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Development of a food compositional database for the estimation of dietary intake of phyto-oestrogens in a group of postmenopausal women previously treated for breast cancer and validation with urinary excretion

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Abstract

The scientific literature contains evidence suggesting that women who have been treated for breast cancer may, as a result of their diagnosis, increase their phyto-oestrogen (PE) intake. In the present paper, we describe the creation of a dietary analysis database (based on Dietplan6) for the determination of dietary intakes of specific PE (daidzein, genistein, glycitein, formononetin, biochanin A, coumestrol, matairesinol and secoisolariciresinol), in a group of women previously diagnosed and treated for postmenopausal breast cancer. The design of the database, data evaluation criteria, literature data entry for 551 foods and primary analysis by LC–MS/MS of an additional thirty-four foods for which there were no published data are described. The dietary intake of 316 women previously treated for postmenopausal breast cancer informed the identification of potential food and beverage sources of PE and the bespoke dietary analysis database was created to, ultimately, quantify their PE intake. In order that PE exposure could be comprehensively described, fifty-four of the 316 subjects completed a 24 h urine collection, and their urinary excretion results allowed for the description of exposure to include those identified as 'equol producers'.

Key words: Phyto-oestrogens: Isoflavones: Dietary analysis databases: Breast cancer

Phyto-oestrogens (PE) are bioactive plant constituents capable of inducing a wide range of oestrogenic effects in humans[1]. They have been reported to possess antioxidant activities, anti-inflammatory properties, vasodilatory effects and may help in the alleviation of menopausal symptoms[2]. There is some evidence of their effect on reducing the age-related decline in bone density[3], and in the reduction of the incidence of CVD and hormone-dependent cancers. Individual studies continue to report reduced cancer risk with increasing PE intake[4], while the conclusions from meta-analysis are more restrained, suggesting both soya and lignan intake may be associated with small reductions in breast cancer risk[5–9]. Consequently, the question of whether or not PE are beneficial or harmful to human health remains unresolved. The answer is likely to be complex and may depend on age at exposure to PE, health status and even the presence or absence of specific gut microflora. Clarity on this issue is needed because global consumption of PE is rapidly increasing[10]. Evidence indicates that women who have been treated for breast cancer may, as a result of their diagnosis, increase their PE intake, perhaps as an alternative to conventional hormone replacement therapy, or because of a belief that PE may help them avoid a recurrence of the disease[11–13]. At this stage, there is no recommended intake for PE and there are concerns about the safety of a high PE intake[14], especially in subgroups of the population such as breast cancer patients.

Abbreviations: Daid, daidzein; Equ, equol; PABA, *para*-aminobenzoic acid; PE, phyto-oestrogen; WINS (UK), Women's Intervention Nutrition Study (UK).

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In order to assess the impact of PE intake on health, a thorough understanding of the PE content of a wide range of foods present in the diet is required. In practice, assessing the impact can be achieved either by replacement of diet[15], dietary supplementation[16], direct analysis of duplicate diets[17], or indirectly through total diet studies[18], FFQ[19] and food compositional databases[20]. Compositional databases require the prior analysis of all items of food consumed by a given population and often must contain many thousands of entries[21]. Further, Internet-based resources offer a wider availability to food composition data[22]. Once a database is fully populated and its use validated against a direct analysis method, then indirect dietary intake estimation, by FFQ, is relatively inexpensive.

Several databases have been constructed containing the PE content of foods and beverages. Some have focused on selected isoflavones[23], while others have included both isoflavones and lignans[22,24–27]. An inventory of PE databases has been published, with the guidance on the selection of best-suited databases and recommendations on their suitability[17]. Estimation of PE intake in Western countries has historically been biased towards underestimations by the inadequacy of coverage of key local foods and the increasing prevalence of soya ingredients in the production of frequently consumed processed foods which are not generally recognised as PE sources. Serum PE measurement is well known to be a poor index of long-term PE intake, while 24 h urinary PE excretion estimates perform much better and are considered to be more reliable[28].

