Dietary Determinants of Plasma Enterolactone[1]

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Abstract

Enterolactone is a lignan produced by fermentation of dietary precursors in the human gut. Because lignan precursors are uniquely found in plant foods, plasma enterolactone concentration may serve as a biological marker of plant food consumption. This cross-sectional study examined associations of dietary intake with plasma enterolactone concentration. Weight-stable, 20–40-year-old volunteers (115 women and 78 men in Seattle, Washington) reporting intake of ≤2.5 or ≥4.5 fruit and vegetable servings/day and no antibiotic use for ≥3 months completed a food frequency questionnaire and 3-day food record. Time-resolved fluoroimmunoassay was used to measure plasma enterolactone. Based on diet records, plasma enterolactone was positively correlated with daily vegetable servings ($r = 0.17$; $P < 0.05$), fiber ($r = 0.36$; $P < 0.0001$), alcohol ($r = 0.24$; $P < 0.001$), caffeine ($r = 0.21$; $P < 0.001$), and daily botanical group servings [*Chenopodiaceae* ($r = 0.15$; $P < 0.05$), *Juglandaceae* ($r = 0.15$; $P < 0.05$), *Leguminosae* ($r = 0.20$; $P < 0.001$), *Pedaliaceae* ($r = 0.20$; $P < 0.001$), and *Vitaceae* ($r = 0.20$; $P < 0.001$)]. Fat-related variables were not correlated with plasma enterolactone. Based on linear regression models, plasma enterolactone increased by 37.0% (SE = 2.3%) for each 10-g increase in fiber and by 6.6% (SE = 0.2%) for each 50-mg serving of caffeine. Participants consuming 0.5–1 alcoholic drink/day had plasma enterolactone concentrations that were 131.4% (SE = 37.6%) higher than those of nondrinkers. Although plasma enterolactone may be useful as a biological measure of exposure to lignan-containing foods, it may be of limited use as a specific biomarker of fruit and vegetable or plant food intake because coffee, tea, and alcoholic beverages also significantly increase its plasma concentration.

Introduction

Lignans are phytochemicals that may influence disease risk through a variety of biological activities including sex hormone modulation and altered proliferation, differentiation, and angiogenesis (1). These diphenolic compounds are closely related to the polymeric lignins and typically occur in the roots, rhizomes, stems, leaves, seeds, and fruits of vascular plants (2). The oilseeds (flax, soy, and rapeseed), whole grain cereals (wheat, oats, and rye), legumes, and various vegetables and fruits, particularly berries, are rich sources of lignans (3–5). Brewed teas, coffees, and wine are also significant sources (4).

Lignans consumed in plant foods are metabolized by colonic bacteria to more biologically active metabolites; both the parent compounds and the metabolites are measurable in various body fluids (6–9). Matairesinol and secoisolariciresinol diglycoside are the two major lignans in grains, vegetables, and fruits and are metabolized by colonic microflora to enterolactone and enterodiol, respectively. Enterodiol is further oxidized to enterolactone. Thus, circulating and excreted concentrations of enterolactone may be useful biomarkers of exposure to lignan-containing foods.

Enterolactone has been shown to have antitumor, antiaromatase, and antiangiogenic effects in human cell lines, offering some biological plausibility for the idea that enterolactone exposure may reduce risk of some cancers (10). Epidemiological studies have explored associations between urinary and circulating lignan concentrations and breast cancer (11–13); however, only a few studies have carefully examined the relationships between diet and lignan concentrations in biological samples (2, 14–18). Urinary lignan excretion is positively associated with dietary fiber intake as well as with diets that are, on average, higher in fiber and carbohydrate and lower in fat. Thus, higher lignan concentrations have been proposed as a marker of dietary patterns that include greater amounts of vegetables and fruits (2, 15, 18). The purpose of this study was to identify the dietary components that are associated with plasma enterolactone concentrations.

