British Journal of Nutrition (2006), **95**, 1150–1158 DOI: 10.1079/BJN20061777

Phyto-oestrogen intake and plasma concentrations in South Asian and native British women resident in England

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(Received 15 September 2005 – Revised 8 February 2006 – Accepted 8 February 2006)

Phyto-oestrogens, naturally occurring hormone-like chemicals in plant food, may play a protective role against hormone-related chronic diseases. South Asian migrants in the UK have a lower incidence of hormone-related cancer than their hosts but the extent to which this difference may be due to phytoestrogen intake is not known. The aim was to compare habitual phytoestrogen intake in first-generation South Asian migrant women and native British women. South Asian (n 221) and native British women (n 50) were recruited from general practitioner lists and were asked to provide monthly 24 h recalls for a period of 1 year. An enhanced phytoestrogen database was compiled using data from a literature search and unpublished data. A sub-sample of South Asian women (n 100) and the native British women (n 40) also provided blood samples every 3 months during the 1-year period. The median daily intakes (μg/d) of isoflavones (184·2 *v.* 333·9) and lignans (110·8 *v.* 148·8) were significantly lower in South Asians than in the native British ($P < 0{\cdot}001$, $P = 0{\cdot}04$ respectively). There were no significant differences in mean plasma isoflavone levels (nmol/l) but plasma enterolactone was significantly lower in the South Asians (13·9 (SD17·5) *v.* 28·5 (SD23·3), $P < 0{\cdot}001$). The main sources of phytoestrogens were bread and vegetables in both ethnic groups. Habitual phytoestrogen intake in South Asian and native British women was below 1 mg/d and was higher in the native British diet. The present study does not support the hypothesis that differences in phytoestrogen intake, or in circulating levels, could explain differences in hormone-related cancer risks between these two populations.

Phytoestrogen: 24-h recall: South Asian diet: Isoflavones: Lignans: Genistein: Daidzein: Enterolactone

A high intake of phyto-oestrogens has been associated with a protective effect against menopause-related signs and symptoms (Nagata *et al.* 2001), female breast cancer (Ingram *et al.* 1997; Pietinen *et al.* 2001; Shu *et al.* 2001; Yamomoto *et al.* 2003), CVD (Vanharanta *et al.* 1999) and decreasing bone mineral density (Erdman *et al.* 2000). However, findings from epidemiological investigations have been inconclusive, with some studies finding no clear protective effect (Key *et al.* 1999; Horn-Ross *et al.* 2002; dos Santos Silva *et al.* 2004; Keinan-Boker *et al.* 2004). One of the major drawbacks in conducting epidemiological investigations has been the difficulty in estimating phytoestrogen intake accurately as there is a lack of complete food composition tables for phytoestrogens. Biomarkers only reflect short-term intake (Nesbitt *et al.* 1999), thus single samples of urine and plasma are of limited value, particularly in Western populations who do not consume high levels of these compounds.

The isoflavones, daidzein and genistein, and the lignans, secoisolariciresinol and matairesinol, are the most widely studied phytoestrogens. Soyabeans and soyabean products are rich in phytoestrogens but much smaller amounts can be found in legumes such as clover, mung beans, alfalfa and peanuts (Adlercreutz & Mazur, 1997). Flaxseed is the richest known source of lignans but it is not a component of any usual diet. Dietary sources of lignans include whole grains, nuts, seeds, berries, fruits and vegetables (Mazur, 1998; Mazur *et al.* 1998).

First-generation UK South Asian migrants from India, Pakistan and Bangladesh are known to have a diet high in pulses and vegetables (Smith *et al.* 1993; dos Santos Silva *et al.* 2002) but low in soya-based products, and a low incidence of hormone-related cancers in comparison to the host population (Winter *et al.* 1999). South Asians are of further interest because of the high inter-individual variation within the population, for example Gujerati Hindus tend to be lifelong vegetarians whereas Pakistani Muslims tend to be lifelong non-vegetarians (McCormack *et al.* 2004). The present study uses multiple 24 h recalls, an enhanced phytoestrogen database, and multiple plasma samples obtained over a 1-year period to compare the long-term habitual intake of phytoestrogens between South Asian and native British women resident in the UK.