Method

Recruitment and experimental design

Between January 2000 and November 2005, a dietary change feasibility study, The Women's Intervention Nutrition Study (UK) (WINS (UK) – stage 1), recruited postmenopausal women previously treated for breast cancer. A set of two hundred and sixty-one 4 d food and drink diaries and sixteen 7 d weighed intake diaries were collected as part of the preliminary screening for eligibility for WINS (UK) – stage 1, and it was noted that subjects reported some soya-rich products in their diets. These diaries were available for re-analysis[29]. All women who had completed the WINS (UK) – stage 1 baseline screening diaries were contacted to ask for their consent to re-analyse their diaries in order to describe PE consumption patterns for this newly funded study. To allow 24 h urinalysis of PE urinary metabolites and comparisons with dietary intake estimations, fifty-five additional subjects were recruited to the PE study using the original WINS (UK) – stage 1 criteria, and summary data on screening, recruitment and participation for the PE study were compiled according to the Consolidated Standards of Reporting Trials guidance[30]. The criteria for eligibility were as follows: postmenopausal and aged 48–78 years at diagnosis with breast cancer, histologically confirmed stage I, II or IIIa breast cancer, not participating in any conflicting studies, not following a special therapeutic diet, no past history of eating disorders, able to speak, read and write English and being geographically accessible for follow-up.

24 h urine collection and urinalysis

Of the fifty-five eligible subjects, fifty-four complied with the urine collection methodology which used 3-litre containers with ascorbic acid as a preservative and PABAcheck tablets (*para*-aminobenzoic acid (PABA); Laboratories for Applied Biology Limited), according to a standard community-based 24 h urine collection protocol. The subjects took one 80 mg tablet with each of the three main meals eaten on the day of urine collection. PABA in urine was measured colorimetrically by absorbance at 540 nm, after alkaline hydrolysis and a diazo coupling reaction with nitrous acid[31] to confirm the completeness of the urine collection. A quality-control sample was run in triplicate within each analytical run. The completeness of the urine collection was assessed in terms of the percentage excretion of the total dose (240 mg) of PABA.

Database

A bespoke version of Dietplan6 (version 6.20c7, 2006; Forestfield Software Limited UK) database was created from both published and primary analysis sources to estimate PE intake. Careful consideration was given to data quality, including its origin and applicability to human dietary intake. A data quality criteria and scoring system for published values in peer-reviewed literature was compiled and used to determine

Table 1. Data quality criteria and scoring system for published literature

Criteria	Score
(1) Data published in peer-reviewed articles or data of equivalent quality	1
(2) Use of analytical reagents or better	1
(3) Use of appropriate high-quality internal standards from recognised sources	1
(4) Use of isotopically labelled standards	1
(5) Clear description of sampling procedure (use of at least three samples)	1
(6) Use of appropriate treated glassware (derivatisation) within the method	1
(7) Clear description of extraction procedure	1
(8) Use of enzymatic or acidic hydrolysis (or measurement of both glucosides and aglycones)	1
(9) Use of HPLC with positive confirmation of peaks by other means (e.g. ESI–MS)	1
(10) Use of LC–MS with appropriate identification of peaks	1
(11) Evidence of baseline chromatographic separation of analytes	1
(12) Evidence of LOD and LOQ of method	1
(13) Clear details on the recovery of the analyte (ideally greater than 60 %)	1
(14) Clear details on the calculation of results	1
(15) Clear presentation of results in terms of expression as aglycone or glucoside	1
(16) Clear presentation of results in terms of dry weight or wet weight	1
(17) Ability to quantify phyto-oestrogen content as aglycone equivalents	1
(18) Evidence of the usage of quality-control procedures during analysis	1
(19) Clear information on inter- and intra-assay variation	1
(20) Results in line with those derived by other analysts	1

