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Citrus Fruit Intake Is Associated with Lower Serum Bilirubin Concentration among Women with the *UGT1A1*28* Polymorphism[1,2,3]

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Abstract

UDP-glucuronosyltransferase (UGT) 1A1 glucuronidates bilirubin, estrogens, and xenobiotic compounds. The *UGT1A1*28* polymorphism results in lower promoter activity due to 7 thymine-adenine (TA) repeats, rather than the more common 6 TA repeats. Previously, we showed that serum bilirubin, a marker of UGT1A1 activity, was lower among individuals homozygous for the *UGT1A1*28* polymorphism (7/7) when randomized to a high fruit and vegetable (F&V) diet, whereas no effect was seen in individuals with the wild-type (6/6) and heterozygous (6/7) genotypes. Our objective here was to determine if we could detect genotype-diet interactions on bilirubin concentrations in an observational study. Healthy non-smoking men (n=146) and women (n=147), recruited from the Seattle area, provided blood samples for genotyping and bilirubin measurements. We used multiple linear regression to assess the relationships between *UGT1A1* genotype, bilirubin concentrations and consumption of specific F&V [cruciferous vegetables, citrus fruits, and soy foods (n=268)] based on FFQ, and F&V from 6 botanical families [*Cruciferae*, *Rosaceae*, *Rutaceae*, *Umbelliferae*, *Solanaceae* and *Leguminosae* (n=261)] based on 3 d food records. We observed a significant interaction of *UGT1A1* genotype and citrus consumption among women. Women with the 7/7 genotype who consumed 0.5 or more daily servings of citrus fruit or foods from the *Rutaceae* botanical family had ~30% lower serum bilirubin than those with the same genotype who consumed less, while 6/6 and 6/7 genotypes did not differ by consumption (P for interaction = 0.006 and 0.03 respectively). These results suggest that citrus consumption may increase UGT1A1 activity among women with the 7/7 genotype.

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Introduction

UDP-glucuronosyltransferases[6] (UGT) conjugate endogenous and exogenous compounds with 5′-diphosphoglucuronic acid to form glucuronidated compounds that are more water-soluble and easily excreted. Two families of human UGT—UGT1A and UGT2B—glucuronidate a wide range of substrates. UGT1A enzymes conjugate estrogens, bilirubin, and xenobiotic compounds, and UGT2B enzymes glucuronidate bile acids, androgens, and drugs; however, there is overlapping substrate specificity among members of both families (1–5). Here we focus on UGT1A1 which metabolizes dietary carcinogens, such as 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), 17β-estradiol, and is the primary UGT that glucuronidates bilirubin (5–7).

Genetic polymorphisms that alter enzyme expression and/or activity and affect carcinogen clearance have been identified in *UGT* (8–10). The *UGT1A1*28* polymorphism, characterized by 7 thymine-adenine (TA) repeats rather than the more common 6 TA repeats, results in lower promoter activity and is the genetic basis for mild unconjugated hyperbilirubinemia associated with reduced hepatic bilirubin glucuronidation (Gilbert syndrome) (11,12). Individuals homozygous for the *UGT1A1*28* variant alleles (7/7) have been shown to have higher circulating concentrations of serum bilirubin (13,14), as well as reduced amounts of conjugated metabolites of the carcinogens benzo(a)pyrene [benzo(a)pyrene-7,8-dihydrodiol(−); (13)] and PhIP [N-hydroxy-PhIP (1)]. These data suggest that individuals with the *UGT1A1*28* alleles may be at increased risk of cancer due to higher or more prolonged carcinogen exposure. Results from population-based case-control studies have shown that pre-menopausal women with the *UGT1A1*28* polymorphism have a higher risk of breast cancer than women with the homozygous wild-type alleles (6/6) (15,16), perhaps due to higher circulating estradiol concentrations associated with reduced UGT1A1 activity (17).

