Critical review of health effects of soyabean phyto-oestrogens in post-menopausal women

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A consensus view of soyabean phyto-oestrogens in clinical interventions in post-menopausal women is presented that is based on data from the EU-funded project Phytohealth. The phyto-oestrogens, primarily genistein and daidzein, were given as soyabean-protein isolates, whole-soyabean foods or extracts, supplements or pure compounds. A comprehensive literature search was conducted with well-defined inclusion or exclusion criteria. For areas for which substantial research exists only placebo-controlled double-blind randomised controlled trials (RCT) conducted on healthy post-menopausal women were included. For emerging areas all available human studies in post-menopausal women were reviewed. In order to make cross comparisons between studies the doses of isoflavones were calculated as aglycone equivalents. There is a suggestion, but no conclusive evidence, that isoflavones from the sources studied so far have a beneficial effect on bone health. The consumption of whole-soyabean foods and soyabean-protein isolates has some beneficial effects on lipid markers of cardiovascular risk. The consumption of isolated isoflavones does not affect blood lipid levels or blood pressure, although it may improve endothelial function. For menopausal symptoms there is currently limited evidence that soyabean-protein isolates, soyabean foods or red-clover (Trifolium pratense L.) extract are effective but soyabean isoflavone extracts may be effective in reducing hot flushes. There are too few RCT studies to reach conclusions on the effects of isoflavones on breast cancer, colon cancer, diabetes or cognitive function. The health benefits of soyabean phyto-oestrogens in healthy post-menopausal women are subtle and even some well-designed studies do not show protective effects. Future studies should focus on high-risk post-menopausal women, especially in the areas of diabetes, CVD, breast cancer and bone health.

Genistein: Daidzein: Bioavailability: Bone health: CVD

The current interest in soyabean and its phyto-oestrogen component in relation to human health has resulted in a substantial number of publications on the potential clinical efficacy of these compounds to improve health in menopausal women. However, although numerous reviews have been presented, to date a consensus on the potential importance of these compounds for menopausal health following a critical grading of the studies and their results has not been conducted. The focus of the current review is specifically to grade the evidence from clinical studies addressing the effects of intervention of soyabean isoflavones (for chemical structures of the aglycones, see

Abbreviations: RCT, randomised controlled trial; SPI, soyabean-protein isolate.
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Fig. 1) fed as soyabean-protein isolates (SPI), whole-soyabean foods or extracts, supplements or pure compounds and reach a consensus on optimal dose, food source and duration of use for each of the health outcomes. This procedure will be conducted in depth for bone health, CVD and menopausal symptoms and current knowledge summarised for other areas of growing interest, including cancer, cognition and diabetes. The comparability of clinical studies is confounded by the variability in phyto-oestrogen composition and dose administered in the intervention studies. Thus, before reviewing the health effects the available evidence on factors influencing the bioavailability of isoflavones will be reviewed, since the intervention studies have used different doses and sources of isoflavones to examine the various health effects.

The consensus view presented is based on data from the EU-funded project Phytohealth, a pan-European network of excellence funded by the EU (QLKII-CT-2002-02453), which has brought together a multidisciplinary team of scientists, including toxicologists, clinicians and nutritionists to evaluate the current research in the phyto-oestrogens field and identify current gaps in knowledge.

Materials and methods
A literature search was conducted (up to June 2005) using MEDLINE (PubMed), EMBASE and the Cochrane Collaboration (The Cochrane Library). The search included the following keywords: phytoestrogens, isoflavones, genistein, daidzein, equol, soy (a); cross-referenced with the key words: post-menopausal, hot flushes, osteoporosis, bone mineral density, bone metabolism, cardiovascular, endothelial function, vascular reactivity, blood pressure, lipid profile, breast, colon, cognition.

In order to make cross comparisons between studies that used a range of sources of isoflavones the doses of isoflavones were calculated as aglycone equivalents. A panel of Phytohealth members reviewed the studies and classified them according to the grading criteria proposed by Harbour & Miller (2001; Table 1).

For areas in which substantial research has been conducted only studies with a high grade (≥1+) have been considered in the formulation of the consensus statement. Only placebo-controlled double-blind randomised controlled trials (RCT) conducted on healthy women >1 year post-menopausal (for hot flushes >6 months and biochemically defined) were included. Evaluation of methodological strength was conducted following the methodology outlined by Jadad et al. (1996). In particular, absence of bias, quality of data collection, quality of reporting of study methodology, adequate power calculations, clear characterisation of dose and composition of phyto-oestrogen product were considered. Additional inclusion criteria were applied for each specific health outcome. For bone health only studies with a duration ≥6

