Urinary Isoflavonoid and Lignan Excretion on a Western Diet: Relation to Soy, Vegetable, and Fruit Intake[1]

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Abstract

Dietary isoflavone and lignan phytoestrogens are potential chemopreventive agents. This has led to a need to monitor exposure to these compounds in human populations and to determine which components of a mixed diet contribute to the exposure. Typically, urinary isoflavonoid excretion is associated with soy consumption and that of lignans is associated with whole grains. However, other plant foods are known to contain phytoestrogen precursors. The purpose of this study was to examine the association between urinary isoflavonoid and lignan excretion and intakes of vegetables and fruits (V&F). Isoflavonoids (genistein, daidzein, *O*-desmethylangolensin, and equol) and lignans (enterolactone, enterodiol, and matairesinol) were measured in urine collected for 3 days from 49 male and 49 female volunteers (age, 18–37 years) reporting a wide range of habitual V&F intakes. Dietary intakes were assessed using 5-day diet records and a food frequency questionnaire. V&F groupings (total V&F, total V, total F, soyfoods, and V&F grouped by botanical families) were used to assess the relationship between V&F intake and urinary isoflavonoid and lignan excretion. Pearson correlations were performed. Intake of soyfoods was correlated significantly with urinary genistein ($r = 0.40$; $P = 0.0001$), *O*-desmethylangolensin ($r = 0.37$; $P = 0.0002$), daidzein ($r = 0.34$; $P = 0.0007$), and the sum of isoflavonoids ($r = 0.39$; $P = 0.0001$). There was no association between equol excretion and soy intake or between the isoflavonoids and any other V&F groupings. In addition, isoflavonoid excretion was correlated positively with intake of high-fat and processed meats, particularly among men who did not consume soy. This suggests that, even in the United States, on a Western diet, soyfoods are the primary contributors to isoflavone intake; however, additional "hidden sources" of soy may also contribute to exposure. In contrast, a variety of fiber-containing foods contributed to lignan excretion; the sum of the urinary lignans, enterodiol, enterolactone, and matairesinol, was associated with intake of total F ($r = 0.27$; $P = 0.008$), total V&F ($r = 0.25$; $P = 0.01$), soyfoods ($r = 0.28$; $P = 0.006$), and dietary fiber ($r = 0.36$; $P = 0.0003$). Overall, urinary phytoestrogens (isoflavonoids + lignans) were significantly higher in "high" compared with "low" V&F consumers. Compared with the "low" V&F group, the "high" group consumed diets that were, on average, higher in fiber and carbohydrate and soyfoods and lower in fat; thus, the urinary phytoestrogens may also be a useful marker of healthier dietary patterns.

Introduction

Epidemiological data indicate that diets high in plant foods, particularly V&F,[5] are associated with a lower risk of many cancers (1). Numerous classes of compounds present in V&F have demonstrated chemopreventive effects. Of these, isoflavonoid and lignan phytoestrogens are of significant interest because they have the capacity to influence cancer risk via a variety of mechanisms, including hormone-dependent pathways (2). This makes them potential candidates for prevention of sex steroid hormone-dependent cancers.

Isoflavones are highly concentrated in soybeans and soy products (3, 4), and urinary excretion is associated strongly and directly with soy protein intake under controlled dietary conditions (5). In observational studies of populations that usually consume soy (*e.g.,* Asian and Asian-American populations), soyfood intake and urinary isoflavonoid excretion are correlated positively (6–9). Among Western populations, plasma and urinary isoflavones have been reported to be significantly higher in women consuming vegetarian and macrobiotic diets compared with omnivorous diets (10). However, the relationship between habitual soyfood intake and urinary excretion of isoflavonoids has not been examined in the continental United States in a larger sample of individuals, who are consuming a Western diet, typically low in soy.

Precursors of the mammalian lignans, enterodiol and enterolactone, are most concentrated in flaxseed but are also

[5] The abbreviations used are: V&F, vegetable(s) and fruit(s); FFQ, food frequency questionnaire; DMA, *O*-desmethylangolensin; QC, quality control; CV, coefficient of variation.

present in legumes, whole grains and other seeds, and vegetables (11, 12). Urinary lignan excretion has been associated positively with various dietary fiber sources (13) and certain vegetable groups (6). We have shown previously that consumption of high intakes of cruciferous vegetables and carrots and spinach as part of a low-fiber, low-phytochemical diet significantly increased mean daily excretion of the mammalian lignans, enterolactone and enterodiol (14).

The purpose of this study was to examine the cross-sectional association between urinary isoflavonoid and lignan excretion and intakes of V&F in a healthy adult population. We hypothesized that dietary soy intake would be associated positively with urinary isoflavonoid excretion and that V&F intake and dietary fiber intake from V&F would be associated with urinary lignan excretion. The work presented here was part of a larger University of Minnesota Cancer Prevention Research Unit project designed to identify biomarkers of V&F intake.

Materials and Methods

Two hundred ninety-five members of the University of Minnesota community responded to advertisements requesting volunteers to participate in a nutrition study. Of these, 103 were recruited. They were nonsmokers, 18–37 years of age, who reported daily V&F intakes of either ≥5 servings (designated as a "high" intake) or ≤2 servings (designated as a "low" intake) during an initial telephone interview. Ninety-eight of the participants, 24 men and 25 women with high intakes and 25 men and 24 women with low intakes of V&F, collected dietary data and provided three 24-h urine collections. Ninety-one % of the participants were Caucasian; the remaining 9% were of Mexican-American, African-American, Chinese, Japanese, or Indian (subcontinent) descent. The participants were not informed of the exact focus of the study to avoid biasing the report of V&F intake.