ESI, electrospray ionisation; LOD, limit of detection; LOQ, limit of quantification.

the robustness of published data (Table 1). A literature search returned 171 relevant peer-reviewed articles published up to and including 31 January 2006. Of these published articles, forty-four contained duplicate information. The remaining publications (*n* 127) were assessed against the twenty data quality criteria and those scoring 13/20 or more were deemed of suitable quality for database inclusion. Information from ninety-seven papers was entered into the Dietplan6 nutrient database for each PE relevant to the present study. Data presented in the glucoside form were converted to aglycone equivalents. Multiple analytical results (e.g. soya milk) were averaged for inclusion. Of the 519 individual foods identified, 312 had existing McCance and Widdowson codes[32] that corresponded to coding used within Dietplan6; the remainder were issued with unique PE study codes. For common composite foods for which the PE content of individual ingredients was available, ingredient analysis of standard recipes was used to provide an overall PE content estimate. Foods were identified, however, that had no published PE data. In these instances, additional foods were analysed by LC–MS/MS (*n* 34; Table 2), by an established and well-validated method[16–18] scoring 20/20 in the quality criteria. These primary analysis data were then entered into the database.

Chemical analyses

Analysis of urine for PE and metabolites was conducted by LC–MS/MS[33] (Table 3). An internal standard mix (40 µl) and acetate buffer (175 µl) were combined and urine (500 µl) was added, followed by β-glucuronidase solution (10 µl). Mixtures were incubated overnight at 37°C and centrifuged before LC–MS/MS analysis of the hydrolysate on a Waters Ultima triple quadrupole mass spectrometer (Waters Corporation). Duplicate transitions were measured in negative electrospray ionisation mode for detection and confirmation of sixteen PE and their metabolites: genistein; dihydrogenistein; 6-hydroxy-*O*-desmethylangolensin; daidzein (Daid); dihydrodaidzein; equol (Equ); *O*-desmethyl angolensin; 8-hydroxydaidzein; 3-hydroxydaidzein; glycitein; desmethylglycitein; biochanin A; formononetin; coumestrol; enterolactone; enterodiol. The limit of detection was 10 µg/l of urine (about 40 nmol/l). PE metabolites were obtained from Plantech (Plantech) and ^{13}C-labelled internal standards were supplied by Dr N. Botting (University of St Andrews)[16,33].

Statistics

Descriptive statistics were used to describe subject characteristics and PE intakes. Spearman's rank correlations and