### Table 1. Grading criteria applied to the studies assessed in the present review

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1++</td>
<td>High-quality meta-analysis, systematic reviews of RCT or RCT with a very low risk of bias</td>
</tr>
<tr>
<td>1+</td>
<td>Well-conducted meta-analysis, systematic reviews of RCT or RCT with a low risk of bias</td>
</tr>
<tr>
<td>1−</td>
<td>Meta-analysis, systematic reviews of RCT or RCT with a high risk of bias</td>
</tr>
<tr>
<td>2++</td>
<td>High-quality systematic reviews of case-control or cohort studies or high-quality case-control or cohort studies with a very low risk of confounding, bias or chance and a high probability that the relationship is causal</td>
</tr>
<tr>
<td>2+</td>
<td>Well conducted case-control or cohort studies with a low risk of confounding, bias or chance and a moderate probability that the relationship is causal</td>
</tr>
<tr>
<td>2−</td>
<td>Case-control or cohort studies with a high risk of confounding, bias or chance and significant risk that the relationship is not causal</td>
</tr>
<tr>
<td>3</td>
<td>Non-analytical studies, e.g. case reports, case series</td>
</tr>
<tr>
<td>4</td>
<td>Expert opinion</td>
</tr>
</tbody>
</table>

RCT, randomised controlled trials.
months were included, and for cardiovascular health and menopausal symptoms only studies with a duration ≥4 weeks were included. For menopausal symptoms only studies purposefully designed for this outcome were included.

For emerging areas, including cognitive function, breast and colo-rectal health and diabetes, all available human studies in post-menopausal women (or mixed menopausal status studies) were considered and reviewed (level of evidence of 2+ and above). All papers reviewed by the panel were tabulated, with the studies that matched the criteria for inclusion in the consensus paper presented within the text. The panel then weighted the evidence and a statement was formulated for each health effect and assigned a grade, based on the quality of the studies used. Grading was assigned according to the methodology outlined in Table 2 (Agency for Health Care Policy and Research, 1992).

Results and discussion

Bioavailability and sources

The importance of understanding factors influencing absorption and metabolism as these factors may influence potential clinical effects under study. During the course of studies on health effects the isoflavone test compounds are consumed in various forms, together with other foods, in various doses and by different age-groups. All these variables could influence the bioavailability and therefore the final outcome of the study. This section examines how the outcome of human intervention studies could be affected, either positively or negatively, by bioavailability factors. Examination of the data (Tables 4–10) reveals that a range of sources of isoflavones has been used: pure compound (tablets or capsules), extracts of soyabean germ or red clover (Trifolium pratense L.), soyabean flour, soya beverages, isoflavone-enriched soyabean protein, soyabean-protein drinks or SPI. Many of the studies were long term and during each study the overall diet was not fully monitored, implying that the effect of foods on bioavailability also needs to be considered.

Variables in isoflavone bioavailability: food matrix and storage. One of the most important factors is to measure the amount of isoflavone in the food or supplement at the moment of consumption, since storage and processing influence the amount of isoflavone in the final product and the stability of the various isoflavones is different depending on the structure (Mahungu et al. 1999; Xu et al. 2002; Eisen et al. 2003; Lee et al. 2003). Other factors in the diet, such as fibre, can also affect the bioavailability (Tew et al. 1996). The source of the isoflavones and hence the food matrix in which the compound is delivered seems to be less important, and generally studies have found no difference in the bioavailability between cooked soyabeans, textured vegetable protein, tofu or tempeh (Xu et al. 2000) or soya-milk powder or soyabean germ (Zheng et al. 1999).

Variables in isoflavone bioavailability: chemical nature of the isoflavone. The chemical form of the isoflavone may affect the bioavailability, and there is some evidence to suggest that bioavailability is different following consumption of fermented soyabean products (containing mainly aglycones) compared with ingestion of non-fermented soyabean products (containing the naturally-occurring isoflavone glucosides (Izumi et al. 2000; Setchell et al. 2001)). This aspect of aglycone v. glycoside is an important factor in designing an intervention study. The pharmaco-kinetic values from sixteen studies are shown in Table 3. There is a linear increase in peak mean plasma concentration for daidzein between 1 and 31 μmol/kg body weight (R² 0.958) and for genistein between 0.2 and 59 μmol/kg body weight (R² 0.974). There is also a correlation, although weaker, for the glucosides (R² 0.716 and R² 0.730 respectively). The area under the curve, urinary excretion and faecal excretion show no significant correlations with dose for any of the compounds between the selected studies.

Variables in isoflavone bioavailability: effect of age. No difference has been found in the pharmaco-kinetics of either genistein or daidzein between pre- and post-menopausal women (Setchell et al. 1997, 2003a; Lu & Anderson, 1998; Faughnan et al. 2004).