Estimation of V&F intake during the initial telephone interview was based on respondents' reports of the total number of V&F servings eaten per day and descriptions of a typical day's food intake. One serving was defined as 120 ml (1/2 cup) cooked or raw fruit or vegetable, 240 ml (1 cup) raw lettuce, 60 ml (1/4 cup) dried fruit, or 180 ml (6 fluid ounces) of real juice. For the purposes of this study, juice could account for no more than two servings of the total V&F servings/day.

Exclusion criteria included: history of gastrointestinal disorders; food allergies; a weight change of >4.5 kg within the past year; major changes in eating habits within the past year (*e.g.,* eliminating a food group or nutrient; changing from an omnivorous to vegetarian diet or *vice versa*); exercise regimens requiring significant dietary changes; antibiotic use within the past 3 months; body weight >150% of ideal weight-for-height (Ref. 15; *i.e.,* for women: ideal = 100 lb + 5 lb for each inch >60; for men: ideal = 106 lb + 6 lb for each inch over 60); current treatment for a diagnosed disease; alcohol intake more than two drinks per day (two drinks are equivalent to 720 ml of beer, 240 ml of wine, or 90 ml of hard liquor). Initially, nutritional supplement use also was an exclusion criterion; however, because a large number of respondents reported using supplements, this criterion was abandoned. Nutritional supplements included vitamin and mineral supplements and herbal and protein supplements.

Each subject participated in the study over a 7-day period. On day 1, body weight and height were measured. Five-day food records were kept on days 2 through 6. Twenty-four-h urine collections were obtained on days 4, 5, and 6. A FFQ and a demographic and health questionnaire were completed. Information requested on the health questionnaire included: age, ethnicity, smoking habits, nutritional supplement use, alcohol intake, history of weight change, and menstrual history and oral contraceptive use for women.

The study design was approved by the Institutional Review Board: Human Subjects Committee at the University of Minnesota. Informed written consent was obtained from all participants prior to the start of the study.

Dietary Assessment

FFQ. All participants completed a modified self-administered, semiquantitative Willett FFQ assessing dietary intake over the past year (16, 17). The 153-item FFQ included 33 vegetables, 18 fruits, and 8 juices. V&F intake was quantified from the FFQ by summing the frequency of consumption across all V&F items using the portion sizes specified on the FFQ. Servings of total vegetables, total fruits, and total V&F were determined using 59 line items, whereas soy intake was determined using the single line item "tofu or soybeans." The items "vegetables or noodle soups" and "chowder or cream soups" were excluded from the total vegetable servings.

Diet Records. Participants recorded food intake for 5 days while they consumed their habitual diets. Nutrient calculations were performed using the Minnesota Nutrient Data System software (version 2.4; Food Database version 6A, Nutrient Database version 21, 1992), developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN (18). Intakes of V&F and of subgroups of V&F were calculated from the food records using a V&F classification scheme developed by the University of Minnesota Cancer Prevention Research Unit (19). Briefly, this classification scheme includes all edible plant tissues consumed in United States diets, excluding herbs, spices, nuts, seeds, and grains (except for sweet corn, which was counted as a vegetable). Four main groups were used: Total V&F; Total Vegetables; Total Fruits; and one category orthogonal to Total V&F, Botanical Groupings. Soy intake was determined using 18 soyfood line items. Standardized serving sizes of V&F were established, similar to those specified in the Dietary Guidelines for Americans (19).

Urinary Isoflavonoid and Lignan Analysis

Urinary isoflavonoid and lignan excretion was measured in a 3-day composite urine: 24-h urines collected on days 4–6. Each 24-h urine was collected separately into bottles containing 1 g of ascorbic acid. Urines were stored at 4°C until processed. Pooled aliquots of the three 24-h collections were stored at −20°C after addition of 10% sodium azide (final w/v, 0.1% sodium azide) until analysis.

Isoflavonoids (equol, DMA, daidzein, and genistein) and lignans (enterodiol, enterolactone, and matairesinol) were extracted from urine by ion-exchange chromatography and measured by gas chromatography/mass spectrometry according to the method of Adlercreutz *et al.* (20). The urine samples (1/900th of the 3-day collection) were passed over Bond Elute LRC C-18 columns (Chrom Tech, Inc., Apple Valley, MN) and washed with 5 ml of 0.15 mol/l acetate buffer (pH 3.0), and the adsorbed phytoestrogens were eluted with 4 ml of methanol. Then, the samples were diluted to 70% methanol with 1.6 ml water, applied to a 3-cm DEAE-Sephadex A-25 (Sigma Chemical Co., St. Louis, MO) anion-exchange column in the acetate form, and washed with 4 ml 70% methanol and 10 ml 0.2 M acetic acid in 70% methanol; the conjugated phytoestrogens were eluted with 10 ml of 0.3 M LiCl in 70% methanol. (Free,

Table 1 Daily nutrient and V&F intakes from 5-day food records for "high" *versus* "low" V&F consumers

Daily intakes	High V&F group[a] (n = 49)	Low V&F group[a] (n = 49)	P
V&F, servings/day	7.9 ± 3.2[b]	4.1 ± 1.9	0.0001[c]
Energy, kcal	2576 ± 819	2496 ± 647	0.6
Carbohydrate, % of kcal	58.1 ± 10.2	51.1 ± 7.7	0.0002
Protein, % of kcal	14.1 ± 2.9	14.0 ± 2.4	0.8
Fat, % of kcal	28.6 ± 9.1	33.8 ± 7.5	0.003
Dietary fiber, g/1000 kcal	11.5 ± 4.5	7.8 ± 3.5	0.0001
Dietary fiber from V, g/1000 kcal	2.2 ± 1.2	1.7 ± 1.1	0.04
Dietary fiber from F, g/1000 kcal	2.2 ± 1.5	0.6 ± 0.7	0.0001
Dietary fiber from grains, g/1000 kcal	7.1 ± 3.8	5.5 ± 2.4	0.014
Soy, servings/day	0.08 ± 0.24	0.01 ± 0.06	0.03[c]

[a] High and low V&F intake classification based on initial telephone report.
[b] Mean ± SD.
[c] P for t test analysis of square-root transformed data.