Abbreviations: GP, general practitioner; LER, low energy reporter.

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Method

Selection of study subjects

The South Asian subjects were recruited from a population-based sample of healthy women living in England who were participating as controls in a case–control study on diet and breast cancer (dos Santos Silva *et al.* 2002). Almost all residents in the UK are registered with a general practitioner (GP), and GP lists in London and the West Midlands were used as the framework to select the study sample using random number tables and SANGRA, a computer program especially devised to identify South Asian names (Nanchanal *et al.* 2001). Ethnicity was further confirmed by the GP and by the women themselves. Only first-generation migrant women who were born in the Indian subcontinent or in East Africa were eligible for the study. Women were asked to take part in the study sequentially as they were recruited as healthy controls into the case–control study. The native British women, defined as Caucasian women of British origin resident in the UK, were recruited from a GP list in London. Native British women who participated in the study did not have spouses or partners who were South Asian. South Asian and British participants were only included in the study if they were aged between 25 and 75 years, had no history of any cancer (except non-melanoma skin cancer) and had no history of chronic disorders requiring highly specialised diets (e.g. renal failure, liver disorders) or mental disorders.

We estimated that with our sample of 200 South Asians we would require a sample of approximately fifty British-native women to ensure that the study would have at least 80 % power to detect a difference of 0·5 between two log-normal intake means at the 5 % significance level. Similarly, we estimated that with our sample of 100 South Asians we would require a sample of approximately forty native British women to ensure that the study would have 80 % power to detect a difference of 0·5 between any two log-normal plasma means at the 5 % significance level.

The subjects were not informed of the specific aims of the study, but were told in general terms that it was a study on eating habits. The first 100 South Asian women who agreed to take part in the blood collection formed the sub-group. This sub-sample of the total South Asian population and all the native British women were asked to provide, in addition to monthly 24 h recalls, a fasting sample of blood every 3 months for the duration of the study. Subjects who had taken antibiotics in the last 6 months were excluded from the blood collection phase as these drugs have been shown to destroy the gut microflora needed to metabolise phytoestrogens (Kilkkinen *et al.* 2002). Ethical approval was obtained from all relevant committees and written informed consent was obtained from each participant.

Multiple 24 h recalls for a 1-year period

An initial face-to-face interview was conducted in the subject's home by a research dietitian (D. B.), who explained the procedure of the 24 h recall and also obtained information on baseline socio-demographic characteristics. Subsequent interviews were conducted over the telephone by experienced nutritionists (D. B. and L. S.), with the aid of interpreters when necessary. The 24 h recalls were conducted on all days of the week (including weekends) and for every calendar month for a period of 1 year. The subjects were not aware when they would be contacted. Detailed descriptions and recipes of the food consumed during the previous 24 h were obtained. The subjects were prompted to recall commonly forgotten foods such as snacks and beverages. A study-specific coding and portion size manual was also developed for South Asian foods, using serving spoons commonly used by South Asians (Bhakta *et al.* 2005). The recalls were coded and entered into a nutrient database program, independently by the two nutritionists, and any inconsistencies between the two were discussed with the rest of the study team. The Ministry for Agriculture, Fisheries and Foods nutrient database 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 (Judd *et al.* 2000).

Blood samples and laboratory assays

Four 2 ml blood samples (one every 3 months) were collected from each of the subjects in the sub-sample during the 1-year period within which the 24 h recalls were conducted. Samples were collected in heparinised tubes during early morning after a 10 h fasting period. 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, centrifuged at 2000 rpm for 10 min and then the plasma aliquots were stored at $-70^{\circ}C$. We measured plasma daidzein, genistein and enterolactone, a metabolite of the lignans secoisolariciresinol and matairesinol.

The plasma samples from the study subjects were sent on dry ice to H. Adlercreutz's laboratory in Helsinki (Finland). Multiple samples from each participant were pooled prior to laboratory assays, to provide more reliable estimates by decreasing intra-individual random variability.