Many phytochemicals induce UGT (18–20). We showed previously, in observational (21) and controlled feeding studies (22), that serum bilirubin concentrations were lower among individuals with the 7/7 genotype when exposed to fruits and vegetables (F&V); whereas, no effect was seen in individuals with the wild-type (6/6) and heterozygous (6/7) genotypes. In the present cross-sectional study, we also use serum bilirubin as an endogenous marker of UGT1A1 activity. Citrus fruit, cruciferous vegetables and soy (and/or phytochemicals contained in these foods) induce UGT activity (23–29). Our objective was to determine whether habitual consumption [as assessed by FFQ] of these foods and recent consumption [as measured by 3 d food records (3DFR)] of foods from several botanical families that contain phytochemicals that induce UGT (19) (*Cruciferae*, *Rosaceae*, *Solanaceae*, *Leguminosae*, *Rutaceae* and *Umbelliferae*), are associated with serum bilirubin concentrations. We also tested whether associations between diet and bilirubin differ by *UGT1A1* genotype.

Subjects and Methods

Subjects

293 healthy, non-smoking men (*n*=146) and women (*n*=147), aged 20–40 y, were recruited from the Seattle area via advertisements in university newspapers, flyers displayed in campus buildings, and targeted mailings of individuals identified from the Washington State Department of Licensing. Participants completed an eligibility questionnaire and were excluded based on the following criteria: 1) medical history of gastrointestinal, hepatic, or renal disorders; 2) current or planned pregnancy or lactation; 3) major dietary and/or weight change

[6]Abbreviations used: AhR, aryl hydrocarbon receptor; ER, estrogen receptor; FHCRC, Fred Hutchinson Cancer Research Center; F&V, fruit and vegetable; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; TA, thymine-adenine; UGT, UDP-glucuronosyltransferase; UM-NCC, University of Minnesota Nutrition Coordinating Center; 3DFR, 3 d food record

(>4.5 kg) in the past year; 4) antibiotic use within the past 3 mo; 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/d (720 mL beer, 240 mL wine or 90 mL hard liquor); 9) no interest in participating in the subsequent controlled feeding trial; 10) exercise regimens that require or result in significant short-term dietary changes. Participants were asked to discontinue use of all multivitamins and dietary supplements 1 wk prior to their participation in the study. The study was approved by the Institutional Review Board at the Fred Hutchinson Cancer Research Center (FHCRC) and informed written consent was obtained from all participants.

Data and sample collection

Participants completed a FFQ reporting on their dietary intake within the past 3 mo, food records on 3 consecutive d and a health and demographics questionnaire. Body weight and height were measured and a 12 h fasting blood sample was drawn from all participants to provide peripheral leukocyte DNA for *UGT* genotyping and serum for bilirubin measurements.

FFQ

Dietary data were collected from 274 (94%) participants using a FFQ developed by the FHCRC Nutrition Assessment Shared Resource. Completed FFQ were analyzed for daily servings of specific foods and daily consumption of nutrients using the database from the University of Minnesota Nutrition Coordinating Center (UM-NCC), Minneapolis, MN (19) and F&V (30). Servings of all F&V were adjusted for serving size and usual frequency of consumption. Daily servings (113 g) of cruciferous vegetables were calculated by summation of adjusted daily servings (as described above) of broccoli, cabbage, cauliflower, Brussels sprouts and coleslaw. Daily servings of citrus fruits were calculated by summation of adjusted daily servings of orange (1 orange), grapefruit (1/2 grapefruit), orange juice (177 mL), and grapefruit juice (177 mL). Daily servings of soy products were calculated by summation of daily servings (adjusted for serving size) of tofu (86 g), miso soup (237 mL), and soy milk (237 mL).

Food records

3DFR were collected from 289 (99%) participants and analyzed as previously described (31). Participants were trained by a registered dietitian on how to keep food records. The 3DFR were analyzed by trained nutritionists using the UM-NCC Nutrition Data System for Research that incorporates a comprehensive food product list and nutrient database (32) to estimate daily intake of total F&V (together and separately), as well as those defined by botanical family, based on standard serving sizes (228 g raw, 113 g cooked or canned, 118 mL juice, etc.). We focused on daily servings of plants from the *Cruciferae*, *Rosaceae*, *Solanaceae*, *Leguminosae*, *Rutaceae* and *Umbelliferae* families because they include foods which contain phytochemicals previously shown to induce UGT (20). Cruciferous vegetables (i.e., *Cruciferae*), such as broccoli and cabbage, are rich in sulfur-containing glucosinolates. Foods in the *Umbelliferae* (e.g., carrots, parsley), *Solanaceae* (e.g., tomato, eggplant) and *Rutaceae* (e.g., citrus fruits) families contain carotenoids and many flavonoids. Fruit, such as apples and berries, in the *Rosaceae* family are also abundant in flavonoids, and legumes (i.e., *Leguminosae*), such as lentils and beans, are rich in lignans.

