of fruit and vegetable intakes. *Cancer Epidemiol Biomarkers Prev* 2000;9:939–44. [PubMed: 11008912]
27. Patterson RE, Kristal AR, Tinker LF, Carter RA, Bolton MP, et al. Measurement characteristics of the Women's Health Initiative food frequency questionnaire. *Ann Epidemiol* 1999;9:178–87. [PubMed: 10192650]
28. Kim DH, Shim SB, Kim NJ, Jang IS. Beta-glucuronidase-inhibitory activity and hepatoprotective effect of *Ganoderma lucidum*. *Biol Pharm Bull* 1999;22:162–4. [PubMed: 10077435]
29. Willett, W. *Nutritional Epidemiology*. Second Edition. New York City: Oxford University Press; 1998.
30. Chang JL, Bigler J, Schwarz Y, Li SS, Li L, et al. UGT1A1 polymorphism is associated with serum bilirubin concentrations in a randomized, controlled, fruit and vegetable feeding trial. *J Nutr* 2007;137:890–7. [PubMed: 17374650]
31. Anderson GD. Sex differences in drug metabolism: cytochrome P-450 and uridine diphosphate glucuronosyltransferase. *J Gend Specif Med* 2002;5:25–33. [PubMed: 11859684]
32. Gratz M, Kunert-Keil C, John U, Cascorbi I, Kroemer HK. Identification and functional analysis of genetic variants of the human beta-glucuronidase in a German population sample. *Pharmacogenet Genomics* 2005;15:875–81. [PubMed: 16272959]
33. Niermann GL, Watson GL. Growth hormone and insulin-like growth factor-I enhance beta-glucuronidase gene activation by androgen in mouse kidney. *Mol Cell Endocrinol* 1999;153:47–55. [PubMed: 10459853]
34. Kremer M, Judd J, Rifkin B, Auszmann J, Oursler MJ. Estrogen modulation of osteoclast lysosomal enzyme secretion. *J Cell Biochem* 1995;57:271–9. [PubMed: 7759564]
35. Triggiani M, Granata F, Oriente A, De Marino V, Gentile M, et al. Secretory phospholipases A2 induce beta-glucuronidase release and IL-6 production from human lung macrophages. *J Immunol* 2000;164:4908–15. [PubMed: 10779801]
36. Shimoi K, Nakayama T. Glucuronidase deconjugation in inflammation. *Methods Enzymol* 2005;400:263–72. [PubMed: 16399354]
37. Browning LM. n-3 Polyunsaturated fatty acids, inflammation and obesity-related disease. *Proc Nutr Soc* 2003;62:447–53. [PubMed: 14506893]
38. Chondrogianni N, Fragoulis EG, Gonos ES. Protein degradation during aging: the lysosome-, the calpain- and the proteasome-dependent cellular proteolytic systems. *Biogerontology* 2002;3:121–3. [PubMed: 12014830]
39. Young JC, Kenyon EM, Calabrese EJ. Inhibition of beta-glucuronidase in human urine by ascorbic acid. *Hum Exp Toxicol* 1990;9:165–70. [PubMed: 2375883]
40. Sperker B, Tomkiewicz C, Burk O, Barouki R, Kroemer HK. Regulation of human beta-glucuronidase by A23187 and thapsigargin in the hepatoma cell line HepG2. *Mol Pharmacol* 2001;59:177–82. [PubMed: 11160851]
41. Sokolove PM, Wilcox MA, Thurman RG, Kauffman FC. Stimulation of hepatic microsomal beta-glucuronidase by calcium. *Biochem Biophys Res Commun* 1984;121:987–93. [PubMed: 6743323]