Table 2. Phyto-oestrogen content in new foods

No.	mg/kg as consumed	Genistein	Daidzein	Glycitein	Biochanin A	Formononetin	Coumestrol	Secoisolariciresinol	Matairesinol
1	Black currants	< 0·02	< 0·02	< 0·02	0·068	< 0·02	< 0·02	< 0·3	< 0·2
2	Ciabatta	< 0·07	< 0·07	< 0·07	< 0·07	< 0·07	< 0·07	< 1	< 0·7
3	Couscous	< 0·09	< 0·09	< 0·09	< 0·09	< 0·09	< 0·09	< 2	< 0·9
4	Butter croissant	< 0·08	< 0·08	< 0·08	< 0·08	< 0·08	< 0·08	< 2	< 0·8
5	Crumpet	< 0·05	< 0·05	< 0·05	< 0·05	< 0·05	< 0·05	< 1	< 0·5
6	Fruit cake	< 0·09	< 0·09	< 0·09	< 0·09	< 0·09	< 0·09	6	< 0·9
7	Ginger cake	< 0·08	< 0·08	< 0·08	< 0·08	< 0·08	< 0·08	< 2	< 0·8
8	Ginger nut biscuits	< 0·5	< 0·5	< 0·5	< 0·1	< 0·1	< 0·1	< 0·5	< 1
9	Ginger root	0·3	0·1	0·1	< 0·01	< 0·01	< 0·01	24	< 0·1
10	Sesame mochi	< 0·07	< 0·07	0·3	< 0·07	< 0·07	< 0·07	< 1	< 0·7
11	Black rice mochi	< 0·07	< 0·07	< 0·07	< 0·07	< 0·07	0·3	< 1	< 0·7
12	Soreen cake	< 0·08	< 0·08	< 0·08	< 0·08	< 0·08	< 0·08	< 2	< 0·8
13	Mange tout steamed	0·01	0·01	7	0·01	0·1	< 0·01	< 0·3	< 0·1
14	Mange tout raw	0·05	0·02	10	0·01	0·1	< 0·01	< 0·2	< 0·1
15	Victorian chutney	< 0·1	< 0·1	0·9	< 0·1	< 0·1	0·2	3	< 1
16	Marmite	< 0·1	< 0·1	< 0·1	< 0·1	< 0·1	< 0·1	< 2	< 1
17	Marzipan	< 0·09	< 0·09	< 0·09	< 0·09	< 0·09	< 0·09	< 2	< 0·9
18	Millet	< 0·09	< 0·09	< 0·09	< 0·09	< 0·09	< 0·09	< 1	< 0·9
19	Mini naan	< 0·07	< 0·07	< 0·07	< 0·07	< 0·07	< 0·07	< 1	< 0·7
20	Parsley	0·3	< 0·01	< 0·01	< 0·01	< 0·01	< 0·01	0·7	< 0·1
21	Pine nuts	< 0·1	< 0·1	< 0·1	< 0·1	< 0·1	< 0·1	< 2	< 1
22	Pumpkin seeds	< 0·1	< 0·1	< 0·1	< 0·1	< 0·1	< 0·1	< 2	< 1
23	Quorn fried	< 0·04	< 0·04	< 0·04	< 0·04	< 0·04	< 0·04	< 0·7	< 0·3
24	Quorn raw	< 0·03	< 0·03	< 0·03	< 0·03	< 0·03	< 0·03	< 0·5	< 0·3
25	Sesame oil	< 0·1	< 0·1	< 0·1	< 0·1	< 0·1	< 0·1	< 0·2	< 1
26	Burgen soya bread	149	84	26	< 0·07	< 0·07	< 0·07	< 1	< 0·7
27	Sushi	< 0·04	0·2	0·3	< 0·04	< 0·04	< 0·04	1	< 0·4
28	Vegetable sausage cooked	35	27	14	3	0·05	< 0·05	0·9	< 0·5
29	Vegetable sausage raw	27	22	11	3	0·04	< 0·04	0·8	< 0·4
30	Wheatgerm mg/l in beverages as consumed	< 0·09	< 0·09	< 0·09	< 0·09	< 0·09	0·2	0·2	< 0·9
31	Green tea	0·013	< 0·01	< 0·01	< 0·01	< 0·01	< 0·01	0·45	0·05
32	Cappuccino	< 0·01	< 0·01	< 0·01	< 0·01	< 0·01	< 0·01	< 0·01	< 0·01
33	Dandelion coffee	< 0·01	< 0·01	< 0·01	< 0·01	< 0·01	< 0·01	< 0·01	< 0·01
34	Horlicks	< 0·01	< 0·01	< 0·01	< 0·01	< 0·01	< 0·01	< 0·01	< 0·01

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Table 3. Urinary phyto-oestrogen (PE) excretion (μg/d)*