Determination of UGT1A1 promoter genotypes and serum bilirubin measurements

Genotyping for the *UGT1A1*28* polymorphism was performed as previously described (22). Serum total and direct (conjugated) bilirubin concentrations were quantified using a Cobas MIRA Plus centrifugal analyzer (Roche Diagnostic Systems). Indirect (unconjugated) bilirubin was calculated as the difference between total and direct bilirubin.

Statistical analysis

Four individuals (1%) did not provide any dietary information and were excluded from analyses. Six (2%) additional participants were excluded because they had *UGT1A1* genotypes other than 6/6, 6/7, or 7/7. Of the remaining 283 study participants, 261 (92%) and 268 (95%) completed 3DFR on 3 consecutive d within 1 wk prior to serum collection and FFQ, respectively. Serum bilirubin concentrations were log-transformed prior to analysis to normalize distributions. Daily servings of total F&V and total botanicals were divided into three groups (<4, 4–5, and >5) and used as grouped linear variables in statistical models. Daily servings of all other dietary variables were split dichotomously (<1/2 or 1/2+) for analysis. Multiple linear regression analysis was used to determine whether demographic characteristics [age (<30 or 30+ y), sex, race (Caucasian, Asian or other), and duration of physical activity (≤6 or >6 h/wk)] and *UGT1A1* genotype (used as a grouped linear variable to test for significant linear trend) were associated with serum bilirubin concentrations. Differences in daily intake of each food group by *UGT1A1* genotype (used as a categorical variable—6/6, 6/7 or 7/7) were assessed using linear regression.

Multiple linear regression analysis was also used to assess the association of F&V consumption (independent variables; total F&V or botanicals and each F&V variable tested individually) with each serum bilirubin measure. These analyses were adjusted *a priori* for *UGT1A1* genotype, sex, and total F&V or energy (for total F&V and total botanicals) intake. The following covariates were assessed as potential confounders or predictors in all regression models: age (continuous), body wt (continuous), duration of physical activity (≤6 or >6 h/wk), race/ethnicity (Caucasian, Asian or other), total energy intake (continuous), and season (winter, spring, summer, or fall; for 3DFR variables only). Predictors were included in models if the *P*-value for the test that the regression coefficient(s) for the variable term(s) was equal to zero was significant ($P<0.05$). Confounders were included if their addition to the regression model changed the main effect(s) estimate(s) by 10% or greater. To determine whether dietary effects on bilirubin concentrations differed by genotype, interactions of each dietary variable (coded as grouped linear) with *UGT1A1* genotype (coded as grouped linear) was assessed by testing the null hypothesis that the regression coefficient(s) for the interaction term(s) was equal to zero. Statistical tests were two-sided and $P<0.05$ was considered statistically significant. All statistical analyses were performed using Stata 9.0 (StataCorp).

Results

Sex, race, and *UGT1A1* genotype were associated with total, direct, and indirect serum bilirubin (Table 1). Men had statistically significantly higher serum bilirubin concentrations than women ($P \leq 0.001$). These measures were also statistically significantly higher among Asians than Caucasians ($P < 0.01$); whereas, total and indirect bilirubin concentrations were significantly lower among races other than Caucasian ($P < 0.05$). There was a statistically significant linear trend of increasing total, direct, and indirect bilirubin ($P < 0.001$) with higher numbers of *UGT1A1*28* alleles. Age was not associated with total and indirect bilirubin; however, individuals ≥ 30 y had slightly lower concentrations of direct (conjugated) bilirubin than those who were <30 y ($P = 0.02$), after adjusting for all other covariates. Duration of physical activity was not associated with any bilirubin measures. There were no statistically significant differences in mean daily intake of each F&V group or botanical family by *UGT1A1* genotype (Supplemental Table 1).