42. Speleman F, Vervoort R, van Roy N, Liebaers I, Sly WS, et al. Localization by fluorescence in situ hybridization of the human functional beta-glucuronidase gene (GUSB) to 7q11.21 --> q11.22 and two pseudogenes to 5p13 and 5q13. *Cytogenet Cell Genet* 1996;72:53–5. [PubMed: 8565635]
43. Kunert-Keil C, Sperker B, Bien S, Wolf G, Grube M, et al. Involvement of AP-2 binding sites in regulation of human beta-glucuronidase. *Naunyn Schmiedebergs Arch Pharmacol* 2004;370:331–9. [PubMed: 15526106]
44. Tomatsu S, Fukuda S, Sukegawa K, Ikeda Y, Yamada S, et al. Mucopolysaccharidosis type VII: characterization of mutations and molecular heterogeneity. *Am J Hum Genet* 1991;48:89–96. [PubMed: 1702266]

Table 1Characteristics and daily dietary intakes of participants^{a,b}

	Total (n=279)	Women (n=142)	Men (n=137)	P-value ^c
Age, y	27.6 ± 5.65	27.8 ± 5.49	27.5 ± 5.82	0.65
Race, n (%)				
Caucasian	207 (74)	105 (74)	102 (74)	0.66
Asian	43 (15)	24 (17)	19 (14)	
Other	29 (10)	13 (9)	16 (12)	
BMI, kg/m ²	23.5 ± 2.88	22.9 ± 2.78	24.0 ± 2.88	0.0013
Energy, kcal/d	1840 ± 686	1682 ± 582	2000 ± 747	0.0001
Total fat, % energy	31.7 ± 7.34	30.8 ± 7.49	32.5 ± 7.11	0.05
Total protein, % energy	15.5 ± 2.67	15.8 ± 2.67	15.2 ± 2.66	0.10
Carbohydrate, % energy	53.8 ± 8.76	54.9 ± 8.89	52.7 ± 8.52	0.04
Total dietary fiber (g/day)	21.7 ± 11.2	21.9 ± 10.1	21.5 ± 12.2	0.79
Alcohol intake (g/day)	4.78 ± 8.78	3.77 ± 4.89	5.81 ± 11.4	0.06
Food groups, servings/day				
Total fruits	2.38 ± 2.15	2.25 ± 1.75	2.51 ± 2.48	0.34
Total vegetables	1.99 ± 1.49	2.30 ± 1.45	1.68 ± 1.47	0.0006

^aDietary data from 3-month food frequency questionnaire (FFQ) to represent habitual intake.^bMean ± SD presented except for race.^cP-value for testing whether values differed by sex (P<0.05, significant); P-values generated from a two-sample t-test (continuous variables) or Chi-square test (race).

Table 2Mean β -glucuronidase activity (SD), by characteristics of participants^{a,b}

Characteristic	β -glucuronidase activity ($\mu\text{g/ml/hr}$)		
	Total	Women	Men
Sex			
Men	6.34 $\pm$ 2.69	-	-
Women	5.11 $\pm$ 2.15	-	-
P-value	<0.0001		
Age			
<30 years	5.49 $\pm$ 2.14	5.20 $\pm$ 2.07	5.80 $\pm$ 2.18
$\geq$ 30 years	6.16 $\pm$ 3.18	4.92 $\pm$ 2.30	7.75 $\pm$ 3.60
P-value	0.07	0.48	0.0002
Race			
Caucasian	5.46 $\pm$ 2.34	4.81 $\pm$ 1.96	6.23 $\pm$ 2.56
Asian	6.51 $\pm$ 2.91	5.81 $\pm$ 2.29	7.52 $\pm$ 3.59
Other	6.16 $\pm$ 2.66	6.60 $\pm$ 2.97	5.82 $\pm$ 2.46
P-value	0.03	0.009	0.14
BMI, kg/m^2			
<25, underweight or normal	5.39 $\pm$ 2.27	4.94 $\pm$ 1.94	5.98 $\pm$ 2.58
$\geq$ 25 overweight	6.62 $\pm$ 2.91	5.82 $\pm$ 2.86	7.26 $\pm$ 2.75
P-value	0.0005	0.06	0.35

^a Unadjusted means of back-log-transformed β -glucuronidase activity and are presented with standard deviations (SD)^b P-value for testing association between characteristics and β -glucuronidase activity; P-values were generated from a two-sample t-test or ANOVA (race).

