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 PABA 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.

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

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

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(55)(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; entero- lactone; enterodiol. The limit of detection was 10 µg/l of urine (about 40 nmol/l). PE metabolites were obtained from Plantech (Plantech) and β-C-glucuronidated internal standards were supplied by Dr N. Botting (University of St Andrews)(16,55).

### Statistics

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

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**Table 2. Phytosterol content in new foods**

<table>
<thead>
<tr>
<th>No.</th>
<th>mg/kg as consumed</th>
<th>Genistein</th>
<th>Daidzein</th>
<th>Glycitein</th>
<th>Biochanin A</th>
<th>Formononetin</th>
<th>Coumestrol</th>
<th>Secoisolariciresinol</th>
<th>Matairesinol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Black currants</td>
<td>&lt;0·02</td>
<td>&lt;0·02</td>
<td>&lt;0·02</td>
<td>0·068</td>
<td>&lt;0·02</td>
<td>&lt;0·02</td>
<td>&lt;0·3</td>
<td>&lt;0·2</td>
</tr>
<tr>
<td>2</td>
<td>Ciabatta</td>
<td>&lt;0·07</td>
<td>&lt;0·07</td>
<td>&lt;0·07</td>
<td>&lt;0·07</td>
<td>&lt;0·07</td>
<td>&lt;0·07</td>
<td>&lt;0·1</td>
<td>&lt;0·7</td>
</tr>
<tr>
<td>3</td>
<td>Couscous</td>
<td>&lt;0·09</td>
<td>&lt;0·09</td>
<td>&lt;0·09</td>
<td>&lt;0·09</td>
<td>&lt;0·09</td>
<td>&lt;0·09</td>
<td>2</td>
<td>&lt;0·9</td>
</tr>
<tr>
<td>4</td>
<td>Butter croissant</td>
<td>&lt;0·08</td>
<td>&lt;0·08</td>
<td>&lt;0·08</td>
<td>&lt;0·08</td>
<td>&lt;0·08</td>
<td>&lt;0·08</td>
<td>&lt;2</td>
<td>&lt;0·8</td>
</tr>
<tr>
<td>5</td>
<td>Crumpet</td>
<td>&lt;0·05</td>
<td>&lt;0·05</td>
<td>&lt;0·05</td>
<td>&lt;0·05</td>
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<td>&lt;1</td>
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<tr>
<td>6</td>
<td>Fruit cake</td>
<td>&lt;0·09</td>
<td>&lt;0·09</td>
<td>&lt;0·09</td>
<td>&lt;0·09</td>
<td>&lt;0·09</td>
<td>&lt;0·09</td>
<td>6</td>
<td>&lt;0·9</td>
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<tr>
<td>7</td>
<td>Ginger cake</td>
<td>&lt;0·08</td>
<td>&lt;0·08</td>
<td>&lt;0·08</td>
<td>&lt;0·08</td>
<td>&lt;0·08</td>
<td>&lt;0·08</td>
<td>&lt;2</td>
<td>&lt;0·8</td>
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<tr>
<td>8</td>
<td>Ginger nut biscuits</td>
<td>&lt;0·5</td>
<td>&lt;0·5</td>
<td>&lt;0·5</td>
<td>&lt;0·1</td>
<td>&lt;0·1</td>
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<td>&lt;0·5</td>
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</tr>
<tr>
<td>9</td>
<td>Ginger root</td>
<td>0·3</td>
<td>0·1</td>
<td>0·1</td>
<td>&lt;0·01</td>
<td>&lt;0·01</td>
<td>&lt;0·01</td>
<td>24</td>
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<tr>
<td>10</td>
<td>Sesame mochi</td>
<td>&lt;0·07</td>
<td>&lt;0·07</td>
<td>&lt;0·07</td>
<td>&lt;0·07</td>
<td>&lt;0·07</td>
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<td>&lt;1</td>
<td>&lt;0·7</td>
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<tr>
<td>11</td>
<td>Black rice mochi</td>
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<td>&lt;0·07</td>
<td>&lt;0·07</td>
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<td>&lt;0·08</td>
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<tr>
<td>13</td>
<td>Mange tout steamed</td>
<td>0·01</td>
<td>0·01</td>
<td>7</td>
<td>0·01</td>
<td>0·1</td>
<td>&lt;0·01</td>
<td>&lt;0·3</td>
<td>&lt;0·1</td>
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<tr>
<td>14</td>
<td>Mange tout raw</td>
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<td>0·02</td>
<td>0·01</td>
<td>0·01</td>
<td>0·1</td>
<td>&lt;0·01</td>
<td>&lt;0·2</td>
<td>&lt;0·1</td>
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<tr>
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<td>Victorian chutney</td>