A time-resolved fluoroimmunoassay method was used to quantify plasma levels of daidzein, genistein and enterolactone. Plasma levels of enterolactone were taken here as a biomarker for lignan intake. Laboratory researchers were kept blind regarding the ethnic origin and type of diet of the study subjects. The immunological part of the assays was analysed in duplicate and the mean values calculated. The intra-assay CV of the time-resolved fluoroimmunoassay method has been 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; Stumpf *et al.* 2000; Wang *et al.* 2000). The time-resolved fluoroimmunoassay method has been previously validated in relation to the 'gold standard' GC–MS, with high correlation coefficients observed between the levels estimated by the two methods for each one of the three phytoestrogens (r 0·87–0·99) depending upon concentration; Stumpf *et al.* 2000; Wang *et al.* 2000).

Development of a phytoestrogen database

A search was conducted on medical scientific literature (Medline) and the Internet using the search words 'phytoestrogens', 'isoflavones', 'lignans', 'genistein', 'daidzein', 'secoisolariciresinol' and 'matairesinol'. All publications were then

reviewed and cross-referenced. Data specific for South Asian foods consumed in the UK were also made available (H. Adlercreutz, personal communication). A set of strict criteria was used to assign values for the phytoestrogen content of foods. Phytoestrogen values were only used if they were estimated using the 'gold standard' GC–MS method, derived from the average of more than one food sample, and they 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 including traditional UK South Asian foods (Bingham *et al.* 1998). This was not possible for lignan values as the primary sources of data for this were the publications by Adlercreutz and Mazur (Adlercreutz & Mazur, 1997; Mazur, 1998; Mazur *et al.* 1998), 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 the 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 value for a similar food was assigned when possible, an approach commonly used by researchers in the compilation of nutrient databases (de Kleijn *et al.* 2001). 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 (Bhakta, 2003). All values were converted to μg per 100 g food and values were reported to the nearest one decimal point.

Phytoestrogen analyses were not available for a large proportion (51 %) of the foods in the database because the levels are thought at present to be nil or negligible, in food groups such as fish and fish products, sugar syrups and confectionery, and animal fats. A set of twelve 24 h recalls obtained from six individuals (a total of seventy-six recalls) was analysed using the enhanced phytoestrogen database to assess its completeness. We found that on average 21 % of foods in the recalls were not assigned values for isoflavones but only 1 % of these foods were considered to be potential sources of isoflavones. The number of missing values for lignans was higher (36 %), but only 15 % of these foods (e.g. lettuce, sweetcorn, nuts, pears) were considered potential sources of lignans.

Statistical analyses

Crude nutrient intake means are shown in the tables but all *P* values were adjusted for energy (per 1000 kcals/d). Medians and interquartile ranges of untransformed data for phytoestrogen intake are presented. Although inappropriate (because of the skewness of the distribution), arithmetic means and standard deviations are presented to allow comparison with other published data and to show the range of intakes and plasma levels of phytoestrogens. Multiple linear regression was used to compare means of intakes and plasma levels between the study populations after data were log-transformed and adjusted for socio-demographic variables. The data were stratified by low energy reporters (LER) and non-LER status, using the Goldberg cut-off limits (energy intake:BMR$>$1·13) to identify participants as LER (Goldberg *et al.* 1991). ANOVA was used to compare log mean phytoestrogen intake between the seasons in participants who had completed recalls in all four seasons (South Asians *n* 185, British *n* 30). Spearman correlation was used to assess the relationship between estimated phytoestrogen intake and plasma levels.

Results

Participation rates

Out of the initial 272 South Asian participants, 221 (81 % response rate) completed the dietary survey with fifty-one (19 %) failing to complete at least four recalls (the number determined as the minimum required to estimate habitual intake for most of the nutrients for the present study (Bhakta, 2003)). Forty-nine (98 %) out of the fifty British participants completed four or more recalls. The average number of recalls completed by all the participants was 10·8 (SD 3·3). One hundred South Asian women (out of a total of 118, response rate 85 %) and forty out of the fifty (80 %) native British women provided three or more blood samples, of which, seventy-five (75 %) of the South Asian and twenty-nine (73 %) British provided four blood samples.

