Development and validation of a food frequency questionnaire to estimate the intake of genistein in Malaysia

Anne R. Fernandez¹, Siti Zawiah Omar², and Ruby Husain¹

¹Department of Physiology, Faculty of Medicine, University of Malaya and ²Department of Obstetrics and Gynecology, University Malaya Medical Centre, Kuala Lumpur, Malaysia

Abstract

Objective: To develop and validate a food frequency questionnaire (FFQ) to estimate the genistein intake in a Malaysian population of pregnant women. Method: A single 24-h dietary recall was obtained from 40 male and female volunteers. A FFQ of commonly consumed genistein-rich foods was developed from these recalls, and a database of the genistein content of foods found in Malaysia was set up. The FFQ was validated against 7-d food diary (FD) kept by 46 pregnant women and against non-fasting serum samples obtained from 64 pregnant women. Reproducibility was assessed by comparing the responses on two FFQs administered approximately 1 month apart. Results: The Pearson correlation coefficient between FFQ1 and FD was 0.724 and that between FFQ2 and FD was 0.807. Classification into the same or adjacent quintiles was 78% for FFQ1 versus FD and 88% for FFQ2 versus FD. A significant dose – response relation was found between FFQ-estimated genistein intake and serum levels. Conclusion: The FFQ developed is a reliable, valid tool for categorising people by level of genistein intake.

Introduction

Isoflavones are phytoestrogens with myriad biological properties (Setchell et al., 2001). Epidemiological studies suggest that diets high in isoflavones are associated with beneficial effects against cardiovascular risk factors (de Kleijn et al., 2002; Goodman-Gruen & Kritz-Silverstein, 2001), reduced blood pressure (He et al., 2005; Nagata et al., 2003) and improved vascular reactivity (van der Schouw et al., 2002).

Genistein, a type of isoflavone, is found mainly in soybeans and soy-based products, which are widely consumed by people of Asian descent (Adlercreutz et al., 1995; Dai et al., 2001). Tofu, a soy-based product, was first used in China (Shurtleff & Aoyagi, 2007), with the influx of Chinese immigrants to Malaysia in the 1800s, it was introduced to other immigrants and the local population. Today, Malaysia is a multiracial nation of 28.7 million people in three main ethnic groups, Malay, Chinese and Indian, who consume soy and its products regularly, having assimilated each other’s cooking styles and ingredients into their daily diet.

Numerous in vitro studies show that genistein can counteract oxidative stress (Breinholt et al., 1999; Kapiotis et al., 1997) and inflammatory activation (Hamalainen et al., 2007), the main pathophysiological processes responsible for preeclampsia. We therefore postulated that genistein has a beneficial effect in the prevention of preeclampsia. To test this hypothesis, we needed a tool to estimate the intake of genistein in the population of interest. Food frequency questionnaires (FFQs) are one of the most economical, practical methods for investigating relations between dietary factors and disease. They may not allow estimation of absolute nutrient or non-nutrient compound intake but are more accurate than other methods for estimating the average intake of a particular compound with large day-to-day variation and few significant food sources. They also make it possible to obtain retrospective information on the pattern of food intake during a certain period (in our case, pregnancy), imposing less of a burden on the respondent than other dietary assessment methods (Thompson & Subar, 2008).

A number of FFQs have been designed or modified (and validated) to measure genistein and other isoflavones. The dietary intake of phytoestrogens by Dutch women (Boker et al., 2002) and the intake of soy food by women in China (Liu et al., 2004) were estimated with newly developed FFQs, and a modified FFQ was used to assess the usual phytoestrogen intake of American women (Horn-Ross et al., 2006). None of these FFQs, however, reflects the intake of a multiracial population such as that of Malaysia. The aim of this study was to develop a FFQ to estimate the genistein intake of pregnant women in Malaysia and to evaluate its reliability and validity.

Methods

The study was conducted between November 2007 and October 2008 at the University Malaya Medical Centre and the University of Malaya, located in a suburb of Kuala Lumpur, the capital of Malaysia. The participants were mainly pregnant women recruited at the University Malaya Medical Centre and a small convenience sample of faculty staff members of the University.

