Original Article

Urinary isoflavonoids as a dietary compliance measure among premenopausal women

Adrian A Franke PhD, Yukiko Morimoto MS, RD, Linda M Yeh, MS RD[1] and Gertraud Maskarinec MD, PhD[1]

Cancer Research Center of Hawaii, Honolulu, Hawaii, U.S.A.

Clinical trials investigating the effects of soy food intake require high compliance with a dietary protocol. Measuring soy isoflavonoids in bodily fluids is the most objective method currently available in determining compliance to a soy diet. In the present study, we investigated how frequently the urinary isoflavonoid excretion rate (UIER) should be analyzed to provide a reasonably accurate measurement of dietary compliance without being a burden on the participants. Nineteen premenopausal women who were on a daily soy diet protocol collected first-morning urine samples over one menstrual cycle. Spearman rank order correlation coefficients (r) between the UIERs of total isoflavonoids for a single day, for a week, and for all weeks combined with the monthly UIER were high for all samples (single day: r = 0.89; Week 1: r = 0.89; Week 2: r = 0.85; Week 3: r = 0.75; all weeks combined UIER: r = 0.94) and remained high after stratification by ethnicity, body mass index, and equol-excretor status. According to these results, the analysis of UIERs from a single or weekly first-morning sample provides a highly accurate and more feasible method of determining dietary compliance among women with regular soy consumption than that from urine samples collected every morning during one month.

Key Words: compliance, soy, urinary isoflavonoids, biomarker, intervention

Introduction

Soy consumption has been linked to a reduced risk of various diseases, including cardiovascular disease, osteoporosis, and prostate and breast cancer.[1-3] Previous studies indicate that isoflavones of soy may play a role in providing such health-protective effects.[4-6] Clinical trials investigating the effects of soy food intake require high compliance with a nutritional protocol. Measuring isoflavonoids in bodily fluids is the most objective method currently available in determining dietary compliance because they are highly specific to soy foods and positively correlated with soy food consumption.[7-9] Moreover, urinary analysis of isoflavonoids is preferred over plasma for its non-invasiveness, its good correlation with circulating levels, and its reflection of soy intake over a longer period of time.[10] These advantages are particularly important considering the constantly changing systemic isoflavonoid amounts over time after soy or isoflavonoid exposure. We conducted the present study as part of a large intervention trial investigating the effects of regular soy consumption on breast cancer risk;[11] 19 pre-menopausal women completed a daily soy diet protocol during one menstrual cycle and collected daily urine samples. We then compared the mean urinary isoflavonoid excretion rates (UIER) for a single first-morning samples (daily)and for weekly first-morning samples (weekly) with the UIER for first-morning samples collected during the whole menstrual cycle (monthly) to determine if measuring samples less than daily will adequately reflect dietary soy intake and to establish a practical method for measusring dietary compliance.

Materials and Methods

Twenty subjects were recruited from 110 premenopausal women participating in the intervention group of the Breast, Estrogen, and Nutrition (BEAN) Study described in more detail elsewhere.[11,12] The BEAN Study was a two-year, randomized dietary intervention study conducted in Honolulu, Hawaii; the intervention group consumed two daily servings of soy foods, providing approximately 45mg isoflavones. The soy food choices (and their isoflavone contents in mg aglycone equivalents) included 1/2 cup or 118g tofu (25mg), ¾ cup or 178ml soymilk (27mg), 1/4cup roasted soy nuts (26mg), 29g soy protein powder (26mg), and 50g soy protein bars (20mg). The original project and the current substudy were approved by the Committee on Human Studies of the University of Hawaii; all participants provided informed consents for both studies. Participants collected first-morning urine samples for one month except for the days of menstruation. Starting on the first morning after menstrual bleeding had stopped, each woman collected 2mL of first-morning urine samples prior to any

Correspondence address: Dr Adrian A. Franke, Cancer Research Center of Hawaii, 1236 Lauhala Street, Honolulu, HI 96813, U.S.A.
Tel: + (808) 586-3008 ; Fax: + (808) 586-2973
Email: adrian@crch.hawaii.edu
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food intake and continued to do this until the next menstrual bleeding occurred. Immediately after collection, the urine samples were stored in the participant's home freezer. If the participant forgot to collect urine one day or if spot bleeding occurred, she discarded the unused vial and resumed urine collection the next day.

