British Journal of Nutrition (2003), **89**, Suppl. 1, S19–S23
DOI: 10.1079/BJN2002792
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6496-V

Phyto-oestrogen levels in foods: the design and construction of the VENUS database

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The objective of the Vegetal Estrogens in Nutrition and the Skeleton (VENUS) project was to evaluate existing data on dietary exposure to compounds with oestrogenic and anti-oestrogenic effects present in plant foods as constituents or contaminants, and to identify and disseminate *in vitro* and *in vivo* methodologies to analyse the effects of such compounds on bone. To permit the assessment of exposure to isoflavones in European populations (Italy, the UK, Ireland, The Netherlands), the VENUS database of phyto-oestrogen levels in foods was established. Data on the isoflavone (genistein and daidzein) content of 791 foods, including almost 300 foods commonly consumed in Europe, were collected. Levels of coumestrol, formononetin and biochanin A in a limited number of foods were also included. Lignan levels (secoisolariciresinol and matairesinol) in 158 foods were incorporated into the database, which also contains information on the references sourced for the compositional data, on the analytical methods used by each author and on the number of foods analysed in each reference. The VENUS database was constructed in Microsoft® Access 2000, which is widely available as part of Microsoft® Office Professional. This paper outlines the procedures used for the selection and evaluation of existing literature data for incorporation into the database. In addition, the design of the database is described, along with the data entry and quality control procedures used in its construction. Limitations of the data are discussed and guidelines for its use are provided.

Isoflavones: Food composition data: Lignans

Introduction

Over the past 20 years, a large number of epidemiological studies have been conducted to investigate diet and the risk of developing different types of cancer. The most consistent finding so far is the association observed between consumption of vegetables and fruit and reduced risk of cancers of the digestive and respiratory tracts (Riboli & Norat, 2001). It has been suggested that the protective effects of plant foods may in part be due to plant oestrogenic compounds, or phyto-oestrogens. Epidemiological investigations strongly support this hypothesis because the highest levels of these compounds in the diet are found in countries or regions with low cancer incidence (Adlercreutz, 1995). Phyto-oestrogens are classified as isoflavones, coumestans and lignans, and they are common components of food items such as grains, beans, fruits and nuts. Isoflavones are found primarily in soyabeans and foods made from soya.

Phyto-oestrogens display oestrogen-like activity because of their structural similarity to human oestrogens and because they exhibit high-affinity binding for the oestrogen receptor β (Sirtori, 2001), thus enabling them to exert both oestrogenic and anti-oestrogenic effects. It has been shown in postmenopausal women that habitually high intakes of dietary isoflavones are associated with higher bone mineral density values at both the spine and the hip region (Mei *et al.* 2001). It is possible that a diet rich in isoflavones may help to reverse the state of secondary hyperparathyroidism associated with oestrogen withdrawal and hence

Abbreviations: IFR, Institute of Food Research; USDA, United States Department of Agriculture; VENUS, Vegetal Estrogens in Nutrition and the Skeleton.

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lower the rate of bone turnover in postmenopausal women, thus reducing the risk of osteoporosis (Valtueña *et al.* 2003).

The clinical implications of these and other findings from small trials are exciting, but, as yet, no results from large-scale intervention trials are available. In addition, some negative effects of phyto-oestrogens have been reported, most notably the proliferative effects of soya isoflavones (daidzein and genistein) on oestrogen-dependent human breast cancer cells (Allred *et al.* 2001). While these effects cannot be extrapolated to man, they do suggest that there may be possible risks associated with the addition of phyto-oestrogens to diets at physiologically relevant concentrations. Barnes (2003) has critically reviewed the available studies and concluded that an oral isoflavone dose of 50 mg/d should be considered safe in most population groups. He considered that up to 2 mg/kg of body weight per day for participants of clinical trials could be reasonably considered safe where participants were carefully monitored.

Taking this into consideration, and in the absence of definitive data from human intervention trials, the evidence base to recommend the widespread use of isoflavones by population groups for long-term health gain or prevention of chronic disease is insufficient at this time. However, research in the field of phyto-oestrogens continues at a rapid pace, and it seems likely that the weight of the evidence may in time favour increasing the levels of these plant constituents in the diets of Western populations. The use of phyto-oestrogens as functional ingredients is one way of achieving these increases. In this event, it will become increasingly important to be able to assess the impact of the addition of these bioactive ingredients to commonly consumed foods in terms of the risks and benefits to consumers. For this reason, it is imperative that current exposure levels to phyto-oestrogens (at baseline) are established in Western populations.