Materials and Methods

Study Population

Participants were 115 female and 78 male volunteers recruited from the greater Seattle area. All were nonsmokers, ages 20–40 years, who reported consuming either ≤2.5 or ≥4.5 servings/day of fruits and vegetables during an initial telephone interview. Participants were not informed of the exact purpose of the study to avoid biasing the dietary self-report. Exclusion criteria included Crohn's disease, ulcerative colitis, inflammatory bowel disease, diabetes, liver disease, weight change of >4.5 kg within the past year, major changes in eating habits within the past year, exercise regimens requiring significant short-term dietary changes, antibiotic use within the past 3 months, body

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weight > 150% of ideal, current use of prescription medications (excluding topical agents), chronic nonsteroidal anti-inflammatory drug use, alcohol intake of >2 drinks/day (2 drinks were equivalent to 720 ml of beer, 240 ml of wine, or 90 ml of spirits), occupational exposure to smoke (tobacco, wood, and so forth) or organic solvents, chronic exposure to second-hand tobacco smoke, and intake of pharmacological doses of dietary supplements. Participants were instructed to avoid all medication for seven days prior to and during the study. The Institutional Review Board of the Fred Hutchinson Cancer Research Center approved all procedures.

Data and Sample Collection

Participants completed a demographic, health history, and FFQ,[4] as well as food records, on 3 consecutive days. Twelve-h fasting blood samples were obtained on the day of the third food record and the following day and were used to measure plasma enterolactone. Details of dietary assessment and plasma enterolactone measures are given below.

FFQ. The FFQ used in this study is the same instrument used in the Women's Health Initiative (19), with a reference period of "over the past 3 months". This questionnaire contains 99 food items, with 19 questions about food purchasing and preparation practices and 2 summary questions that asked about the frequency of consuming all servings of fruit (excluding juice) and all vegetables (excluding salad or potatoes). The nutrient database is derived from the University of Minnesota Nutrition Coordinating Center (20), and the algorithms for analysis are described elsewhere (21). Daily vegetable and fruit intakes were calculated using the "5-A-Day" method (22). Total vegetables was calculated as "servings of vegetables, not including salad or potatoes" plus servings of salad and potatoes (not fried); total fruit was calculated as "servings of fruit, not including juices." Botanical groupings were not derivable from the FFQ information.

Food Records. A trained nutritionist instructed participants in how to collect food records and reviewed returned records for accuracy and completeness. Records were analyzed using the University of Minnesota Nutrition Data System, (Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN; Food Database version 12A, release date November 1996; Nutrient Database version 27, release date November 1996). Servings/day of fruits and vegetables were calculated using a scheme developed by the Nutrition Assessment Shared Resource at the Fred Hutchinson Cancer Research Center (22). Briefly, this classification scheme includes all edible plant tissues included in the Nutrition Data System Food Database, excluding herbs, spices, and grains, except for sweet corn. Servings/day of fruits and vegetables are calculated based on standardized serving sizes similar to those specified in the Dietary Guidelines for Americans (23) and classified both by culinary form (*e.g.,* juice, fresh, fried) and into 63 botanical families. In this study, we limited analysis to the 18 botanical groups for which there were at least 30 values greater than 0, namely, *Cucurbitaceae* (*e.g.,* squash and melons), *Cruciferae* (*e.g.,* broccoli, cauliflower, cabbage, and so forth), *Roseaceae* (*e.g.,* apples, pears, stone-fruits, strawberries, raspberries, and so forth), *Solanaceae* (*e.g.,* tomatoes, peppers, potatoes, and so forth), *Leguminosae* (*e.g.,* beans, peas, and so forth), *Rutaceae* (citrus fruits), *Liliaceae* (*e.g.,* onions, leeks, and garlic), *Compositae* (lettuces), *Lauraceae* (avocado), *Chenopodiaceae* (*e.g.,* spinach, beets, Swiss chard, and so forth), *Vitaceae* (grapes), *Umbelliferae* (*e.g.,* carrots, celery, and parsley), *Musaceae* (bananas), *Juglandaceae* (walnuts and pecans), *Ericaceae* (*e.g.,* blueberries, cranberries, and lingonberries), *Oleaceae* (olives), *Pedaliaceae* (sesame seeds), and *Agaricaceae* (cultivated button mushrooms).