All urine samples were analyzed for total and individual isoflavonoid levels (daidzein, genistein, glycitein, equol, and O-desmethylangolensin) using high-pressure liquid chromatography with photo diode-array detection.[13] Inter-assay variability for daidzein (mean = 13.0 µM), glycitein (mean =1.6µM), genistein (mean=3.4 µM), and O-desmethylangolensin (ODMA) (mean=1.1µM) were 12%,6%,9%, and 22%, respectively. Absorbance readings were obtained with a spectrophotometer (Bio Spec 1601, Shimadzu; Columbia, MD). To standardize for urinary volume, creatinine concentration for each urine sample was determined using the Jaffe reaction based Quantitative Colorimetric Determination Creatinine Kit, Procedure No. 555 from Sigma Diagnostics (St. Louis, MO) with an interassay variability of 4.2% and UIERs were calculated as nmol/mg creatinine.

The first-morning urine samples from each subject were prepared to yield one monthly pool (monthly), one single day pool (daily), and two to four weekly pools (weekly). The monthly pool consisted of a combination of all first-urine samples from each participant, whereby a volume equivalent to 100µg creatinine from each sample was used. The weekly pools consisted of a combination of three first-morning samples for each week using a volume equivalent to 100µg creatinine from each day. This included one weekend and two weekday-urine samples at least one day apart from each other. Each woman had between one to four weekly pools. Single day samples were taken from the same day, in midweek, as one of the three samples used in one of the weekly pools.

Statistical analysis was performed using Microsoft® Excel 2000/XLSTAT©-Pro (Version 7.0, 2003, Addinsoft, Inc., Brooklyn, NY); the significance level was set at $P<0.05$. Spearman rank order correlation coefficients (r) were calculated for total isoflavonoids and for individual isoflavonoids to assess the degree of correlation between the gold standard, the monthly UIER, and the UIER for a single day, each week, and all weeks combined. In addition, we stratified the women into subgroups by ethnicity (Asian vs. non-Asian), body mass index (BMI, <25 kg/m^2 vs. ≥25 kg/m^2), and equol-exretor status (excretors vs. non-excretors). Individuals who convert the isoflavone daidzein to the metabolite equol are hypothesized to derive greater benefits from soy consumption.[14]

Results

Nineteen of 20 women, aged 44.4 ± 2.7 years, completed the study and collected between 17 and 25 urine samples. Seven women were Caucasian, one was Filipino, three were Japanese, six were of mixed ethnicity, and two women did not specify. Their body mass index (BMI) was 25.3 ± 6.0 kg/m^2. The mean UIERs for the different time periods were very similar and showed highly significant correlations with the monthly UIER (Table 1, Fig. 1). The correlations for the all weeks combined sample with the monthly UIER were generally higher than those for the single week samples. The correlation coefficients for total isoflavonoids varied between 0.75 and 0.94 and the magnitude was similar for single isoflavonoids except for equol (r = 0.98). As shown in the scatter plots (Fig. 2), one or two women had a UIER above 50nmol/mg creatinine and another few had a UIER below 10 nmol/mg creatinine. The correlation for total isoflavonoids between a single day and the monthly UIER (Fig. 2) was slightly weaker than for the all weeks combined samples with the monthly UIER; however, the correlation was still very high (r = 0.89). The study included seven women who excreted detectable amounts of equol. Their equol excretion rate tended to be higher than their ODMA excretion rate, and their UIER for ODMA and daidzein was lower than the UIER of non-equol producers (data not shown).

