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A newly constructed and validated isoflavone database for the assessment of total genistein and daidzein intake

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The principal phyto-oestrogens (PO) in food are isoflavones, lignans, coumestans and prenylated flavonoids, with isoflavones and lignans being the most commonly found in UK diets. Until recently obtaining accurate data on the PO content of foods was hampered by lack of suitable analytical methods and validation techniques. Furthermore, although PO data exist for some foods, these foods may not be available in the UK. The aim of the present study was to construct a new, comprehensive isoflavone (total genistein + daidzein) database. Using data, mainly from recent GC–MS analysis, for approximately 300 foods available in the UK, and extensive recipe calculations, a new database was constructed containing approximately 6000 foods allocated an isoflavone value. By analysing 7 d weighed food diaries, the database was subsequently used to estimate isoflavone intake in two groups of healthy volunteers, omnivores (*n* 9) and vegetarians (*n* 10). Mean isoflavone intake in the vegetarian and omnivorous group was 7·4 (SEM 3·05) and 1·2 (SEM 0·43) mg/d, respectively. Mean intake for the total group was 4·5 (SEM 1·89) mg/d. Main food sources of isoflavones for the vegetarian group were soya milk (plain), meat-substitute foods containing textured vegetable protein and soya protein isolate, soya mince, wholemeal bread and rolls, white bread and rolls, croissants and pitta breads, beans, raisins and soya sauce. Main food sources of isoflavones for the omnivorous group were soya yogurts, wholemeal bread and rolls, white bread and rolls, garlic bread, nan bread and brown bread, sultanas and scones.

Isoflavone database: Recipe calculations: Dietary intake

There is increasing interest in phytochemicals and their role in disease prevention. Phytochemicals include dietary phyto-oestrogens (PO) such as the isoflavones genistein and daidzein, present in fruits, nuts, peas and beans, lentils, chickpeas and soya (Setchell *et al.* 1987; Franke *et al.* 1995; Mazur *et al.* 1996; Reinli & Block, 1996; Liggins *et al.* 1998, 2000*a*,*b*, 2002; Mazur & Adlercreutz, 1998), lignans, which are present in cereals, beans, peas, tea (black and green), wine and strawberries (Thompson *et al.* 1991; Obermeyer *et al.* 1995; Adlercreutz & Mazur, 1997; Nesbitt & Thompson, 1997; Bingham *et al.* 1998; Mazur & Adlercreutz, 1998, Mazur *et al.* 1998*a*,*b*, 2000; Glitso *et al.* 2000) and prenylated flavones found in hops (Rong *et al.* 2000).

Soya is recognised as the major dietary source of PO (Bingham *et al.* 1998; Mazur & Adlercreutz, 1998, 2000; United States Department of Agriculture & Agricultural Research Service, 2004) and soya-based products have been shown to contain significant quantities of the isoflavones genistein and daidzein (Murphy *et al.* 1999; Pillow *et al.* 1999). Since genistein and daidzein are the most prevalent dietary isoflavones, values for their amounts in foods were used in the construction of the new isoflavone database.

There are a number of factors which can affect the isoflavone concentration in plants such as plant species and strain, crop year and geographical location (Franke *et al.* 1995). Processing can also affect isoflavone concentrations (Wang *et al.* 1990).

Although several databases have been constructed containing the isoflavone content of foods (Pillow *et al.* 1999; Horn-Ross *et al.* 2000; United States Department of Agriculture & Agricultural Research Service, 2004; Vegetal Estrogens in Nutrition and Skeleton (VENUS), 2005), there is no current isoflavone database comprehensive enough to be used in epidemiological studies in the UK which assess the dietary impact of isoflavones on the health of a population.

The new isoflavone database has been constructed using a combination of techniques including results from previous analyses (Knight *et al.* 1998; Mazur & Adlercreutz 1998; Pillow *et al.* 1999; Horn-Ross *et al.* 2000), more recent analyses (Liggins *et al.* 1998, 2000*a*,*b*, 2002), recipe calculations and

Abbreviations: EI, energy intake; FC, food code; M&W, McCance & Widdowson; PO, phyto-oestrogen; VENUS, Vegetal Estrogens in Nutrition and Skeleton.
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nutrient information obtained from several major supermarket chains in Tayside, UK, including Sainsburys, Asda, Tesco, Morrisons, Iceland and Farmfoods. Foods from supermarkets were sampled and ingredient lists and variations in ingredient lists were checked by telephone calls to supermarket head offices and stores in other areas within the UK. Nutrient information was also obtained from UK food manufacturers relating to over 1600 bakery and frozen products.