It has been estimated that phyto-oestrogens levels are low in Western diets, typically <1 mg/d, compared with oriental diets, which range from 20 to 50 mg/d (Mazur & Adlercreutz, 2000). The richest sources of isoflavones in Japan are tofu, miso, natto and fried tofu (Wakai *et al.* 1999) and these foods are consumed relatively infrequently in Western countries. Studies on the actual intakes of dietary phyto-oestrogens in population groups are scarce, mainly because of the dearth of reliable phyto-oestrogen composition data. Isoflavone intakes were recently assessed in postmenopausal women in the USA as part of The Framingham Study (de Kleijn *et al.* 2001). The median daily total intake of isoflavones was 154 μg (99–235 μg), and the main dietary sources were beans and peas. These data are in contrast to the results of Horn-Ross *et al.* (2001), who found (also using a food frequency questionnaire in US women) that intakes on average were >3 mg/d and the main dietary sources were tofu, doughnuts, soya milk and white bread. One of the reasons for the large discrepancy in the phyto-oestrogen intake values obtained in these studies is likely to be the different tables of phyto-oestrogen levels in foods used by the researchers to quantify phyto-oestrogen intakes.

To date, two databases containing phyto-oestrogen levels in foods have been publicly available. The Institute of Food Research (IFR) established a database in Visual Foxpro, called NOTIS^{PLUS} (http://www.ifr.bbsrc.ac.uk/phytochemicals/Links.htm) and this was the first commercially available database on the levels of bioactive compounds in plant foods. The second and more recently constructed database (1998) is the United States Department of Agriculture (USDA)–Iowa State University Isoflavones Database (http://www.nal.usda.gov/fnic/foodcomp/Data/isoflav/isoflav.html).

At the outset of the Vegetal Estrogens in Nutrition and the Skeleton (VENUS) project in 1998, the participants decided that neither the NOTIS^{PLUS} nor the USDA–Iowa State University database contained sufficiently up-to-date information, particularly about foods commonly consumed in European countries, to enable the intakes of dietary phyto-oestrogens in Europe to be estimated, and that a separate VENUS database was required. Therefore, as a primary objective, the Concerted Action research group decided to establish an electronic database containing phyto-oestrogen levels in foods from literature sources, including foods rich in phyto-oestrogens and foods that contained low levels of phyto-oestrogens but were commonly consumed in European diets. The VENUS database was then applied to recent population-based food consumption data for Italy, Ireland, the UK and The Netherlands. The procedures used and results obtained from this project are described fully in a separate paper in this supplement (Van Erp-Baart *et al.* 2003). Briefly, the VENUS database served as a starting point for the establishment of individual national databases in the four countries, which accounted for variations in the national food supply and local recipe databases. The data obtained are a useful baseline assessment of dietary exposures to isoflavones in three Northern European countries and one Southern European country using the most up-to-date food consumption data and isoflavone compositional data available at this time.

The current paper describes the selection and evaluation of the data included in the VENUS database, the design of the database, and the data entry and quality control procedures used in its construction.

Methods

Selection and evaluation of analytical data

The data used to compile the VENUS database were gathered from peer-reviewed literature published before June 2000, and from laboratories participating in the VENUS Concerted Action project. As a starting point for the VENUS database, the literature sources used in the compilation of the USDA–Iowa State University Isoflavones Database and the IFR NOTIS^{PLUS} database were collected. The literature used in both of these databases was selected using strict criteria described in the documentation accompanying the databases: prior to selection, papers were evaluated for five general categories including analytical method, quality control, sampling procedure, sample handling and number of samples handled.

Additional literature searches were carried out to collect data that were published after the compilation of these databases, and project participants provided some unpublished data on foods commonly consumed in Europe. In particular, Dr Sheila Bingham's group in the Dunn Nutrition Unit, Cambridge, UK, supplied previously unavailable data on isoflavone levels in 300 commonly consumed foods (Liggins *et al.* 2000*a*,*b*). The entire body of literature, which comprised 47 references, was reviewed by VENUS participants (M Faughnan, personal communication, 2000; K Wähälä *et al.*, personal communication, 2001) who were experienced in the analysis of these compounds in foods. On the basis of their recommendations, 33 references were selected for inclusion in the VENUS database, as listed in the References section. References were excluded on the basis of either inadequate or outmoded analytical methods, poor or poorly described quantification of results or unclear expression of results. The majority of data excluded was due to the inability to quantify phyto-oestrogen content in foods as aglycone equivalents. Three publications were excluded from the database due to out-dated methods where it was difficult to determine the reliability of the data. One set of data was excluded because of the use of a base hydrolysis step in the analytical work-up of samples, which is known to be destructive to the compounds. A further reference was excluded owing to discrepancies within the values reported.

Conversion of reported analytical data into a form suitable for inclusion in the VENUS database

Genistein and daidzein values were entered into the VENUS database as aglycone equivalents because, following ingestion, the glucoside forms of isoflavones are converted to free (aglycone) forms for absorption in the gut. Presenting isoflavone values as total additions of free and glucoside values seriously overestimates their true aglycone concentration in food. However, many literature values of isoflavones are presented as glucosides. Thus, appropriate molecular weights were applied to the glucoside values in the literature to obtain aglycone equivalents for entry to the VENUS database.

Values for foods in the literature were often given on a dry weight basis, and conversion factors from a number of sources, mainly *McCance & Widdowson's The Composition of Foods* (Holland *et al.* 1995), were used to convert these to wet weights.