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<td>&lt;0·1</td>
<td>&lt;0·9</td>
<td>&lt;0·1</td>
<td>&lt;0·1</td>
<td>0·2</td>
<td>3</td>
<td>&lt;1</td>
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<tr>
<td>16</td>
<td>Marmite</td>
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<td>&lt;0·1</td>
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<tr>
<td>17</td>
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<td>&lt;0·09</td>
<td>&lt;0·09</td>
<td>&lt;0·09</td>
<td>&lt;0·09</td>
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<td>&lt;2</td>
<td>&lt;0·9</td>
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<tr>
<td>18</td>
<td>Millet</td>
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<td>&lt;0·09</td>
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<tr>
<td>19</td>
<td>Mini naan</td>
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<td>&lt;0·01</td>
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<td>21</td>
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<td>&lt;0·1</td>
<td>&lt;0·1</td>
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<td>&lt;1</td>
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<td>22</td>
<td>Pumpkin seeds</td>
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<td>&lt;0·1</td>
<td>&lt;0·1</td>
<td>&lt;0·1</td>
<td>&lt;0·1</td>
<td>&lt;0·1</td>
<td>2</td>
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<tr>
<td>23</td>
<td>Quorn fried</td>
<td>&lt;0·04</td>
<td>&lt;0·04</td>
<td>&lt;0·04</td>
<td>&lt;0·04</td>
<td>&lt;0·04</td>
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<tr>
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<td>&lt;0·03</td>
<td>&lt;0·03</td>
<td>&lt;0·03</td>
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<tr>
<td>25</td>
<td>Sesame oil</td>
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<td>&lt;0·1</td>
<td>&lt;0·1</td>
<td>&lt;0·1</td>
<td>&lt;0·1</td>
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<td>&lt;1</td>
</tr>
<tr>
<td>26</td>
<td>Burgen soya bread</td>
<td>149</td>
<td>84</td>
<td>26</td>
<td>&lt;0·07</td>
<td>&lt;0·07</td>
<td>&lt;0·07</td>
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<td>&lt;0·7</td>
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<tr>
<td>27</td>
<td>Sushi</td>
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<td>0·2</td>
<td>0·3</td>
<td>&lt;0·04</td>
<td>&lt;0·04</td>
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<td>1</td>
<td>&lt;0·4</td>
</tr>
<tr>
<td>28</td>
<td>Vegetable sausage cooked</td>
<td>35</td>
<td>27</td>
<td>14</td>
<td>3</td>
<td>0·05</td>
<td>&lt;0·05</td>
<td>0·9</td>
<td>&lt;0·5</td>
</tr>
<tr>
<td>29</td>
<td>Vegetable sausage raw</td>
<td>27</td>
<td>22</td>
<td>11</td>
<td>3</td>
<td>0·04</td>
<td>&lt;0·04</td>
<td>0·8</td>
<td>&lt;0·4</td>
</tr>
<tr>
<td>30</td>
<td>Wheatgerm mg/l in beverages as consumed</td>
<td>&lt;0·09</td>
<td>&lt;0·09</td>
<td>&lt;0·09</td>
<td>&lt;0·09</td>
<td>&lt;0·09</td>
<td>&lt;0·09</td>
<td>0·2</td>
<td>&lt;0·9</td>
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<tr>
<td>31</td>
<td>Green tea</td>
<td>0·013</td>
<td>&lt;0·01</td>
<td>&lt;0·01</td>
<td>&lt;0·01</td>
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<td>&lt;0·01</td>
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<td>32</td>
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<td>&lt;0·01</td>
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<tr>
<td>33</td>
<td>Dandelion coffee</td>
<td>&lt;0·01</td>
<td>&lt;0·01</td>
<td>&lt;0·01</td>
<td>&lt;0·01</td>
<td>&lt;0·01</td>
<td>&lt;0·01</td>
<td>&lt;0·01</td>
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<tr>
<td>34</td>
<td>Horlicks</td>
<td>&lt;0·01</td>
<td>&lt;0·01</td>
<td>&lt;0·01</td>
<td>&lt;0·01</td>
<td>&lt;0·01</td>
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</tr>
</tbody>
</table>
Table 3. Urinary phyto-oestrogen (PE) excretion (µg/d)*