				Individual PE and metabolites (μg/d)												
				Gen and metabolite		Daid and metabolites					Gly and metabolite					
No.	PE intake from food (μg/d)	Volume (litres)	PABA†	Gen	DHG	Daid	DHD	3OH-D	Equ	ODMA	Gly	DmGly	Form	End	Enl	Total PE (μg/d)
1	15 162·4	2·82	103	<LOD‡	<LOD	90	<LOD	59	<LOD	31	<LOD	203	<LOD	68	1700	2151
2	14 184·6	2·60	94	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	52	<LOD	<LOD	486	538
3	21 756·6	1·97	88	<LOD	<LOD	92	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	324	416
4	12 428·9	1·42	94	33	30	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	112	1088	1263
5	22 101·7	1·48	95	32	<LOD	61	<LOD	<LOD	18	24	<LOD	<LOD	<LOD	56	1116	1307
6	16 835·2	2·71	97	<LOD	<LOD	371	<LOD	<LOD	27	<LOD	<LOD	<LOD	27	87	3339	3851
7	36 857·9	2·17	96	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	59	1758	1817
8	3738·2	1·26	83	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	40	<LOD	40
9	4547·1	2·67	98	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	77	77
10	8086·4	1·46	98	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
11	12 345·0	2·39	92	36	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	36
12	13 929·8	1·68	90	23	<LOD	463	<LOD	<LOD	<LOD	40	<LOD	<LOD	18	275	2304	3123
13	8343·2	2·63	102	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	203	2768	2971
14	12 597·3	2·38	94	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	274	2030	2304
15	21 428·8	3·17	109	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	95	95
16	7363·7	2·85	96	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
17	49 083·4	2·95	93	1991	121	7779	1027	56	41	1159	693	<LOD	<LOD	<LOD	<LOD	12 867
18	5115·6	2·60	102	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	333	458	791
19	71 971·0	1·84	106	<LOD	59	<LOD	322	<LOD	<LOD	103	<LOD	<LOD	<LOD	3365	9754	13 603
20	1944·2	2·80	104	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	532	532
21	10 141·1	1·95	93	5345	123	5850	289	<LOD	1361	177	396	<LOD	<LOD	<LOD	1244	14 785
22	33 575·6	3·14	81	3458	342	3141	1135	31	<LOD	339	<LOD	<LOD	<LOD	91	<LOD	8537
23	17 053·0	1·50	92	<LOD	<LOD	260	<LOD	<LOD	<LOD	53	<LOD	<LOD	<LOD	44	293	650
24	13 595·2	2·25	109	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	259	<LOD	259
25	49 094·0	1·50	112	2672	365	2231	822	<LOD	<LOD	95	18	<LOD	<LOD	135	2190	8528
26	27 534·9	2·70	97	<LOD	<LOD	<LOD	103	<LOD	27	<LOD	<LOD	57	<LOD	<LOD	378	565
27	22 339·8	3·75	89	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
28	15 017·1	2·90	103	249	<LOD	818	<LOD	<LOD	<LOD	128	<LOD	<LOD	<LOD	<LOD	452	1647
29	17 148·7	3·10	107	143	<LOD	78	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	221
30	8320·1	2·71	95	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LO,D	<LOD	73	1463	1536
31	44 739·7	2·50	99	7555	178	6328	335	48	<LOD	258	50	<LOD	<LOD	<LOD	80	14 832
32	760·1	2·49	80	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	35	1467	1502
33	13 833·8	1·56	100	555	225	1097	448	<LOD	<LOD	37	87	<LOD	<LOD	<LOD	1296	3745
34	10 410·8	2·04	91	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
35	1195·8	1·89	80	289	<LOD	818	<LOD	<LOD	<LOD	43	38	<LOD	<LOD	53	584	1825
36	6907·6	6·16	94	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	942	942
37	37 792·0	2·83	79	34	<LOD	464	<LOD	<LOD	42	45	<LOD	<LOD	<LOD	382	6523	7490
38	7192·4	3·15	99	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	491	491
Mean	18 328·2	2·472	96	590	38	788	118	5	40	67	34	8	1	156	1190	3035
SD	15 617·5	0·866	8	1610	92	1869	285	16	220	197	128	35	5	545	1914	6916

Gen, genistein; Daid, daidzein; Gly, glycitein; PABA, *para*-aminobenzoic acid; DHG, dihydrogenistein; DHD, dihydrodaidzein; 3OH-D, 3-hydroxydaidzein; Equ, equol; ODMA, *O*-desmethyl angolensin; DMGly, desmethylglycitein; Form, formononetin; End, enterodiol; Enl, enterolactone; LOD, limit of detection.