Variables in isoflavone bioavailability: frequency of ingestion. No significant differences have been observed in the pharmaco-kinetics of [1³C]daidzein or [1³C]genistein after 2 weeks wash-out or after 7 d of soyabean consumption (Setchell et al. 2003b). After 1 month of daily soya-milk feeding there is a decrease in the urinary excretion of genistein and daidzein, whereas that of equol increases; however, this effect is only detected in women and not in men (Lu & Anderson, 1998).

Metabolism as a confounder or additional variable in efficacy studies: equol production. The ability to convert daidzein to equol is observed in about 30–50% of individuals. An analysis by Setchell et al. (2002a) has shown that
the ability to produce equol is associated with an increased benefit of isoflavones on bone mineral density. Equol production is influenced by intestinal microflora composition, gut transit time and the redox potential of the colon (Setchell et al. 1984). During the first months of life the plasma and urine levels of equol are lower than those in adults, probably because of an immature gut flora (Setchell et al. 1997). Ingestion of daidzin results in a higher equol production than after ingestion of the aglycone daidzein alone (Setchell et al. 1997; Lampe et al. 1998; Zubik & Meydani, 2003), and equol production is more prevalent in subjects with a high consumption of carbohydrates and dietary fibre and a low dietary fat:fibre ratio (Lampe et al. 1998; Watanabe et al. 1998; Rowland et al. 1999).

Metabolism as a confounder or additional variable in efficacy studies: chemical forms in the plasma. In most subjects the plasma curves for isoflavones show a double peak consisting of a sharp early peak followed by a later larger and more rounded peak, representing a combination of entero-hepatic recycling, together with absorption in the duodenum–jejunum followed by (higher) absorption in the colon (King & Bursill, 1998; Setchell, 1998; Day et al. 2000). Isoflavones are deconjugated during absorption (Day et al. 2000; Setchell et al. 2002b; Wilkinson et al. 2003) and are found in plasma as conjugates of sulfate and glucuronic acid. Genistein glucuronide exhibits a later time to reach maximum plasma concentration than genistin sulfate, daidzin sulfate and daidzein glucuronide, and both genistin conjugates show a longer half-life than the corresponding daidzin conjugates (Shelnutt et al. 2002). The frequency of isoflavone consumption has no effect on the proportion of glucuronides, sulfates and aglycones of genistin and daidzein in plasma or urine (Zhang et al. 2003). The relative biological activities of the isoflavone conjugates in the plasma are dependent on the chemical structure (Turner et al. 2004).

Bioavailability in human intervention studies. It can be concluded that understanding the bioavailability of the isoflavones in a given foodstuff is essential before embarking on a study of the clinical effects. Most importantly, it is essential to measure accurately the amount and form of isoflavones at the point of consumption during the study, and to check this information at regular intervals. Taken together, factors such as glycoside v. aglycone, food source, the ‘equol producer’ state of the volunteers and the influence of other foods eaten during the study can determine whether a significant biological or clinical effect is observed, or not.

Health effects: bone

Consensus statement for effects on bone: grade A recommendation. As a result of limited relevant studies and differences in methodological approaches there is a suggestion, but no conclusive evidence, that isoflavones from the sources studied so far have a beneficial effect on bone health. A recommendation cannot therefore be made at the current time and there is a need for further high-quality long-term (>1 year) studies to clarify the effect of phyto-oestrogens on bone.

Summary of data for bone health. Bone health is a major concern as women age, and femur or vertebral fractures may severely affect the quality of life. Hormone-replacement therapies have been the first line of treatment of hormone-related osteoporosis, but major side effects preclude their universal use. The observation that South-East Asian women report a lower occurrence of osteoporosis has led to the hypothesis that soyabean or soyabean phyto-oestrogens are a possible alternative option for the prevention of osteoporosis. Thirty-one studies have examined the effects of phyto-oestrogens on bone mass or bone turnover, or both, but only six studies met the inclusion criteria (Potter et al. 1998; Clifton-Bligh et al. 2001; Morabito et al. 2002; Chen et al. 2003; Gallagher et al. 2004; Kreijkamp-Kaspers et al. 2004; Table 4). Three of these studies used pure compounds or extracts and three studies provided foods containing soyabean protein or SPI.

Of the three studies that used soyabean isoflavone extracts or pure genistin, two are suggestive of an effect on bone mineral density at doses ranging from 35 to 54 mg aglycone equivalents. Only one of the three studies performed with SPI shows an effect on bone mineral density at a dose of 56 mg aglycone equivalents, while the two other studies show no effect with doses ranging from 4 to 103 mg aglycone equivalents. The age since menopause does not appear to influence the effect of phyto-oestrogens on bone mineral density or bone mineral content in all studies.

