A Semiquantitative Food Frequency Questionnaire Is a Valid Indicator of the Usual Intake of Phytoestrogens by South Asian Women in the UK Relative to Multiple 24-h Dietary Recalls and Multiple Plasma Samples[1]

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ABSTRACT We investigated the relative validity of an interview-administered FFQ to estimate phytoestrogen intake among South Asian women in the UK. A population-based sample of 108 healthy South Asian women completed random repeated monthly 24-h recalls [with a subsample (n = 58) also providing multiple plasma samples] over a period of 1 y followed by administration of the FFQ. The FFQ produced slightly higher estimates of phytoestrogen intake than the 24-h recalls, but the percentage of women classified into the same ± 1 quartile by the 2 methods was high for all phytoestrogens (from 81 to 94%) with only a small percentage (<5%) being misclassified into extreme opposite quartiles. Energy-adjusted Spearman correlations coefficients between the estimates obtained by the FFQ and the 24-h recalls were 0.55 for genistein, 0.60 for daidzein, 0.70 for secoisolariciresinol, and 0.63 for matairesinol (all $P < 0.001$). Spearman correlation coefficients between the FFQ estimates and plasma levels were 0.21 ($P = 0.12$) for genistein, 0.32 ($P = 0.02$) for daidzein and 0.10 ($P = 0.43$) for enterolactone; the corresponding values for the 24-h recalls compared with plasma levels were 0.43 ($P < 0.001$), 0.40 ($P = 0.002$), and 0.08 ($P = 0.50$), respectively. The method of triads was used to estimate the validity coefficients (VCs) between the estimates provided by each assessment method and "true intake." The FFQ had the highest VC for lignans (0.91 vs. 0.73 for 24-h recalls and 0.11 for plasma samples) and satisfactory VCs for both genistein (0.46 vs. 0.95 and 0.45, respectively) and daidzein (0.67 vs. 0.83 and 0.45, respectively). This FFQ is thus a relatively valid tool with which to estimate phytoestrogen intake among South Asian women in the UK. J. Nutr. 135: 116–123, 2005.

KEY WORDS: • *phytoestrogens* • *food frequency questionnaire* • *24-h recalls* • *plasma phytoestrogens* • *validation*

Phytoestrogens, naturally occurring plant compounds with hormone-like activity, have been the source of recent media and scientific interest because of their potential protective role against menopause-related signs and symptoms (1–3), female breast cancer (4–7), cardiovascular diseases (8,9), and decreasing bone mineral density (10,11). However, the overall epidemiologic evidence remains inconclusive (12–17). The inconsistency of results may be related to the different study designs (prospective vs. case-control), the different measures of phytoestrogen intake (dietary assessment vs. biomarkers), and the different populations (oriental vs. Western) being investigated (17).

The most widely studied phytoestrogens are the isoflavones, daidzein and genistein, and the lignans, secoisolariciresinol and matairesinol. Isoflavones are found in high quantities in soybeans and soybean products and in much smaller amounts in other legumes such as clover, mug beans, alfalfa, and peanuts (18). Lignans are more widely distributed in plant foods; flaxseed is the richest source, and other dietary sources include whole grains, nuts, seeds, berries, fruits, and vegetables (19,20).

The FFQ is often the method of choice in epidemiologic studies of diet and chronic diseases because of its ability to estimate long-term usual intake. This tool is also easy to use and relatively inexpensive; hence, it can be applied to the large numbers of subjects required in most epidemiologic research. Few FFQs have been validated for isoflavone intake

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(21–23) and none for lignan intake. Dietary (including FFQ) estimates of phytoestrogen intake are either made by considering the phytoestrogen content of all relevant foods (12), or indirectly by using soy intake as a proxy measure for isoflavone intake (4). As an alternative, some epidemiologic studies relied on single plasma samples (7) or on spot (24) or 24-h urinary samples (6) to estimate levels of phytoestrogens in the body, but because of their short half-lives (usually between 72 and 96 h) these biomarkers may not necessarily represent long-term intake (25–27). Biomarkers are also affected by factors such as gender, gut microflora activity, recent use of antibiotics, genetic traits that may influence phytoestrogen metabolism, and other metabolic factors.

South Asian populations from the Indian subcontinent consume a diet rich in pulses, vegetables and fruits but poor in soy-based products (28) and have a low incidence of hormone-related cancers (29). We recently developed an interview-administered FFQ to specifically measure fruit and vegetable intake in South Asian women in the UK; the FFQ was validated for macro- and micronutrient intakes (30). The present study investigates the validity of this FFQ for estimating long-term usual phytoestrogen intake in a population that does not traditionally consume soy compared with multiple 24-h dietary recalls and multiple plasma samples.