Total F&V consumption was not statistically significantly associated with any measure of serum bilirubin (Supplemental Table 2). However, among the individual F&V groups, there was a statistically significant interaction between *UGT1A1* genotype and citrus fruit ($P = 0.006$; **Table 4**) and *Rutaceae* ($P = 0.03$; **Table 5**) consumption associated with total and indirect (data not shown) bilirubin among women but not men: women with the 7/7 genotype who

consumed 0.5 or more daily servings of citrus fruit or *Rutaceae* had lower (~30%) total and indirect bilirubin concentrations than those who consumed less. In contrast, bilirubin measures did not differ by consumption of these food groups among men or women with the 6/6 and 6/7 genotypes. Multiple regression models that included citrus consumption, *UGT1A1* genotype, race, age, total F&V consumption, and the citrus -*UGT1A1* genotype interaction terms accounted for ~30% (R^2 values) of the variation in total and indirect bilirubin, respectively.

Discussion

We observed a statistically significant interaction between *UGT1A1* genotype and consumption of citrus, which was associated with lower serum bilirubin among women with the 7/7 genotype. These results are consistent with previous studies that showed bilirubin concentrations were lower among individuals with the 7/7 genotype who were exposed to cruciferous vegetables (21) or a combination of soy, citrus fruit, and cruciferous vegetables (22).

Phytochemicals found in citrus fruits increase UGT activity (28,33–35). Small changes in UGT activity may result from direct phytochemical binding to the enzyme's active site; however, the primary mechanism is hypothesized to be through induction of *UGT* gene expression (19). Many phytochemicals can regulate multiple transcription factors. Quercetin, a flavonoid found in citrus fruit, activates the nuclear factor-erythroid 2-related factor 2 transcription factor, that binds to the antioxidant response element (36,37), and induces *UGT1A1* expression (38, 39). Therefore, consumption of citrus fruit associated with greater bilirubin glucuronidation may be a result of induced *UGT1A1* expression/activity.

Previously, in a study in which participants were recruited on the basis of high and low F&V consumption, we observed that *Cruciferae* intake was associated with lower bilirubin concentrations among individuals with the 7/7 genotype (21); however, we did not observe this relationship in the current study. Although the range of *Cruciferae* consumption among participants with the 7/7 genotype in the current study is slightly wider than that among those in the previous study [(0–1.0) versus (0.2–0.7), respectively], mean intake of *Cruciferae* was ~60% lower. This level of consumption in our current study was possibly too low to reliably detect differences in total serum bilirubin concentrations among participants with the 7/7 genotype. In addition, among individuals with the 7/7 genotype, mean total bilirubin concentration in the current study was ~30% lower than in the previous study, which may have also affected our ability to detect differences.

As previously reported, women had lower serum bilirubin than men, independent of *UGT1A1* genotype (21,22,40,41), presumably due to estrogen- and/or progesterone-mediated *UGT1A1* transcription (42–44). Results from several studies suggest that estrogen receptor (ER)-mediated increases in transcriptional activity of the arylhydrocarbon receptor (AhR) affect *UGT1A1* (45–47). Phytochemicals in citrus foods activate the AhR (48–51) to increase *UGT1A1* transcription (39). Thus, lower bilirubin concentrations among women may result from increased *UGT1A1* expression associated with ER/AhR cross-talk.

The mechanism for the effects of F&V consumption only among women with the 7/7 genotype is unclear. Extra TA repeats associated with the *UGT1A1*28* polymorphism result in low promoter activity due to reduced binding of TATA-binding protein (11,52) and other transcription factors, including the AhR (53). Recently, it was proposed that the AhR can serve as a positive transcriptional co-regulator of the ER (54). Perhaps, increased AhR/ER cross-talk among women who consume F&V can somewhat overcome the transcriptional defect associated with the *UGT1A1*28* polymorphism.

The collection of detailed dietary and demographic and behavioral data from study participants is the primary strength of this study. Changes in UGT expression/activity can be detected within an h of phytochemical exposure (55,56); thus, recent consumption from 3DFR may be most relevant for detecting associations between F&V intake and bilirubin concentrations. However, because study participants tend to overestimate F&V consumption when reporting their usual intake on FFQ (30), the FFQ may further separate the low- and high-consumers and this may have increased our power to detect differences in bilirubin concentrations by citrus fruit consumption compared to those associated with *Rutaceae* consumption. This may have contributed to the stronger association with citrus fruit consumption.