Development of the FFQ

A single 24-h dietary recall was administered to 40 volunteers, who represented the three main ethnic groups of Malaysia.
(13 Malays, 16 Chinese and 11 Indians) and consisted of 30 pregnant women with a minimum of 7 years of formal education and 10 male and female faculty staff members with secondary and tertiary education. In a personal face-to-face interview with the researcher, the participants were asked to report all food and beverages consumed on the previous day. The answers were recorded on a recall form adapted from one used by the Department of Nutrition and Dietetics at the Medical Centre. A three-pass recall method was used, the first pass to obtain a list of foods consumed, the second to add descriptions of the food and the third for missing items.

As the Malaysian Nutrition Composition database (Institute of Medical Research, 2000) had no data on the genistein content of food items, a new database was needed. The scientific literature was searched for the genistein content of food items (van der Schouw et al., 2005) by collecting articles published in refereed journals that contained analytical data on genistein in foods (Arai et al., 2000; Horn-Ross et al., 2000; Liggins et al., 2000a,b; 2002; Mazur, 1998; Natchimuthu, 2004; Reini & Block, 1996; Seow et al., 1998; United States Department of Agriculture, 2002; Wang & Murphy, 1994). All values were converted to milligrams of genistein per 100 g of food and reported to the nearest decimal point (van der Schouw et al., 2002). When more than one value was found for the genistein content of a food item, the mean value was calculated. The genistein content of seven food items (shallots, carrots, pear, cauliflower, tomatoes, sunflower seed and white rice) was reported as zero or “not detected” in some studies and as a small amount in others, perhaps due to differences in the methods used for detection and quantification (Mazur, 1998); in these cases, the zero values were not included in calculating the mean. For most food items, especially legumes and nuts, the cooked values were considered for calculation. The final genistein value assigned to the “soy drink” item on the FFQ was the mean value for the different soy beverages available on the Malaysian market. The genistein content in soy milk beverages ranged from 186 to 4005 ng/ml. The products included soy milk packed in cartons purchased in supermarkets and freshly made soy milk purchased from randomly chosen restaurants and stalls.

A list of food items containing genistein was drawn up from the information obtained from the 24-h dietary recall and the newly constructed database. This list was augmented with other food items rich in genistein consumed uniquely by each ethnic group but not captured in the initial step. This process yielded a list of 64 items, which were then organised into major food groups: soy products, legumes and nuts, vegetables, fruits and cereal, including cereal products. To the list of food items, we added categorised open-ended responses: vegetables and cereal, including cereal products. To the list of food items, we added categorised open-ended responses: fruits and cereal, including cereal products. To the list of food items, we added categorised open-ended responses: vegetables and cereal, including cereal products.

To ensure that the list represented foods eaten by the population of the study, it was pre-tested on 22 pregnant women who had not participated in the 24-h dietary recall and in pre-testing the list of foods. Participants were encouraged to call the researcher if they had any questions regarding the procedure.

For the purpose of calculation, each food item was assigned a predefined portion size, obtained from the Malaysian Nutrition Composition Database (Institute of Medical Research, 2000), as suggested in a previous study involving pregnant women (Glynn et al., 2007). The genistein content of each food item in the FFQ was determined individually by multiplying the frequency weight (e.g. intake once a day received a weighting factor of 1, and intake four times a week received a weighting factor of 4/7 = 0.57) by the genistein content of the predefined portion size (Cade et al., 2001). The genistein intake from all the food items in the FFQ was then summed to estimate the total genistein intake of the participant. The genistein content of each food item listed in the 7-d FFQ was validated against serum levels of genistein, which is a more objective marker.

Validation and reproducibility of the FFQ

The newly developed FFQ was validated against a 7-d food diary (FD). As dietary assessment methods such as FFQs and FDs are based solely on subjective self-reports, the validity of the FFQ was also tested against serum levels of genistein, which is a more objective marker.