MATERIALS AND METHODS

Selection of study subjects. Study subjects were drawn from a population-based sample of healthy South Asian women living in England who participated as controls in a case-control study on diet and breast cancer (31). This sample was randomly recruited from General Practitioners' lists from London and West Midlands. Practically all residents in England and Wales are registered with a General Practitioner. A realistic desirable level of precision in dietary validation studies is a correlation of ~0.50–0.70 (32). Thus, assuming a true correlation of ~0.60 between the levels of dietary intake estimated by 24-h recalls and those estimated by FFQ, 80% power and a 5% significance level, a sample of ~100 women was required to ensure that the lower limit of the 95% CI of the observed correlation coefficient was at least 0.40. Women were asked to take part in the validation study sequentially as they were recruited as healthy controls into the main case-control study until we had ~100 women who had completed at least nine 24-h recalls during the 1-y period. Participants were included in the study if they were between 25 and 75 y old, had no history of any cancer (except nonmelanoma skin cancer), were first-generation South Asian migrants born in the Indian subcontinent or East Africa, and had no history of chronic disorders requiring highly specialized diets (e.g., renal failure, liver disorders) or mental disorders. Subjects who had taken antibiotics in the last 6 mo were excluded from the validation study because these drugs were shown to destroy the gut microflora needed to metabolize phytoestrogens (33). A total of 133 women enrolled in the case-control study were invited to participate in this study; 108 women agreed to participate and were able to complete the 1-y follow-up (81% response rate). For funding and other logistic reasons, collection of multiple plasma samples throughout the study period began later and was possible only for the last 58 women who participated in the validation study. The study was approved by all relevant ethics committees and written consent was obtained from all participants.

Random repeated 24-h dietary recalls. Subjects were interviewed by a research dietitian (D.B.) or a nutritionist (L.S.) over the telephone and asked to provide a recall of their dietary intake for the previous 24 h. The 24-h recalls were conducted on random days of the week (including weekends) every calendar month for a period of 1 y. Specially trained interpreters were used for languages not covered by the nutritionists. Detailed descriptions of the foods consumed during the previous 24 h were obtained, including those that are commonly forgotten such as snacks and beverages, and information on the main ingredients of composite dishes recorded. A study-specific coding and portion size manual was developed for South Asian foods using serving spoons commonly used by South Asians (30). The recalls were coded and entered in COMPEAT, a nutrient database program (34), independently by the 2 researchers; any inconsistencies between the 2 were discussed with the rest of the study team. The Ministry for Agriculture, Fisheries and Foods nutrient database available in COMPEAT was enhanced by the addition of data from a more recently published compilation of the nutrient composition of dishes commonly consumed by South Asian migrants in the UK (35).

Blood samples and laboratory assays. Four 2-mL blood samples (1 every 3 mo) were collected from each of the 58 participating subjects during the period when the 24-h recalls were conducted. Samples were collected in heparinized tubes in the early morning after a 10-h fast. The duration of fasting and use of antibiotics was checked before each blood sample was taken. The blood samples were kept in a refrigerator for a maximum of 24 h and then centrifuged at 2000 rpm for 10 min; aliquots of serum were stored at −70°C. At the end of the study, plasma samples from all study subjects were sent on dry ice to Professor Adlercreutz's laboratory in Helsinki (Finland), where the multiple samples from each participant were pooled before laboratory assays. Pooling samples from each subject before assay provided more reliable estimates by decreasing intraindividual random variability. The samples were analyzed in duplicate as a normal laboratory procedure and as part of one single run. The time-resolved fluoroimmuno-assay (TR-FIA)[7] was used to quantify plasma levels of daidzein, genistein, and enterolactone (36,37). The measurement of plasma levels of enterodiol was deemed unnecessary because it is highly correlated with plasma levels of enterolactone (unpublished data); thus, plasma levels of enterolactone were taken here as a biomarker for lignan intake. The intra-assay CV of the TR-FIA method was shown to be low (3.2–4.5% for daidzein, 3.2–4.1% for genistein, and 4.6–6.0% for enterolactone depending upon the concentrations) (36,37). The TR-FIA method was validated previously in relation to the "gold standard" GC-MS, with high correlation coefficients observed between the levels estimated by the 2 methods for each one of the 3 phytoestrogens (r = 0.87–0.99) depending upon concentration (36,37).