Glucuronidated compounds are rapidly excreted. It is possible that changes in bilirubin concentrations, used here as a surrogate for UGT activity, are diluted compared to those occurring in relevant tissues, such as the liver and intestine, which are in direct contact with phytochemicals released during F&V digestion. Perhaps the most significant limitation of this study is the small sample size among strata of women and men with the rare 7/7 genotype, which probably limited our ability to distinguish differences in bilirubin glucuronidation. Our decision to categorize continuous F&V intake may have also decreased our power to detect associations between bilirubin concentrations and F&V consumption; however, similar associations were observed using continuous citrus fruit intake. Another potential issue is the large number of comparisons performed during the statistical analysis, which increases the likelihood of observing a significant association due to chance. A total of 33 statistical tests were performed. With an α-significance level of 0.05, ~2 associations would have been statistically significant by chance alone, and, thus, these results should be interpreted with caution. However, our result for citrus fruit was at the $P = 0.006$ level, and that level of significance among any one of 33 comparisons has a likelihood of occurrence by chance of less than 20%.

UGT1A1 glucuronidates several compounds, such as estradiol and PhIP, that have been associated with cancer; thus, changes in UGT1A1 expression/activity could affect cancer risk. Several studies have suggested that individuals with the *UGT1A1*28* polymorphism are at increased risk of certain cancers (15,16,57,58) and toxicity from certain drugs and have a reduced ability to clear potential carcinogens (1,8,13,59,60). The results of this study suggest that women with the 7/7 genotype who consume F&V, particularly citrus fruit, may have higher UGT1A1 activity than women with the 7/7 genotype who do not consume F&V. Our results suggest that certain components in F&V may increase UGT1A1 activity among individuals with this variant genotype and thus potentially improve clearance of certain carcinogens and influence cancer susceptibility.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Concentrations of serum total, direct, and indirect bilirubin among study participants grouped by demographic characteristics and *UGT1A1* genotype

	Distribution	Total bilirubin[1]	Direct bilirubin[1]	Indirect bilirubin[1]
	n (%)		μmol/L	
Total	283 (100.0)			
Sex[2]				
Women	142 (50.2)	11.8 (11.1, 12.6)	1.7 (1.6, 1.8)	10.1 (9.5, 10.7)
Men	141 (49.8)	15.3 (14.4, 16.3)	1.9 (1.8, 2.1)	13.3 (12.5, 14.2)
Age[3], y				
<30	199 (70.3)	13.8 (13.1, 14.5)	1.9 (1.8, 2.0)	11.8 (11.2, 12.5)
30+	84 (2m9.7)	12.7 (11.8, 13.8)	1.7 (1.6, 1.8)	11.0 (10.1, 11.9)
Race[2]				
Caucasian	199 (70.3)	13.3 (12.6, 14.0)	1.8 (1.7, 1.9)	11.4 (10.8, 12.1)
Asian	45 (15.9)	15.9 (14.2, 17.7)	2.1 (1.9, 2.3)	13.8 (12.3, 15.4)
Other	33 (11.7)	11.4 (10.0, 13.0)	1.7 (1.5, 1.9)	9.7 (8.5, 11.1)
Missing	6 (2.1)			
Physical activity, h/wk				
≤6	191 (67.5)	13.7 (13.0, 14.5)	1.8 (1.7, 1.9)	11.9 (11.2, 12.5)
>6	81 (28.6)	12.8 (11.8, 13.9)	1.8 (1.7, 1.9)	11.0 (10.1, 11.9)
Missing	11 (3.9)			
UGT1A1 genotype[4]				
6/6	144 (50.9)	10.7 (10.1, 11.4)	1.5 (1.4, 1.6)	9.2 (8.6, 9.7)
6/7	109 (38.5)	15.6 (14.9, 16.5)	2.0 (1.9, 2.1)	13.6 (12.9, 14.3)
7/7	30 (10.6)	23.0 (20.7, 25.4)	2.6 (2.4, 2.9)	20.3 (18.2, 22.6)

[1] Bilirubin data were transformed ln(x+1). Back-transformed means and 95% CI (in parentheses) are presented.