Mean serum β -glucuronidase activity ($\mu\text{g/ml/hr}$) by daily intakes of botanical families as calculated from average of 3-days of food records (3-DFR)^{a,b,c}

Table 3

Daily intakes	β -glucuronidase activity ($\mu\text{g/ml/hr}$)					
	Total		Women		Men	
	Adjusted Mean (SD)	% Difference from lowest category ^d	Cutoffs	Adjusted Mean (SD)	Cutoffs	Adjusted Mean (SD)
Total botanicals (all 63 botanical families)						
Q1, ≤ 3.86	6.60 (3.09)	ref	Q1, ≤ 4.14	6.59 (2.94)	Q1, ≤ 3.56	6.96 (3.31)
Q2, $>3.86 - \leq 5.39$	7.18 (3.39)	8.82%	Q2, $>4.14 - \leq 5.52$	6.59 (3.26)	Q2, $>3.56 - \leq 5.22$	7.55 (3.42)
Q3, $>5.39 - \leq 7.17$	6.17 (2.99)	-6.46%	Q3, $>5.52 - \leq 7.13$	5.39 (2.66)	Q3, $>5.22 - \leq 7.34$	7.08 (3.17)
Q4, >7.17	6.05 (2.86)	-8.3%	Q4, >7.13	5.49 (2.59)	Q4, >7.34	6.82 (3.22)
P for trend	0.06			0.01		0.69
P for interaction by sex ^f	0.36					
<i>Cruciferae</i> (crucifers e.g., broccoli, cabbage)						
Median, ≤ 0.06	6.57 (3.60)	ref	Median, ≤ 0.15	6.29 (3.53)	Median, ≤ 0.03	7.07 (3.71)
Median, > 0.06	6.41 (3.46)	-2.34%	Median, >0.15	5.82 (3.16)	Median, >0.03	7.17 (3.66)
P comparing 2 groups ^e	0.64			0.26		0.84
P for interaction by sex ^f	0.53					
<i>Rutaceae</i> (citrus e.g., oranges, grapefruit, lemons)						
Median, ≤ 0.33	6.60 (3.57)	ref	Median, ≤ 0.28	6.18 (3.43)	Median, ≤ 0.33	7.22 (3.65)
Median, >0.33	6.38 (3.45)	-3.39%	Median, > 0.28	5.90 (3.23)	Median, >0.33	7.02 (3.67)
P comparing 2 groups ^e	0.49			0.50		0.69
P for interaction by sex ^f	0.73					
<i>Solanaceae</i> (tomatoes, peppers; potatoes excluded)						
Q1, ≤ 0.34	6.08 (2.81)	ref	Q1, ≤ 0.34	5.91 (2.86)	Q1, ≤ 0.35	6.44 (2.69)
Q2, $>0.34 - \leq 0.69$	6.60 (3.18)	8.55%	Q2, $>0.34 - \leq 0.62$	6.18 (2.99)	Q2, $>0.35 - \leq 0.77$	7.83 (3.71)
Q3, $>0.69 - \leq 1.20$	6.73 (3.50)	10.6%	Q3, $>0.62 - \leq 1.10$	6.21 (3.13)	Q3, $>0.77 - \leq 1.29$	6.43 (3.06)
Q4, >1.20	6.73 (3.17)	10.7%	Q4, >1.10	5.89 (2.82)	Q4, >1.29	8.06 (3.59)
P for trend	0.15			0.97		0.14
P for interaction by sex ^f	0.63					
<i>Leguminosae</i> (legumes, alfalfa sprouts)						