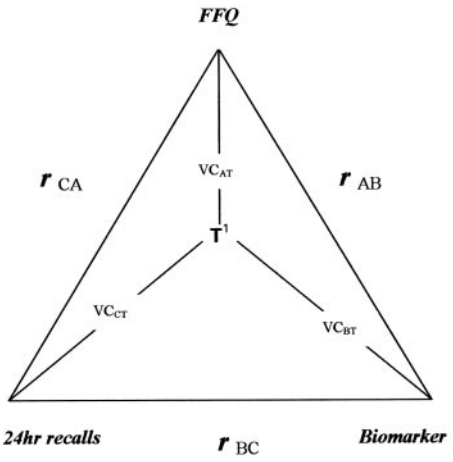
Administration of the FFQ. Approximately 1 mo after the completion of the multiple 24-h recalls and the blood collection, the participants were visited in their home and administered the FFQ to assess their dietary intake over the previous year. All interviews were conducted by the same research dietitian (D.B.) using interpreters, when necessary. The compilation of the FFQ is described in detail elsewhere (30). Briefly, a list of foods commonly consumed by at least 20% of the subjects in each subethnic group was compiled from data obtained from previous surveys of frequency of food consumption. Foods and dishes were included if they were consumed once a week and were likely to contribute to individual intervariation. A total of 207 questions were asked, 23 about breads and cereals, 26 about dairy products, 30 about meat and fish, 40 about vegetables and vegetable dishes, 21 about pulses, 23 about fruits, 5 about nuts and seeds, 17 about sweets and snacks, 9 about soups, sauces and spreads, and 13 about beverages including tea, coffee, and alcohol. These questions also covered foods popular in the British diet (e.g., pizza, lasagna) to take into account the assimilation of some of these foods into the migrant South Asian diet as well as soy and soy products. Questions on the type of fat and the method of cooking were also included as part of the questionnaire. Participants were asked about the frequency (per day, week, month, or never) of consumption; for seasonal foods such as mangoes, the women were asked to estimate their average intake when the food was in season. Portion size was collected using natural units, household portion sizes, average portion sizes (38), and previously validated photographs (39). A total of 277 food items were used to analyze the FFQ dietary information. This exceeds the actual number of questions asked because for 31 of the 46 South Asian traditional composite dishes, a choice of 3–4 recipes was available. A special program was developed whereby the closest recipe was selected in COMPEAT on the basis of the participant's religion, region of origin, use of fat, and method of cooking. This FFQ was validated

[7] Abbreviations used: NSP, nonstarch polysaccharides; TR-FIA, time-resolved fluoroimmuno-assay; VC, validity coefficient.

previously against multiple 24-h recalls for macronutrient intake (30). The percentage of women classified in the same ±1 quartile by the 2 methods was 74% for total energy and 85, 85, 96, and 90% for energy-adjusted fat, carbohydrates, proteins, and nonstarch polysaccharides (NSP), respectively. The Spearman correlation coefficients for energy-adjusted fat, carbohydrate, protein, and NSP were 0.45, 0.59, 0.76, and 0.71, respectively.

Development of a phytoestrogen database. A search of the medical scientific literature (Medline) and the Internet was conducted using the search words "phytoestrogens," "isoflavones," "lignans," "genistein," "diadzein," "secoisolariciresinol," and "matairesinol." All publications were then reviewed and cross-referenced. Data on the phytoestrogen content of some South Asian foods were also made available (unpublished data). Values for the phytoestrogen content of foods were used only if the following were true: *1*) the method of analysis was obtained using the "gold standard" GC-MS method; *2*) the values were derived from the average of >1 food sample; and 3) the values were reported separately for cooked and raw weight. Preference was given to phytoestrogen values from analyses that had been conducted on foods consumed in the UK (40). This was not possible for many of the lignan values because the primary source of data for this were the publications by Adlercreutz and Mazur (18–20), which were based on foods consumed in Finland. Values expressed on a dry weight basis were converted to a wet weight basis either by using the moisture content provided by the author, or by assuming commonly expected moisture content for the particular food. When there were no direct analyses conducted for food items consumed by the population under study, the phytoestrogen level for a similar food was assigned, an approach commonly used by researchers in the compilation of nutrient databases (41). Composite recipes were broken down into their constituent ingredients and the total phytoestrogen value assigned was calculated from the proportional phytoestrogen contribution of each ingredient to the recipe (42). All values were converted to μg/100 g food and reported to the nearest one decimal point (see the Appendix). Phytoestrogen data were not available for a large proportion of the foods in the database (51%) because the levels are thought at present to be nil or negligible. Multiple 24-h recalls from 6 individuals selected at random showed that 21% of foods in the recalls were not assigned values for isoflavones but only 1% belonged to food groups considered to be potential sources of isoflavones. For lignans, the number of missing values was 36%, 15% from potential sources of lignans. Of the 277 food items listed in the FFQ, 51 items (22%) had no values assigned because the phytoestrogen content was assumed to be negligible. Of the remaining 226 items, 221 (98%) were assigned isoflavone levels and 192 (84%) were assigned lignan levels.