[2] Significant associations with all bilirubin measures ($P \leq 0.01$) after adjusting for all other variables in table using multiple linear regression

[3] Significant association with direct bilirubin concentration ($P = 0.02$) after adjusting for all other variables in table using multiple linear regression

[4] Significant linear associations with all bilirubin measures ($P < 0.001$) after adjusting for all other variables in table using multiple linear regression with grouped linear *UGT1A1* genotype

Table 2

Total serum bilirubin among women and men stratified by *UGT1A1* genotype and daily servings of foods assessed by FFQ: interaction of genotype and diet[1]

	Women				Men			
Food group servings, n/d	**6/6**	**6/7**	**7/7**	***P* for interaction[2]**	**6/6**	**6/7**	**7/7**	***P* for interaction[2]**
	μmol/L				μmol/L			
Total fruits & vegetables								
<4	10.5 (9.4, 11.7) n=36	13.9 (12.6, 15.4) n=19	18.4 (15.2, 22.4) n=7	0.41	11.9 (10.7, 13.2) n=41	18.5 (16.7, 20.4) n=32	18.7 (23.8, 34.6) n=9	0.21
4–5	9.7 (8.9, 10.5) n=11	13.4 (12.5, 14.4) n=12	18.6 (16.1, 21.5) n=3		12.0 (10.9, 13.2) n=11	17.4 (16.0, 18.8) n=3	25.2 (21.5, 29.6) n=3	
>5	9.0 (7.8, 10.4) n=20	13.0 (11.6, 14.6) n=21	18.8 (14.8, 23.8) n=4		12.1 (10.2, 14.4) n=17	16.4 (14.0, 19.1) n=16	22.2 (16.7, 29.4) n=3	
Soy products								
<0.5	9.9 (9.0, 10.8) n=49	13.7 (12.5, 14.9) n=33	18.9 (16.0, 22.4) n=9	0.68	11.6 (10.6, 12.7) n=54	17.9 (16.5, 19.3) n=41	27.5 (23.5, 32.1) n=14	0.23
0.5+	9.9 (8.5, 11.5) n=18	13.2 (11.7, 14.9) n=19	17.5 (13.6, 22.6) n=5		13.2 (10.9, 16.0) n=15	17.1 (13.9, 21.1) n=10	22.3 (14.6, 34.1) n=1	
Citrus fruit								
<0.5	9.3 (8.4, 10.4) n=39	14.2 (12.9, 15.6) n=26	21.5 (17.9, 25.8) n=8	0.006	11.6 (10.3, 13.1) n=34	18.6 (16.6, 20.8) n=24	29.8 (24.1, 36.9) n=7	0.19
0.5+	10.7 (9.5, 12.1) n=28	12.7 (11.4, 14.0) n=26	15.0 (12.1, 18.5) n=6		12.1 (10.7, 13.7) n=35	17.2 (15.4, 19.1) n=27	24.4 (19.9, 29.9) n=8	
Cruciferous vegetables								
<0.5	10.3 (9.4, 11.4) n=46	14.1 (13.0, 15.4) n=32	19.4 (16.4, 22.9) n=10	0.93	11.5 (10.5, 12.6) n=57	17.8 (16.4, 19.3) n=41	27.3 (23.2, 32.1) n=12	0.21
0.5+	9.0 (7.8, 10.5) n=21	12.3 (10.9, 13.9) n=20	16.7 (13.0, 21.5) n=4		13.7 (11.1, 16.9) n=12	18.1 (15.3, 21.5) n=10	24.1 (17.4, 33.2) n=3	

[1] Bilirubin data were transformed ln(x+1). Back-transformed means and 95% CI (in parentheses) are presented.

[2] Adjusted for race, age, and total energy (total fruits & vegetables) or total fruits & vegetables (soy products, citrus fruit, and cruciferous vegetables) using multiple linear regression

Table 3

Total serum bilirubin among women and men stratified by *UGT1A1* genotype and daily servings of foods assessed by 3DFR: interaction of genotype and diet[1]