FFQ validation against the 7-d FD

The participants were 76 pregnant women recruited at the antenatal clinic who expressed interest in taking part in the study. They were different women from those who had participated in the 24-h dietary recall and in pre-testing the list of foods.

At recruitment, each woman completed the newly developed FFQ at an in-person interview (FFQ1) with a researcher. The FFQ enquired about the frequency of intake of the items since the beginning of pregnancy. As women in the first trimester of pregnancy often have a poor appetite and feel nauseated (Trindade de Castro et al., 2010), only women in the second and third trimesters of pregnancy were recruited for this part of the study. Visual aids were provided to define the type of food item. The women were then given the FD to complete at home, with verbal and written instructions on how to fill it in. They were asked to record in as much detail as possible the types of food and beverages consumed daily over 7 d, including weekends. Participants were encouraged to call the researcher if they had any questions regarding the procedure.

For the purpose of calculation, each food item was assigned a predefined portion size, obtained from the Malaysian Nutrition Composition Database (Institute of Medical Research, 2000), as suggested in a previous study involving pregnant women (Glynn et al., 2007). The genistein content of each food item in the FFQ was determined individually by multiplying the frequency weight (e.g. intake once a day received a weighting factor of 1, and intake four times a week received a weighting factor of 4/7 = 0.57) by the genistein content of the predefined portion size (Cade et al., 2001). The genistein intake from all the food items in the FFQ was then summed to estimate the total genistein intake of the participant. The genistein content of each food item listed in the 7-d FFQ was validated against serum levels of genistein, which is a more objective marker.

Reproducibility of the FFQ

On the subsequent check-up, women returned the completed FD and answered the same 19-item FFQ administered by the same researcher (FFQ2), thus minimising interviewer bias. The interval between FFQ1 and FFQ2 was 3–4 weeks for all women.

FFQ validation against serum genistein level

The participants in this validation study were 64 pregnant women who had been recruited for a case-control study involving patients
Table 1. Food list based on information from 24-h recall augmented by other genistein-rich foods.

<table>
<thead>
<tr>
<th>Soya products</th>
<th>Legumes and nuts</th>
<th>Vegetables</th>
<th>Fruits</th>
<th>Cereals and cereal products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japanese tofu</td>
<td>Baked beans</td>
<td>Mung bean sprouts</td>
<td>Strawberries</td>
<td>Wheat bran</td>
</tr>
<tr>
<td>Chinese tofu</td>
<td>Red beans</td>
<td>Long beans</td>
<td>Dates</td>
<td>Semolina</td>
</tr>
<tr>
<td>Soya bean curd</td>
<td>Chick peas</td>
<td>Snow peas</td>
<td>Mangoes</td>
<td>Brown rice</td>
</tr>
<tr>
<td>Tauchewa</td>
<td>Black chick peas</td>
<td>Shallots</td>
<td>Honey dew</td>
<td>White rice</td>
</tr>
<tr>
<td>Fuchuok</td>
<td>Mung beans (Green gram)</td>
<td>Carrots</td>
<td>Pears</td>
<td>Egg noodles</td>
</tr>
<tr>
<td>Taupok</td>
<td>Pigeon peas (Red gram)</td>
<td>Chinese chives</td>
<td>Prunes</td>
<td>Instant noodles</td>
</tr>
<tr>
<td>Tempeh</td>
<td>Peanuts</td>
<td>Broccoli</td>
<td>Raisins</td>
<td>Brown bread</td>
</tr>
<tr>
<td>Taucheong</td>
<td>Kidney beans</td>
<td>Cauliflower</td>
<td>Coconut</td>
<td>White bread</td>
</tr>
<tr>
<td>Soya sauce</td>
<td>Broad beans</td>
<td>French beans</td>
<td>Mandarin oranges</td>
<td>Wholemeal bread</td>
</tr>
<tr>
<td>Soya milk</td>
<td>Pistachios</td>
<td>Potatoes</td>
<td></td>
<td>Muesli</td>
</tr>
<tr>
<td>Soya noodles</td>
<td>Cashew nuts</td>
<td>Egglplants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soya bean sprouts</td>
<td>Sunflower seeds</td>
<td>Red cabbage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soya beans (boiled or roasted)</td>
<td>White kidney beans</td>
<td>Cucumbers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Textured vegetable protein</td>
<td></td>
<td>Mushrooms</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Food list based on information from 24-h recall augmented by other genistein-rich foods.