Stratification by ethnicity, BMI, or equol-exretor status did not alter the correlation between daily and weekly UIERs with the monthly UIER (data not shown). Interestingly, the mean monthly UIER of the women with a BMI less than 25kg/m^2 was higher than the mean UIER of those with a BMI equal to and greater than 25kg/m^2 (28.2 vs. 18.9nmol/mg creatinine). The women with high BMI had lower weekly UIERs during weeks 2 and 3, while the low BMI women maintained high UIERs throughout the study period. However, the correlation between BMI and monthly UIER was not statistically significant (r = -0.38, P = 0.11). .

Discussion

This comparison of the UIERs from various collection periods found that urinary isoflavonoid excretion of both single first-morning and weekly first-morning samples was highly correlated with the monthly first-morning UIER for total and individual isoflavonoids. Furthermore, we observed these results using spot urine samples of first-morning collections, instead of 24-hour urine collection, after adjusting the subjects' isoflavone excretion levels by urinary creatinine levels. Based on these results, collecting single and weekly urine samples can be considered a reasonably accurate and more practical approach than collecting urine every morning during one month to monitor dietary compliance in soy intervention studies.

One previous study described that the UIER from one overnight sample was significantly correlated with a second sample collected 48 hours later.[15] This suggests that collecting a single urine sample provides adequate information for recent soy intake. Our current study went further to investigate how frequently UIER should be assessed to obtain a reasonably accurate measurement while still being feasible to participants. Equol was excreted in 37% of the women, which was similar to the reported proportion (~30%) of excretors in omnivorous populations.[16-18] The lower UIER for ODMA and daidzein in the equol excretors is very likely due to their preferred metabolic pathway converting daidzein to equol. Nonetheless, equol-excretor status should not affect the total isoflavone excretion levels of our subjects because equol, ODMA, and daidzein have been all included in the UIER calculation.

Table 1. Urinary isoflavonoid excretion rates for monthly (all days combined), weekly (2 weekdays and weekend day of one week combined), and single day samples and their respective correlations with the monthly rate

N=19		Mean[a]	SD[a]	Range[a]	r	*P*
Total Isoflavones	Monthly	25.8	18.2	6.7 - 76.8	1	
	Week 1	23.6	16.5	1.8 - 45.7	0.89	< 0.001
	Week 2	22.5	15.5	4.8 - 66.2	0.85	< 0.001
	Week 3	27.4	23.2	3.7 - 94.6	0.75	< 0.001
	All Weeks	23.9	18.4	1.8 - 94.6	0.94	< 0.001
	Single	22.4	18.3	1.7 - 67.3	0.89	< 0.001
Daidzein	Monthly	12.1	10.9	2.7 - 46.4	1	
	Week 1	10.4	9.9	0.8 - 45.0	0.94	< 0.001
	Week 2	10.6	9.8	2.0 - 39.0	0.69	< 0.001
	Week 3	13.4	14.6	0.4 - 58.8	0.77	0.001
	All Weeks	11.4	11.3	0.4 - 58.9	0.91	0.001
	Single	9.6	10.3	0.5 - 31.3	0.87	< 0.001
Genistein	Monthly	5.7	5.0	1.4 - 21.9	1	
	Week 1	5.0	4.5	1.2 - 19.5	0.87	< 0.001
	Week 2	5.1	4.4	1.2 - 19.6	0.83	< 0.001
	Week 3	7.1	7.0	0.2 - 26.6	0.82	< 0.001
	All Weeks	5.6	5.3	0.2 - 26.6	0.94	< 0.001
	Single	5.2	5.6	0.5 - 20.5	0.86	< 0.001
Glycitein	Monthly	1.8	1.4	0.3 - 6.2	1	
	Week 1	1.6	1.2	0.2 - 5.6	0.91	< 0.001
	Week 2	1.6	1.2	0.2 - 5.2	0.71	0.001
	Week 3	1.9	1.8	0.4 - 7.7	0.60	0.015
	All Weeks	1.7	1.4	0.1 - 7.7	0.85	< 0.001
	Single	1.6	1.2	0.1 - 4.6	0.59	0.007
ODMA[c]	Monthly	4.1	3.4	0.4 - 14.4	1	
	Week 1	4.2	3.0	0 - 10.3	0.87	< 0.001
	Week 2	3.3	2.6	0.5 - 10.9	0.88	< 0.001
	Week 3	3.4	3.6	0.7 - 14.3	0.93	< 0.001
	All Weeks	3.7	3.0	0 - 14.3	0.95	< 0.001
	Single	3.5	2.7	0.7 - 9.0	0.88	< 0.001
Equol[b]	Monthly	5.6	2.2	1.8 - 8.0	1	
	Week 1	6.3	3.3	1.9 - 9.3	0.98	< 0.001
	Week 2	5.5	2.9	1.5 - 9.7	0.99	< 0.001
	Week 3	4.1	1.9	1.5 - 7.3	0.99	< 0.001
	All Weeks	4.8	2.0	1.5 - 9.7	0.99	< 0.001
	Single	6.7	4.2	0.7 - 13.8	0.89	< 0.001