Socio-demographic variables

Significant differences ($P<0·05$) in height, marital status, parity, formal education, house ownership and smoking status were observed between South Asians (*n* 221) and native British women (*n* 49) who participated in the 24 h diet survey (Table 1). No differences in age, marital status, social class, education or house ownership were observed between those subjects who participated in the diet survey and the sub-sample who participated in the blood collection in either South Asians or British population (data not shown in Table 1). Age, height, BMI, marital status, parity, education, house ownership and smoking status were significantly different between South Asians and the British sub-sample who provided blood samples.

Energy and nutrient intake

Mean energy, carbohydrate, Fe/Ca, fibre and calcium intake was significantly higher in the South Asian population than in the native British (Table 2). Protein, folate, vitamin D, vitamin B_{12} and Zn were significantly lower in the South Asian diet than in the native British, and there was no significant difference in fat intake.

Phytoestrogen intake

Total mean isoflavone and lignan intakes were below 1 mg/d in both the South Asian and the British groups, with wide inter-individual variation in intakes (Table 3). Statistically significant differences were observed between South Asians and native British for genistein, daidzein, secoisolariciresinol and matairesinol. When combined, total isoflavone and lignan intake was significantly higher in the native British than in the South Asian population. Participants who were 'suspected' of underestimating their energy intake (LER) reported lower intakes of phytoestrogens than non-LER in both the South Asian and British groups (data not shown). Similar gradients

Table 1. Baseline characteristics of the study subjects by ethnic group
(Mean values and standard deviations)

	Multiple 24 h recall dietary survey					Sub-sample who participated in the plasma collection				
	South Asian (*n* 221)		Native British (*n* 49)			South Asian (*n* 100)		Native British (*n* 40)		
	Mean	SD	Mean	SD	*P* values	Mean	SD	Mean	SD	*P* values
Age (years)*	52·7	8·8	50·5	9·0	NS	53·9	8·3	49·1	8·7	<0·001
Height (cm)	154·3	6·1	162·6	6·8	<0·001	154·5	5·2	162·2	6·8	<0·001
Weight (kg)	67·5	12·5	70·7	14·1	NS	67·5	11·1	69·2	12·9	NS
BMI (kg/m^2)	28·3	5·0	26·7	5·2	NS	28·3	4·3	26·4	5·0	0·05
Waist:hip ratio	0·81	0·1	0·79	0·1	NS	0·81	0·1	0·78	0·1	NS
Marital status (% married)	88		61		<0·001	87		58		0·002
Parity†	3·2	1·8	2·1	1·4	0·001	3·3	1·6	2·1	1·5	<0·001
No formal education (%)	17		0		0·001	16		0		0·02
Current social class (%)‡§					NS					NS
Non-manual (I, II IIInm)	62		71			63		77		
Manual (IIIm, IV, V)	38		29			36		23		
House ownership (%)	86		63		<0·001	90		63		<0·001
Never smoked (%)	99		41		<0·001	99		40		<0·001

* Measured at the start of the study/first 24 h recall.
† Number of live-born children.
‡ Measured as either the social class of the woman or her partner, whichever was the highest.
§ Unclassified category, due to missing data, excluded (South Asians *n* 5, native British *n* 2).

were, however, observed in both LER and non-LER: in each stratum total isoflavone and total lignan intake was higher in the British than in South Asians (data not shown). No significant differences were observed between the average habitual phytoestrogen intake of South Asian vegetarians and non-vegetarians (isoflavone median intake (interquartile range): vegetarians 168 (109–283) *v.* non-vegetarians 193 (133–272) μg/d, $P=0·1$); lignan median intake (interquartile range): vegetarians 100 (70–192) *v.* non-vegetarians 111 (81–174) μg/d, $P=0·4$).

The main source of isoflavones in the British population was from bread, whereas both the bread and vegetables were identified as the important sources in the South Asian diet (Table 4). The main source of lignans in both populations was from bread, with fruit and vegetables making a smaller contribution. Legumes and dishes containing legumes were included in the 'Vegetable and vegetable dishes' food group.

Habitual intakes of genistein, daidzein, secoisolariciresinol and matairesinol did not vary with season in the South Asian or the native British population (Table 5).