Plasma Enterolactone. Plasma enterolactone concentrations were measured on a Wallac 1420 Victor Multilabel Counter (Perkin-Elmer Life Sciences, Gaithersburg, MD) using a time-resolved, fluoroimmunoassay kit (Perkin-Elmer Life Sciences). Our only modification to the packet insert was the inclusion of 4-methylumbilliferone glucuronide as an internal standard to estimate sample recovery. The plasma enterolactone assay had interassay percentage coefficients of variation of 17% at higher concentrations (97 nmol/liter) and 26% at lower concentrations (38 nmol/liter). Intra-assay percentage coefficients of variation were 15% (97 nmol/liter) and 13% (38 nmol/liter). Values less than 1.2 nmol/liter were considered to be below the detection limit, and we assigned a value of 0.6 nmol/liter to plasma samples with enterolactone concentration <1.20 nmol/liter after correction for 4-methylumbilliferone recovery.

Statistical Analysis

The 2-day average of plasma enterolactone measures was used to characterize each participant. To normalize distributions, plasma enterolactone concentrations and nutrient measures (with the exception of cholesterol and those expressed as nutrient density) were log-transformed, and dietary measures calculated as frequencies/day were transformed using a $\ln(x + 1)$ transformation. Results are either given back-transformed into original units to ease interpretation or described in the text to appropriately reflect the logarithmic transformation used in the analysis.

ANOVA was used to examine associations of age, sex, and BMI with plasma enterolactone concentrations. Pearson partial correlation coefficients, adjusted for age, sex, and BMI, were used to measure associations between dietary measures and plasma enterolactone concentrations. In addition to demographic characteristics, plant food variable analyses were controlled for percentage of energy as fat; fat-related variable analyses were controlled for total fruit and vegetable servings/day and fiber in grams/day; and tea, coffee and alcohol analyses were adjusted for percentage of energy as fat, fruit and vegetable servings/day, and fiber in grams/day. Finally, multiple linear regression was used to calculate change in average plasma enterolactone concentrations associated with differences in dietary and demographic characteristics.

Results

Plasma enterolactone concentrations ranged from 0.6–155.3 nmol/liter, and concentrations in blood samples drawn on the two consecutive days were similar [Pearson $r = 0.84$, mean difference = 2.7 (SE = 23.5) nmol/liter, paired t test $P = 0.24$]. Seventeen percent ($n = 67$) of samples were below the detection limit of 1.2 nmol/liter. Table 1 gives mean plasma enterolactone concentrations by sex, age, and BMI, each adjusted for the other characteristics. Although differences did not reach statistical significance, plasma enterolactone tended to be higher in women, older persons, and persons with low-normal body weight.

Table 2 gives associations between FFQ dietary measures and plasma enterolactone concentrations. Of the plant food

[4] The abbreviations used are: FFQ, food frequency questionnaire; BMI, body mass index; OR, odds ratio; CI, confidence interval.

Table 1 Comparison of plasma enterolactone concentrations by age, gender, and BMI in 193 healthy 20–40-year-old participants

	Percentage	Geometric mean[a] (SD)
Total ($N = 193$)		13.97 (15.95)
Sex		
Male ($n = 78$)	40.4	11.01 (2.10)
Female ($n = 115$)	59.6	13.34 (2.45)
Age (yrs)		
20–29 ($n = 125$)	64.8	10.39 (1.92)
30–40 ($n = 68$)	35.2	14.12 (2.71)
BMI[b]		
Low normal ($n = 144$)	74.6	15.66 (1.57)
High normal ($n = 43$)	22.3	11.16 (1.95)
Overweight ($n = 6$)	3.1	10.17 (4.71)

[a] Plasma enterolactone values were transformed $\ln(x + 1)$. Back-transformed values and SDs are presented in nmol/liter. Each characteristic is controlled for others in the table.
[b] Calculated as weight (kg)/height (m^2). For women: low normal, <23.1; high normal, 23.1–27.3; and overweight, 27.3–32.2. For men: low normal, <24.3; high normal, 24.3–27.8; and overweight, 27.8–31.1 (34).

measures, total vegetables, total fruit and vegetables, and grams of insoluble fiber/day were significantly associated with plasma enterolactone concentration. Fat-related variables were inversely correlated with plasma enterolactone but failed to retain significance when expressed as measures of nutrient density. Both caffeine and alcohol intakes were significantly associated with plasma enterolactone. Associations did not differ by type of alcoholic beverage; however, when the partial correlations for alcohol (in grams) were adjusted for beer ($r = -0.07$), liquor ($r = -0.14$), and wine ($r = 0.21$), it was beer or liquor that explained the relationship with enterolactone. Adjustment for wine left the partial correlation between enterolactone and alcohol both unchanged and statistically significant.