Table 2 Urinary lignan and isoflavonoid levels (μmol/day) for 98 participants

	Mean ± SD	Range	Geometric mean ± SD[a]
Enterolactone	3.64 ± 4.59	0.14–32.75	2.12 ± 2.25
Enterodiol	1.25 ± 3.44	0.02–29.31	0.45 ± 0.58
Matairesinol	0.98 ± 0.13	0.00–1.19	0.07 ± 0.06
Lignans[b]	4.96 ± 6.28	0.23–36.90	2.99 ± 2.96
Genistein	0.55 ± 1.22	0.03–7.18	0.22 ± 0.25
Daidzein	1.68 ± 4.36	0.04–29.48	0.56 ± 0.72
DMA	0.57 ± 2.62	0.03–19.49	0.13 ± 0.14
Equol	0.25 ± 1.02	0.00–9.36	0.08 ± 0.08
Isoflavonoids[c]	3.05 ± 8.23	0.17–55.06	1.13 ± 1.29
Phytoestrogens[d]	7.98 ± 13.20	0.54–90.42	4.79 ± 4.32

[a] Back-transformation of mean and SD of log-transformed data.
[b] Sum of lignans = enterolactone + enterodiol + matairesinol.
[c] Sum of isoflavonoids = DMA + daidzein + genistein + equol.
[d] Sum of lignans and isoflavonoids.

unconjugated forms of lignans and isoflavonoids are lost during this part of the extraction; however, in humans, these account for only ~2% of the compounds; Ref. 7.) The samples were dried under nitrogen to evaporate the methanol, and 1 ml of 1.5 M acetate buffer (pH 3.0) and 8 ml of water were added. These 10-ml fractions were again passed over Bond Elute LRC C-18 columns, as above. Next, deuterated internal standards of the unconjugated compounds (synthesized by Drs. T. Hase, K. Wähälä, and T. Mäkelä, Department of Chemistry, University of Helsinki in collaboration with Dr. H. Adlercreutz, Department of Clinical Chemistry, University of Helsinki) were added. The samples were incubated overnight with β-glucu-ronidase (*Helix pomatia* extract; Sigma) in 0.15 M acetate buffer (pH 4.1) at 37°C. The following morning, samples were drawn through Bond Elute C-18 columns, washed with 5 ml water, eluted with 4 ml methanol, and applied to 5-cm QAE-Sephadex A-25 (Sigma) anion-exchange column in the acetate form. Two fractions were collected; the first fraction containing the lignans and equol was eluted with 4 ml methanol, and the second fraction containing DMA, daidzein, and genistein was eluted with 7 ml of 0.2 M acetic acid in methanol. The first fraction was purified further on a 4-cm QAE-Sephadex A-25 (Sigma) column in the carbonate form and washed with 4 ml 80% methanol, and the lignans and equol were eluted with 5 ml of 0.1 M acetic acid in 80% methanol. Samples were dried under nitrogen and stored in 0.5 ml methanol at −20°C until deriva-tized for analysis. Trimethyl-silyl derivatives of the samples were analyzed by gas chromatography-mass spectrometry in the selective ion monitoring mode on a Hewlett-Packard (Wilmington, DE) 5890 and 5971A quadrupole gas chroma-tography-mass spectrometry instrument.

The urine samples were analyzed in six batches with QC urine samples included in duplicate in each batch. Twenty-four-h urine collections from three individuals were pooled to provide the QC urine pool. The mean values and interassay imprecision for the QC urine samples were as follows: entero-diol, 1.82 μmol/day (CV, 6.7%); enterolactone, 4.81 μmol/day (CV, 3.2%); matairesinol, 0.08 μmol/day (CV, 10.0%); equol 0.11 μmol/day (CV, 7.9%); DMA, 0.08 nmol/day (CV, 13.4%); daidzein, 2.41 μmol/day (CV, 3.2%); and genistein, 0.12 μmol/day (CV, 16.0%). Intraassay imprecision in each run was 10% or less for all compounds.

Statistical Analysis

Recruiting participants on the basis of initial "low" or "high" self-reported V&F intakes provided two relatively distinct pop-ulations based on data from the FFQ; however, significant overlap between the two groups did occur (21). We compared nutrient intakes and urinary lignans and isoflavonoids between low and high V&F intake groups and between men and women using an unpaired *t* test. Intakes of food groupings were square-root transformed [square-root (servings/day)], dietary fiber was converted to g of dietary fiber/1000 kcal, and all urinary phy-toestrogen excretion data were log-transformed [ln(nmol/day)] prior to analysis. Unadjusted Pearson correlations were used to assess the relationships between intakes of various food group-ings and urinary phytoestrogen excretion. Similar correlations were obtained when we adjusted for energy intake; therefore, we report only the unadjusted correlations.

Results

The high and low V&F groups were established based on estimates from initial, self-reported V&F intakes, low (≤2 servings/day) and high (≥5 servings/day) V&F intake. As described previously (21), the high and low groups were similar for almost all general characteristics (*i.e.,* age, body mass index, ethnicity, plasma cholesterol, and dietary supplement use), with the exception of alcohol intake; there was a lower proportion of drinkers in the high V&F group. There were significant differ-ences in overall diet between the two groups. On the basis of the analysis of 5-day food records, the high group compared with the low group consumed a higher percentage of energy from carbohydrate and lower percentage of energy from fat, despite similar intakes of total energy and percentage of energy from protein (Table 1). As expected, V&F intake was higher in the high V&F group, and this group also had higher intakes of soyfoods and dietary fiber from all sources.