Table 2. Dietary intake assessed using multiple 24 h recalls for 1 year*
(Mean values and standard deviations)

	South Asians (*n* 221)		Native British (*n* 49)		
	Mean	SD	Mean	SD	*P* value†
Energy (kJ/d)	6672·5	1534·1	5875·8	1037·9	0·002
Fat (g/d)	66·4	20·8	59·9	14·7	NS
Carbohydrate (g/d)	211·3	47·5	165·3	34·9	<0·001
Protein (g/d)	52·1	14·3	60·0	10·3	<0·001
Fibre (NSP) (g/d)	16·5	4·9	10·6	3·6	<0·001
Folate (μg/d)	176·1	48·2	198·8	51·5	<0·001
Vitamin D (μg/d)	0·8	0·6	2·1	1·0	<0·001
Vitamin B_{12} (μg/d)	1·7	0·8	3·3	1·6	<0·001
Fe (mg/d)	10·5	2·9	8·5	2·4	0·04
Ca (mg/d)	742·9	219·7	595·1	162·9	0·006
Zn (mg/d)	6·5	1·7	6·5	1·6	<0·001

* For details of procedures, see p. 1151.
† Adjusted for height, marital status, parity, education, house ownership, smoking status and total energy intake.

Phytoestrogen plasma concentrations

Mean plasma genistein was higher in South Asians, and mean plasma daidzein was lower than in the native British but these differences were not statistically significant (Table 6). Plasma enterolactone levels were significantly lower in South Asians than in the native British. No significant differences in plasma phytoestrogens were observed between the South Asian vegetarians and non-vegetarians (data not shown).

Correlation of plasma phytoestrogens with intake

A positive significant relationship was observed between plasma genistein and estimated habitual intake using 24 h recall data for all subjects, and for South Asians alone (Table 7). The relationship between plasma genistein and intake for the native British population was positive but not significant. A similar pattern was also evident for daidzein with significant positive relationships between intake and plasma levels when all subjects were combined and for South Asians only, but the relationship between plasma daidzein and intake for the native British population was weak. Non-significant weak relationships were observed between plasma enterolactone levels and lignan intake for all subjects combined, for South Asians alone and for British alone.

Discussion

This is the first study, to our knowledge, to assess phytoestrogen intake in the South Asian population in the UK. We used

Table 3. Phytoestrogen intake assessed using multiple 24 h recalls for 1 year*
(Mean values and standard deviations)

	South Asians (*n* 221)				Native British (*n* 49)				
Phytoestrogen (μg/d)	Mean	SD	Median	IQ range	Mean	SD	Median	IQ range	*P* value†
Genistein	190·3	570·8	108·2	75·8–146·5	192·0	94·3	176·8	118·6–239·6	<0·001
Daidzein	131·5	308·5	75·1	44·8–132·2	174·0	83·3	153·3	109·8–215·0	<0·001
Secoisolariciresinol	128·0	76·9	102·5	71·6–170·6	169·4	122·4	138·6	81·5–212·2	0·05
Matairesinol	9·0	4·9	8·1	5·1–12·1	13·4	10·3	10·4	6·4–16·3	0·001
Total isoflavones‡	321·7	877·0	184·2	121·1–277·6	366·0	175·5	333·9	227·0–448·7	<0·001
Total lignans§	137·1	81·5	110·8	76·8–182·4	182·8	131·6	148·8	87·4–228·6	0·04

IQ, interquartile.
* For details of procedures, see p. 1151.
† Adjusted for height, marital status, parity, education, house ownership and smoking status.
‡ Genistein and daidzein combined.
§ Secoisolariciresinol and matairesinol combined.

multiple 24 h recalls, which were collected over a period of 1 year, multiple blood samples rather than a single one and an enhanced database to estimate habitual phytoestrogen intake. We observed a low (below 1 mg/d) habitual phytoestrogen intake in South Asians and in the native British population and the present finding is consistent with other studies in populations who do not traditionally consume soya (Maskarinec *et al.* 1998; Horn-Ross *et al.* 2000; de Kleijn *et al.* 2001).