Statistical analysis. All statistical analyses were performed using STATA 7.0 (43). An a priori level of significance was set at $P = 0.05$. Comparisons of sociodemographic, anthropometric, and dietary data were made among the 3 groups: those who completed multiple 24-h recalls and FFQ only ($n = 50$); those who provided multiple 24-h recalls, the FFQ and plasma samples ($n = 58$); and those in the main study who were not included in the validation study ($n = 375$) using ANOVA and a post-hoc t test. Values in the text are arithmetic means ± SD unless specified otherwise. The validity of the FFQ intake was assessed in terms of its ability to accurately rank individuals according to their phytoestrogen intake rather than in terms of its ability to accurately estimate absolute levels of nutrient intake. Thus, quartile agreement between the 2 dietary methods was assessed by calculating the percentage of women classified in the same, the same or adjacent, and in extreme opposite quartiles (quartile 1 and quartile 4). Spearman correlation coefficients were also calculated to assess the level of agreement between the FFQ and multiple 24-h recalls in assessing levels of phytoestrogen intake. This was calculated for unadjusted (crude) and energy-adjusted data. For energy-adjusted analyses, residuals from a normal errors regression model of the nutrient on total energy intake (both on a logarithmic scale) were used (32). Finally, the method of triads (**Fig. 1**) was used to compute validity coefficients (VCs) between "true" phytoestrogen intakes and those estimated by each of the 3 assessment methods (FFQ, 24-h recalls, and plasma samples) from the correlation coefficients observed between each pair of methods (44). The method of triad



$$VC_{AT} = \sqrt{(r_{AB} * r_{CA} / r_{BC})}$$
$$VC_{BT} = \sqrt{(r_{AB} * r_{BC} / r_{CA})}$$
$$VC_{CT} = \sqrt{(r_{CA} * r_{BC} / r_{AB})}$$

FIGURE 1 The graphical representation of the "method of triads" (46) as used in this study.

assumes that the correlations among the 3 measures are explained completely by each being linearly related to the true intake, and that the measurement errors are mutually independent. Bootstrap sampling methods, which require no assumptions regarding the theoretical probability distributions of the estimated VCs, were used to estimate 95% CIs around the VCs (45). A STATA routine (43) was written to randomly resample from the observed dataset 10,000 times, each sample of size $n = 58$. When the estimated VC and the upper limit of the CI were >1 (referred to as a Heywood case) they were set at 1.0.

RESULTS

Women who participated in the validation subsamples had sociodemographic characteristics similar to the whole group of randomly selected population-based controls for the case-control study (**Table 1**). There were no differences in adult height or waist-hip ratio among the groups, but those in the validation study had a higher mean BMI ($P = 0.009$). The groups had similar subethnic composition (Table 1), reflecting the main South Asian population subgroups in the UK. There were no other differences among the groups.

The number of monthly 24-h recalls completed by each participant was 13 ± 2.1, with all participants having completed at least 9 recalls. The median interval between the recalls was 5.5 wk (range 4.4–9.2 wk), and 91% of the women had at least 1 recall that included a Saturday or Sunday. There were also no differences in the intakes of macronutrients (Table 1) or of phytoestrogens [not shown in table, genistein ($P = 0.1$), daidzein ($P = 0.2$), secoisolariciresinol ($P = 0.7$) and matairesinol ($P = 0.4$)], as estimated by multiple 24-h recalls, between women who participated in the validation study and those who did not.

The FFQ provided higher estimates of absolute intake than multiple 24-h recalls for each of the 4 phytoestrogens examined (**Table 2**). However, a high proportion of the study subjects were classified in the same or adjacent quartiles by the 2 dietary assessment methods, ranging from 82% for genistein to 94% for secoisolariciresinol. FFQ misclassification into ex-

TABLE 1

Characteristics of participants in the validation study[1]

	Validation study		
	Multiple 24-h recalls and FFQ	Multiple 24-h recalls, FFQ and plasma samples	Subjects in the main study
n	108	58	483
Age,[2] *y*	53.2 ± 8.7	53.2 ± 8.8	53.4 ± 9.0
Height, *cm*	153.5 ± 6.4	154.3 ± 5.5	154.4 ± 6.1
Weight, *kg*	68.1 ± 11.9	67.2 ± 11.1	66.1 ± 12.4
BMI, *kg/m*^{2}	28.9 ± 4.7	28.2 ± 4.3	27.8 ± 4.9*
Waist/hip ratio	0.81 ± 0.1	0.81 ± 0.1	0.82 ± 0.1
Married, *n (%)*	95 (88)	52 (90)	409 (85)
Parity,[3] *n*	3.0 ± 1.6	3.1 ± 1.5	3.4 ± 1.5
Formal education, *%*	90 (83)	49 (84)	400 (83)
Current social class,[4,5] *n (%)*			
Nonmanual (I, II, III nm)	67 (64)	36 (64)	258 (55)
Manual (IIIm, IV, V)	38 (36)	20 (36)	214 (45)
Subethnicity, *n (%)*			
Gujerati Hindu	55 (51)	30 (52)	209 (43)
Punjabi Hindu	12 (11)	5 (9)	54 (11)
Punjabi Sikh	20 (19)	11 (19)	105 (22)
Pakistani Muslim	21 (19)	12 (20)	115 (24)
Nutrient intake[6]			
Energy, *kJ/d*	6836.6 ± 1553.4	6871.7 ± 1567.6	6852.5 ± 383.9
Protein, *g/d*	53.5 ± 14.5	53.8 ± 14.5	53.4 ± 14.6
Carbohydrate, *g/d*	212.9 ± 48.1	212.9 ± 46.2	215.0 ± 49.3
Fat, *g/d*	69.2 ± 20.2	69.9 ± 21.4	69.0 ± 20.9
Englyst fiber (NSP), *g/d*	16.6 ± 4.7	16.3 ± 4.5	16.7 ± 4.8