In Table 2, we present the means, geometric means, and ranges of urinary lignan and isoflavonoid excretion for the 98 participants in the study. In Table 3, we divide the participants according to their V&F intake. On average, urinary lignan excretion among the high V&F intake group was 50% higher than that of the low intake group (P = 0.04); however, the variability in excretion was great in both groups and individual lignan differences between the groups were not statistically significant. The sum of all compounds (lignans + isofla-vonoids) also was significantly higher in the high V&F intake group (P = 0.016).

Thirty-two of the participants (33%) reported regular use

Table 3 Urinary lignan and isoflavonoid excretion (μmol/day) in "high" *versus* "low" V&F consumers

	High V&F group[a] (n = 49)	Low V&F group[a] (n = 49)
Enterolactone	2.53 ± 2.84[b]	1.77 ± 1.73
Enterodiol	0.56 ± 0.84	0.36 ± 0.36
Matairesinol	0.07 ± 0.14	0.06 ± 0.05
Lignans[c]	3.69 ± 4.03	2.42 ± 2.03
Genistein	0.24 ± 0.30	0.21 ± 0.20
Daidzein	0.56 ± 0.86	0.56 ± 0.60
DMA	0.15 ± 0.20	0.11 ± 0.09
Equol	0.08 ± 0.09	0.08 ± 0.08
Isoflavonoids[d]	1.20 ± 1.61	1.06 ± 0.97
Phytoestrogens[e]	5.95 ± 5.82	3.85 ± 2.97

[a] High and low V&F intake classification based on initial telephone report.
[b] Back-transformation of mean and SD of log-transformed data.
[c] Sum of lignans = enterolactone + enterodiol + matairesinol; significantly different between high and low intake groups, $P = 0.04$.
[d] Sum of isoflavonoids = DMA + daidzein + genistein + equol.
[e] Sum of lignans and isoflavonoids; significantly different between high and low intake groups, $P = 0.016$.

Table 4 Urinary lignan and isoflavonoid excretion in men compared with women

	Men (n = 49) μmol/day	Women (n = 49) μmol/day	P[a]
Enterolactone	2.29 ± 2.43[b]	1.96 ± 2.09	0.5
Enterodiol	0.48 ± 0.68	0.41 ± 0.50	0.5
Matairesinol	0.07 ± 0.06	0.06 ± 0.05	0.4
Lignans[c]	3.28 ± 3.35	2.73 ± 2.62	0.4
Genistein	0.29 ± 0.38	0.17 ± 0.14	0.01
Daidzein	0.83 ± 1.24	0.37 ± 0.38	0.002
DMA	0.17 ± 0.24	0.09 ± 0.07	0.007
Equol	0.09 ± 0.11	0.07 ± 0.06	0.4
Isoflavonoids[d]	1.59 ± 2.13	0.80 ± 0.62	0.003
Phytoestrogens[e]	5.86 ± 5.80	3.91 ± 2.99	0.03

[a] *P*s comparing men *versus* women.
[b] Back-transformation of mean and SD of log-transformed data.
[c] Sum of lignans = enterolactone + enterodiol + matairesinol.
[d] Sum of isoflavonoids = DMA + daidzein + genistein + equol.
[e] Sum of lignans and isoflavonoids.

of dietary supplements. Of these, 28 (88%) used a multivitamin or an individual nutrient supplement (*e.g.,* calcium, iron, vitamins C and E). Four men and no women reported consuming a supplement other than a multivitamin or individual micronutrient; they used supplements containing bioflavonoids, garlic, and glandular extracts. One man also used an alfalfa supplement. His urinary enterolactone and daidzein levels were among the highest, −32.7 and 29.5 μmol, respectively. Eliminating these four men did not change any of the results of the analyses. Thirty-eight % of the women (40% of high and 32% of low V&F intake group; nonsignificant difference) reported current use of oral contraceptives. Lignan and isoflavone excretion was not significantly different between oral contraceptive users and nonusers.

Urinary lignan excretion was similar in men and women; however, urinary excretion of all of the isoflavonoids, except equol, was higher in men than women (Table 4). This was not due to differences in soy intake. Prevalence of soy consumption, estimated from the 5-day food records, was comparable in men and women; 41% of men and 37% of women consumed soy products. Also, among the individuals consuming soy, daily intake of soy was not different between men and women, 0.3 and 0.2 servings/day, respectively ($P = 0.3$).

We examined the association between soy intake and isoflavonoid excretion, and the sum of the urinary isoflavonoids was correlated more strongly with intake of soyfoods as determined by the 5-day food record ($r = 0.39$; $P = 0.0001$) compared with FFQ ($r = 0.25$; $P = 0.014$; Fig. 1). When we excluded individuals with no reported soy intake, the correlation between isoflavonoid excretion and soyfood intake measured by food record was even stronger ($r = 0.53$; $P = 0.0008$; $n = 37$); however, it disappeared when soy intake was measured by FFQ ($r = 0.04$; $P = 0.8$; $n = 25$). Associations between the individual isoflavonoids and soy intake measured by food record and FFQ, respectively, were as follows: genistein: $r = 0.40$, $P = 0.0001$ and $r = 0.25$, $P = 0.015$; daidzein: $r = 0.34$, $P = 0.0007$ and $r = 0.20$, $P = 0.046$; and DMA: $r = 0.37$, $P = 0.0002$ and $r = 0.23$, $P = 0.025$. Excluding nonconsumers of soy from the analyses also improved the associations between the individual isoflavonoids and soy intake measured by food record (genistein: $r = 0.60$, $P = 0.0001$; daidzein: $r = 0.50$, $P = 0.0015$; and DMA: $r = 0.47$, $P = 0.0032$) but not by FFQ. There was no association between equol excretion and soy

intake or between the isoflavonoids and any other V&F groupings, including legumes and mature beans. Soy intake measured by FFQ was correlated with soy intake measured by food records ($r = 0.46$, $P = 0.0001$), despite the fact that the FFQ only asked about intake of "tofu and soybeans" over the past year and that the food record reflected 5 days.