[a] Values in nmol/mg creatinine; [b] Results of 7 women; [c] O-desmethylangolensin (daidzein metabolite)

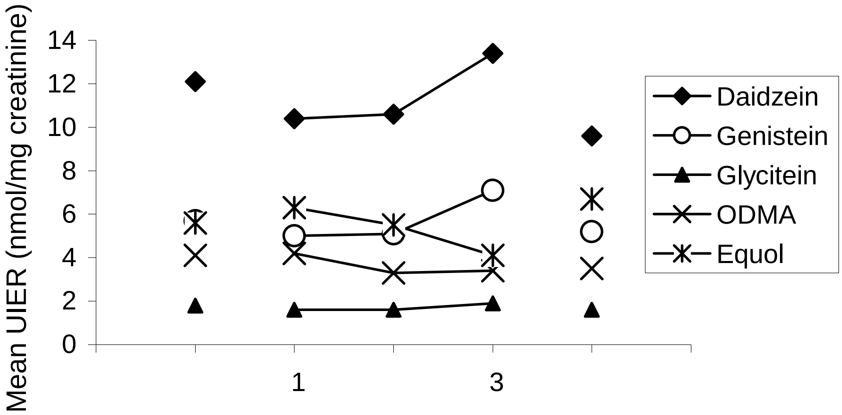
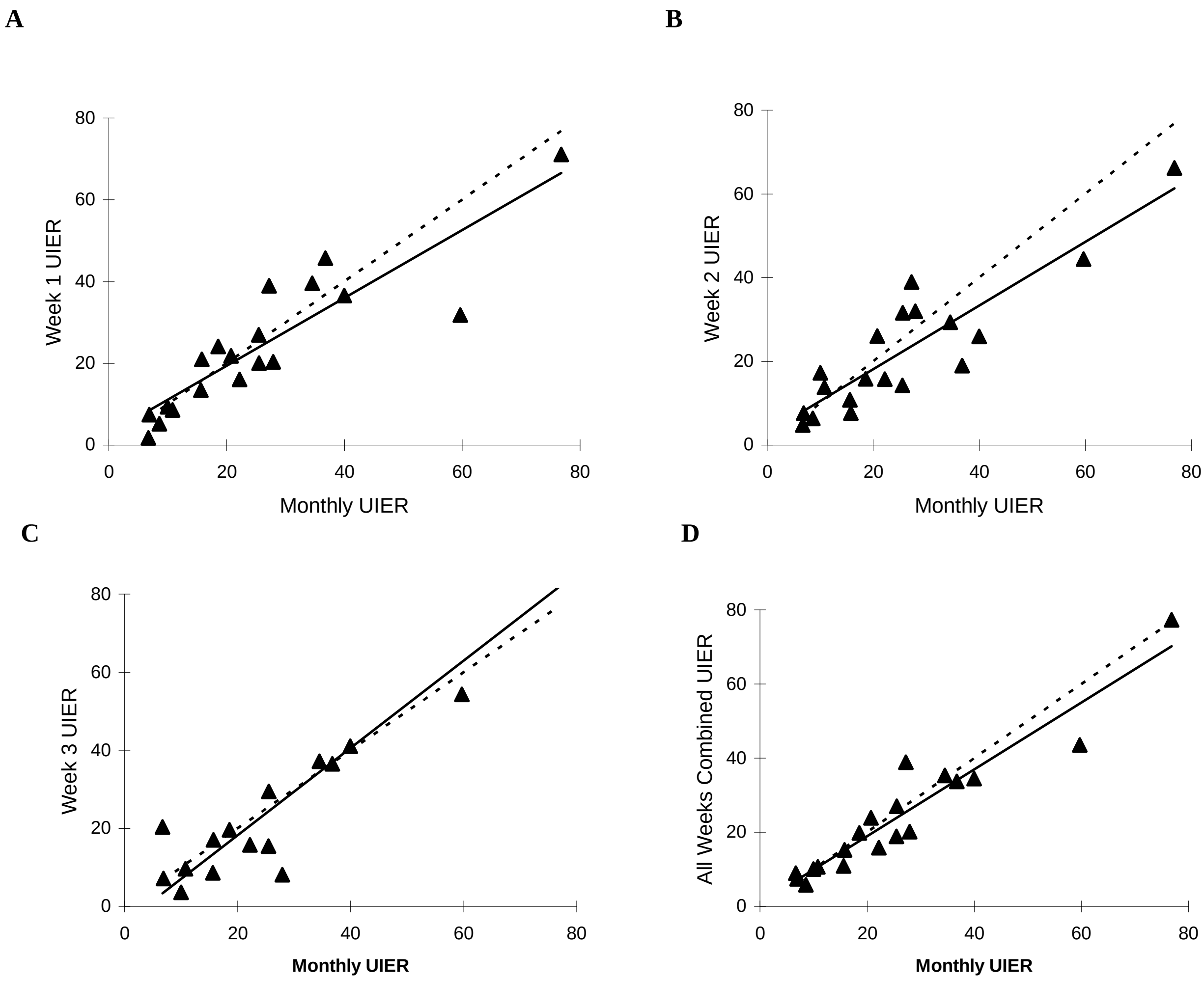