Only three of the identified studies included biomarkers of bone formation or bone resorption. One study, which used SPI (Gallagher et al. 2004), shows no effect on bone markers, and the study that used genistin (54 mg) shows
Table 4. Effects of soyabean isoflavones on bone biomarkers and bone density

<table>
<thead>
<tr>
<th>Reference</th>
<th>Age (years)*</th>
<th>Subjects completed</th>
<th>Study design</th>
<th>Duration (months)</th>
<th>Isoflavone source</th>
<th>Daily dose (mg aglycone equivalents)</th>
<th>Bone biomarkers</th>
<th>Bone density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soyabean protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SPI</td>
<td>2, 32, 60</td>
<td>BF (BGP, BALP)</td>
<td>No effect</td>
</tr>
<tr>
<td>Gallagher et al.</td>
<td>40–62</td>
<td>50</td>
<td>Pa</td>
<td>9</td>
<td>SPI</td>
<td>35, 56</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>(2004)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SPI</td>
<td>NTx</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Potter et al.</td>
<td>49–83</td>
<td>66</td>
<td>Pa</td>
<td>6</td>
<td>SPI</td>
<td>35, 56</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>(1998)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SPI</td>
<td>NA</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Kreijkamp-Kaspers et al.</td>
<td>60–75</td>
<td>175</td>
<td>Pa</td>
<td>12</td>
<td>Soyabean-germ extract</td>
<td>25, 50</td>
<td>NA</td>
<td>No effect</td>
</tr>
<tr>
<td>(2004)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SPI</td>
<td>NTx</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Chen et al.</td>
<td>48–62</td>
<td>175</td>
<td>Pa</td>
<td>12</td>
<td>RC extract</td>
<td>17, 35, 53</td>
<td>BR (fDPD): no effect</td>
<td>No effect</td>
</tr>
<tr>
<td>(2003)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SPI</td>
<td>99</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Clifton-Bligh et al.</td>
<td>57 (so 5)</td>
<td>46</td>
<td>Pa</td>
<td>6</td>
<td>RC extract</td>
<td>17, 35, 53</td>
<td>BR (fDPD): no effect</td>
<td>No effect</td>
</tr>
<tr>
<td>(2001)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RC extract</td>
<td>99</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Morabito et al.</td>
<td>47–57</td>
<td>90</td>
<td>Pa</td>
<td>12</td>
<td>Genistein</td>
<td>54</td>
<td>BF (BGP, BALP)</td>
<td>0.5% for BMD for hip and spine</td>
</tr>
<tr>
<td>(2002)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Genistein</td>
<td>99</td>
<td>No effect</td>
<td>No effect</td>
</tr>
</tbody>
</table>

Pa, parallel; BF, bone formation; BGP, bone Gla protein (osteocalcin); BALP, bone alkaline phosphatase; BR, bone resorption; NTx, urinary N telopeptide; NA, not available; BMD, bone mineral density; BMC, bone mineral content; fPyd, free pyridinoline; fDpd, free deoxypyridinoline; SPI, soyabean-protein isolate; RC, red clover (Trifolium pratense L); †, increase; ‡, decrease.

*Values shown are ranges and/or means and standard deviations.

an increase in bone formation biomarkers and a reduction in bone resorption biomarkers.

Health effects: cardiovascular effects

Consensus statement for cardiovascular effects: grade A recommendation. On the basis of the available evidence, the panel has concluded that the consumption of whole-soyabean foods and SPI has some beneficial effects on lipid markers of cardiovascular risk in healthy post-menopausal women. The consumption of isolated isoflavones does not affect blood lipid levels or blood pressure, although it may improve endothelial function.

Adequately-powered human intervention studies that can definitively establish the benefits of either encapsulated isoflavones or isoflavone-fortified foods are needed, particularly in women at high risk.

Summary of data for cardiovascular effects. Epidemiological evidence from human subjects suggests that high soyabean consumption, the main dietary source of isoflavones, is cardioprotective. To date the predominant interest has been in relation to the hypercholesterolaemic effects, stimulated by the significant lipoprotein effects observed in the meta-analysis of Anderson et al. (1995). More recently, two further meta-analyses have investigated the effects of soyabean foods, soyabean protein and soyabean-isoflavone extracts on serum lipids (Weggemans & Trautwein, 2003; Yeung & Yu, 2003). In a meta-analysis of seventeen randomised trials (Yeung & Yu, 2003) it has been found that isoflavone extracts (encapsulated) have no effects on serum lipids. Furthermore, since the studies of soyabean foods and SPI were heterogeneous in design and not statistically well-powered it is, therefore, not possible to draw conclusions on the lipid-lowering benefits of isoflavones contained in soyabean. Weggemans & Trautwein (2003) have analysed the independent effects of soyabean-associated isoflavones on plasma LDL- and HDL-cholesterol in ten randomised controlled studies and have concluded that differences in isoflavone content of soyabean protein are not associated with concentrations of these lipoproteins. Thus, currently, there is uncertainty about extent of the contribution of soyabean isoflavones to the cholesterol-lowering effects that are reported in controlled trials of soyabean and soyabean products. However, data from in vitro and animal experiments are emerging that suggests that isoflavones may be cardioprotective by mechanisms independent of blood lipids (see review, Hall et al. 2004), but these underlying mechanisms are only partly understood. As a result, more recently, attention has focused on the potential effects of phyto-oestrogens on blood pressure, in vivo measures of vascular function such as flow-mediated dilation and novel biomarkers of CVD risk, and to date these studies have not been systematically reviewed.