* No coumestrol, biochanin A, 6-hydroxy-*O*-desmethylangolensin or 8-hydroxydaidzein was detected in any urine sample.

† Percentage recovery of 3 × 80 mg of PABA.

‡ Less than 10 μg/l

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Bland–Altman plots were used to assess the relationship between estimated PE intakes using the 4 d diaries and urinary PE excretion results[34]. Statistical analyses were conducted using SPSS (version 12, annex 6; SPSS, Inc.).

Results

The dietary analysis database created in the present study used a more critical evaluation of the published literature than has been the case for previous databases[26] and complies with the guidance on reporting clinical studies of soya intervention[35]. The present study further benefited from the inclusion of the primary analysis of an additional thirty-four food and beverage items known to be consumed by the study population and for which PE content data were lacking. Considerable recoding of the food and beverage items listed in the diaries was required to allow the PE study data to be read alongside the macronutrient assessment data within each report from 551 separately coded foods (see the Supplementary material, available online). The process of recoding the food items was also indicated when the actual food or beverage was not explicitly listed within the database, but was deemed dietetically similar. For example, PE fraction data were available for fresh tomatoes. Giving due consideration to the fact that food processing and cooking generally reduce the PE content, through loss of water (dehydration) or boiling (aqueous extraction)[36], tinned tomatoes and grilled tomatoes were recoded accordingly, so as to represent their potential contribution to the intake of the respective PE fractions.

Recovery of 85–110 % of the PABA dose from the urine is generally accepted as complete urine collection. Values of less than 70 % indicate incomplete urine collection[37]. There was a trend ($P<0·1$) for the older women in the present study to excrete slightly less PABA in the 24 h urine collection. This is consistent with the literature, although the reduction in excretion is only small[38]. Taking this into account, the cut-off was revised to 100 ± 22 % recovery of the PABA dose; thirty-eight of the fifty-four urine collections were thus deemed adequate, with an average recovery of 95 (SD 8) %.

The urinalysis results are shown in Table 3. Biochanin A, 8-hydroxydaidzein and coumestrol were not detected in any samples and have, therefore, been excluded from the data table. The mean total PE excretion was 3·0 (SD 6·9) mg/d (n 38, <0·01–15 mg/d)

Of the fifty-four women (15 %) who provided a 24 h urine collection, eight were found to be Equ producers, based on the detection of more than 10 μg/l (41·3 nmol/l) of Equ. Of these eight women, two (4 %) excreted Equ at levels greater than 1000 nmol/d (4198 and 5618 nmol Equ/d) and could be described as good Equ producers on this basis. The remaining six excreted 74–173 nmol Equ/d and were, therefore, poor Equ producers. Of the six Equ producers, five provided complete urine samples, as confirmed by PABA analysis, hence one of the high Equ producers was excluded from subsequent analysis. Setchell & Cole[39] has designated a formula based on a nmol/l urinary $\log_{10}$ Equ:Daid ratio of $> -1·75$ as indicating Equ production, with expected distributions of 25 % in non-vegetarian adults and 59 % in vegetarian adults after a Daid challenge. This is a more intuitive approach that can better predict which subjects would be high Equ excreters given a suitable Daid challenge. Of the fifty-four subjects, twenty-two had detectable urinary Daid or Equ levels; it was necessary to enter a non-zero value (0·05 μg/l) in place of <limit of detection (10 μg/l) in order to use the formula. It can clearly be seen that seven (15 %) of the fifty-four were then classified as Equ producers (Fig. 1). As many subjects had excreted neither Equ nor Daid, this dataset only supports the hypothesis that 25 % of subjects are Equ producers in contrast to those (seven (32 %) out of twenty-two) whose urine contained isoflavones.