Figure 1. Mean urinary isoflavonoid excretion rates for each isoflavonoid over a time period of 3 weeks in comparison to the monthly excretion rate and a single day sample



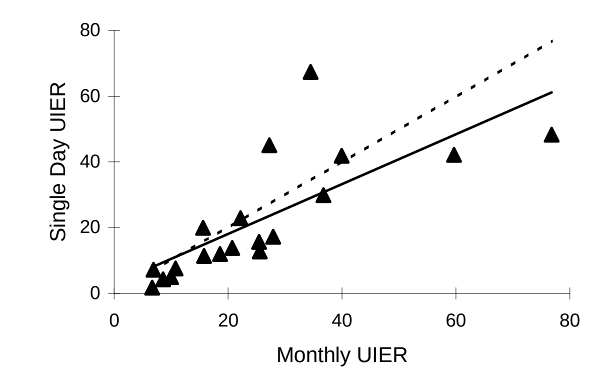


Figure 2. Correlations of total urinary isoflavonoid excretion rates from Week1 (A), Week 2 (B), Week 3 (C), all weeks combined (D), and a single day sample (E) to the monthly excretion rate (expressed as nmol/mg creatinine). The dotted line represents the agreement line. The solid line is the linear regression line for the correlation of weekly or single day with the monthly rate.

One of the major limitations of the current study was its small sample size of an exclusively female population with relatively high soy consumption. These characteristics narrow the generalizability of the study's findings. Furthermore, the small sample size made the statistical analysis more susceptible to the effects of extreme values and uneven distribution. In particular, the high correlations for equol were due to the presence of many non-excretors whose undetectable values were treated as "0" during the calculation of correlation coefficients. The higher correlations of equol relative to the other isoflavonoids might also have been due to its slower pharmacokinetics resulting in less variability over time.