...with preeclampsia and healthy pregnant women, which was conducted simultaneously.

Non-fasting blood samples (10 ml) were collected after administration of the newly developed FFQ. Samples were collected in serum separator tubes (Becton Dickson Vacutainer Systems, Franklin Lakes, NJ), which were then placed upright at room temperature for at least 30 min to allow the blood to clot. The samples were centrifuged at 1500 × g for 10 min, and separated serum was aliquoted into cryovials and stored at -70°C until analysis. Blood samples that were not centrifuged immediately were stored in a refrigerator for not more than 24 h (Bhakta et al., 2005).

A sensitive, convenient, time-resolved fluoroimmunoassay was used to analyse serum genistein (Wang et al., 2000) at the Institute for Preventive Medicine, Nutrition and Cancer, University of Helsinki, Finland. The research reagents for the genistein kit were from Labmasters, Turku, Finland.

Ethics statement

The guidelines of the Declaration of Helsinki were followed, and all procedures involving human patients were approved by the Medical Ethics Committee of the University of Malaya Medical Centre (IRB reference number 595.7). Verbal or written informed consent was obtained from all participants.

Statistical analysis

All statistical analyses were performed with SPSS 13.0 for Windows (SPSS Inc., Chicago, IL). The means and standard deviations (SDs) of estimated genistein intake from FFQ1, FFQ2 and FD were calculated. As the estimates were not normally distributed, the data were subjected to square root transformation (which gave the most normalised distribution) before analysis.

The Pearson correlation coefficient was used to evaluate the correlation between FFQ- and FD-estimated intakes of genistein. Intraclass correlation coefficients were calculated to assess the reproducibility of the FFQ.

Continuous (non-transformed) genistein intakes estimated from FFQ1, FFQ2 and FD were further divided into quintiles separately for each method, and the level of agreement in the FFQ1, FFQ2 and FD was determined by calculating the percentage of women categorised in the same, adjacent or another quintile. Participants were considered to have been classified correctly if they were categorised into the same quintile or within one quintile by both methods. Kappa statistics were also used to measure the agreement between the two methods of dietary assessment, based on quintile category. Kappa values from 0 to 0.20 indicate poor agreement, 0.21 to 0.40 fair agreement, 0.41 to 0.60 moderate agreement, 0.61 to 0.80 good agreement and 0.81 to 1 very good agreement (Viera & Garrett, 2005).

The Bland–Altman method (Bland & Altman, 1986) was used to evaluate the agreement between FFQ1 and the FD. This is an approach for assessing agreement between two methods when a new measurement is compared to an established one. It consists essentially of a plot of differences between methods and their mean, which also allows investigation of relations between measurement error (difference between methods) and true values (mean measurements).

We validated the FFQ against the mean (±standard error of mean) serum levels of genistein. As the distribution of serum genistein concentrations in the population sample was markedly skewed, Spearman rank correlation was used to assess the relations between estimated genistein intake and serum genistein levels. The data were divided into quintiles, the mean values for each quintile were determined, and a trend test was performed to find whether there was a significant dose–response relation between estimated genistein intake and serum genistein. For all tests, p < 0.05 was considered significant.

Results

A total of 33 food items containing genistein was derived from the 24-h dietary recall. Addition of other genistein-rich foods resulted in a 64-item food list (Table 1). The contributions of food items to FFQ estimates are listed in Table 2. The contribution analysis revealed that 19 food items, which included 11 soya-based products, accounted for 99.41% of genistein intake. Each of the other food items contributed less than 0.01% of the total genistein intake and were therefore not included in the FFQ.