Methods

Initially the sum of genistein and daidzein values were allocated to a range of foods on the database, many of which were identified from over 1000 food diaries, 24 h dietary recalls and food-frequency questionnaires obtained during several studies over a 4-year period (Ritchie *et al.* 2004*a*,*b*,*c*; Heald *et al.* 2005).

A database should be constructed with, as far as possible, one method of analysis being used throughout (Ziegler, 2001) and the analysis should be recent, reliable and sensitive (Ovaskainen *et al.* 1996; Ziegler, 2001). For these reasons, values of genistein and daidzein used in construction of the UK isoflavone database were mainly obtained from recent analyses carried out by Liggins *et al.* (1998, 2000*a*,*b*, 2002) using GC–MS. Although some HPLC values for the isoflavone content of foods were used in the database construction, HPLC analysis of foods was not considered sensitive, reliable and accurate enough to be used with great confidence. For example, in Table 1, the results for analyses performed by researchers (Adlercreutz & Mazur, 1997; Mazur & Adlercreutz, 1998; Liggins *et al.* 2002) largely concur; however, those for green split peas and mung bean sprouts differ considerably from values published by Franke *et al.* (1995). The database also included brand names and recipe dishes (Ovaskainen *et al.* 1996).

Construction of new isoflavone database

Since genistein and daidzein are the principal isoflavones in the diet (Pillow *et al.* 1999), comprising more than 90 % of the intake of oestrogenic isoflavones, the sum of levels of these compounds in foods was allocated to corresponding foods in the database. In order to distinguish values to be used for the database construction, all the isoflavone data gathered from research papers underwent quality assurance (Ovaskainen *et al.* 1996; Ziegler, 2001) involving a number of steps.

For foods analysed, did the author specify the following?

- type of food and country of origin;
- McCance & Widdowson (M&W) code;
- if food was raw or cooked;
- if the results were for dry weight or wet weight (as is);
- the type of internal standard used;
- method of analysis (including CV quoted, multiple sample analyses for accuracy, adequate level of detection).

It was essential that all these criteria were fulfilled in order for results to be considered for inclusion in the database (exceptions were ‘new user foods’ without M&W codes).

Table 2 is an example of the quality assurance carried out.

Comparison of genistein and daidzein concentrations in cereals by different methods of analysis was also performed and values are presented in Table 3.

Recipe calculations

All isoflavone values quoted represent the sum of genistein + daidzein and are in μg per 100 g for wet weight.

The same mathematical formula was used for calculating isoflavone content of bakery products involving the ratio of protein content of the required bakery product. For example, brown rolls crusty (A)/protein content of the same bread

Table 1. Comparison of genistein and daidzein values obtained by different studies for selected vegetables (adapted from Liggins *et al.* 2000*b*)

Food	Study	Daidzein (μg per 100 g)	Genistein (μg per 100 g)
Soyabeans boiled*	Liggins *et al.* (2002)	15 000	32 000
Soyabeans boiled*	Mazur *et al.* (1998*a*)	20 000	32 000
Miso*	Liggins *et al.* (2002)	59 000	67 000
Miso*	Adlercreutz & Mazur (1997)	19 000	31 000
Bean sprouts (mung)*	Liggins *et al.* (2002)	39 000	6800
Bean sprouts (mung)†	Adlercreutz & Mazur (1997)	800	1900
Bean sprouts (mung)†	Franke *et al.* (1995)	nd	nd
Broad beans*	Liggins *et al.* (2002)	7	6
Broad beans†	Mazur & Adlercreutz (1998)	24	tr
Broccoli, calabrese*	Liggins *et al.* (2002)	nd	nd
Broccoli, calabrese†	Adlercreutz & Mazur (1997)	5	7
Carrots*	Liggins *et al.* (2002)	nd	nd
Carrots†	Adlercreutz & Mazur (1997)	2	2
Cranberry*	Liggins *et al.* (2002)	5	21
Cranberry†	Adlercreutz & Mazur (1997)	0	0
Green split peas*	Liggins *et al.* (2002)	13	35
Green split peas†	Mazur *et al.* (1998*a*)	8	0
Green split peas†	Franke *et al.* (1995)	7300	nd
Mushrooms*	Liggins *et al.* (2002)	1	21
Mushrooms†	Adlercreutz & Mazur (1997)	20	12

nd, not detected; tr, isoflavone identified but could not be quantified.
* Concentrations expressed on a wet-weight basis.
† Concentrations expressed on a dry-weight basis.