[1] Values are arithmetic means ± SD unless indicated otherwise. * Different from the other 2 groups, $P = 0.009$.
[2] Measured at the start of the study/first 24-h recall.
[3] Mean number of live-born children.
[4] Measured as either the social class of the women or her partner, whichever was the highest.
[5] Unclassified category excluded ($n = 2$ in column 1, $n = 2$ in column 2, $n = 11$ in column 3).
[6] Absolute nutrient intakes estimated by monthly 24-h recalls.

treme opposite quartiles was very low (<5%) for individual phytoestrogens. Crude Spearman's correlations coefficients between levels of intake estimated by the 2 dietary assessment methods ranged from 0.51 for genistein to 0.67 for secoisolariciresinol ($P < 0.001$ for each). The correlation coefficient for each of the 4 phytoestrogens increased slightly after adjustment for total energy (range: 0.55 for genistein to 0.70 for secoisolariciresinol, $P < 0.001$ for each).

Both the FFQ and 24-h recalls identified the food groups "Vegetable and Vegetable Dishes" (which included recipes containing pulses, legumes and vegetables) and "Breads" as being the main sources of isoflavones; these 2 food groups

TABLE 2

Level of agreement between multiple 24-h dietary recalls and FFQ as estimates of phytoestrogen intake among South Asian women in the UK[1]

Phytoestrogens	24-h recalls Arithmetic mean ± SD	FFQ Arithmetic mean ± SD	24-h recalls Median (IQ range)	FFQ Median (IQ range)	Spearman correlation, *r*		Level of quartile agreement, %[2]		
					Unadjusted	Energy-adjusted	Same	Same ± 1	Opp[3]
	μg/d								
Genistein	245.0 ± 791.9	261.1 ± 476.6	109.7 (73.8–142.7)	170.6 (94.8–224.3)	0.51	0.55	42	82	4
Daidzein	159.5 ± 418.5	209.8 ± 294.9	80.4 (45.4–132.3)	158.2 (59.6–234.6)	0.59	0.60	45	84	3
Secoisolariciresinol	131.4 ± 79.9	196.5 ± 136.4	101.4 (72.7–178.4)	142.3 (81.4–327.2)	0.67	0.70	46	94	3
Matairesinol	9.3 ± 5.2	11.7 ± 7.7	8.1 (5.1–12.4)	8.7 (5.5–17.6)	0.60	0.63	34	88	1
Isoflavones[4]	404.5 ± 1208.7	470.9 ± 766.2	194.2 (119.7–284.2)	326.7 (157.9–458.2)	0.53	0.57	42	81	5
Lignans[4]	140.7 ± 84.7	208.2 ± 143.9	109.8 (78.3–191.2)	149.3 (87.2–346.2)	0.66	0.70	44	93	3

[1] $n = 108$. IQ, interquartile.
[2] Based on unadjusted data.
[3] Percentage level of agreement in extreme opposite quartiles (quartile 1 and quartile 4).
[4] Isoflavones, genistein and daidzein combined; lignans, secoisolariciresinol and matairesinol combined.

TABLE 3

Sources of phytoestrogens in the diet of South Asian women in the UK[1]

Food group[2]	Isoflavone intake		Lignan intake	
	24-h recalls	FFQ	24-h recalls	FFQ
	μg/d (%)			
Breads	163.9 (41)	237.5 (50)	97.8 (70)	156.4 (75)
Flours, grains, and cereals	6.9 (2)	7.5 (2)	5.1 (4)	6.0 (3)
Breakfast cereals	0.9	4.4 (1)	0	0.1
Milk and milk products	8.4 (2)	0	0	0
Cheese and cheese dishes	0.3	0.2	0.2	0.1
Meat and meat dishes	0.4	0.4	0.8	1.1 (1)
Fish and fish dishes	0	0	0.1	0.1
Vegetable and vegetable dishes	218.5 (54)	212.8 (45)	15.3 (11)	18.7 (9)
Fruit and fruit juices	0.8	1.1	7.2 (5)	13.7 (7)
Nuts and seeds	0.2	0.1	1.6 (1)	2.5 (1)
Snacks	1.0	2.5 (1)	2.3 (2)	8.4 (4)
Biscuit, cakes, and pastries	3.2 (1)	4.2 (1)	0.1	0.4
Alcohol	0	0	0.1	0.1
Miscellaneous	0	0.2	10.1 (7)	0.6
Total	404.5 (100)	470.9 (100)	140.7 (100)	208.2 (100)

[1] Values are means (% of mean total intake from each food group), $n = 108$.
[2] Food groups that were not identified as sources of phytoestrogens were egg and egg dishes, animal fats, vegetable fat, oils, and sugars, syrups and confectionery.

accounted for ~95% of total dietary intake (**Table 3**). The food group "Breads" was by far the main source of lignans according to both dietary assessment methods accounting for ~70–75% of total intake, with "Vegetable and Vegetable Dishes" accounting for ~10%.