Of the sixty-three studies conducted to date forty studies have used soyabean foods or soyabean-protein extracts or
SPI and twenty-three have used isoflavone extracts from soyabean or other sources (e.g. red clover). Following grading of evidence three soyabean food studies, thirteen SPI studies and fifteen isoflavone-extract studies were included for further review. These data suggest that soyabean food and soyabean protein interventions may have a beneficial effect on lipoprotein status (Table 5), while there is limited data to support a lipid-lowering effect of isoflavone extracts. The data on the effects of soyabean foods and soyabean protein on blood pressure are equivocal, but it is clear that there is no evidence for an effect of isoflavone extracts on blood pressure.

Although there is growing interest in the potential direct effects of isoflavones on the arterial wall, only limited studies matching the present criteria have been conducted to date and the available data are inconclusive (Table 6). Increased flow-mediated dilation (Cuevas et al. 2003), decreased brachial artery peak flow velocity (Steinberg et al. 2003) and improved peripheral vascular resistance (Teede et al. 2001) have been demonstrated following soyabean protein, although not all studies are in agreement in their findings (Blum et al. 2003b). Some isoflavone extract studies have found positive effects on in vivo endothelial function measurements (Nestel et al. 1997, 1999; Squadrito et al. 2002, 2003; Teede et al. 2003). Other studies, however, have found no effect of isoflavones on in vivo endothelial function (Simons et al. 2000; Lissin et al. 2004). Other, more novel, biomarkers of CVD risk have been assessed in some studies included in the present analysis, such as inflammatory factors, coagulatory and fibrinolytic factors and markers of LDL oxidation (Table 7; Jenkins et al. 2002; Steinberg et al. 2003; Krebs et al. 2004; Hall et al. 2005; Teede et al. 2005). However, the number of these studies is very low and no conclusions can be drawn from the data available to date.

Health effects: climacteric symptoms

Consensus statement on climacteric symptoms: grade A recommendation. There is currently limited evidence that SPI, soyabean foods or red-clover extract are effective in reducing menopausal symptoms. Soyabean-isoﬂavone extracts may be effective in reducing hot flushes but the effect is about half that observed with hormone-replacement therapy and similar to that of other non-hormonal pharmacological therapies.

Summary of data for climacteric symptoms. Epidemiological evidence suggests that there is wide international variability in the reporting of menopausal symptoms. In particular, populations consuming soyabean as a staple have a substantially reduced incidence compared with Western populations (Lock, 1993). Three systematic reviews on the effects of isoflavones and other herbal remedies on menopausal symptoms have so far been published (Huntley & Ernst, 2003, 2004; Krebs et al. 2004). However, one review suggests some efficacy of soyabean preparations (Huntley & Ernst, 2004), the second does not support the hypothesis that isoflavones from a range of sources improve menopausal symptoms (Krebs et al. 2004), while the third focuses on reviewing trials using herbal remedies but only includes red clover as a source of isoflavones (Huntley & Ernst, 2003). The effects of all sources of phyto-oestrogens on menopausal symptoms were therefore further evaluated using the criteria set out earlier (Table 8). The main outcome measure in the studies was the change in the number of hot flushes following intervention, which was either qualitatively assessed (e.g. using the Kupperman (1953) or Greene (1998) climacteric scale) or quantified using diaries. Some of the studies also quantified the effects of phyto-oestrogen sources on the vaginal mucous, either subjectively by questioning the subject’s perception of vaginal dryness or objectively using a vaginal maturation index (as a measure of oestrogenicity).