The Spearman's correlation coefficients measured in the present study (Tables 4 and 5) are virtually identical to the best recent examples[19], with recent diet to urine values of 0·54 (isoflavones and total PE) and 0·40 (lignans) and urinary to FFQ correlations for total isoflavones (Daid, genistein and Equ) of 0·57 (95 % CI), increasing to 0·72 for the 24 h recall[40]. Literature correlations reflecting the reproducibility of the FFQ of 0·67–0·81 and validity correlations (FFQ compared with dietary) of 0·67–0·79 with urinary validity correlations of 0·41–0·51 (isoflavones) and 0·16–0·21 (lignans) are reported[25]

Discussion

The volume of collected urine was 2·52 (SD 0·88) litres (n 54, 1·26–6·16 litres) and, while this was considerably higher than volumes measured in similar studies, it was largely unchanged

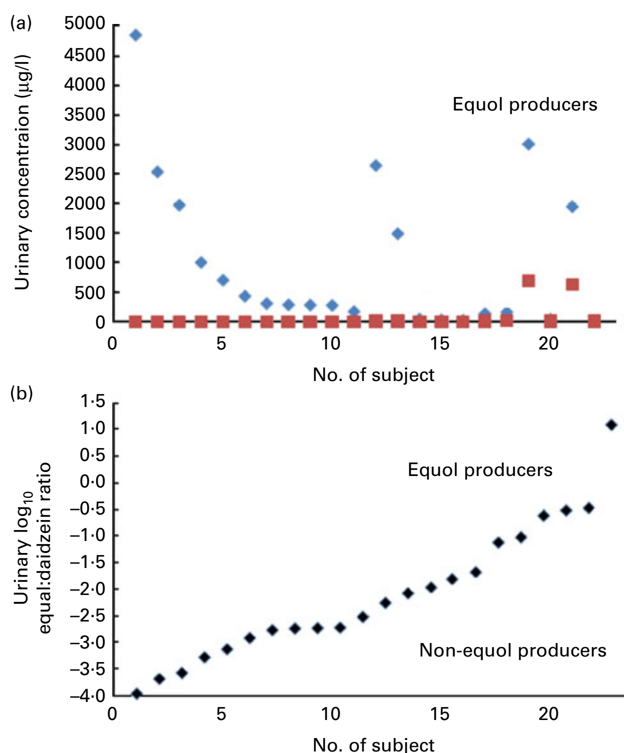

Fig. 1. Comparison of equol production status by (a) concentration and (b) $\log_{10}$ transformation of the metabolite:precursor ratios. ◆, Daidzein; ■, equol. (A colour version of this figure can be found online at http://www.journals.cambridge.org/bjn).

Table 4. Spearman's correlations between phyto-oestrogen (PE) intake (μg/1000 kcal/d) measured using 4 d diaries and 7 d weighed intake data

Dietary PE	Spearman's ρ
Daidzein	0·723**
Genistein	0·763**
Glycitein	0·714**
Formononetin	−0·070
Biochanin A	0·225
Coumesterol	0·563*
Matairesinol	0·622*
Secoisolariciresinol	0·640*
Total PE	0·749**

* $P<0·05$ ** $P<0·1$.

by the elimination of the incomplete collections (2·47 (SD 0·87) litres; *n* 38, 1·26–6·16 litres). This indicates that low collection volume was not the major factor in the low completion rate.

The urinalysis results and 7 d weighed intakes contributed to the validation of the 4 d food and drink diary as a data collection tool. Variations between subjects in terms of absorption, distribution, metabolism and excretion of PE fractions and their excretion products are an important consideration when interpreting PE urinalysis results overall. While an attempt to control for such inter-individual variations could be made by comparing averaged dietary intake over 4 d with the urinalysis results, it would not be possible to individually predict the rate at which PE fractions and their excretion products would appear in urine. This may warrant attempts to collect urine over a longer period in future studies.