Plasma levels of genistein and daidzein correlated fairly well with the intake estimates produced by the multiple 24-h recalls ($r = 0.43$, $P < 0.001$ and $r = 0.40$, $P = 0.002$, respectively), but less so with those obtained by the FFQ ($r = 0.21$, $P = 0.12$ and 0.32, $P = 0.02$, respectively) (**Table 4**). The correlations between plasma levels of enterolactone and estimates of total lignan intake were low for both multiple 24-h recalls ($r = 0.08$, $P = 0.50$) and the FFQ ($r = 0.10$, $P = 0.43$). The proportion of subjects classified in the same or adjacent quartile by both plasma levels and multiple recalls was good (62–81%) with only a small proportion (3–7%) misclassified in the opposite quartile. The level of quartile agreement between the FFQ and plasma levels was fair (67–72% in the same or adjacent quartile, 7–10% misclassified in the opposite quartile).

The FFQ had the highest VC for intake of lignans (0.91) and reasonable VCs for intakes of daidzein (0.67) and genistein (0.46) (**Table 5**). Multiple 24-h recalls had higher VCs than the FFQ for genistein (0.95) and daidzein (0.83) but lower for lignans (0.73). Plasma samples had the lowest VCs of the 3 methods of assessment, particularly for lignans (0.45, 0.45, and 0.11 for genistein, daidzein, and lignans, respectively).

DISCUSSION

The validation of dietary instruments is an important and essential step in the design of any nutritional epidemiologic study (46). The present study aimed at assessing the validity of an FFQ to measure habitual intake of phytoestrogens among South Asian women in the UK relative to multiple 24-h recalls and multiple plasma samples. The energy-adjusted correlation coefficients between estimates of phytoestrogen intake produced by the FFQ and those produced by multiple 24-h recalls (0.55–0.70) were similar to those for other nutrients

TABLE 4

Level of agreement between plasma phytoestrogens and dietary intake among South Asian women in the UK[1]

	Plasma phytoestrogens Arithmetic mean ± SD	24-h recalls Arithmetic mean ± SD	Spearman's correlation		Level of quartile agreement			FFQ Arithmetic mean ± SD	Spearman's correlation		Level of quartile agreement		
			r	*P*	Same	Same ± 1	Opp[2]		*r*	*P*	Same	Same ± 1	Opp[2]
	nmol/L	*μg/d*			%			*μg/d*			%		
Genistein	18.3 ± 20.7	245.0 ± 791.9	0.43	<0.001	34	71	3	286.5 ± 587.5	0.21	0.12	28	72	7
Daidzein	7.8 ± 11.0	159.5 ± 418.5	0.40	0.002	35	81	3	229.1 ± 352.9	0.32	0.02	29	72	10
Enterolactone[3]	13.7 ± 18.8	140.7 ± 84.7[4]	0.08	0.50	31	62	7	224.4 ± 149.9[4]	0.10	0.43	31	67	7

[1] $n = 58$.
[2] Percentage level of agreement in extreme opposite quartiles (quartile 1 and quartile 4).
[3] Enterolactone measured in the plasma is a metabolite of lignan intake and is produced exclusively by the gut microflora.
[4] Lignan intake, secoisolariciresinol and matairesinol combined.

TABLE 5

Validity coefficients for phytoestrogens[1]

	Validity coefficients (95% CI)[2]		
	24-h recalls vs. T	FFQ vs. T	Biomarker vs. T
Genistein	0.95 (0.60–1.00)	0.46 (0.13–0.78)	0.45 (0.14–0.76)
Daidzein	0.83 (0.54–1.00)	0.67 (0.32–0.96)	0.45 (0.17–0.75)
Lignans[3]	0.73 (0.22–1.00)	0.91 (0.22–1.00)	0.11 (0.02–0.41)

[1] $n = 58$; T, "true intake."
[2] CIs set at 1.00 when a Heywood case.
[3] Lignan intake equal to secoisolariciresinol and matairesinol intakes combined. Plasma enterolactone taken as a biomarker of lignan intake.

(32) and in other validation studies for phytoestrogens (21–23). The FFQ and multiple 24-h recalls also identified similar sources of phytoestrogens in the diet, with the food groups Breads and Vegetable and Vegetable Dishes emerging as the main sources in the UK South Asian diet. We also applied the triad method of evaluating the 3 forms of assessment for the estimation of phytoestrogen intake. The FFQ performed adequately for the estimation of genistein and daidzein and proved to be the best assessment method to estimate lignan intake.