In addition, values were converted to μg/100 g edible portion, as many foods were included in the database that contain low levels of phyto-oestrogens but, because they are commonly consumed in appreciable quantities in European diets, their contributions to the overall intakes may be significant.

Database design

The VENUS database was constructed in MS Access® 2000, because it is widely available as part of the Microsoft® Office Professional suite of software, it is easy to use and data can be exported to MS Excel® or other analytical software for performing calculations as the user requires. Five tables were set up to contain fields of data as follows:

1. Table 1: Isoflavone levels (μg/100 g) in foods. Data fields: Food ID, Name of food, Daidzein, Genistein, Total isoflavones, Formononetin, Biochanin A, Coumestrol, Reference ID.
2. Table 2: Lignan levels (μg/100 g) in foods. Data fields: Lignan ID, Food ID, Name of food, Secoisolariciresinol, Matairesinol, Total lignans, Reference ID.
3. Table 3: List of references. Data fields: Reference ID, Author, Year, Journal.
4. Table 4: Methods used in food analysis. Data fields: Reference ID number, Analytical method, Acid hydrolysis (yes/no), Internal standard (yes/no), Quality control (yes/no), Number of samples.
5. Table 5: Molecular weights of isoflavones and lignans.

The Total isoflavones column refers to either a value obtained from the literature if one was provided, or the simple addition of genistein and daidzein values, if no total value was available. Missing values were identified as blank cells to differentiate them from zero or undetectable levels, which were marked 'n/d' for 'not detected'.

The database was designed for use as a relational database; therefore, corresponding food ID numbers and reference ID numbers were used across the five tables to facilitate the establishment of relationships between tables, as the user requires. The entire database is contained in one file (current size 396 kB).

Quality control

Each calculation was checked independently in-house on an individual basis, including the conversions from glucoside values to aglycone equivalents, the conversions from dry weights to wet weights and the standardisation of values to μg/100 g edible portion. In addition, the accuracy of the data entry was checked on a line-by-line basis in-house and a VENUS partner checked a random selection of data, in particular the data most relevant to Western food patterns (H Brants, personal communication, 2000).

Discussion

The current paper describes the rationale, design, construction and content of the VENUS database of phyto-oestrogen levels in foods. The VENUS database was established primarily to facilitate the estimation of exposure levels to phyto-oestrogens in four European countries, including Italy, The Netherlands, Ireland and the UK, using recent food consumption data collected for adults in these countries. Owing to the relative dearth of data on lignan levels in foods relative to the wide variety of foods commonly consumed in the four countries, it was decided to limit the assessment of dietary exposure to phyto-oestrogens to isoflavones only.

Van Erp-Baart *et al.* (2003), in a separate paper in the current supplement, fully describe the methodology and procedures used in the application of the VENUS database for the estimation of dietary exposure to isoflavones. In each of the four countries, these protocols were strictly adhered to. The food consumption data used in each country was of a similar high quality, as it consisted of food diary data collected in individuals on between two and seven days. In addition, the researchers in the four countries agreed on the selection of the appropriate food categories to include in the analysis and guidelines for the compilation of national food lists containing isoflavones for each country. The data show that the mean total isoflavone intake in the four countries was < 1 mg/d (Van Erp-Baart *et al.* 2001). As this is the primary use for which the VENUS database was designed and intended, we consider that it has been a successful starting point in the estimation of baseline exposure to vegetal oestrogens in European populations, against which future assessments based on updated versions of the VENUS database and/or new food consumption data can be compared.

It is important to point out the uses for which the VENUS database was not intended. It was not designed for the determination of individual phyto-oestrogen intakes. Hence, it is not appropriate to use the VENUS database for the prescription of individual diets (e.g. in human intervention studies). In addition, the database is not suitable for food labelling purposes. There are certain limitations to the database that users should be aware of:

1. The quantity of data available is limited overall in comparison to the number of foods consumed.
2. In relation to certain manufactured foods, particularly meat products and meat-containing mixed dishes and snacks, there is a dearth of information on the type of soya ingredients used in their manufacture and therefore the isoflavone contents of these foods are difficult to ascertain accurately by calculations from recipe databases.
3. There is variable quality in the analytical data available, particularly in the data obtained from older studies.
4. There is natural variation in soyabean isoflavone values between seasons, crops and varieties, and it has not been possible to factor this into the VENUS database.

Users of the VENUS database, in their interpretation of database values, should consider these limitations. However, it is important to remember that the database has been designed to evolve with the addition of new data and the deletion of old or irrelevant data, as new analytical techniques for the determination of more isoflavones in a wider range of relevant foods are continuously developed and optimised.

The database has been made available as a resource on-line at www.venus-ca.org.

Acknowledgements

This review has been carried out with financial support from the Commission of the European Communities, Food and Agroindustrial Research programme CT-98-4456 'Dietary exposure to phyto-oestrogens and related compounds and effects on skeletal tissues (VENUS)'. It does not reflect its views and in no way anticipates the Commission's future policy in this area.

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