Table 2. Example of table used for carrying out quality assurance of data

Food	Type	Raw or cooked, ± skin	Dry or wet weight	Standard	Reference	Method	Genistein (µg per 100 g)	Daidzein (µg per 100 g)	Sec (µg per 100 g)	Biochanin A (µg per 100 g)
Chickpeas	ns	ns	DW	Synthetic ^{2}H-labelled IS	Adlercreutz & Mazur (1997) (unpublished results)	GC–MS	76·3	11·4	8·4	
Chickpeas	Garbanzo	Raw	FD	Internal stock solution	Franke *et al.* (1995)	HPLC				1520
Chickpeas	Jackrabbit	ns	DW	Synthetic ^{2}H-labelled IS	Mazur & Adlercreutz (1998*a*)	ID GC–MS - SIM	18·8	10·4		
Chickpeas	Whole dried	Raw	FD	Authentic synthetic standards	Liggins *et al.* (1998)	GC–MS - SIM	76·6	47·5		
Chickpeas*	Whole dried	Cooked	FD	Authentic synthetic standards	Liggins *et al.* (1998)	GC–MS - SIM	24·1	nd		

Sec, secoisolariciresinol; ns, not specified; DW, dry weight; IS, internal standard; FD, freeze-dried; ID, isotope dilution; SIM, single ion monitoring; nd, not detected.
* These values were used since chickpeas are eaten cooked, wet (as is) weight is specified and method of analysis is reliable.

type, for example, brown bread (B) × isoflavone concentration of bread type, as measured by Liggins *et al.* (2000*a*,*b*, 2002). It was assumed that isoflavone content in bakery products is proportional to protein content. See Table 4 and Appendix 1.

Calculations (Appendix 1) for estimation of isoflavone content of composite dishes were carried out in a similar manner to those used for beef sausages (Table 5), fish pie and carrot and bean salad dishes.

Three brand-name soya milks were analysed for total genistein + daidzein content (by M. S. M.) using GC–MS. These foods were entered onto the database as 'new user' foods (see Table 6).

The isoflavone content of sweetened soya milk (M&W food code (FC) 12 043) = (A + B)/2 µg per 100 g;

So, the estimated isoflavone content of sweetened soya milk = 8155 µg per 100 g.

The isoflavone content of plain soya milk was calculated by averaging isoflavone values for soya milk (published and unpublished values). The values used are listed in Table 7.

Isoflavone (genistein + daidzein) estimated values for foods

These were obtained in two ways:

- by calculation using a recipe;
- by comparison with isoflavone values for similar foods which had been analysed.

For example, the isoflavone content of raisins (M&W FC 14 242) = 183·6 µg per 100 g (A);

The isoflavone content of currants (M&W FC 14 074) = 224·5 µg per 100 g (B);

So, the estimated isoflavone content of sultanas = content (A + B)/2 = 204·1 µg per 100 g;

The isoflavone content of database addition (M&W FC 14 263) = 204·1 µg per 100 g.

Further examples of estimated isoflavone values and corresponding foods used in allocation of isoflavone levels are listed in Table 8.

Isoflavone (genistein + daidzein) for 'second generation' soya-containing foods

Several foods containing soya extract which were consumed by volunteers did not appear on the database (i.e. no M&W FC) and subsequently had to be added. Furthermore, there was no reference to their isoflavone content in the literature. Isoflavone content of the food item was estimated using nutrient information present on food wrappers detailing the percentage composition. A 'new user food' code was also designated. An example of this is given in Appendix 2.

Assessment of dietary isoflavone intake using the database: rationale

Once constructed, it was necessary to test the database. The validity of the database was tested using the method of duplicate diet analysis (Ritchie *et al.* 2004*a*). The accuracy of the database was tested by using it to assess dietary isoflavone intake in healthy volunteers consuming a range of intakes (vegetarians and omnivores), i.e. two groups, with different anticipated isoflavone intakes.

Table 3. Comparison of genistein and daidzein concentrations (μg per100 g) obtained by different studies for selected cereals (adapted from Liggins *et al.* 2002)

Food	Study	Daidzein (μg per 100 g)	Genistein (μg per 100 g)
Bread, white	Liggins *et al.* (2002)	135·6	157·2
Bread, white	Horn-Ross *et al.* (2000)	606·0	
Bread, wholegrain	Liggins *et al.* (2002)	373·1	456·7
Bread, wholegrain	Horn-Ross *et al.* (2000)	155·8	141·8
Oatmeal	Liggins *et al.* (2002)	nd	nd
Oatmeal	Horn-Ross *et al.* (2000)	0 or trace	0 or trace
Oatmeal	Adlercreutz & Mazur (1997)	3·5	6·0

nd, not detected.