The high inter-individual variability in UIER may be due to the timing of soy food consumption and urine collection. Given the short half-life (5-8 hours) of isoflavonoids, varying time differences between soy food intake and urine sample collection leads to a large variability in UIER.[19-21] In addition, non-compliance with the dietary protocol and other possible factors, such as inter-individual differences in bioavailabiliy caused by in the diet or the variability of the gut microflora, might have been present. Moreover, although all soy food choices of this study were similar in isoflavone content, each individual's soy food selection may have in part contributed to the inconsistency in UIER. The subjects were also allowed to consume additional soy foods, including fermented soy products (miso and natto) containing predominantly isoflavone aglycones that are absorbed at different efficiencies than non-fermented soy foods containing predominantly isoflavone glycosides.[17,19,22]

The low excretion rates seen in several of the subjects may be due to an inadequate soy intake or to low absorption of isoflavones into enterocytes.[17,23] For example, a high consumption of insoluble fiber increases fecal bulk and transit time and may interfere with the absorption of isoflavones due to a lesser amount of isoflavones being exposed to the gut microflora and overall less time for isoflavones to be absorbed. Similarly, consuming other foods concurrently with the soy products (e.g., consuming tofu in a meal) or different soy products (protein drink vs. roasted soybeans) may reduce or increase isoflavone absorption. Among these factors, a declining dietary compliance towards the end of the study period is likely to have contributed to the lower monthly UIER observed in the high BMI subgroup.

Despite the observed inter-individual variability in UIER, the overall high correlations suggest that measuring single day or weekly UIERs provides an adequate tool to complement traditional methods, such as food diaries, 24-hour dietary recalls or food frequency questionnaires, to monitor adherence to a daily soy diet protocol. However, in populations who do not regularly consume soy different methods are necessary because urinary isoflavonoids reflect primarily the intake within the past 48 hours. Since it is unrealistic to expect healthy, free-living subjects to collect urine samples every day, especially for a long term study, our findings offer good support that a single day or weekly urine sample can provide a reasonably accurate measurement of daily adherence to soy food consumption. Whether our finding applies to other studies (cross-sectional, longitudinal, Asian versus non-Asian populations) remains to be determined.

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Adrian A Franke PhD, Yukiko Morimoto MS, RD, Linda M Yeh, MS RD[1] and Gertraud Maskarinec MD, PhD[1]

Cancer Research Center of Hawaii, Honolulu, Hawaii, U.S.A.

尿液异黄酮作为测定绝经期前妇女饮食是否一致的指标

调查摄入大豆食品作用效果的临床试验要求日常饮食高度一致。测定体液内大豆异黄酮含量是现有的决定大豆饮食是否一致的最客观的方法。本研究中，我们调查了应该以多高的频率来分析尿液异黄酮分泌率(UIER)来达到既相当准确的测定饮食是否一致的目的，又不增加参与者的负担。19 位正在每天食用大豆食品的绝经期前的妇女收集每天早上的第一次尿液样本达一个月经周期。对所有样本来说，单独一天，一星期，结合所有星期的总异黄酮 UIER 与月度总异黄酮 UIER 之间斯皮尔曼等级相关性高(一天: r = 0.89; 第一星期 1: r = 0.89; 第二星期: r = 0.85; 第三星期: r = 0.75; 结合所有星期 UIER: r = 0.94)，而且按种族，BMI 和尿中可检测到的植物雌激素分为亚组后，斯皮尔曼等级相关性仍然高。根据这些研究结果，对决定这些有规律的食用大豆食品的妇女饮食是否一致，分析单独一天或一星期的早上第一次尿液样本 UIERs 的方法是高度精确的，比分在一个月内每天所取尿液样本 UIERs 更加可行。