Validation and reproducibility of the FFQ

The FFQ was validated against both the 7-d FD and the serum levels of genistein. Of the 76 pregnant women recruited for the validation against the FD, 46 returned completed FDs, corresponding to a response rate of 60%. The average age (±SD) was 29.8 ± 6.2, and 17 were Malay, 16 Chinese and 13 Indian. In the study to validate the FFQ against serum levels, there were 64 participants with an average age (±SD) of 29.6 ± 5.3. Of these, 38 were Malay, 16 Chinese and 10 Indian.
Table 2. Food items selected by the contribution analysis.

<table>
<thead>
<tr>
<th>Food item</th>
<th>% of total genistein intake</th>
<th>Cumulative % of total genistein intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chinese tofu</td>
<td>25.74</td>
<td>25.74</td>
</tr>
<tr>
<td>Soya bean curd/taufoofoah</td>
<td>19.83</td>
<td>45.57</td>
</tr>
<tr>
<td>Japanese tofu</td>
<td>12.38</td>
<td>57.95</td>
</tr>
<tr>
<td>Soya milk</td>
<td>17.64</td>
<td>75.59</td>
</tr>
<tr>
<td>Tempeh</td>
<td>9.66</td>
<td>85.25</td>
</tr>
<tr>
<td>Soya bean sprouts</td>
<td>4.21</td>
<td>89.46</td>
</tr>
<tr>
<td>Fuchook</td>
<td>3.21</td>
<td>92.67</td>
</tr>
<tr>
<td>Taukwa</td>
<td>3.12</td>
<td>95.79</td>
</tr>
<tr>
<td>Taupok</td>
<td>1.85</td>
<td>97.64</td>
</tr>
<tr>
<td>Taucheong</td>
<td>0.56</td>
<td>98.2</td>
</tr>
<tr>
<td>Mung bean sprouts</td>
<td>0.44</td>
<td>98.64</td>
</tr>
<tr>
<td>Red beans</td>
<td>0.23</td>
<td>98.87</td>
</tr>
<tr>
<td>Soya sauce</td>
<td>0.18</td>
<td>99.05</td>
</tr>
<tr>
<td>Long beans</td>
<td>0.16</td>
<td>99.21</td>
</tr>
<tr>
<td>White rice, long grain, boiled</td>
<td>0.08</td>
<td>99.29</td>
</tr>
<tr>
<td>White bread</td>
<td>0.06</td>
<td>99.35</td>
</tr>
<tr>
<td>Wholemeal bread</td>
<td>0.03</td>
<td>99.38</td>
</tr>
<tr>
<td>Baked beans</td>
<td>0.02</td>
<td>99.4</td>
</tr>
<tr>
<td>Brown rice, whole grain</td>
<td>0.01</td>
<td>99.41</td>
</tr>
</tbody>
</table>

Table 3. Pearson correlation coefficient, kappa statistics and percentage agreement between FFQ1 and FD and FFQ2 and FD.

<table>
<thead>
<tr>
<th></th>
<th>Pearson correlation coefficient R</th>
<th>Kappa statistic</th>
<th>Same quintile</th>
<th>Adjacent quintile</th>
<th>Two quintiles apart</th>
<th>Three quintiles apart</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFQ1 and FD</td>
<td>0.724*</td>
<td>0.305*</td>
<td>45</td>
<td>33</td>
<td>18</td>
<td>4</td>
</tr>
<tr>
<td>FFQ2 and FD</td>
<td>0.807*</td>
<td>0.315*</td>
<td>45</td>
<td>43</td>
<td>10</td>
<td>2</td>
</tr>
</tbody>
</table>

*p < 0.001.

As the FFQ was administered by the interviewer, all the women answered all the questions on FFQ1 and FFQ2, with no incomplete answers or excluded questionnaires.

**FFQ validation against FD**

The estimated genistein intake (mean ± SD) was 13.7 ± 9.2 mg/d from FFQ1, 13.3 ± 9.7 mg/d from FFQ2 and 9.9 ± 8.4 mg/d from the FD. The Pearson correlation coefficient for estimated genistein intake between FFQ1 and FD was 0.724 and that between FFQ2 and FD was 0.807, indicating a good correspondence between the different methods of estimation. The intraclass correlation coefficient was 0.927 (p < 0.001), indicating excellent reproducibility.