Among the individuals who reported no soy intake, urinary isoflavonoid levels ranged from 0.21 to 18.80 μmol/day (Fig. 1). Review of the food records of nonconsumers of soy with high urinary isoflavonoids revealed that these individuals ate a number of prepared, meat-based, mixed dishes, of which vegetable protein was an ingredient. We examined the association between isoflavonoid excretion and intake of other food groupings (estimated by the FFQ) to determine to what extent other foods may have contributed to isoflavone excretion. The only foods for which intake was associated with excretion of total isoflavonoids, as well as genistein and daidzein individually, were processed meats ($r = 0.23$, $P = 0.02$) and high-fat meats ($r = 0.22$, $P = 0.03$). Interestingly, r values for associations between equol excretion and processed and high-fat meats were also significant, 0.31 ($P = 0.002$) and 0.27 ($P = 0.007$), respectively. There was no significant association between meat intake and DMA. Although the prevalence of high-fat meat consumption was not significantly different between men and women, among individuals consuming high-fat meats, men tended to have higher intakes ($P = 0.047$). The association between isoflavonoid excretion and high-fat meat intake was strongest in men who did not consume soy.

We also examined the association between urinary isoflavonoid excretion and soy intake data from the FFQ, grouped by intakes of "never or less than once per month" ($n = 73$) and "greater than once per month" ($n = 25$). The latter group was comprised of individuals consuming soy "one to three times per month" ($n = 16$), "once per week" ($n = 3$), "two to four times per week" ($n = 4$), and "once per day" ($n = 2$). The group consuming soy more than once a month had significantly higher urinary excretion of DMA ($P = 0.03$), daidzein ($P = 0.02$), genistein ($P = 0.01$), and the sum of the isoflavonoids ($P = 0.006$). Equol excretion did not differ between the groups.

The sum of the urinary lignans (enterodiol + enterolactone + matairesinol) was significantly correlated with intake of total V&F ($r = 0.25$; $P = 0.01$), total fruit ($r = 0.27$; $P = 0.008$), and soyfoods ($r = 0.28$; $P = 0.006$), as measured by food
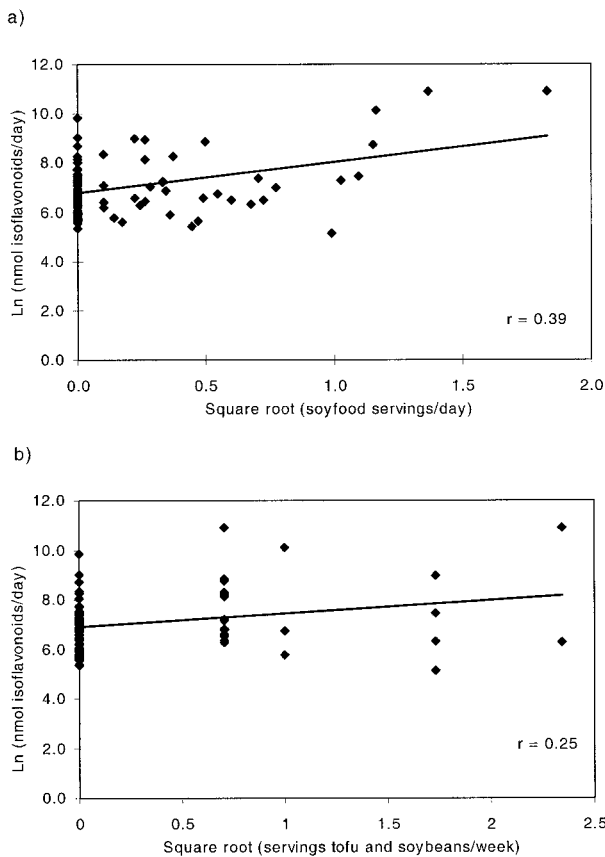
a)

b)

Fig. 1. Urinary isoflavonoid excretion (sum of DMA, daidzein, genistein, and equol) *versus* soyfood intake as measured by food record (*a*) and FFQ (*b*).

Table 5 Pearson correlations between urinary lignans and intake of dietary components as measured by food record

Dietary component	Enterolactone	Enterodiol	Lignans[a]
Total V&F	0.19[b]	0.23[c]	0.25[c]
Total vegetables	0.06	−0.03	0.07
Total fruit	0.20[c]	0.29[b]	0.27[b]
Dietary fiber	0.24[c]	0.36[b]	0.36[c]
Dietary fiber from vegetables	0.03	0.01	0.06
Dietary fiber from fruit	0.18	0.28[b]	0.24[c]
Dietary fiber from grains	0.22[c]	0.34[b]	0.34[b]

[a] Sum of lignans = enterolactone + enterodiol + matairesinol.
[b] *P* for Pearson correlation <0.01.
[c] *P* for Pearson correlation <0.05; correlations without superscripts are not significant.

were not statistically significant when dietary fiber was estimated by FFQ.

Discussion

We measured urinary phytoestrogen excretion in 98 young healthy men and women with low and high V&F intakes and examined the relationship between urinary phytoestrogens and dietary intake of specific food groups in this population. There was a wide range in urinary phytoestrogen levels; daily excretion ranged from 0.2 to 37 μmol/day and 0.2 to 55 μmol/day for lignans and isoflavonoids, respectively. The data shed light on some unexpected associations between urinary phytoestrogens and food. They also provide evidence that, as with other aspects of colon biology, there may be differences in isoflavone excretion between men and women.