This study is unique in that mean plasma phytoestrogen levels were obtained from 4 plasma samples collected over a period of 1 y, whereas previous studies relied on 1 sample (21,23). Thus, this study was able to estimate plasma levels more precisely by taking into account daily and seasonal fluctuations, which is particularly relevant given the short half-lives of these compounds (25–27). The 24-h recalls were also collected over a period of 1 y; therefore, their estimates provided an accurate measure of usual long-term intake. The low burden of the random telephone interview ensured a high overall response rate (81%) and the representativenesss of the women who participated in the validation subsamples, whereas the use of more demanding methods (e.g., 7-d weighed intake records) would have been difficult in this migrant population with relatively low education and literacy levels. The distribution of South Asian main subethnic groups in the validation study was similar to those in the main study group (Table 1) and in the general population of England and Wales (31). There was difficulty in recruiting interpreters for Bangladeshis for the 24-h dietary recalls, making it unfeasible to include South Asian migrants who originated from Bangladesh; however, this group accounts for only 8% of the South Asian population in the UK (47).

Telephone interviews are also more cost efficient than face-to-face interviews and other studies did not note any significant difference in the reporting of daily energy intake by these 2 methods (48,49). Women who participated in the validation study had a higher mean BMI than those who did not, but the intakes of macronutrients and phytoestrogens did not differ between the groups.

A phytoestrogen database for UK South Asian diets was specifically compiled for this study using standard criteria. The estimated phytoestrogen intake in the UK South Asian population was <1 mg/d, much lower than intakes reported in Japanese populations (daidzein 14.5 mg/d, genistein 23.4 mg/d) (21) but similar to populations who do not consume soy; for example, Keinan-Boker and colleagues (50) reported a daily intake in Dutch women of 0.15 mg/d for daidzein, 0.16 mg/d for genistein, 0.07 mg/d for matairesinol, and 0.93 mg/d for secoisolariciresinol. Plasma phytoestrogen levels in this study were also much lower than those in Japanese populations (geometric mean: daidzein 119.9 nmol/L, genistein 475.3 nmol/L) (21). Similarly low plasma levels were reported in other Western populations; for example, one study reported geometric mean plasma levels in U.S. women of 2.1 nmol/L for daidzein, 5.7 nmol/L for genistein, and 20.2 nmol/L for enterolactone (51).

The correlation coefficients in this study between intake estimates produced by FFQs and dietary records (0.55–0.70) and between FFQs and biochemical markers (0.30–0.40) were of similar magnitude to those in other validation studies (21–23). Higher correlations (>0.70) between plasma isoflavones and estimated phytoestrogen intake were observed in a study by Verkasalo and colleagues (52) but the relatively high correlations were probably a reflection of the nonrandom selection of the study sample who were chosen on the basis of their reported soy intake. The low correlation coefficients between plasma levels of enterolactone and 24-h dietary recalls and FFQ estimates for matairesinol and secoisolariciresinol could be explained in part by the fact that matairesinol is metabolized into enterolactone, whereas secoisolariciresinol is metabolized into enterodiol by the gut microflora. Although enterolactone and enterodiol plasma levels correlate well, it may be insufficient to use only one of the mammalian lignans as a biomarker for both plant precursors.

A potential source of error in this study concerns the missing data in the phytoestrogen database, particularly for lignans. The FFQ actually performed better than 24-h multiple recalls and plasma samples at estimating lignan intake according to the "triad method." This may be because the FFQ required only 277 foods to be assigned phytoestrogen values, whereas it numbered >800 for multiple 24-h dietary recalls. Although the percentage of missing lignan values for food items in the FFQ (16%) was lower than for 24-h recalls (36%), only 11% of the unassigned foods in the FFQ and 15% in the 24-h recalls were considered potentially high sources of lignans.

It is possible that the FFQ overestimated the consumption of a number of food groups, particularly in the amount of phytoestrogens from the Breads and Fruit and Fruit Juices group. Overestimation can result from either too large a portion size or too high a frequency. Portion sizes for chapatis in the Breads group were assessed in the 24-h multiple recalls as "small, medium, or large"; for the FFQ, 3 photographs were used to categorize chapatis into small, medium, and large. The weights assigned for each portion size of small, medium, and large were similar. Portion size for fruit was determined using "natural units" (e.g., 1 apple) for both the multiple 24-h recalls and the FFQ. Thus, it is likely that the frequency, rather than portion size was overestimated in the FFQ. The problem of overestimation of frequency, particularly for fruit, occurs in dietary questionnaires (32). However, it is also true that multiple 24-h recalls tend to underestimate intake (46); thus, "true intake" may lie somewhere in between.

Biomarkers had the poorest performance according to the method of triads for estimating phytoestrogen intake. Published VCs for nutrients are relatively scarce; however, some studies evaluated 3 assessment tools for β-carotene with varying results. Ocke and Kaaks (53) reported the highest VC for dietary records, whereas the FFQ performed the best in the study by Kabagame and colleagues (54). Duares et al. (55) reported the highest VCs for plasma biomarkers. The disparity between the estimates obtained from studies could be due to differences in the methods used, random errors resulting from differences in sample sizes, or differences in the structure and size of the questionnaires used in these studies.