Daily intakes	β -glucuronidase activity ($\mu\text{g/ml/hr}$)					
	Total			Women		
	Adjusted Mean (SD)	% Difference from lowest category ^d	Cutoffs	Adjusted Mean (SD)	Cutoffs	Adjusted Mean (SD)
Q1, ≤ 0.29	6.21 (2.93)	ref	Q1, ≤ 0.31	6.07 (2.87)	Q1, ≤ 0.26	6.68 (3.05)
Q2, $>0.29 - \leq 0.68$	6.89 (3.31)	11.0%	Q2, $>0.31 - \leq 0.71$	6.54 (3.26)	Q2, $>0.26 - \leq 0.67$	7.38 (3.35)
Q3, $>0.68 - \leq 1.49$	6.39 (3.12)	2.98%	Q3, $>0.71 - \leq 1.54$	6.16 (3.14)	Q3, $>0.67 - \leq 1.39$	7.08 (3.31)
Q4, >1.49	6.51 (3.12)	4.8%	Q4, >1.54	5.58 (2.59)	Q4, >1.39	7.38 (3.53)
P for trend	0.76			0.33		0.41
P for interaction by sex ^f	0.15					
<i>Liliaceae</i> (onions)						
Q1, ≤ 0.07	6.73 (3.18)	ref	Q1, ≤ 0.09	6.12 (2.97)	Q1, ≤ 0.06	7.28 (3.11)
Q2, $>0.07 - \leq 0.19$	6.63 (3.07)	-1.46%	Q2, $>0.09 - \leq 0.19$	6.36 (2.98)	Q2, $>0.06 - \leq 0.19$	6.78 (3.02)
Q3, $>0.19 - \leq 0.39$	6.86 (3.39)	1.93%	Q3, $>0.19 - \leq 0.36$	6.18 (3.12)	Q3, $>0.19 - \leq 0.41$	8.27 (3.89)
Q4, >0.39	5.83 (2.77)	-13.3%	Q4, >0.36	5.53 (2.64)	Q4, >0.41	6.16 (2.85)
P for trend	0.07			0.27		0.31
P for interaction by sex ^f	0.63					
<i>Compositae</i> (lettuce)						
Non-consumer	6.50 (4.10)	ref	Non-consumer	5.99 (3.92)	Non-consumer	7.19 (4.20)
Consumer	6.14 (2.66)	-5.48%	Consumer	8.06 (3.31)	Consumer	5.61 (2.37)
P comparing 2 groups ^e	0.63			0.15		0.07
P for interaction by sex ^f	0.04					
<i>Cucurbitaceae</i> (squash, melons, cucumbers)						
Median, ≤ 0.02	6.52 (3.49)	ref	Median, ≤ 0.06	6.22 (3.25)	Non-consumer	6.97 (3.85)
Median, >0.02	6.46 (3.54)	-0.95%	Median, > 0.06	5.74 (3.36)	Consumer	7.27 (3.47)
P comparing 2 groups ^e	0.85			0.24		0.54
P for interaction by sex ^f	0.30					
<i>Roseaceae</i> (apple)						
Q1, ≤ 0.09	6.49 (3.09)	ref	Q1, ≤ 0.16	6.02 (2.81)	Q1, ≤ 0.04	7.29 (3.55)
Q2, $>0.09 - \leq 0.51$	6.52 (3.12)	0.43%	Q2, $>0.16 - \leq 0.55$	6.56 (3.07)	Q2, $>0.04 - \leq 0.48$	7.13 (3.25)
Q3, $>0.51 - \leq 1.07$	6.90 (3.19)	6.40%	Q3, $>0.55 - \leq 1.11$	5.93 (2.92)	Q3, $>0.48 - \leq 1.00$	7.47 (3.34)
Q4, >1.07	5.83 (2.91)	-10.1%	Q4, >1.11	5.45 (2.75)	Q4, >1.00	6.51 (3.02)