Urinary lignan and phytoestrogen excretion (lignans and isoflavonoids combined) were greater in individuals with higher V&F intakes, despite the high variability in excretion in both groups. Intake of a number of dietary constituents differed between the low and high V&F groups. Not only were there differences in V&F intake, as expected, but the overall patterns of energy intake differed. In addition, mean dietary fiber intakes from grains, as well as from V&F, were higher in the high V&F group. Adlercreutz *et al.* (13) have shown previously that there are differences in lignan and isoflavone excretion among groups consuming very different diets (*e.g.,* macrobiotic, vegetarian, and omnivorous diets). Our data suggest that, even among individuals consuming a Western diet, urinary phytoestrogen excretion can reflect differences in dietary patterns.

Consistent with some of our earlier observations on gut function, we found sex differences in isoflavone excretion; in this study, men had higher urinary isoflavone excretion of genistein, daidzein, and DMA but not equol. Among Singapore Chinese (76 men and 71 women), Seow *et al.* (22) reported no difference in mean isoflavone values (measured in a spot urine) for men and women. Furthermore, previously, we did not detect sex differences in isoflavonoid excretion when individuals were given a high-isoflavone soy challenge (23). Although there was no statistically significant sex difference in prevalence of soy consumption or in amounts of soy consumed, a tendency toward higher soy or meat (see below) consumption among men in the present study may account for these differences. At the same time, there are recognized sex differences in colonic function (24) that may influence isoflavone metabolism under habitual dietary conditions of moderate soy intake.

The stronger association between urinary isoflavone excretion and soyfood intake as measured by food records compared with FFQ is hardly unexpected for the following reasons:

record. It was not associated with total vegetable, and none of these associations were significant for intakes of these food groupings as measured by FFQ. Similar associations were found for the individual lignans (Table 5).

In vitro work (11), correlative studies (13), and recent food analyses (2) have identified V&F sources of lignans. On this basis, we examined the relationship between lignan excretion and 10 botanical groupings of V&F: *Umbelliferae, Ericaceae, Liliaceae, Compositae, Cruciferae, Cucurbitae, Leguminosae, Rosaceae, Rutaceae,* and *Solanaceae.* Of these, only intakes of *Cucurbitaceae* (*i.e.,* squash, melons, and others; $r = 0.21$; $P = 0.04$) and *Rosaceae* (*i.e.,* stone fruits and berries, such as strawberries and raspberries, and others; $r = 0.23$; $P = 0.02$) were associated with the sum of urinary lignans.

We also examined the association between intakes of various dietary fiber sources and lignan excretion (Table 5). Secoisolariciresinol and matairesinol, precursors of the mammalian lignans, are subunits involved in lignin formation; therefore, foods high in dietary fiber, particularly lignin, may contribute to urinary lignan excretion. When dietary fiber was estimated by food record, each of the following: total dietary fiber, dietary fiber from fruits, and dietary fiber from grains (determined by difference: total dietary fiber minus fiber from V&F), but not dietary fiber from vegetables, were associated significantly with lignan excretion. In particular, total dietary fiber intake per 1000 kcal was strongly correlated with lignan excretion ($P = 0.0003$; Fig. 2). However, these associations
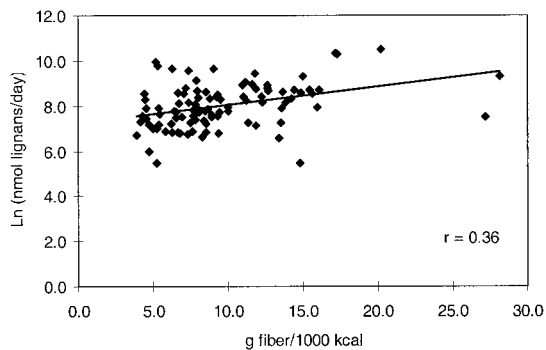
Fig. 2.   Urinary lignan excretion (sum of enterolactone, enterodiol, and matairesinol) *versus* dietary fiber intake as measured by food record.

(*a*) there were 18 soyfood entries, including traditional and second-generation soyfoods, available in the Nutrient Data System database for use in food record analysis, whereas the FFQ relied on one line-item; (*b*) the 5 days when records were kept overlapped with the 3 days of urine collection, whereas the FFQ asked about food intake over the past year; and (*c*) the half-life of plasma isoflavones in the body is approximately 6–8 h, and therefore urinary or plasma isoflavonoid concentrations reflect recent exposure to soy (25). Nonetheless, given the correlation between soyfood intake and isoflavone excretion ($r = 0.4$), even soyfood intake estimated by the 5-day food record explains only 16% of the variance in total isoflavonoid excretion.

The high isoflavone excretion among some individuals with no reported soy intake (Fig. 1) also suggests that, on a typical American (United States) diet, other food sources contribute substantially to isoflavone intake. The most likely candidates are processed foods containing soy flour or soy protein; isoflavones from these sources are difficult to detect by the usual self-report methods of monitoring soyfood intake. Thus, a comprehensive database of isoflavone content of foods must include not only traditional (first-generation) soyfoods (*e.g.,* tofu, tempeh, and soymilk), second-generation soyfoods (*e.g.,* soy-based meat analogues), but also prepared food items (*e.g.,* meat products with added soy protein) to capture total dietary isoflavone exposure. There are some non-soy-based foods that are also sources of isoflavones. Daidzein and genistein have been identified in legume sprouts, such as clover and alfalfa (4), and in some alcoholic beverages (26). Equol is present in low amounts in cow's milk and other dairy foods (27, 28).

Isoflavonoid excretion was associated with processed and high-fat meat intake, particularly among men who had low soyfood intakes. These findings may reflect, in part, the addition of soy protein to processed meats. Depending on the meat product, soy flour, soy protein concentrate, or soy protein isolate are added at various levels as processing aids (29). Present United States laws permit addition of up to 3.5% soy flour or soy protein concentrate to cooked or fresh sausages before product category names have to be changed. Up to 30% hydrated soy protein is permitted in commercially made hamburger patties; however, a 20% level is more acceptable to consumers (30). Murphy *et al.* (31) have reported isoflavone levels in USDA commodity beef patties in the range of 1–2 mg/100 g of edible portion.