Two technical difficulties were encountered in the current study in the calculation of the VCs. First, some of the estimated VCs (and/or their upper CIs) were >1.0 (referred to as Heywood cases). These could be the result of sampling fluctuation when the VC is just below 1.0 or may result from the violation of the assumption behind the method of triads, i.e., that the 3 methods of assessment are uncorrelated (44). This assumption is likely to be valid for the estimates of intake obtained with either the FFQ or 24-h recalls relative to those produced by biomarkers, but not necessarily between estimates obtained by the FFQ and those derived from the 24-h recalls. The FFQ was administered after the completion of the multiple 24-h recalls; according to some researchers (32,46), this sequence may lead to an overestimation of validity because the subjects could possibly have been sensitized to their food intake and improve their accuracy in completing a questionnaire. This criticism is fair if applied to intensive and highly demanding reference methods such as 7-d weighed food records, in which the subjects are likely to remember the items eaten after 1 wk. However, in this study, the 24-h recalls were collected monthly, at random times with minimal burden to the respondent; thus, it is unlikely that subjects were able to remember what they had reported on several occasions throughout the year. Because correlated errors between the FFQ and 24-h recalls cannot be ruled out, the VCs reported in this study could have been overestimated and should be regarded as upper limits of the true VCs. The second technical problem that was encountered was that some of the resampled correlation coefficients were negative; on those occasions, calculation of the VC was impossible. This also occurs due to random sampling fluctuations, particularly if the true VC of the marker is low because the likelihood of negative sample correlations using the bootstrap method increases. The number of negative sample correlations was low for daidzein (1%) and genistein (5%) but higher for enterolactone (25%). Negative sample correlations were excluded when estimating VCs and their corresponding 95% CIs.

In summary, this study shows that our FFQ can be used as a valid tool with which to rank subjects according to their phytoestrogen intake for future epidemiologic studies in South Asian women in the UK.

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APPENDIX

Compilation of a list of the phytoestrogen concentration of some foods commonly consumed by South Asian women in the UK[1]

Food group	Dry weight d	Dry weight g	Dry weight s	Dry weight m	% DM	Wet weight d	Wet weight g	Wet weight s	Wet weight m	Reference d	Reference g	Reference s	Reference m
	μg/100 g					*μg/100 g*							
Breads													
White bread, average	229.7	266.3	nd	nd	59	135.6	157.2	nd	nd	40	40	42	42
Wholemeal bread	638.7	781.8	611.4	34.2	58.4	373.1	456.7	357.1	20.0	40	40	42	42
Chapati, made with fat[2]	2.4	14.6	32.9	2.6	63	1.5	9.2	20.7	1.6	40	40	18	18
Chapati, no fat[2]	2.4	14.6	32.9	2.6	72	1.7	10.5	23.6	1.9	40	40	18	18
Grains and cereals													
White rice, boiled	7.9	11.9	16	tr	32.5	2.6	3.9	5.2	tr	40	40	18	18
Vegetable and vegetable dishes													
Cauliflower, boiled	nd	nd	97	tr	10.9[3]	nd	nd	10.6	tr	40	40	18	18
Broccoli, boiled	7.2	9.4	414	23	10.9	0.8	1	45.1	2.5	40	40	18	18
Carrots, old, boiled	nd	nd	192	3	9.1[4]	nd	nd	17.5	0.3	40	40	18	18
Onions, raw	nd	nd	83	8	9.4[5]	nd	nd	7.8	0.8	40	40	18	18
Red kidney beans, boiled	28.2	158	56	0.7	41.9	11.8	66.2	23.4	0.3	unpublished data			
Mung, whole, boiled	9.7	365	171.6	0.3	37.3	3.6	136.1	64	0.1	unpublished data			
Bean sprouts, mung, raw	3900	6800	468	0.9	5.4	206.6	367	25.3	0.1	40	40	18	18
Lentils, red split boiled	3.3	7.1	8.9	0.3	35.7	1.2	2.5	3.2	0.1	unpublished data			
Chickpeas, whole, boiled	11.4	76.2	8.4	0	41.8	4.8	31.9	3.5	0	unpublished data			
Aubergine, cooked	5	10.5	99.7	3	5.4	0.3	0.6	5.4	0.3	40	40	18	18
Tomatoes, raw	nd	48	51.6	6.5	6.8	nd	3.3	3.5	0.4	40	40	18	18
Old potatoes, boiled	0.5	3.7	10	6	17.5	0.1	0.7	1.8	1.1	40	40	18	18
Fruits													
Mangoes, ripe, raw	25.1	21.2	na	na	15.3	3.8	3.2	na	na	40	40	na	na

[1] Abbreviations: d, daidzein; g, genistein; s, secoisolariciresinol; m, matairesinol; % DM, % dry matter; na, none available; nd, none detected; tr, trace.
[2] Values are based on wholemeal flour.
[3] % dry matter as for broccoli.
[4] % dry matter as for turnips.
[5] % dry matter as for salad onions.