Table 2 also gives associations between 3-day food record data and plasma enterolactone concentrations. Almost all plant food-related measures were statistically significantly associated with plasma enterolactone, with the strongest associations for fiber. There was no association between fruit servings/day and plasma enterolactone. Fat-related variables were not correlated with plasma enterolactone concentrations. Consistent with findings from the FFQ, both caffeine consumption and alcohol consumption (in grams and percentage of energy) were associated with plasma enterolactone. Table 3 shows that of the plant foods categorized into botanical groups, servings/day from *Chenopodiceae*, *Juglandaceae*, *Luguminosae*, *Pedaliaceae*, and *Vitaceae* were statistically significantly associated with plasma enterolactone.

Table 4 gives results of a multiple regression analysis predicting change in average plasma enterolactone as a function of demographic characteristics, total fiber, alcohol intake, and caffeine intake from 3-day record data. This model explained 22% of the variability in plasma enterolactone concentration ($P < 0.0001$). Plasma enterolactone was 47.3% (SE = 7.7%) higher among women, decreased 6.2% (SE = 0.2%) for each unit of BMI, and increased 6.7% (SE = 0.2%) for each 50 mg of caffeine and 37.0% (SE = 2.3%) for each 10-g increase in total fiber. As alcohol consumption reached 0.5–1 drink/food record day, plasma enterolactone concentrations exceeded those of nondrinkers by 131.4% (SE = 37.6%). However, above this threshold, the proportional increases were more modest. There were no heavy drinkers in this sample due to our eligibility criteria. The coefficient for *Pedaliaceae*, when added to this model, predicts a 63.2% (SE = 20.6%) increase in plasma enterolactone per serving but failed to reach statistical significance ($P = 0.13$; data not shown).

Table 2 Partial correlations of FFQ and 3-day food record (FR) dietary measures *versus* plasma enterolactone[a] in healthy, 20–40-year-old participants

Dietary measure[a]	Adjusted[b] correlation coefficients	
	FFQ[c]	3-day FR[d]
Plant food-related variables		
Vegetables (servings/day)	0.16[e]	0.17[e]
Fruit (servings/day)	0.11	0.08
Fruit & vegetables (servings/day)	0.15[e]	0.20[f]
Fiber (g)		
Total	0.13	0.36[g]
Water-soluble fiber	0.10	0.35[g]
Insoluble fiber	0.15[e]	0.34[g]
Carbohydrate (g)	−0.01	0.19[f]
(% energy)	0.08	0.11
Vegetable protein (g)	0.03	0.32[g]
(% energy)	0.10	0.25[h]
Fat-related variables		
Protein (g)	−0.21[f]	−0.04
(% kcal)	−0.13	−0.04
Animal Protein (g)	−0.19[f]	−0.05
Fat (g)	−0.16[e]	0.01
(% energy)	−0.07	0.01
Saturated fat (g)	−0.11	0.04
(% energy)	−0.02	0.06
Cholesterol (mg)	−0.18[e]	0.06
Miscellaneous		
Tea & coffee (servings/day)	0.13	
Caffeine (mg)	0.22[h]	0.21[h]
Alcohol (g)	0.20[f]	0.24[h]
(% energy)	0.16[e]	0.21[h]
Wine (servings/day)	0.05	
Beer (servings/day)	0.08	
Liquor (servings/day)	0.08	

[a] Variables are $\ln(x + 1)$ transformed (except for cholesterol and carbohydrate, vegetable protein, protein, fat, and saturated fat when expressed as percentage of energy.
[b] All controlled for age, BMI, and sex. In addition to demographics, plant-related variables are controlled for percentage of energy as fat; fat-related variables are controlled for total fruits and vegetables (servings/day) and fiber (g/day); and miscellaneous variables are controlled for percentage of energy as fat, total fruits and vegetables (servings/day), and fiber (g/day).
[c] $n = 177$.
[d] $n = 193$.
[e] $P < 0.05$.
[f] $P < 0.01$.
[g] $P < 0.0001$.
[h] $P < 0.001$.