In total, twenty-four studies have examined the effect of phyto-oestrogens on the incidence and severity of hot flushes in peri- and post-menopausal women. Following review only sixteen studies met the inclusion criteria (Table 8). Four of these studies used SPI (Albertazzi et al. 1998; Kotsopoulos et al. 2000; Knight et al. 2001; St Germain et al. 2001) in doses ranging from 28 to 85 mg aglycone equivalent (duration range 3–6 months), of which three have observed no effects on the incidence of hot flushes or vaginal dryness (Kotsopoulos et al. 2000; Knight et al. 2001; St Germain et al. 2001). Only one of the four studies has reported a decrease in hot flushes (Albertazzi et al. 1998) and only one study has reported a beneficial effect on vaginal dryness (Kotsopoulos et al. 2000). The study that used a soyabean beverage has reported no change in menopausal symptoms following intervention (Van Patten et al. 2002). Three studies using red clover as a source of isoflavones have indicated no effect either on the incidence of hot flushes or vaginal epithelial cell maturation (Baber et al. 1999; Knight et al. 1999; van de Weijer & Barentsen, 2002). Seven studies have been performed using isolated isoflavones at doses ranging from 32 to 72 mg aglycones equivalents/d (Scambia et al. 2000; Upmalis et al. 2000; Faure et al. 2002; Han et al. 2002; Nikander et al. 2003; Penotti et al. 2003; Petri Nahas et al. 2004) and one using purified genistein (Crisaﬁlli et al. 2004). Six of the studies have observed a significant reduction in the occurrence of hot flushes (Scambia et al. 2000; Upmalis et al. 2000; Faure et al. 2002; Han et al. 2002; Crisafulli et al. 2004; Petri Nahas et al. 2004), but

**Table 5.** An overview of the effects of different soyabean products on lipoprotein status and blood pressure in post-menopausal women*

(The values represent the relative no. of trials showing an effect)

<table>
<thead>
<tr>
<th>Soyabean product</th>
<th>Lipoprotein status</th>
<th>Blood pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soya foods</td>
<td>3/3</td>
<td>0/1</td>
</tr>
<tr>
<td>Soyabean protein</td>
<td>9/13</td>
<td>2/3</td>
</tr>
<tr>
<td>Isoflavone extracts</td>
<td>2/12</td>
<td>0/5</td>
</tr>
</tbody>
</table>

*Trials reviewed: Atkinson et al. (2004a); Blum et al. (2003a,b); Campbell et al. (2004); Cuevas et al. (2003); Desnoches et al. (2004); Dewell et al. (2002); Gardner et al. (2001); Han et al. (2002); Hodgson et al. (1996, 1999a,b); Howes et al. (2000); Lichtenstein et al. (2002); Lissin et al. (2004); Mackey et al. (2000); Nestel et al. (1997, 1999); Nikander et al. (2003); Puska et al. (2002); Simons et al. (2000); Squadrito et al. (2002, 2003); Steinberg et al. (2003); Teede et al. (2001, 2003, 2005); Vigna et al. (2000); Wangen et al. (2001); Washburn et al. (1999).
<table>
<thead>
<tr>
<th>Reference</th>
<th>Age (years)*</th>
<th>Subjects completed</th>
<th>Study design</th>
<th>Duration</th>
<th>Isoflavone source</th>