Daily intakes	β-glucuronidase activity (μg/ml/hr)					
	Total			Women		
	Adjusted Mean (SD)	% Difference from lowest category ^d	Cutoffs	Adjusted Mean (SD)	Cutoffs	Adjusted Mean (SD)
P for trend	0.24			0.20		0.35
P for interaction by sex ^f	0.65					
<i>Umbelliferae</i> (carrots; celery)						
Q1, ≤ 0.003	6.31 (2.91)	ref	Q1, ≤ 0.01	6.02 (2.80)	Q1, ≤0.00	6.85 (3.11)
Q2, >0.003 - ≤0.11	7.11 (3.53)	12.7%	Q2, >0.01 - ≤ 0.20	6.71 (3.26)	Q2, >0.00 - ≤0.07	7.72 (3.56)
Q3, >0.11 - ≤0.34	6.56 (3.16)	3.93%	Q3, >0.20 - ≤ 0.38	5.93 (2.99)	Q3, >0.07 - ≤0.28	7.37 (3.34)
Q4, >0.34	6.15 (2.93)	-2.5%	Q4, >0.38	5.70 (2.77)	Q4, >0.28	6.71 (3.17)
P for trend	0.46			0.39		0.74
P for interaction by sex ^f	0.98					

^aLeast-square means of serum β-glucuronidase activity from multivariate linear regression adjusted for age, gender, race, and BMI; β-glucuronidase activity is presented as back-log-transformed values

^bExamples of common foods are provided for each botanical family

^cTests for trend (2-sided) were calculated by modeling the exposures ordinally.

^d% Difference=(C_i - C₀)*100/C₀; where C₀ is the reference category and C_i are the non-reference categories (i > 0).

^eP for testing whether dichotomized exposure variables were significantly associated with β-glucuronidase activity.

^fP for interaction for testing whether diet response differed by sex.

Table 4

β -glucuronidase activity, by daily nutrient intakes calculated from average of 3-day food records (3-DFR) and 3-month food frequency questionnaire (FFQ)^{a,b,c}

Daily Intakes	β -glucuronidase activity ($\mu\text{g/ml/hr}$)			
	3-DFR		3-month FFQ	
	Mean (SD)	% Difference from lowest category ^d	Mean (SD)	% Difference from lowest category ^d
Total energy (kcal)				
Q1	6.82 (3.20)	ref	6.93 (3.20)	ref
Q2	6.83 (3.33)	0.15%	6.62 (3.10)	-4.49%
Q3	6.37 (3.09)	-6.54%	5.88 (2.84)	-15.1%
Q4	5.91 (2.99)	-13.3%	6.44 (3.19)	-7.11%
P for trend	0.04		0.13	
P for interaction by sex	0.58		0.82	
Total fat (% energy)				
Q1	6.25 (2.99)	0.00%	6.42 (3.12)	0.00%
Q2	6.23 (3.03)	-0.35%	6.54 (3.11)	1.79%
Q3	6.55 (3.12)	4.70%	6.35 (3.20)	-1.18%
Q4	6.86 (3.24)	9.79%	6.56 (3.01)	2.07%
P for trend	0.13		0.89	
P for interaction by sex	0.98		0.41	
Saturated fat (g)				
Q1	6.11 (3.01)	ref	6.68 (3.65)	ref
Q2	6.52 (3.14)	6.66%	5.97 (2.82)	-10.7%
Q3	6.18 (3.10)	1.13%	6.69 (3.30)	0.16%
Q4	7.12 (3.66)	16.5%	6.76 (4.17)	1.26%
P for trend	0.14		0.68	
P for interaction by sex	0.61		0.75	
Total protein (% energy)				
Q1	6.63 (3.07)	0.00%	6.54 (3.13)	0.00%
Q2	6.51 (3.18)	-1.91%	6.39 (3.13)	-2.37%
Q3	6.62 (3.17)	-0.21%	6.85 (3.24)	4.71%
Q4	6.11 (2.99)	-7.87%	6.16 (2.90)	-5.90%
P for trend	0.31		0.62	
P for interaction by sex	0.34		0.98	
Animal protein (g)				
Q1	6.73 (3.21)	ref	6.85 (3.50)	ref
Q2	6.40 (3.22)	-4.95%	6.45 (3.19)	-5.75%
Q3	6.39 (3.12)	-5.05%	6.17 (2.92)	-9.97%
Q4	6.35 (3.12)	-5.61%	6.46 (3.48)	-5.62%
P for trend	0.44		0.36	
P for interaction by sex	0.40		0.53	
Vegetable protein (g)				