The association between equol excretion and meat intake is especially interesting, because typically, only about one-third of individuals have colonic bacteria capable of producing equol from daidzein (23); therefore, we would not expect equol to be

associated with intake of a particular dietary constituent. For example, we found no association between equol excretion and soy intake in this same population. We cannot determine from this study whether the relationship between urinary equol excretion and meat intake reflects a diet-induced isoflavone-metabolizing profile with improved production of equol from daidzein or is due to equol in the meat itself. In a study of 19 Japanese consuming a traditional Japanese diet, Adlercreutz *et al.* (6) also observed an association between equol excretion and intake of meat, as well as total fat. They hypothesized that consuming more fat and meat creates a colonic environment capable of sustaining equol-producing bacteria. This is one explanation. However, with a prescribed soy dose, we found no relationship between fat or animal protein intake and equol-excreter status in 60 men and women (23).

Another possible explanation for the association between high-fat meat intake and equol excretion is that equol is present in the meat of animals on soy-, alfalfa-, or clover-supplemented feed. These legumes all contain daidzein or formononetin, a precursor of daidzein (31, 32). Ruminant microflora very effectively convert daidzein to equol (33), and equol has been detected in bovine milk at concentrations of 45–293 ng/ml, higher than those for daidzein (<5 ng/ml) and genistein (2–30 ng/ml; Ref. 28). In poultry, daidzein and formononetin are also converted to equol (34, 35). It is likely that other animals have similar capacities for isoflavone metabolism. To date, the isoflavone content of muscle tissue has not been examined; however, steroid hormones are present in measurable concentrations in muscle and fat (36). The similarity in structure between isoflavones and sex steroid hormones suggests that these plant compounds might also be present in meat of soy-eating animals. Furthermore, in studies of laboratory animals, isoflavone supplementation results in detectable concentrations of the isoflavones and their metabolites in uterine (37) and mammary tissue (38). Thus, in countries where soy or other isoflavone-containing legumes are an important constituent of animal feed, isoflavones in meat may contribute to dietary exposure in humans.

Despite the rather weak correlation between urinary isoflavone excretion and soy intake by FFQ ($r = 0.25$), urinary isoflavones were higher in the group of individuals consuming soy more than once a month compared with those consuming soy less than once a month or never. A number of studies of populations that typically consume soyfoods have established a strong positive association between soy intake and isoflavone excretion. Soy product intake and urinary isoflavones, from a 3-day urine collection, were significantly correlated in a group of 19 men and women consuming a typical Japanese diet (6). Among Singapore Chinese, urinary daidzein and sum of urinary daidzein, genistein, and glycitein measured in spot urine collections were also associated in a dose-dependent manner with intake of traditional Chinese soyfoods (22). Similarly, among Chinese women in Shanghai, isoflavones in overnight urine collections were also associated with soy intake ($r = 0.5$; $P < 0.001$; Ref. 9). Maskarinec *et al.* (8), studying a multiethnic population of women in Hawaii ($n = 102$), showed an association between isoflavones measured in an overnight urine and soy protein intake, both in the previous 24 h ($r_s = 0.61$; $P < 0.0001$) and in the past year ($r_s = 0.32$; $P < 0.0012$). Franke and Custer (7), also in Hawaii, reported the ability to discriminate, using overnight urine isoflavone levels, between 7 women consuming tofu more frequently than once a week and 16 women eating tofu less than once a week. In our population, more than 70% reported on the FFQ that they consumed soy less than once a month or never. This, coupled with the uni-

dentified sources of isoflavones contributing to urinary isoflavone excretion, reduced the magnitude of the association.

On average, daily urinary isoflavonoid excretion among our study participants was similar to that reported for a multiethnic cohort in the San Francisco Bay area (39) and for Caucasian women in Hawaii (8). Daidzein excretion (geometric means) for our predominantly Caucasian population at the University of Minnesota and Caucasian, Japanese, African-American, and Latina women in the San Francisco Bay Area was 0.56, 0.13, 0.29, 0.32, and 0.48 μmol/day, respectively. Mean daidzein excretion in our population (1.68 μmol/day) was also similar to that of Caucasian women in Hawaii (1.90 μmol/day; calculated from mean nmol/h) and higher than in individuals of Chinese (0.69 μmol/day) or Anglo-Celtic (0.16 μmol/day) origin in Australia (40). These levels are approximately four times lower than in Japanese in Hawaii and two times lower than in Native Hawaiians and Chinese in Hawaii (8).

Under controlled dietary conditions, isoflavone excretion is associated positively with isoflavone intake. For example, daily daidzein excretion was 0.44, 3.65, 5.29, and 9.86 μmol/day with daily soy protein powder intakes of 0, 5, 10, and 20 g (0, 3.25, 6.5, and 13 mg daidzein), respectively (5). With a mean daidzein excretion of 1.68 μmol/day in the present study, we estimate that soy protein intake among our participants would be approximately 1–2 g/day. Soy protein intakes of Caucasians in Hawaii were estimated at 2.8 and 1.8 g/day during the previous 24 h and previous year, respectively (8); in comparison, soy protein intakes in Japanese (6.1 g/day) and Chinese (4.8 g/day) in Hawaii (8) were three to four times higher than in United States Caucasians. Soy protein intake among Chinese in Singapore was only 2 g/day with a corresponding urinary daidzein concentration of 1.9 nmol/mg creatinine (22), whereas soy protein intake among Chinese in Shanghai was 8.5 g/day with a corresponding urinary daidzein concentration of 3.7 nmol/mg creatinine (9). Although differences in methods of collection of dietary intake data and urine (*e.g.,* 24-h *versus* spot *versus* overnight) contribute to variability among studies, there appears to be a relatively consistent relationship between urinary isoflavonoids and self-reported soy intake both within and across study populations.