Table 4. Isoflavone (genistein (G) plus daidzein (D)) values for other bakery products

Food	M&W food code	Protein (%)	Total G + D content (μg per100 g)
White rolls, soft	11124	9·2	320·7
Brown rolls, crusty	11118	10·3	635·7
Brown rolls, soft	11119	10·0	624·5
Wholemeal, rolls	11125	9·0	811·8
Croissants	11120	8·3	289·3
Chapatis made with fat	R11074	8·1	282·3
Chapatis made minus fat	46	7·3	254·5
Nan bread	11086	8·9	310·2
Pitta bread	11090	9·2	310·7
Rye bread*	48	–	33

M&W, McCance & Widdowson.
* Assumes isoflavone value same as that for granary bread (Liggins *et al.* 2002).

Table 5. Genistein (G) plus daidzein (D) values for other meat products

Food	M&W food code	TVP (%)	Potato (%)	Total G + D content (μg per 100 g)
Beef sausages, 'Premium'	19 095	0·85		580
Frankfurters	19 100	0·99		676
Beefburgers, fried	19 029	0·75		512
Beefburgers, low-fat	19 037	0·75		512
Bridie or scotch pie	19 053	0·98		253*
Cornish pastie	19 056	0·98		253*
Fishcakes	16 281		7·2	0·2

M&W, McCance & Widdowson; TVP, textured vegetable protein.
* Assume G + D are found in meat only, which is 40 % of food.

Isoflavone intake in a group of healthy volunteers consuming a vegetarian or an omnivorous diet

The aim of the present study was to determine usual isoflavone intake in healthy vegetarians and omnivores living in the Dundee area using a 7 d weighed food diary.

Study design and methods

Nineteen healthy volunteers (age range 19–76 years), seventeen female and two male were recruited by advertisement (poster) in Ninewells Hospital and Dundee University. The group consisted of nine omnivores, eight females, one male,

Table 6. Phyto-oestrogen (genistein and daidzein) values for branded soya milks (MS Morton, unpublished results)*

Brands of soya milk	Daidzein (μg per 100 g)	Genistein (μg per 100 g)	New user
'So good' (A)	1530	4270	MR19
Tesco sweetened soya milk (B)	2720	7790	MR17
Tesco unsweetened soya milk (C)	5040	12 800	MR18

* Analysis was carried out according to the method of Pumford *et al.* (2002) and Ritchie *et al.* (2004*a*).

Table 7. Isoflavone (total genistein (G) plus daidzein (D)) values used for estimating the content of plain soya milk*

Author	G + D content (μg per 100 g)
Morton *et al.* (2002) (Morton MS, unpublished results)	17 840
Pumford *et al.* (2002)	14 600
Wiseman *et al.* (2002)	10 800
Wiseman *et al.* (2002)	12 600
Horn-Ross *et al.* (2000)	11 322
USDA–IUS database‡	4710†
USDA–IUS database‡	10 501

USDA, United States Department of Agriculture; ISU, Iowa State University.
* Plain soya milk; McCance & Widdowson food code 12 042; mean phyto-oestrogen content 11 800 μg per 100 g.
† Iced soya milk.
‡ United States Department of Agriculture & Agricultural Research Service (2004).

mean age 40 (range 19–76) years and ten vegetarians, nine females, one male, mean age 36 (range 21–56) years. Ethical approval was granted for the study and signed consent was obtained from each subject.

Subject weight and height were recorded at the start and end of the 1 week of weighed food recording (Bingham *et al.* 1988). BMI was calculated for each subject and BMR was calculated using Schofield equations (Schofield *et al.* 1985). Energy intake (EI) from the food diaries (using 'Microdiet'; Downlee Systems Ltd., Downlee Lodge SK23 9UB, UK) was used to calculate the EI:BMR ratio for each volunteer. EI:BMR had to be greater than or equal to 1·2 to indicate subject compliance. Dietary composition was calculated using 'Microdiet' and individual and mean group isoflavone intakes were estimated using Excel (Microsoft Corporation, Redwood, WA, USA) and SPSS (SPSS Inc., Chicago, IL, USA) statistical analysis programs.

Isoflavone intake in the vegetarian and the omnivorous groups were recorded (Table 9) and compared by Student's *t* test. The percentage contribution for each of the major PO-containing foods to mean intake of each group, i.e. vegetarian or omnivore, was calculated and foods were listed in order of greatest dietary isoflavone contributor to lowest contributor (Table 10).

Results

A database containing isoflavone (total genistein + daidzein) concentrations in μg per 100 g allocated to approximately 6000 foods was constructed and placed on the website at http://medicine.st-and.ac.uk/research/docs/ritchie/. To access the database the user name and password are gentian and violet respectively.