<table>
<thead>
<tr>
<th>No.</th>
<th>PE intake from food (µg/d)</th>
<th>Volume (litres)</th>
<th>PABA†</th>
<th>Gen and metabolite</th>
<th>Daid and metabolite</th>
<th>Gly and metabolite</th>
<th>Total PE (µg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15 162-4</td>
<td>2-82</td>
<td>103</td>
<td>&lt; LOD‡</td>
<td>&lt; LOD</td>
<td>&lt; LOD</td>
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</table>

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, desmethyglycitein; Form, formononetin; End, enterodiol; Enl, enterolactone; LOD, limit of detection.

*No coumestrol, biochanin A, 6-hydroxy-O-desmethylandolensin or 8-hydroxydaidzein was detected in any urine sample.
†Percentage recovery of 3 x 80 µg of PABA.
‡Less than 10 µg/l.
Bland–Altman plots were used to assess the relationship between estimated PE intakes using the 4 d diaries and urinary PE excretion results. 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 and complies with the guidance on reporting clinical studies of soya intervention. 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 micronutrient 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 generally reduce the PE content, through loss of water (dehydration) or boiling (aqueous extraction), 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. 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 (36). Taking this into account, the cut-off was revised to 100±22 % recovery of the PABA dose; thirty-eight of the fifty-four urine samples 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 (413 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 has designated a formula based on a nmol/l urinary log10 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, 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. 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.

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.
reported in the literature(4,41–47) . A direct comparison of quan-

twarrant attempts to collect urine over a longer period in

their excretion products would appear in urine. This may

individually predict the rate at which PE fractions and

fractions and their excretion products are an important con-

sideration 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 quant-

tified 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.

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 stat-

istical 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 com-

prehensive 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 soy(48)

and the contribution of low-isoflavone foodstuffs is possibly

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 soy 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 con-

version 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 poten-

tially hyper-exposed subgroup of the population such as

women who have been treated for breast cancer.

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

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<thead>
<tr>
<th>Dietary PE</th>
<th>Spearman’s ρ</th>
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<tr>
<td>Daidzein</td>
<td>0.723**</td>
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<td>Genistein</td>
<td>0.763**</td>
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<td>Glycitein</td>
<td>0.714**</td>
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<td>Formononetin</td>
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<td>Biochanin A</td>
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<td>Coumestrol</td>
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<tr>
<td>Matairesinol</td>
<td>0.622*</td>
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<tr>
<td>Secoisolariciresinol</td>
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<td>Total PE</td>
<td>0.749**</td>
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</table>

* P<0.05 ** P<0.1

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

<table>
<thead>
<tr>
<th>Dietary PE (μg/d)</th>
<th>Urinary PE and metabolite (μg/l)</th>
<th>Spearman’s ρ</th>
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<td>Daidzein</td>
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<td>3-Hydroxydaidzein</td>
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<td>O-DMA</td>
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<td>Equol</td>
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<tr>
<td>Daidzein</td>
<td>Daidzein and metabolites†</td>
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<td>Genistein</td>
<td>Genistein</td>
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<td>Dihydrogenistein</td>
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<td>Total PE</td>
<td>Total phyto-oestrogens</td>
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** P<0.1
† Daidzein and metabolites = daidzein, dihydrodaidzein, 3-hydroxydaidzein, O-desmethylangolensin (O-DMA) and 6-hydroxy-O-DMA.

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 consider-

ation 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.

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.
Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S0007114512004394

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References