The kappa statistics for FFQ1–FD and FFQ2–FD were 0.305 and 0.315, respectively, indicating fair agreement.

When the genistein intake estimated from the FFQ and the FD was divided into quintiles, the proportions of women classified into the same and adjacent quintiles were 78–88%, indicating good performance of the FFQ. None of the women was grossly misclassified into the opposite quintile (Table 3).

The Bland–Altman plot (Figure 1) showed a concentration of data between the limit of agreements (which is computed as the mean difference ± 2SDs), indicating fairly good agreement between the genistein intake estimated from FFQ1 and the FD. The mean line represents the bias between the methods, which is 3.8 mg/d in this case. The positive slope of the trendline shows that the error of agreement increased among participants consuming more genistein. The R² of 0.015 in the Bland–Altman analysis indicates a weak relation between the score and the error (Bishop, 2008).

**FFQ validation against serum levels**

The mean serum genistein concentration was 55.9 (±12.1) nmol/l. Table 4 shows the relations between genistein intake and serum genistein levels for women classified by quintile of intake. Trend tests showed a significant dose–response relation (p < 0.05) between estimated genistein intake and serum genistein across quintiles of dietary intake.

**Discussion**

The FFQ was shown to capture genistein intake accurately and made it possible to rank women according to their intake. The reliability (reproducibility), assessed by intraclass correlation between the results of two FFQs administered to the same participant, was shown to be good.

Malaysians usually eat three main meals a day – breakfast, lunch and dinner, each typically consisting of rice or noodles with meat and vegetables. In a study to identify the food consumption patterns of the Malaysian population, it was found that people of different age groups, sex and social status consumed similar foods, with a subtle difference in food eaten by people living in rural and urban areas (Norimah et al., 2008). Soy and soy-based foodstuffs are particularly rich sources of genistein, while the concentrations in legumes, nuts and vegetables are lower (Committee on Toxicology of Chemicals in Food, Consumer Products and the Environment, 2003). Because of the limited variety of soy products available on the Malaysian market (Malaysia Food.net Glossary, 2003), a single 24-h recall by 40 participants was considered sufficient to obtain information on foods that contribute to genistein intake. Participants of both genders and different educational background were included to ensure that the full variation of the population’s diet was captured.

Contribution analysis showed that soya and soya products alone were responsible for 99% of the genistein intake. A study by Frankenfeld et al. (2003), comparing the validity of a short soya FFQ consisting of 20 items with a comprehensive FFQ consisting of 122 items, suggested that the shorter, more specific FFQ better captured soya consumption than the longer FFQ. Therefore, we considered it plausible that the food list could be shortened without compromising the accuracy of the FFQ. We decided that further pre-testing of the food list on more participants was unnecessary and that a 19-item FFQ consisting predominantly of soya-based products was sufficient.

A sample size of at least 50 and preferably 100 or more is desirable for FFQ validation studies (Cade et al., 2001). Although the recruitment period for participants in the FD validation study was extended several times, the response rate was 60%. This may be attributable to the demanding task of keeping a 7-d FD. The participants may therefore have been a group of highly motivated individuals, thus compromising the validity of the study. It may also indicate that a FD is not the most appropriate method for estimating the intake of nutrients in the Malaysian population.

The performance of our FFQ is comparable to that of other, well-established FFQs that have been used in epidemiological studies. In our study population, the mean genistein intake per day, as estimated from FFQ1, FFQ2 and FD, was 9–14 mg/d. The intake of the Malaysian population therefore falls within the wide international range, which appears to increase gradually from west to east: the mean genistein intake of women was 1.3 mg/d in the United States (Goodman-Gruen & Kritz-Silverstein, 2001), 0.24 mg/d in England (Grace et al., 2004), 30.5 mg/d in Japan (Arai et al., 2000) and 32.6 mg/d in China (Lee et al., 2003).