Discussion

This study of 193 healthy young men and women found statistically significant associations between diet and plasma enterolactone concentrations. A parsimonious, multivariate model, which included consumption of total fiber, alcohol, and caffeine along with demographic characteristics, explained 22% of the variance in plasma enterolactone, whereas dietary fiber intake alone accounted for 13% of this variability. The magnitudes of the correlation coefficients between plant food consumption and plasma enterolactone depended on the measure of plant food consumption and ranged from $r = 0.08$ for fruit (servings/day) to $r = 0.36$ for total fiber (g); however, there were also significant associations between caffeine and alcohol consumption and plasma enterolactone concentration. Thus, whereas plasma enterolactone concentration may be a

Table 3 Partial correlations of botanical food group servings ascertained from a 3-day food record and plasma enterolactone[a] in 193 healthy 20–40-year-old participants

Botanical group (servings/day)[a]	Adjusted[b] correlation coefficients
Agaricaceae (cultivated button mushrooms)	0.07
Chenopodiaceae (spinach, beets, Swiss chard)	0.15[c]
Compositae (lettuces)	−0.07
Cruciferae (broccoli, cauliflower, cabbage, etc.)	0.02
Cucurbitaceae (melon, squash)	0.07
Ericaceae (blueberries, cranberries, lingonberries, etc.)	0.05
Juglandaceae (walnut, pecan)	0.15[c]
Lauraceae (avocado)	0.13
Leguminosae (beans, peas)	0.20[d]
Liliaceae (onion, garlic)	0.05
Musaceae (bananas)	0.05
Oleaceae (olives)	0.07
Pedaliaceae (sesame)	0.20[d]
Rosaceae (apple, pear, strawberry, stone fruits)	0.04
Rutaceae (citrus fruits)	0.08
Solanaceae (tomato, peppers, potatoes)	0.13
Umbelliferae (carrot, celery)	0.05
Vitaceae (grapes)	0.20[d]

[a] Variables are ln(x + 1) transformed.
[b] All controlled for age, BMI, sex, and percentage of energy as fat.
[c] $P < 0.05$.
[d] $P < 0.01$.

Table 4 Predictors of average plasma enterolactone[a] concentrations in multivariate analyses in 193 healthy 20–40-year-old participants using 3-day food record data

	Plasma enterolactone
	% Change (SE)
Dietary variable	
Total fiber (per 10 g)	37.0 (2.3)[b]
Alcohol (g/day)[c]	
<0.1 (n = 87)	Reference value
0.1 to <2 (n = 46)	4.7 (0.9)
≥2–6 (n = 22)	97.0 (24.3)[d]
>6–12 (n = 16)	131.4 (37.6)[e]
>12–24 (n = 17)	78.2 (21.4)[f]
>24 (n = 5)	13.8 (6.6)
Caffeine (per 50 mg)	6.7 (0.2)[f]
Demographic variable	
Age (per decade)	17.9 (2.5)
BMI (per kg/m^2)	−6.2 (0.2)[f]
Female (*versus* male)	47.3 (7.7)[f]

[a] A two-visit average plasma enterolactone was ln (x + 1) transformed. % Change (SE) are back-transformed values.
[b] $P < 0.0001$.
[c] Daily average over the 3 days measured by food records.
[d] $P < 0.01$.
[e] $P < 0.005$.
[f] $P < 0.05$.

potentially sensitive biomarker of plant food consumption, it is not specific.