Table 9. Mean daily dietary genistein (G) plus daidzein (D) intake for vegetarian and omnivorous groups and total volunteer group using 7 d diaries and newly constructed isoflavone database

	G + D intake (mg/d)			
Vegetarian or omnivore*	Mean	Range	SEM	*n*
Vegetarian	7·4	0·04–24·9	3·05	10
Omnivore	1·2	0·2–3·5	0·43	9
Total	4·5	0·04–24·91	1·89	19

* Vegetarian *v.* omnivore, $P=0.07$.

Where a value has been derived from a database containing genistein and daidzein values obtained by a variety of methods (such as in the United States Department of Agriculture database), the reference reflects this. Hence, if HPLC and GC–MS were used, this is represented as GC–MS/HPLC. Food reference codes are based on *McCance & Widdowson's The Composition of Foods*, 4th ed. (Paul & Southgate, 1978) and 5th ed. (Royal Society of Chemistry, 1991), together with the 1980 and 1985 supplements.

The following symbols are used:
N = currently no known value (from recent analysis)
e = estimated value (usually from a similar food where genistein + daidzein concentrations have been measured)
c = calculated value
r = recipe used
c/r = value calculated using a recipe
m = measured value
T = source of value and rationale for using it are outlined (Ritchie, 2003)

Subject compliance

Mean subject weight (kg) at the start and end of the recording period was 67·1 (range 46·5–117) and 67·1 (range 46·6–116·0), which indicated good compliance regarding usual food intake.

Individual isoflavone intakes are represented graphically in Fig. 1.

Table 8. Estimated genistein (G) plus daidzein (D) values for foods not analysed (FN) compared with similar foods which have been analysed (FA) (Liggins *et al.* 1998)

FA for G + D	Measured G + D content (μg per100 g)	FN for G + D	Estimated G + D content (μg per100 g)
Readybrek	0	Porridge with milk	0
Chickpeas, cooked	24·1	Chickpeas, tinned	24·1
Lentils red, split	0·5	Lentils, green and brown	0·5
Carrots, canned	0	Carrots, new, boiled	0
Broccoli, purple	1·8	Broccoli, green	1·8
Tomatoes, raw	3·25	Tomatoes, cherry	3·25
Peppers, green	0	Peppers, red	0
Fruit cocktail in syrup	1·54	Fruit cocktail in juice	1·54
Oranges	0	Grapefruit	0

Table 10. Main food sources of isoflavones (genistein (G) plus daidzein (D)) for each group and percentage contribution to daily average

Vegetarians		Omnivores	
Food	Percentage contribution	Food	Percentage contribution
Soya milk	82	Soya yogurts	41·2
Not Bacon®	6	Wholemeal bread	30·4
Soya mince	5·1	White bread	11
Wholemeal bread	2·7	Wholemeal toast	6·9
White bread	1·9	White toast	3
White toast	0·9	Sultanas	0·9
Wholemeal toast	0·2	Nan bread	0·8
Croissants	0·2	Garlic bread	0·7
Pitta bread	0·1	Muffins	0·2
Biscuits	0·09	Brown bread	0·1
Beans, green and kidney	0·03	Scones	0·1
Raisins	0·03	Pitta bread	0·1
Soya sauce	0·02	Shredded wheat	0·1

Main sources of dietary isoflavones and intake (mg/d) foreach group

The main food sources of isoflavones and subject intakes from these sources (mg/d over the 7 d recording) for the vegetarian group were soya milk (plain) (24·5–15·3), soya mince (3·8), meat-substitute foods containing textured vegetable protein and soya protein isolate (0·4), wholemeal bread and rolls (0·3), white bread and rolls (0·4), bread varieties such as croissants (0·2) and pitta breads (<0·1), beans (<0·1), raisins (<0·1) and soya sauce (<0·01).

The main food sources of isoflavones and subject intakes from these sources (mg/d over the 7d recording) for the omnivorous group were soya yogurts (2·8), wholemeal bread (0·3) and rolls (0·2), white bread (0·3) and rolls (0·2), varieties of bread such as garlic bread (0·1), nan bread (<0·1) and brown bread (0·2), sultanas (<0·1) and scones (<0·1).

Discussion

Assessment of dietary intake of specific nutrients or non-nutrients tends to be based on information obtained from food diaries, dietary recall or food-frequency questionnaires, all of which have to be analysed using a database containing appropriate values for foods consumed. Although dietary intake of isoflavones is correlated with intake of soya and soya-based foods (Verkasalo *et al.* 2001), a number of studies report apparently low dietary isoflavone intakes in Western diets (Jones *et al.* 1989; Verkasalo *et al.* 2001) due to the lack of genistein plus daidzein values for non-soya-based foods or the restricted range of non-soya-based foods included in the dietary assessment (Kirk *et al.* 1999). Examples of non-soya-based foods that contain genistein and daidzein are processed foods, bakery products and composite foods such as salads (containing raisins, rice, chickpeas, haricot beans, butter beans and beansprouts), desserts and mixed vegetable dishes.