The correlations between intakes estimated by the FFQ and the FD were excellent, although the FFQ appeared to overestimate intake, as in other validation studies (Kusama et al., 2005; Shu et al., 2004; Zhang & Ho, 2009). The mean difference in genistein...
intake (or the estimated bias) was 3.8 mg/d between FFQ1 and FD and 3.37 mg/d between FFQ2 and FD. In our study, overestimation of genistein intake may have been due to exaggerated reports of intake of taufoofoo (a dessert made with very soft tofu and served with clear, sweet syrup or caramelised sugar). Although many participants reported consuming it weekly in the FFQ, they did not record it in the FD for that particular week. Self-reported dietary intake can be affected by social desirability and social approval (Hebert et al., 2001). In an attempt to reduce the cognitive burden on participants, the FFQ did not ask whether a food item was consumed as part of a dish or alone. This may have led to double counting and thus overestimation of intake. Another source of overestimated intake from the FFQ could be reduced recording of intake with increasing number of reporting days (recording fatigue) (Vuckovic et al., 2000).

In a meta-analysis (Molag et al., 2007), questions on portion sizes did not necessarily improve the performance of an FFQ. Furthermore, the addition of questions on portion sizes would increase the number of questions in the FFQ, thus imposing a greater burden on participants (Wakai et al., 1999) and perhaps posing a cognitive challenge (Cade et al., 2001). In a study of the effect of portion sizes on the validity of an FFQ, it was concluded that they are less important than frequency of consumption (Tjonneland et al., 1992). Subar et al. (2000) compared the Diet History Questionnaire, Block’s FFQ and Willett’s FFQ and found that between-person bias was due mainly to factors that affect self-reporting, like personality and cultural characteristics, rather than to portion size. In a review (McNutt et al., 2008), it was concluded that FFQs used for a homogeneous population (in our case, Malaysian women of reproductive age) did not require questions on portion size.

Although many researchers consider it appropriate to adjust for energy intake for all nutrients, Hill & Davies (2001) showed that energy is poorly measured in most self-reported dietary instruments, including recall and records. Block (2001) stated that researchers should report crude correlations, without adjusting for energy or measurement errors, simply because the values used for correcting measurement errors may be imperfect themselves. In an FFQ validation study by researchers in Hong Kong (Chan et al., 2008), isoflavone intake values were not adjusted for total energy intake because the nutrient composition of local foods was not available. Instead, these researchers adjusted the crude correlation coefficient for body mass index, which is considered to be an indirect measure of total energy intake. They found little difference between the unadjusted and the adjusted correlation and suggested that variation in isoflavone intake is not related to variation in dietary energy intake. In our study, no adjustment was applied to estimated genistein intake.

The FFQ validation was strengthened by cross-validation with serum levels in another set of participants. Biochemical indicators are influenced not only by dietary intake but also by the timing of blood sampling, the absorption and metabolism of the nutrient and many other factors. A study by Verkasalo et al. (2001) indicated a linear dose–response relation between dietary genistein and its blood levels in a timed blood sample. Unavoidable circumstances compelled us to take a single untimed blood sample in this study. Nevertheless, the demonstration of a correlation between FFQ-estimated intake and this biochemical indicator provides unquestionable documentation of the validity of the FFQ.

Block et al. (1986) mentioned that the steps taken to develop an FFQ might be as important as the FFQ itself. The simple methods used in this study might be useful for other researchers in the field. Our FFQ includes all the food items eaten by the main ethnic groups originated. It has already been used in other countries of the Asian region, especially the countries from which the Malaysian ethnic groups originated. It has already been used in a study of preeclampsia in pregnant women, which will be published shortly.

**Conclusion**

This study shows that our FFQ can be used to measure the intake of genistein accurately among pregnant women in Malaysia. It is short, easy to administer, convenient and provides an inexpensive tool for dietary assessment. It could therefore be used in studies of...
diet–disease relations in South-East Asia. The developmental approach used is simple and may be readily adapted to other populations, study designs and nutrients of interest.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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