Associations of diet with plasma enterolactone were consistent between measures of usual diet over the past 3 months (from a FFQ) and diet measured during the blood collection period (from 3-day food records). However, associations were stronger for the food records, which is consistent with the biology of enterolactone metabolism. In supplementation trials, plasma enterolactone concentrations increased 8–9 h after flaxseed ingestion (8). There is also evidence that plasma lignan concentrations may be sustained to some degree, at least over a period of days (8). Associations with food record data may also be stronger because FFQ measures of usual fruit and vegetable intake have relatively low validity (22).

Overall, our results were generally consistent with those of previous studies. The range of plasma enterolactone concentrations for this study was similar to that reported by Adlercreutz *et al.* (24) using time-resolved fluoroimmunoassay in a study of 224 Finnish subjects. In our sample of 193 healthy men and women, 61.1% of plasma enterolactone values were <20 nmol/liter, which is comparable to the baseline distribution found by others (25).

Our results are consistent with those of Kirkman *et al.* (26), who demonstrated previously that vegetable supplementation, as part of a low-fiber, low-phytochemical diet, significantly increased lignan excretion. Adlercreutz *et al.* (17) also 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. In addition, as compared with omnivores, vegetarians excreted significantly higher amounts of lignans in urine and feces (27, 28) and had higher plasma lignan concentrations (29).

In a recent population-based intervention, increasing fruits and vegetables and decreasing dietary fat resulted in higher serum enterolactone concentrations in a free-living sample (n = 85; Ref. 25). The study design did not allow separation of the effects of reduced fat intake on plasma enterolactone from the effects of increased fruit and vegetable intake; however, it was postulated that reducing dietary fat, independent of fruit and vegetable intake, could increase plasma enterolactone, given that lignan absorption has been shown to decrease in rats when dietary fat increases (30). In our study, we were able to evaluate the effects of numerous fat-related variables on plasma enterolactone. Although many of the fat-related variables appeared to be inversely correlated with plasma enterolactone, controlling for confounders such as energy density, fruit and vegetable intake, and grams of fiber/day abolished the associations.

Some of the details of our results were inconsistent with other studies. Both the 3-day food records and FFQ found vegetables and total vegetables + fruit (no juice), but not fruit alone, to be correlated with enterolactone. This is inconsistent with results of a similar but smaller study (n = 98) using urinary enterolactone measures (2). These investigations found fruit, but not vegetables, to be correlated with urinary enterolactone; however, only simple correlations without adjustments for covariates were presented.

Our findings are not consistent with Finnish reports that berries and other fruits containing substantial amounts of lignan precursors significantly contribute to plasma enterolactone concentrations (4, 5). However, berries were not consumed regularly by the population we sampled. Three-day food record data were specifically analyzed for berries in the botanical classes *Rosaceae* and *Ericaceae.* Participants consumed 379 servings from the *Rosaceae* class, but this includes several other common fruits such as apples and pears. Consumption from *Ericacea* was limited (73 servings) and may have been inadequate to detect associations. Other fruits that have been evaluated and found to be high in the lignan precursors secoisolariciresinol or matairesinol are not frequently or consistently consumed on Western diets (4); examples include lychee, guava, cantaloupe, and avocado. Oranges and lemons are reported to be moderate sources of secoisolariciresinol, yet no association was detected with *Rutaceae* in our study group. The only botanical group representing fruit that was correlated with enterolactone was

Vitaceae, which includes grapes. No precursor content values were available for grapes at the time of this writing, but wine, specifically red wine, is known to contain considerable levels of secoisolariciresinol (4).

Tea and coffee appear to significantly contribute to plasma enterolactone concentrations in this sample, which was expected given the high published food values for lignan precursors in teas and coffees (4). To assess the contribution these beverages make to plasma enterolactone, we used caffeine as a surrogate for coffee and tea consumption in the 3-day food record and FFQ data analyses. However, this surrogate included other sources of caffeine aside from coffee and tea and did not account for precursors in herbal teas without caffeine. The FFQ allowed further investigation of this issue using questions specific to tea and coffee intake. Daily caffeine intake from the FFQ was significantly correlated with plasma enterolactone, but servings of tea and coffee were not. Given that tea contains about four times the enterolactone precursor content of coffee, coffee intake may have diluted the correlation between tea and enterolactone (4). Adjustment for tea and coffee did render the caffeine surrogate nonsignificant.