	Women				Men			
Botanical family servings, n/d	**6/6**	**6/7**	**7/7**	***P* for interaction[2]**	**6/6**	**6/7**	**7/7**	***P* for interaction[2]**
	µmol/L				µmol/L			
Total botanicals								
<4	10.4 (8.9, 12.0) n=18	13.9 (12.2, 15.9) n=12	18.6 (14.2, 24.5) n=2	0.74	11.0 (9.2, 13.1) n=16	17.6 (15.4, 20.2) n=16	28.2 (21.5, 37.1) n=4	0.53
4–5	10.0 (9.2, 10.9) n=8	13.7 (12.7, 14.7) n=11	18.7 (16.0, 21.7) n=7		11.5 (10.4, 12.6) n=10	17.7 (16.3, 19.1) n=6	27.3 (23.3, 32.0) n=4	
>5	9.6 (8.7, 10.7) n=39	13.4 (12.2, 14.7) n=30	18.7 (15.5, 22.5) n=6		11.9 (10.6, 13.4) n=39	17.7 (16.0, 19.7) n=27	26.4 (21.4, 32.5) n=7	
Cruciferae								
<0.5	9.8 (8.9, 10.8) n=50	13.4 (12.3, 14.4) n=41	18.2 (15.5, 21.3) n=12	0.78	11.3 (10.2, 12.5) n=52	17.4 (16.0, 19.0) n=37	26.9 (22.8, 31.9) n=13	0.45
0.5+	10.2 (8.5, 12.2) n=15	14.3 (12.3, 16.5) n=12	20.0 (14.9, 26.8) n=3		13.2 (10.7, 16.4) n=13	18.5 (15.4, 22.2) n=12	25.9 (17.9, 37.5) n=2	
Rosaceae								
<0.5	10.4 (9.2, 11.8) n=26	13.5 (12.2, 14.9) n=23	17.4 (14.3, 21.1) n=8	0.22	11.8 (10.3, 13.5) n=32	18.2 (16.2, 20.4) n=28	28.0 (22.3, 35.1) n=6	0.86
0.5+	9.5 (8.5, 10.6) n=39	13.8 (12.5, 15.2) n=30	19.9 (16.3, 24.2) n=7		11.4 (9.9, 13.0) n=33	17.2 (15.4, 19.3) n=21	26.1 (21.1, 32.2) n=9	
Solanaceae								
<0.5	10.0 (8.9, 11.3)	13.7 (12.1, 15.4) n=17	18.6 (14.7, 23.7) n=5	0.94	10.7 (9.1, 12.6) n=21	18.1 (16.0, 20.5) n=19	30.6 (24.1, 38.9) n=6	0.11

Botanical family servings, n/d	Women 6/6	Women 6/7	Women 7/7	Women *P* for interaction[2]	Men 6/6	Men 6/7	Men 7/7	Men *P* for interaction[2]
	n=30							
0.5+	9.8 (8.8, 11.0) n=35	13.5 (12.4, 14.7) n=36	18.5 (15.6, 22.1) n=10		12.1 (10.8, 13.5) n=44	17.4 (15.7, 19.2) n=30	25.0 (20.5, 30.4) n=9	
Leguminosae								
<0.5	10.1 (8.8, 11.6) n=26	13.2 (11.6, 15.1) n=14	17.3 (13.3, 22.4) n=4	0.47	11.3 (9.8, 13.1) n=27	17.5 (15.5, 19.9) n=21	27.1 (21.2, 34.6) n=6	0.81
0.5+	9.8 (8.8, 10.9) n=39	13.7 (12.6, 14.8) n=39	19.1 (16.2, 22.5) n=11		11.8 (10.5, 13.3) n=38	17.8 (16.1, 19.8) n=28	26.9 (22.1, 32.8) n=9	
Rutaceae								
<0.5	9.3 (8.4, 10.3) n=39	13.7 (12.6, 14.8) n=39	20.0 (17.1, 23.5) n=11	0.03	11.2 (9.9, 12.7) n=37	17.7 (16.0, 19.6) n=29	28.1 (23.0, 34.2) n=9	0.37
0.5+	11.0 (9.6, 12.6) n=26	12.8 (11.2, 14.8) n=14	15.0 (11.4, 19.9) n=4		12.2 (10.6, 14.1) n=28	17.7 (15.6, 20.0) n=20	25.5 (20.0, 32.5) n=6	
Umbelliferae								
<0.5	10.0 (9.1, 10.9) n=55	13.8 (12.7, 14.9) n=39	19.0 (16.3, 22.1) n=14	0.77	11.9 (10.8, 13.2) n=56	17.4 (16.0, 19.0) n=41	25.4 (21.4, 30.2) n=11	0.08
0.5+	9.6 (7.8, 11.8) n=10	12.7 (10.7, 15.1) n=14	16.8 (11.7, 24.3) n=1		10.2 (8.0, 12.9) n=9	18.5 (15.5, 22.0) n=8	33.6 (24.5, 46.1) n=4	

[1] Bilirubin data were transformed ln(x+1). Back-transformed means and 95% CI (in parentheses) are presented.

[2] Adjusted for race, age, and total energy (total botanicals) or total botanicals (models for all other botanical families) using multiple linear regression