The analysis required, of the extensive range of foods consumed in the UK, in order to create a fully comprehensive isoflavone database for use in epidemiological studies would not be feasible in terms of time and costs.

The current database was originally developed for the analysis of food-frequency questionnaires used in a study

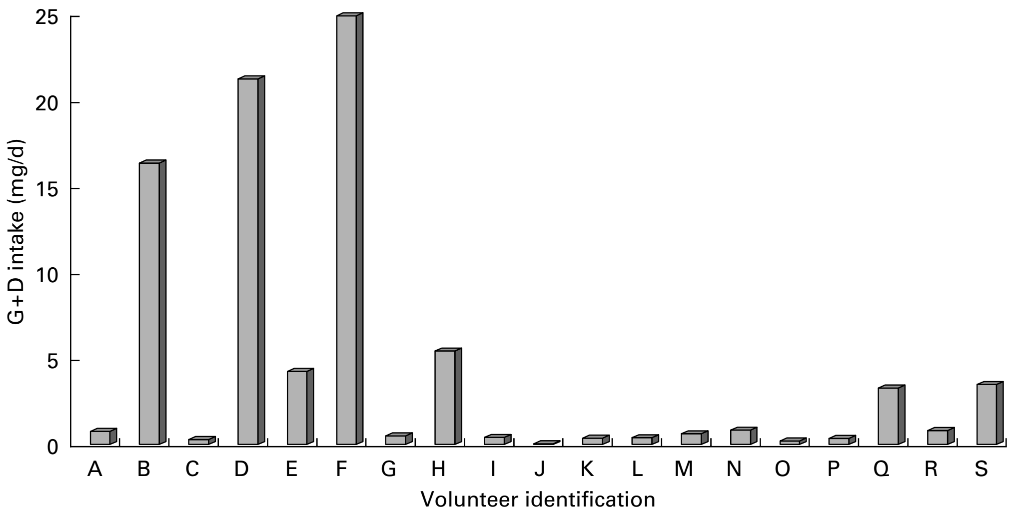

Fig. 1. Individual dietary isoflavone (genistein (G) plus daidzein (D)) intake using 7 d weighed food diaries and isoflavone database. Volunteers A to J were vegetarians; volunteers K to S were omnivores.

investigating isoflavone and prostate cancer risk (Food Standards Agency project number FS2069). It was assumed that this more comprehensive database would provide more accurate information about actual isoflavone intake across a range of intakes and, in particular, in populations not consuming a soya-based diet.

Other methods for assessing total isoflavone intake per d were employed by Clarke and colleagues (Clarke *et al.* 2003, 2004; Clarke & Lloyd, 2004). The researchers analysed vegetarian duplicate diets and food groups from the 1998 Total Diet Survey for isoflavones. There was considerable agreement between the two methods (isoflavone database and food group analysis) used for assessing the contribution on non-soya-based food groups to isoflavone intake. Clarke *et al.* (2004) report the isoflavone contribution of each food group to isoflavone intake from highest to lowest as bread, processed meat, fish products, cereals, fruit products and nuts, which is largely in agreement with the order indicated in the new database.

Comparison with other databases

The isoflavone databases produced by the United States Department of Agriculture and Iowa State University (United States Department of Agriculture & Agricultural Research Service, 2004) and Pillow *et al.* (1999) contain foods eaten in America but not obtainable in the UK, for example, 'Bacon bits', 'Baco's', 'Arrowhead Mills multigrain corn bread', etc. Furthermore the isoflavone content of breads (white bread and wholegrain) in the database produced by Horn-Ross *et al.* (2000*a*) differ considerably from the values obtained by Liggins *et al.* (2002). This is probably because soya flour is added to bread (Liggins *et al.* 2002) and different countries use different amounts in their bakery products. For this reason, isoflavone values assigned by Horn-Ross *et al.* (2000*a*) to bakery products were not used in the database for foods eaten in the UK. The VENUS database contains isoflavone values for foods analysed using a variety of methods, internal standards and methods of detection. The lack of consistency in methods of analysis and restricted number of foods limit the use of this database in epidemiological studies. The advantages and strengths of the newly constructed database are due to the large number of UK foods which have been used in its construction. Many PO values are obtained from the GC–MS analysis, which is both reliable and recent. In addition, many values were derived from recipe calculations relating to the extensive range of foods sampled during the database construction. Furthermore, the database includes a range of bakery products such as toast, muffins, croissants, Chelsea buns, pitta bread and nan bread, a range of salads such as bean salad, Florida salad, potato salad, and coleslaw and a range of combined dishes such as fruit pie and wholemeal pancakes stuffed with vegetables. As such it is the first comprehensive isoflavone database to be constructed for use in UK-based studies assessing isoflavone intake.