Our eligibility screening protocol excluded individuals drinking more than 2 servings of alcohol/day, and this limits our ability to explore a possible dose-response relationship between alcohol intake and plasma enterolactone. The source of alcohol is likely to be an important factor; red wine is known to include high concentrations of lignan precursors (600–1300 μg/liter), whereas white wines offer considerably lower amounts (140–170 μg/liter) (4). Little is known about the lignan content of other alcoholic beverages.

There are several limitations to this study. It was cross-sectional in design, therefore, we were not able to detect how changes in food intake would actually affect plasma enterolactone concentrations. We studied a narrow age range of healthy individuals with high ($\geq$4.5 servings/day) or low ($\leq$2.5 servings/day) fruit and vegetable intake and used stringent exclusion criteria to provide a broad range of fruit and vegetable consumption and avoid the impact of nondietary variants. These aspects of our design may limit the generalizability of our results. The structure of our database did not allow evaluation of the effects that servings of whole grains, which are rich sources of lignans, have on plasma enterolactone; we were only able to describe the effects of fiber. Enterolactone metabolism is not entirely understood. Matairesinol and secoisolariciresinol are known precursors, but numerous others are suspected (4). Therefore, only a limited degree of plasma enterolactone variability can be explained by these two compounds. Additionally, considerable interindividual variability in the production of enterolactone from known precursors has been observed (8, 31). Correlating foods or nutrients with plasma enterolactone may constitute a gross simplification of the relationship. Only 3% and 14% of the variation in serum enterolactone concentration could be accounted for in men ($n = 1168$) and women ($n = 1212$), respectively, in a Finnish study in which the most important determinants of serum enterolactone included not only lignan-containing foods but also constipation (32). Enterolactone production from precursors is dependent on colonic bacteria. Whereas some humans produce little to no enterolactone, the microflora of others have been shown to increase enterolactone produced from a standardized lignan-rich meal over a 1-week period (8). Any component altering intestinal flora or its environment has the potential to affect the degree to which precursors are converted to enterolactone and absorbed. We specifically excluded individuals using antibiotics within 3 months of the study. Colonic bacteria are likely to affect how precursors from foods are associated with plasma enterolactone concentrations, but this could not be measured or controlled.

Identifying determinants of plasma enterolactone concentrations is important in development of disease prevention interventions. Several studies are now reporting associations between enterolactone concentrations and disease risk. A case-control study found men in the highest enterolactone concentration quartile to have 65.3% (95% CI, 11.9–86.3; $P = 0.03$) lower risk of an acute coronary event than men in the lowest enterolactone quartile after adjusting for the strongest predictive risk factors (33). Another case-control study in Finland, including both pre- and postmenopausal women, found serum enterolactone concentrations to be significantly lower ($P = 0.003$) in 194 women with breast cancer as compared with 208 community controls. Serum enterolactone was significantly inversely associated with risk of breast cancer, based on the OR in the highest enterolactone quintile (OR, 0.38; 95% CI, 0.18–0.77; P for trend $= 0.03$) after adjusting for all known breast cancer risk factors (12). Researchers studying postmenopausal, Dutch women (88 breast cancer cases and 268 controls) using urinary enterolactone collected years before cancer diagnosis found no significant associations between enterolactone and breast cancer risk (OR highest *versus* lowest tertile enterolactone, 1.43; 95% CI , 0.79–2.59; Ref. 13).

Identification of a biomarker of exposure to dietary habits related to better health outcomes is crucial if we are to circumvent the problem of reporting error in the investigation of diet and disease relationships. Our results suggest that plasma enterolactone is positively correlated with vegetable servings, fiber, caffeine, and alcoholic beverages. Specific botanical groups including *Chenopodiaceae*, *Juglandaceae*, *Leguminosae*, *Pedaliaceae*, and *Vitaceae* may include particularly significant contributors. Plasma enterolactone may be useful in measuring exposure to this lignan and assessing its effects on health. However, plasma enterolactone may be of limited use as a specific biomarker of fruit and vegetable or plant food intake, given that coffee, tea, and alcoholic beverages are also significant contributors.

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