Daily Intakes	β -glucuronidase activity ($\mu\text{g/ml/hr}$)			
	3-DFR		3-month FFQ	
	Mean (SD)	% Difference from lowest category ^d	Mean (SD)	% Difference from lowest category ^d
Q1	6.74 (3.40)	ref	6.66 (3.46)	ref
Q2	6.40 (3.05)	-5.03%	6.63 (3.23)	-0.51%
Q3	6.74 (3.26)	-0.07%	6.34 (3.14)	-4.82%
Q4	6.06 (3.08)	-10.0%	6.29 (3.27)	-5.59%
P for trend	0.29		0.44	
P for interaction by sex	0.60		0.15	
Total carbohydrates (% energy)				
Q1	6.51 (3.02)	0.00%	6.58 (2.98)	0.00%
Q2	6.92 (3.32)	6.24%	6.14 (2.98)	-6.69%
Q3	6.52 (3.21)	0.18%	6.81 (3.40)	3.48%
Q4	6.03 (2.87)	-7.41%	6.43 (3.11)	-2.16%
P for trend	0.20		0.85	
P for interaction by sex	0.86		0.86	
Total dietary fiber (g)				
Q1	6.84 (3.30)	ref	7.12 (3.62)	ref
Q2	6.47 (3.19)	-5.51%	6.03 (2.93)	-15.3%
Q3	6.39 (2.98)	-6.69%	6.34 (3.16)	-10.9%
Q4	6.04 (3.15)	-11.7%	6.22 (3.40)	-12.6%
P for trend	0.11		0.28	
P for interaction by sex	0.65		0.35	
Alpha-carotene (μg)				
Q1	6.65 (3.13)	ref	7.00 (3.35)	ref
Q2	6.92 (3.31)	3.98%	6.27 (3.03)	-10.4%
Q3	6.27 (3.00)	-5.74%	6.50 (3.02)	-7.19%
Q4	6.06 (2.93)	-8.97%	6.05 (3.08)	-13.5%
P for trend	0.09		0.10	
P for interaction by sex	0.41		0.17	
Beta-carotene (μg)				
Q1	6.57 (3.15)	ref	6.62 (3.32)	ref
Q2	6.78 (3.30)	3.21%	6.83 (3.27)	3.22%
Q3	6.70 (3.31)	1.92%	6.38 (3.03)	-3.61%
Q4	6.03 (2.80)	-8.29%	6.11 (3.02)	-7.76%
P for trend	0.24		0.21	
P for interaction by sex	0.07		0.56	
Ascorbic acid (mg)				
Q1	6.40 (3.01)	ref	6.34 (3.11)	ref
Q2	7.06 (3.43)	10.41%	6.57 (3.26)	3.52%
Q3	6.49 (3.13)	1.52%	6.50 (3.07)	2.55%
Q4	6.06 (2.89)	-5.31%	6.55 (3.48)	3.23%
P for trend	0.28		0.75	