The distribution of the South Asian sub-ethnic groups was similar to those in the general population of England and Wales (Peach, 1996). The Bangladeshi group had to be excluded from the present study because of difficulty in recruitment of appropriate interpreters; however, this group accounts for only 8 % of the South Asian population in the UK. The socio-economic differences between South Asians and the British native women observed in the present study were similar to those reported in the Census (Peach, 1996), with South Asians having, on average, a lower socio-economic status but a higher level of house ownership. Similarly to the present study, previous surveys have shown that South Asians have, on average, a higher parity (Office of Population and Census Survey, 1993), shorter height and higher waist:hip ratios (Department of Health, 2001).

South Asians, who have a lower incidence of hormone-related diseases (Winter *et al.* 1999), despite having a higher intake of legumes and vegetables had a lower habitual phytoestrogen intake compared with the British population. The present study, therefore, does not lend support to the hypothesis that population differences in phytoestrogen intake may partly account for the marked geographical variation in the risk of hormone-related cancers. The differences in estimated phytoestrogen intake between South Asian and British women were essentially related to differences in the consumption of staple breads, as found in other studies in Western populations

Table 4. Sources of phytoestrogens in the South Asian and native British diet*
(Mean values and % of mean total intake from each food group)

	Isoflavone distribution (μg/d)				Lignan distribution (μg/d)			
	South Asians (*n* 221)		Native British (*n* 49)		South Asians (*n* 221)		Native British (*n* 49)	
Food groups†	Mean	%	Mean	%	Mean	%	Mean	%
Breads	162·7	51	340·3	93	95·3	70	110·0	60
Flours, grains and cereals	6·2	2	2·4	1	4·4	3	0·9	
Breakfast cereals	0·8		1·2		0		0	
Milk and milk products	24·3	8	10·7	3	0		0	
Cheese and cheese dishes	0·3		0		0·2		0	
Egg and egg dishes	0		0·1		0		0·1	
Meat and meat dishes	0·4		0·5		0·9		0·4	
Vegetable and vegetable dishes‡	122·1	38	7·0	2	16·3	12	10·5	6
Fruit and fruit juices	0·8		0·8		7·2	5	39·0	21
Nuts and seeds	0·2		0·1		1·2		0·1	
Snacks	0·9		0		2·2	2	0·8	
Biscuit, cakes and pastries	3·0	1	2·9	1	0·1		1·2	1
Alcohol	0		0		0		3·2	2
Miscellaneous	0		0		9·3	7	16·5	9
Total	321·7	100	366·0	100	137·1	99	182·8	99

* For details of procedures, see p. 1151.
† Food groups that were not identified as sources of phytoestrogens were fish and fish dishes, animal fats, vegetable fat and oils, and sugars, syrups and confectionery.
‡ Includes legumes and dishes containing legumes.

Table 5. Seasonal variation in phytoestrogen intake as assessed by multiple 24 h recalls for 1 year*

	Winter		Spring		Summer		Autumn		
Phytoestrogen (μg/d)	Median	IQ range	Median	IQ range	Median	IQ range	Median	IQ range	*P* value
South Asians†									
Genistein	100·2	55·6–147·8	112·9	72·8–112·9	105·7	66·2–149·2	103·3	55·7–149·9	NS
Daidzein	67·3	25·9–132·5	80·8	39·9–145·8	72·5	36·1–133·6	63·8	22·2–117·8	NS
Secoisolariciresinol	87·4	52·9–162·1	96·1	54·0–179·2	103·9	67·2–181·3	96·0	56·6–172·1	NS
Matairesinol	6·4	4·4–11·5	7·1	4·2–11·7	8·3	5·2–12·8	7·3	4·5–12·1	NS
Native British‡									
Genistein	128·9	107·8–181·2	170·5	122·9–255·4	180·0	114·4–242·7	158·3	128·2–223·2	NS
Daidzein	110·2	92·2–188·9	172·3	98·1–213·9	137·3	98·2–205·0	47·4	113·2–218·6	NS
Secoisolariciresinol	109·1	27·2–257·0	168·1	21·1–273·3	69·7	25·9–211·0	143·6	34·1–245·6	NS
Matairesinol	7·3	3·3–19·4	9·9	3·3–17·3	6·2	3·4–15·6	9·2	3·9–16·6	NS

IQ, interquartile.
* For details of procedures, see p. 1151. Winter is December to February; Spring is March to May; Summer is June to August; Autumn is September to November.
† Data based on 185 South Asian women who completed 24 h recalls for all four seasons.
‡ Data based on thirty native British women who completed 24 h recalls for all four seasons.