Data on PE intake in other study populations have been reported in the literature[4,41–47]. A direct comparison of quantified intakes is difficult as some researchers have reported intakes of a limited number of individual PE and the dietary analysis database used to quantify intake is not always explicit.

Table 5. Spearman's correlations between estimated dietary and measured urinary phyto-oestrogen (PE) values

Dietary PE (μg/d)	Urinary PE and metabolite (μg/l)	Spearman's ρ
Daidzein	Daidzein	0·517**
Daidzein	Dihydrodaidzein	0·398**
Daidzein	3-Hydroxydaidzein	0·403**
Daidzein	O-DMA	0·534**
Daidzein	6-OH-O-DMA	0·267
Daidzein	Equol	−0·147
Daidzein	Daidzein and metabolites†	0·492**
Genistein	Genistein	0·507**
Genistein	Dihydrogenistein	0·468**
Glycitein	Glycitein	0·441**
Glycitein	Desmethylglycitein	−0·021
Matairesinol	Enterodiol	0·079
Matairesinol	Enterolactone	0·088
Secoisolariciresinol	Enterodiol	0·287
Secoisolariciresinol	Enterolactone	0·221
Matairesinol + secoisolariciresinol	Enterodiol + enterolactone	0·238
Total PE	Total phyto-oestrogens	0·450**

** $P<0·1$.
† Daidzein and metabolites = daidzein, dihydrodaidzein, 3-hydroxydaidzein, *O*-desmethylangolensin (O-DMA) and 6-hydroxy-O-DMA.

There is considerable methodological variation between these studies. In particular, the derivation of the dietary analysis database used in each study is not always clear. Given that such variation introduces error, a quantitative comparison between study populations is difficult to achieve. While it is beyond the scope of the present study to conduct any statistical comparisons between the study populations described by other researchers and the present results, a review of the published data does reinforce the need to ensure that a comprehensive and quality-assured dietary analysis database is used in such studies. The need to comprehensively analyse foodstuffs for more than just their isoflavone PE content is also evident. In general, the British are low consumers of soya[48] and the contribution of low-isoflavone foodstuffs is potentially under-represented in existing dietary analysis databases.

Thus, assessing intake and exposure should consider other PE fraction sources with known biological activity. A high intake of dietary lignans, for example, has been associated with a reduced risk of breast cancer in a large cohort study of French women, a population that does not consume a diet rich in soya products[49]. The techniques for quantifying lignan intake are not comprehensively described and the quality of the database used to analyse intake warrants review. However, these findings suggest an emerging role for dietary lignans with respect to breast cancer.

The interpretation of correlations with the urinalysis results is complicated by the known inter-individual variations in the enterohepatic circulation and the efficiency of microbial conversion of PE into other bioactive oestrogenic metabolites in the gut. Identifying 'equol producers' is important, however, in the assessment of exposure to PE, particularly in a potentially hyper-exposed subgroup of the population such as women who have been treated for breast cancer.

Conclusion

The present study provide a unique opportunity to create a bespoke dietary analysis database to measure PE consumption. It was informed by the dietary consumption patterns of women who had previously been treated for postmenopausal breast cancer and who were, as such, potential high consumers of PE compared with the general population. Quantifying true dietary intake is always challenging as the very attempts to measure it can alter actual and reported consumption. However, when validated methods for recording dietary intake are used, the methods to quantify intake rely upon a comprehensive nutrient database so that consumption can be meaningfully assessed.

Women who have been treated for breast cancer are likely to have different PE intakes which reflect differences in individual food preferences and, quite possibly, variations in the women's existing knowledge of PE themselves. The availability of a comprehensive database for reliably measuring PE intake is a valuable resource for researchers and healthcare professionals who wish to measure intake and discuss in detail the contribution of these bioactive compounds to the health of the population.

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Supplementary material

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