Daily Intakes	β -glucuronidase activity ($\mu\text{g/ml/hr}$)			
	3-DFR		3-month FFQ	
	Mean (SD)	% Difference from lowest category ^d	Mean (SD)	% Difference from lowest category ^d
P for interaction by sex	0.39		0.77	
Beta-cryptoxanthin (μg)				
Q1	6.24 (2.97)	ref	6.32 (3.04)	ref
Q2	6.85 (3.14)	9.69%	6.46 (3.07)	2.25%
Q3	6.64 (3.11)	6.31%	6.48 (3.10)	2.48%
Q4	5.95 (3.01)	-4.61%	6.74 (3.57)	6.68%
P for trend	0.46		0.44	
P for interaction by sex	0.29		0.01	
Lycopene (μg)				
Q1	6.43 (2.97)	ref	6.62 (3.14)	ref
Q2	5.94 (2.97)	-7.73%	6.12 (2.99)	-7.58%
Q3	7.03 (3.39)	9.28%	6.90 (3.39)	4.23%
Q4	6.49 (3.02)	0.82%	6.25 (3.31)	-5.53%
P for trend	0.37		0.97	
P for interaction by sex	0.67		0.29	
Gamma-tocopherol (mg)				
Q1	5.89 (3.11)	ref	5.86 (3.12)	ref
Q2	6.57 (3.16)	11.6%	6.36 (3.10)	8.59%
Q3	6.69 (3.25)	13.6%	6.19 (2.91)	5.67%
Q4	6.66 (3.18)	13.1%	7.40 (3.92)	26.3%
P for trend	0.13		0.03	
P for interaction by sex	0.29		0.23	
Alpha-tocopherol (mg)				
Q1	6.47 (3.19)	ref	6.65 (3.42)	ref
Q2	6.68 (3.18)	3.36%	6.83 (3.40)	2.63%
Q3	6.82 (3.46)	5.43%	6.25 (3.01)	-6.06%
Q4	6.06 (2.95)	-6.33%	6.24 (3.32)	-6.28%
P for trend	0.43		0.35	
P for interaction by sex	0.33		0.58	
Caffeine (mg)				
Q1	6.28 (3.14)	ref	6.12 (3.02)	ref
Q2	6.39 (2.93)	1.70%	6.63 (3.19)	8.21%
Q3	6.67 (3.30)	6.11%	6.75 (3.17)	10.2%
Q4	6.62 (3.25)	5.30%	6.39 (3.06)	4.34%
P for trend	0.39		0.53	
P for interaction by sex	0.43		0.87	
Calcium (mg)				
Q1	6.88 (3.19)	ref	7.07 (3.43)	ref
Q2	6.66 (3.34)	-3.13%	6.69 (3.16)	-5.39%
Q3	6.38 (3.23)	-7.24%	5.73 (2.81)	-19.0%

Daily Intakes	β -glucuronidase activity ($\mu\text{g/ml/hr}$)			
	3-DFR		3-month FFQ	
	Mean (SD)	% Difference from lowest category ^d	Mean (SD)	% Difference from lowest category ^d
Q4	5.79 (3.06)	-15.8%	6.25 (3.37)	-11.6%
P for trend	0.03		0.07	
P for interaction by sex	0.49		0.54	
Iron (mg)				
Q1	7.05 (3.55)	ref	7.34 (3.80)	ref
Q2	7.03 (3.30)	-0.23%	6.65 (3.16)	-9.40%
Q3	6.03 (2.98)	-14.4%	5.83 (2.79)	-20.6%
Q4	5.74 (2.93)	-18.6%	6.10 (3.22)	-16.9%
P for trend	0.005		0.02	
P for interaction by sex	0.54		0.56	
Magnesium (mg)				
Q1	7.09 (3.47)	ref	7.42 (4.03)	ref
Q2	6.64 (3.17)	-6.30%	6.48 (3.17)	-12.7%
Q3	5.98 (2.88)	-15.7%	6.43 (3.17)	-13.3%
Q4	6.06 (3.28)	-14.6%	5.59 (3.18)	-24.7%
P for trend	0.03		0.01	
P for interaction by sex	0.88		0.54	
Phosphorous (mg)				
Q1	6.96 (3.47)	ref	7.20 (3.88)	ref
Q2	6.95 (3.42)	-0.07%	6.56 (3.28)	-8.88%
Q3	5.76 (2.74)	-17.3%	5.91 (2.87)	-17.9%
Q4	6.24 (3.70)	-10.3%	6.03 (3.87)	-16.2%
P for trend	0.06		0.05	
P for interaction by sex	0.78		0.99	

Abbreviations: FFQ= Food frequency questionnaire; 3-DFR=3-day food records

^aLeast-square means from multivariate linear regression adjusted for energy, age, gender, race, and BMI. Fat, protein, and carbohydrates, expressed in % energy, were already adjusted for energy.

^b β -glucuronidase activity was back-log-transformed.

^cTests for trend (2-sided) were calculated by modeling the exposures ordinally.

^d% Difference=($C_i - C_0$)*100/ C_0 ; where C_0 is the reference category and C_i are the non-reference categories ($i > 0$).