(van Erp-Baart *et al.* 2003). Isoflavone levels in manufactured white, brown and wholemeal bread are relatively high (Bingham *et al.* 1998) because of soya flour added during the manufacturing process. Chapattis, the main staple bread for South Asians, does not have soya flour added. We were unable to conduct our own direct phytoestrogen analyses for chapattis, but the estimated phytoestrogen content calculated from the recipe (wholewheat flour, water and oil, the proportions depending upon the sub-ethnic group) suggests that this food is not a high source of phytoestrogens. We did not observe a high intake of flaxseed in any of our subjects.

No significant differences in phytoestrogen intake were observed over the seasons in the British or the migrant South Asian population. Yamomoto *et al.* (2001) similarly found no differences in seasonal intake of isoflavones in Japan, where the diet is traditionally high in soya. However, it is not possible to rule out completely seasonal variation in phytoestrogen intake as lignan values for some of the fruits, which are consumed seasonally (e.g. pears, peaches, lychees, apricots), were not available.

A unique feature of the present study is the use of multiple plasma samples to estimate habitual phytoestrogen levels. Most studies have only used single samples (Gooderham *et al.* 1996; Arai *et al.* 2000; Wang *et al.* 2000, Yamomoto *et al.* 2001), whereas Zeleniuch-Jacquotte *et al.* (1998) recommend an optimal collection of three plasma samples to accurately estimate habitual phytoestrogen intake. All subjects in the present study provided at least three plasma samples.

Table 6. Plasma levels of phytoestrogens by ethnic group (Mean values and standard deviations)

	South Asians (*n* 100)		Native British (*n* 40)		
Phytoestrogens (nmol/l)	Mean	SD	Mean	SD	*P* value†
Genistein	18·0	20·4	14·8	11·1	NS
Daidzein	8·0	10·3	11·0	11·0	NS
Enterolactone	13·9	17·5	28·5	23·3	<0·001

* For details of procedures, see p. 1151.
† Adjusted for age, height, marital status, parity, education, house ownership and smoking status.

Average concentration of plasma isoflavones was not significantly different between the South Asians and the British, despite their different intakes. The plasma levels of isoflavones were relatively low in both the South Asian and native British population compared with populations who traditionally consume soya (Arai *et al.* 2000; Yamomoto *et al.* 2001). Only one study, to our knowledge, has examined isoflavone levels in a South Asian population in the USA, and levels of urinary isoflavones were low in both the native US and the South Asian populations (Kamath *et al.* 1999). One recent study in English women (Verkasalo *et al.* 2001), which used single plasma samples, reported mean plasma levels, daidzein (arithmetic mean 8·1 nmol/l) and genistein (arithmetic mean 27·8 nmol/l) similar to those observed in the British women in the present study.

The correlations observed between plasma daidzein and genistein levels and dietary intake in the present study are similar to those found in other studies (0·34–0·42; Arai *et al.* 2000; Yamomoto *et al.* 2001). Higher correlations (0·7–0·8) between isoflavone plasma levels and intake have been observed by Verkasalo *et al.* (2001) but, as stated by the authors, this is probably a reflection of the non-random selection of the study sample.

Daidzein and genistein intake assessed using 24 h recalls was significantly higher in the native British than in the South Asians, but this difference was not reflected in plasma levels. It is possible that isoflavones added commercially to ready-made food items (such as bread and pastries), which was consumed in higher quantities in the native British population than in the South Asian, are not as biologically available or of low biological activity. It is also possible that biomarkers for isoflavones are of limited use to predict habitual intake because of their short half-life. This may be particularly so for populations who do not traditionally consume soya, where regular intake of isoflavones to obtain steady-state plasma levels may not be achieved and thus fasting blood measurements may only reflect a weak relationship with recent isoflavone intake.