<th>Isoflavone dose (mg aglycone equivalents/d)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blum et al. (2003b)</td>
<td>55</td>
<td>24</td>
<td>RDBP CO</td>
<td>6 weeks per arm</td>
<td>SPI</td>
<td>Not reported</td>
<td>NC in arterial diameter or vasodilatation</td>
</tr>
<tr>
<td>Cuevas et al. (2003)</td>
<td>47–70</td>
<td>18</td>
<td>RDBP CO</td>
<td>4 weeks per arm; no wash-out</td>
<td>SPI</td>
<td>80 (60% genistein, 30% daidzein, 10% glycitein)</td>
<td>↑ in FMD</td>
</tr>
<tr>
<td>Steinberg et al. (2003)</td>
<td>55 (SD 1)</td>
<td>28</td>
<td>RDBP CO</td>
<td>6 weeks per arm</td>
<td>SPI, isoflavone-depleted soyabean protein, or total milk protein</td>
<td>Total isoflavones 107 (genistein 55, daidzein 47, glycitein 5)</td>
<td>↓ in brachial artery peak flow velocity</td>
</tr>
<tr>
<td>Teede et al. (2003)</td>
<td>50–75</td>
<td>83</td>
<td>RDBP Pa</td>
<td>3 months</td>
<td>SPI v. casein drink</td>
<td>118 (genistein 76, daidzein 37, glycitein 5)</td>
<td>NC in arterial compliance nor FMD</td>
</tr>
<tr>
<td>Lissin et al. (2004)</td>
<td>62 (SD 8)</td>
<td>40</td>
<td>RDBP Pa</td>
<td>6 weeks</td>
<td>90 mg SI extract/d in tablets</td>
<td>Genistein 40-9, daidzein 40-9, glycitein 8-2</td>
<td>NC in FMD ↑ in endothelium-independent vasodilation.</td>
</tr>
<tr>
<td>Nestel et al. (1997)</td>
<td>54 (SD 6)</td>
<td>14</td>
<td>RSBP CO</td>
<td>15 weeks per arm</td>
<td>80 mg SI extract/d in tablets</td>
<td>Genistein 45, daidzein 33, glycitein 2</td>
<td>↑ in arterial compliance but NC in forearm blood flow or arterial pressure (including seven pre-menopausal women as well as fourteen PMW; no separate analysis).</td>
</tr>
<tr>
<td>Nestel et al. (1999)</td>
<td>56 (SD 7)</td>
<td>17</td>
<td>RDBP Pa</td>
<td>15 weeks</td>
<td>RC extract in tablets</td>
<td>Placebo, 40 mg isoflavone and 80 mg isoflavone in tablets sequentially for 5 weeks each (n 14), or placebo for 15 weeks (n 3)</td>
<td>40 mg tablet; genistein 4, daidzein 3-5, biochanin A 24-5, formononetin 8</td>
</tr>
<tr>
<td>Simons et al. (2000)</td>
<td>50–70</td>
<td>20</td>
<td>RDBP CO</td>
<td>8 weeks per arm</td>
<td>Isoflavone tablets (source not specified)</td>
<td>80</td>
<td>NC in FMD</td>
</tr>
<tr>
<td>Squadrito et al. (2002)</td>
<td>52–60</td>
<td>60</td>
<td>RDBP Pa</td>
<td>6 months</td>
<td>Genistein</td>
<td>54</td>
<td>↑ in nitrites or nitrates ↓ in endothelin-1 ↑ in brachial artery FMD</td>
</tr>
<tr>
<td>Squadrito et al. (2003)</td>
<td>52–60</td>
<td>79</td>
<td>RDBP Pa</td>
<td>12 months</td>
<td>Genistein</td>
<td>54</td>
<td>↑ in nitrites or nitrates ↓ in endothelin-1 ↑ in brachial artery FMD</td>
</tr>
<tr>
<td>Teede et al. (2003)</td>
<td>54 (SD 0-7)</td>
<td>34</td>
<td>RDBP CO</td>
<td>6 weeks per arm</td>
<td>80 mg RC isoflavon/d in tablets</td>
<td>Biochanin 80 (n 40) or formononetin 80 (n 40) v. placebo</td>
<td>↑ in arterial compliance following formononetin in thirty-four PMW NC in FMD</td>
</tr>
</tbody>
</table>