Soyfood consumption is still relatively low and uncommon in the continental United States, and monitoring exposure to isoflavones using either dietary report or biological measures poses a number of challenges: (*a*) short-term dietary recall and recording techniques are not very useful because within-person variability in intake may exceed between-person variability; (*b*) the half-lives of the isoflavones genistein and daidzein are short (25); therefore, if isoflavone exposure is monitored using urinary or plasma markers, intermittent soy consumption may be severely under- or overestimated; (*c*) metabolism of isoflavones is inextricably linked to the health of colonic bacterial populations and the effects of diet and drugs on the colonic environment. The isoflavone metabolites, DMA and equol, have longer half-lives and therefore, theoretically, might be better markers. Only ~30% of individuals consuming Western diets, however, are equol excreters; therefore, measuring equol is not a viable alternative. There was no indication from our data that the association between soy intake and DMA excretion was any stronger than that between soy consumption and genistein and daidzein excretion. Barnes *et al.* (41) also have observed that plasma concentrations of genistein and daidzein decreased over 14 days of soy protein supplementation, suggesting either a metabolic adaptation to the isoflavone load or altered excretion.

This could result in an underestimation of isoflavone exposure among regular soy consumers.

Lignans are more ubiquitous in the Western diet than are isoflavones. They are closely related to the polymeric lignins, occur typically in vascular plants, and are found in roots and rhizomes and the woody parts, stems, leaves, seeds, and fruits. The two dietary lignans, secoisolariciresinol and matairesinol, derived from coniferyl alcohol (42), are immediate precursors of the mammalian lignans, enterodiol and enterolactone, respectively (43); however, studies suggest that there are probably additional precursors (2, 44). The oilseeds (flax, soy, and rapeseed), whole-grain cereals (wheat, oats, and rye), legumes, and various vegetables and fruit are rich sources of lignans (11).

In our study, the association between V&F intake and urinary lignan excretion was due exclusively to an association between fruit intake and lignan excretion; there was no association with vegetable intake. This was surprising because Adlercreutz *et al.* (6) reported that, in Japanese men and women, lignan excretion was correlated significantly with intakes of green and yellow vegetables, pulses and beans, and boiled soybeans. Although some soyfoods do contain lignan precursors (2), the soyfood-lignan association that we observed here most probably reflects a tendency for individuals consuming the higher amounts of V&F also to be consuming a higher-fiber diet and more soyfoods (Table 1). We demonstrated previously that vegetable supplementation, as part of a low-fiber, low-phytochemical diet, significantly increased lignan excretion, and we hypothesized that the woody portions of the cruciferous vegetables and carrots were contributing to the lignan exposure (14). However, in the present study, dietary fiber from vegetables was not associated with urinary lignans. *Cucurbitaceae* and *Rosaceae* were the only 2 of 10 V&F botanical groupings that were correlated with lignan excretion. These are botanical families that contain a number of fruits commonly consumed on a Western diet, and both families include fruits with edible seeds (*e.g.,* cucumbers, summer squash, strawberries, and raspberries) and edible skins (*e.g.,* cucumbers, summer squash, stone fruit, apples, pears, and others). Seeds, such as sesame and pumpkin seeds, and the skins and seeds of berries are reported to be high in lignans (2); thus, it is likely that the *Cucurbitaceae* and *Rosaceae* families may be rich sources of lignans.

Intake of total dietary fiber and fiber from grains was most strongly associated with lignan excretion (Fig. 2 and Table 5). Adlercreutz *et al.* (13, 45) have demonstrated similar associations. In addition, they reported that certain groups consuming special diets have higher urinary lignan levels. Compared with omnivores, vegetarians excreted significantly higher amounts of lignans in urine and feces (10, 46). Urinary enterolactone levels (geometric means) in the high V&F group in our study (2.53 μmol/day) were similar to those in omnivores in Boston and Helsinki (2.05 and 2.46 μmol/day, respectively) and women eating an omnivorous diet containing <10 g fiber/day (2.55 μmol/day; Ref. 47). Levels were lower than for Bostonian lactovegetarians (4.17 μmol/day) and macrobiotics (17.68 μmol/day; Ref. 10) and a multiethnic population in California (4.31 μmol/day; Ref. 39). Our low V&F group had the lowest enterolactone level (1.77 μmol/day). Enterodiol excretion followed a similar pattern.

Despite the fact that lignan precursors are more widespread than isoflavones in plant foods, the associations we observed between diet and lignan excretion were weaker than those for diet and isoflavones, when food intake was assessed by 5-day records. There were no significant relationships between diet and lignan excretion when intake was measured by

FFQ. This probably reflects: (*a*) the measurement error inherent in assessing diet by FFQ (48); (*b*) the variation in lignan content of high-fiber foods; (*c*) the seasonal and varietal variation in lignan content within a food (49); and (*d*) the effects of food processing on lignan precursor availability. Like the isoflavones, enterolactone and enterodiol have relatively short half-lives in the body (47), and their production relies on colonic bacteria (10, 43); therefore, differences in intestinal environment among individuals may also contribute to the observed variation and difficulty establishing associations between diet and lignan excretion. Apart from the work of Adlercreutz *et al.* (10, 13, 45, 46) and our present contribution, the association between diet and lignan excretion in observational studies has not been investigated; more work is needed in this area.

In conclusion, urinary isoflavone excretion was associated positively with intake of soyfoods, even in a population that does not regularly consume soy; however, "hidden sources" of isoflavones appeared to contribute significantly to isoflavone exposure in this United States population. Lignan excretion was associated with fruit, but not vegetable consumption, with dietary fiber intake from fruit and grains and with intake of certain botanical families of V&F. Together, urinary lignans and isoflavonoids are associated with a higher intake of V&F and may serve as a useful overall marker of plant food intake.

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