Dietary intake of isoflavones

Analysis of food diaries using the database estimated a dietary isoflavone intake of 1·2 (range 0·2–3·5) mg/d for the omnivorous group and a mean dietary intake for the total group of 4·45 mg/d. This is higher than the value of <1 mg/d quoted by Jones *et al.* (1989), as a result of HPLC analysis of diet samples obtained during the 1987 British Total Diet Survey.

Since 1989, the addition of soya products to food items in supermarkets has increased and it is probable that the Total Diet Survey underestimates the mean intake of isoflavones in the UK. Horn-Ross *et al.* (2000) assessed the mean isoflavone intake in 447 non-Asian women (age 50–79 years) in the San Francisco Bay area, USA, using a modified food-frequency questionnaire and a newly constructed database. Mean intakes of genistein and daidzein were estimated as 1·5 and 1·3 mg/d, respectively, resulting in a total isoflavone intake of genistein plus daidzein equal to 2·8 mg/d.

This value is similar to that of 3 mg/d proposed by Clarke *et al.* (2003) based on analysis of samples obtained from the 1998 UK Total Diet Survey. This intake was obtained exclusively from bread and processed meats. It is also in agreement with 3·5 mg as being the upper range of low isoflavone intakes reported in the present study. During validation of the database Ritchie *et al.* (2004*a*) indicated that, at low isoflavone intakes, the database underestimates intake by 1·2–2·2 mg. By adding this to the mean intake of 1·2 mg/d, estimated using the database, isoflavone intake at low intakes (mainly due to breads) agrees with the value of 3 mg quoted by Clarke *et al.* (2003).

Using the newly constructed database, a mean vegetarian isoflavone intake of 7·4 mg/d was estimated. This was expected since vegetarian diets were assumed to contain more plant-based foods and possibly more food items consisting of meat substitutes and likely to contain soya.

Mean daily intakes of 10·5 mg/d were measured in thirty-five duplicate vegetarian diets by Clarke *et al.* (2003) using LC-MS. Reasons for the difference between the estimated mean isoflavone intake for the vegetarian group in the present study and the measured intake may include the small sample size in the present study. In addition, one vegetarian in the present study did not eat bread and wheat products. These foods are major sources of PO in non-Asian diets (Horn-Ross *et al.* 2000).

The main food sources of isoflavones for each group in the present study were soya milks and yogurts, soya and textured vegetable protein-based foods, breads, dried fruit. This is in agreement with work carried out by Horn-Ross *et al.* (2000*b*) who found that tofu, doughnuts, soya milk and bread were primary sources of PO for post-menopausal women in the USA.

The standard errors of the mean associated with estimated isoflavone intake were high (Table 9) due to the small sample size and large range of estimated intakes. However, the aim of the present study was to construct a new, more comprehensive isoflavone database and test it, both to find out the number of foods requiring isoflavone values and how the estimated intakes compared with published values. For the present study 5570 foods on the database were initially assigned an isoflavone value, of which 4887 occur as distinct foods. A total of 4852 foods are allocated a value of zero isoflavone due to estimates, measured values or isoflavone content unknown.

By adding estimated values, the final database contains 5951 foods assigned an isoflavone value.

There is good correlation (r 0·98) between estimated isoflavone intake using the database and measured isoflavone intake from duplicate diet analysis (Ritchie *et al.* 2004*a*). Hence, results of duplicate diet analysis demonstrate the ability of the database to assess accurately dietary intakes of isoflavones.

This new database makes a valuable contribution to the requirement for a comprehensive isoflavone database available for use in epidemiological studies assessing the effect of isoflavone intake on health. As more information on the isoflavone content of foods becomes available, the database can be updated and added to. The construction of the new database and its availability, however, provides the initial step for future studies involving a more accurate assessment of dietary intakes of phytochemicals and their effects on health and disease prevention.

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Appendix 1. Isoflavone (genistein plus daidzein) content for bakery products

White bread, toasted; food code 11106

Genistein plus daidzein (G + D) content of white bread (WB) is 292·8 μg per 100 g.

So, G + D content of WB, toasted = (% protein WB, toasted × 292·8)/% protein WB

$$= \frac{9{\cdot}3 \times 292{\cdot}8}{8{\cdot}4} = 352{\cdot}2\ \mu\text{g per } 100\ \text{g}.$$

Brown bread, toasted; food code 11073

G + D content of brown bread (BB) is 524·6 μg per 100 g.