Plasma enterolactone levels were significantly lower in South Asians compared with native British and this was also reflected in the pattern of intake. The difference is unlikely to be due to laboratory error as the staff were kept 'blind' to the hypothesis and the analyses were conducted in one

Table 7. Correlation between plasma levels of phytoestrogens and dietary intake*
(Mean values and standard deviations)

	Plasma levels (nmol/l)		24 h recalls (μg/d)			
	Mean	SD	Mean	SD	Spearman's correlation	*P* value
Genistein						
All (*n* 140)	17·1	18·2	225·6	638·8	0·33	0·0001
South Asian (*n* 100)	18·0	20·4	238·3	754·0	0·41	<0·0001
Native British (*n* 40)	14·8	11·1	193·8	98·3	0·24	0·1
Daidzein						
All (*n* 140)	8·8	10·5	163·8	347·0	0·36	<0·0001
South Asian (*n* 100)	8·0	10·3	158·9	407·8	0·35	0·0003
Native British (*n* 40)	11·0	11·0	176·0	84·0	0·06	0·7
Enterolactone						
All (*n* 140)	18·0	20·4	147·3	96·5	0·10	0·2
South Asian (*n* 100)	13·9	17·5	136·8	78·6	0·10	0·1
Native British (*n* 40)	28·5	23·3	173·6	128·5	0·08	0·6

* For details of procedures, see p. 1151.

single run. The plasma enterolactone levels observed in the South Asians were similar to those observed in Canadian males (Gooderham *et al.* 1996). No other study, to our knowledge, has measured plasma enterolactone levels in South Asians. Ethnic differences in enterolactone levels have also been observed in the study conducted by Horn-Ross *et al.* (1997). In fact, the mean plasma enterolactone levels observed in South Asians in the present study are one of the lowest reported in the literature to date (H. Adlercreutz, personal communication). The levels are lower than in native Finnish subjects who have been on antibiotics in the previous 12–16 months (geometric mean 16·3 nmol/l; Kilkkinen *et al.* 2002) and lower than in men who have had an acute coronary event (18·2 nmol/l; Vanharanta *et al.* 1999).

Kilkkinen *et al.* (2001) could only explain 3–14 % of the variation in plasma enterolactone in a Finnish study. Determinants of plasma enterolactone included not only lignan-containing foods but also constipation. Whereas some human subjects produce little to no enterolactone, the microflora of others have been shown to increase enterolactone produced from a standardised lignan-rich meal over a 1-week period (Nesbitt *et al.* 1999). Several researchers have proposed that differences in the concentrations of the lignan levels *in vivo* may be due to the composition of the colonic microflora (Axelson *et al.* 1982; Hutchins *et al.* 2000), differences in intestinal transit time (Axelson *et al.* 1982; Hutchins *et al.* 1995) or the redox level of the large intestine (Hutchins *et al.* 1995), all factors that may be related to the habitual diets of the subjects. Rowland *et al.* (2000) have suggested that different substrates in the diet may affect the colonic environment. However, large inter-individual variation has been noted even in subjects who have stable, long-standing habitual dietary patterns (Hutchins *et al.* 2000). It has also been suggested that the variability observed in lignan excretion in human subjects may be due to the existence of several alternate pathways for lignan metabolism (Hutchins *et al.* 2000) or the existence of mammalian lignan metabolites that are not routinely measured (Jacobs *et al.* 1999). It is also possible that lignan intake could be underestimated because there may be new enterolignan precursors, other than secoisolariciresinol and matairesinol. One recent study reported the inclusion of two new recently identified enterolignan precursors, lariciresinol and pinoresinol, which increased the overall lignan intake 3-fold (Milder *et al.* 2005).

McKeigue *et al.* (1989) have reported on marked differences in colonic metabolism in South Asians in the UK compared with the host population. Of the sixty-one South Asian subjects, thirty-eight contained detectable concentrations of unconjugated primary bile acids, whereas these compounds were undetectable in the native British subjects (*n* 36) because degradation was complete. It is possible that this altered colonic metabolism in South Asians may be related to lower plasma enterolactone levels compared with the native British population.

In summary, we found that phytoestrogen intake in South Asian and native British women was lower than 1 mg/d, and was lower among South Asians than the native British.

Acknowledgements

This study was funded by the Cancer Research Campaign (now Cancer Research UK) (grant no. SP2315).

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