So, G + D content of BB, toasted = (% protein BB, toasted × 524·6)/% protein BB

$$= \frac{10{\cdot}4 \times 524{\cdot}6}{8{\cdot}5} = 641{\cdot}9\ \mu\text{g per } 100\ \text{g}.$$

Wholemeal bread, toasted; food code 11117

G + D content of wholemeal bread (WHB) is 829·8 μg per 100 g.

So, G + D content of WHB, toasted = (% protein WHB, toasted × 829·8)/% protein WHB

$$= \frac{10{\cdot}8 \times 829{\cdot}8}{9{\cdot}2} = 974{\cdot}1\ \mu\text{g per } 100\ \text{g}.$$

White rolls, crusty; food code 11123

G + D content of white rolls, crusty (WRC) is 292·8 μg per 100 g (assume same as WB).

So, G + D content of WRC = (% protein WRC × 292·8)/% protein WB

$$= \frac{9{\cdot}3 \times 292{\cdot}8}{8{\cdot}4} = 379{\cdot}9\ \mu\text{g per } 100\ \text{g}.$$

Protein contents are from the 5th edition of *McCance and Widdowson's The Composition of Foods* (Royal Society of Chemistry, 1991).

Table 4 contains genistein and daidzein values for other bakery products.

Isoflavone (genistein plus daidzein) values for meat products

Pork sausages (brand name); food code 19091

Pork and beef sausages 'Economy' contain 0·85 % soya concentrate.

Assume that the G + D content of soya concentrate is the G + D content of textured vegetable protein (TVP).

G + D content of TVP = (68 600 + 68 000)/2 = 68 300 μg per 100 g.

In 100 g sausages, weight of TVP = 0·85 g.

G + D content of 100 g TVP = 68 300 μg.

So, in 0·85 g of TVP we have 0·85/100 × 68 300 μg G + D.

G + D content of 100 g sausages = 580 μg per 100 g.

The same formula was used for beef sausages 'Premium', beef olives, beefburgers, bridie or scotch pie, and Lorne sausage. The foods and associated G + D values are listed in Table 5.

Isoflavone (genistein plus daidzein) values for fish products

Recipe for fish pie; McCance & Widdowson food code 16294

200 g cooked cod
150 ml milk
400 g mashed potato
15 g margarine
level teaspoon salt
15 g flour
Total weight of ingredients 780 g.
Only ingredient with G + D (measured) is potatoes (400 g).
Content of potato in fish pie (%) = 400/780 = 51·28 %.
G + D content of potato (old and new) = (0·74 + 3·75)/2 = 2·6 μg per 100 g.

G + D content for total fish pie = (2·6 × 51·28)/100 μg per 100 g

= 1·3 μg per 100 g.

Assume 10·1 % weight loss on cooking and G + D content rises by similar amount, then the G + D content of fish pie = 1·3 + (10·1 × 1·3) = (1·3 + 0·13) μg per 100 g = 1·5 μg per 100 g.

The same mathematical formula was used to calculate the G + D content of other fish products such as fishcakes (see Table 5).

Isoflavone (genistein plus daidzein) values for retail products

Recipe for carrot and nut salad with French dressing; food code 15 288 (Tesco)

42 g carrot
35 g French dressing
15 g groundnuts
8 g sultanas
Total weight 100 g.

Groundnut content (%) = 15; G + D content of groundnuts is 20·9 μg per 100 g.

Sultanas content (%) = 8; G + D content of sultanas is 204·1 μg per 100 g.

So, in 100 g salad G + D content = ((15 × 20·9)/100 + (8 × 204·1)/100) μg G + D

= (3·1 + 16·3) μg G + D

Phyto-oestrogen content of carrot and nut salad = 19·4 μg per 100 g.

Appendix 2. Genistein plus daidzein content of Streaky Strips®

Streaky Strips®, food code MR11, contain 43 % textured vegetable protein (TVP; wheat gluten and soya protein concentrate), soya and maize protein, and soya protein isolate (SPI; assume 14 %)

We can make the following assumptions:

50 % TVP is wheat protein and 50 % is soya protein;

Genistein plus daidzein (G + D) content is 50 % G + D content of soya flour (Riaz, 'Soya and Health 2000').

100 g Streaky Strips® contains 23 g TVP and 14 g SPI.

G + D content of TVP = 68 600 μg per 100 g.

G + D content of SPI = 105 000 μg per 100 g.

So, total G + D content = ((23 × 68 600) + 14 × 10 5000))/100

Phyto-oestrogen content of Streaky Strips® = (15 778 + 14 700) μg per 100 g = 30 478 μg per 100 g.