SPI, soyabean-protein isolate; SI, soyabean protein; RC, red clover (Trifolium pratense L.); RDBP, randomised double-blind placebo-controlled; RSBP, randomised single-blind placebo-controlled; Pa, parallel; CO, cross-over trial; FMD, flow-mediated dilatation; NC, no change; PMW, post-menopausal women; ↑, increase; ↓, decrease.

*Values shown are ranges or means and standard deviations.
Table 7. Effects of soyabean isoflavones on circulating inflammatory and haemostatic factors

<table>
<thead>
<tr>
<th>Reference</th>
<th>Age (years)*</th>
<th>Subjects completed</th>
<th>Study design</th>
<th>Duration of study</th>
<th>Isoflavone source</th>
<th>Isoflavone dose (mg aglycone equivalents/d)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hall et al. (2005)</td>
<td>45–70</td>
<td>117</td>
<td>RDBP CO</td>
<td>8 weeks per arm</td>
<td>50 mg/d; soyabean isoflavone-enriched cereal bars</td>
<td>Genistein 33, daidzein 17</td>
<td>↓ in CRP NC in ICAM-1, VCAM-1, E-selectin, vWF, MCP-1 or endothelin-1</td>
</tr>
<tr>
<td>Huntley &amp; Ernst (2004)</td>
<td>117 PMW, twenty-six peri-menopausal women</td>
<td>43.5 mg/d; RC isoflavone tablets</td>
<td>Biochanin A 26, formononetin 16, genistein 1, daidzein 0-5</td>
<td>NC in fibrinogen ↓ in PAI-1 (but peri-menopausal only)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jenkins et al. (2002)</td>
<td>62 (so 2; including twenty-three men)</td>
<td>18</td>
<td>RSBP CO</td>
<td>4 weeks per arm</td>
<td>Three diets: low-isoavone or high-isoavone soya foods; or control dairy and egg-protein diet</td>
<td>Mean daily intake: low-isoavone (10), high-isoavone (73)</td>
<td>High isoavone: ↑ in IL-6 NC in CRP, serum amyloid A or TNFα</td>
</tr>
<tr>
<td>Krebs et al. (2004)</td>
<td>24</td>
<td>25 g soyabean protein v. milk protein</td>
<td>NA</td>
<td></td>
<td></td>
<td>NC in soluble IL-2 receptor, E-selectin, P-selectin, ICAM-1 or VCAM</td>
<td></td>
</tr>
<tr>
<td>Squadrito et al. (2002)</td>
<td>52–60</td>
<td>60</td>
<td>RDBP Pa</td>
<td>6 months</td>
<td>54 mg genistein/d in tablets (n 30) v. placebo (n 30)</td>
<td>Genistein 54</td>
<td>↑ in nitrates or nitrates ↓ in endothelin-1</td>
</tr>
<tr>
<td>Squadrito et al. (2003)</td>
<td>52–60</td>
<td>79</td>
<td>RDBP Pa</td>
<td>12 months</td>
<td>54 mg genistein/d in pills (n 27) v. HRT or placebo</td>
<td>Genistein 54</td>
<td>↑ in nitrates or nitrates ↓ in endothelin-1</td>
</tr>
<tr>
<td>Steinberg et al. (2003)</td>
<td>55 (so 1)</td>
<td>28</td>
<td>RDBP CO</td>
<td>6 weeks per arm</td>
<td>SPI, isoflavone-depleted soyabean protein or total milk protein</td>
<td>Total isoavones 107 (genistein 55, daidzein 47, glycitein 5)</td>
<td>NC in NO products, endothelin-1, E-selectin, VCAM-1, ICAM-1</td>
</tr>
<tr>
<td>Teede et al. (2003)</td>
<td>54 (so 0-7; including forty-six men)</td>
<td>34</td>
<td>RDBP CO</td>
<td>6 weeks per arm</td>
<td>80 mg/d; RC isoflavone tablets</td>
<td>Biochanin 80 (n 40) or formononetin 80 (n 40) v. placebo</td>
<td>↓ in VCAM-1 following formononetin in thirty-four PMW</td>
</tr>
<tr>
<td>Teede et al. (2005)</td>
<td>50–75</td>
<td>40</td>
<td>RDBP Pa</td>
<td>3 months</td>
<td>SPI drink v. casein drink</td>
<td>Total isoavones 118 (76 mg genistein, 37 mg daidzein, 5 mg glycitein)</td>
<td>↓ in factor VIIc NC in fibrin, PAI-1 and vWF</td>
</tr>
</tbody>
</table>

SPI, soyabean-protein isolate; RC, red clover (Trifolium pratense L.); RDBP, randomised double-blind placebo-controlled; RSBP, randomised single-blind placebo-controlled; Pa, parallel; CO, cross-over; NA, not available; HRT, hormone-replacement therapy; PMW, post-menopausal women; NC, no change; CRP, C-reactive peptide; BP, blood pressure; vWF, von Willebrand factor, PAI-1, plasmigogen-activator inhibitor-1; MCP-1, monocyte chemoattractant protein-1; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intracellular adhesion molecule-1; ↓↑, increase; ↓↓, decrease.*Values shown are ranges or means and standard deviations.
Table 8. Effects of soyabean isoflavones on climacteric symptoms

<table>
<thead>
<tr>
<th>Reference</th>
<th>Age (years)*</th>
<th>Subjects completed</th>
<th>Study design</th>
<th>Duration (weeks)</th>
<th>Isoflavone source</th>
<th>Isoflavone dose (mg aglycone equivalents/d)</th>
<th>Hot flushes</th>
<th>Other climacteric signs and symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albertazzi et al. (1998)</td>
<td>53 (range 45–2)</td>
<td>79</td>
<td>Pa</td>
<td>12</td>
<td>SPI</td>
<td>76</td>
<td>↓45%</td>
<td>→ on vaginal maturation index</td>
</tr>
<tr>
<td>St Germain et al. (2001)</td>
<td>50 (range 42–50)</td>
<td>58</td>
<td>Pa</td>
<td>24</td>
<td>SPI</td>
<td>28–38</td>
<td></td>
<td>→ on other climacteric symptoms</td>
</tr>
<tr>
<td>Kotsoopoulos et al. (2000)</td>
<td>59 (range 50–75)</td>
<td>75</td>
<td>Pa</td>
<td>12</td>
<td>SPI</td>
<td>71</td>
<td></td>
<td>↓ on vaginal dryness</td>
</tr>
<tr>
<td>Knight et al. (2001)</td>
<td>53</td>
<td>20</td>
<td>Pa</td>
<td>12</td>
<td>SPI</td>
<td>85</td>
<td></td>
<td>→ on vaginal maturation index</td>
</tr>
<tr>
<td>Van Patten et al. (2002)</td>
<td>55 (so 6-3)</td>
<td>123</td>
<td>Pa</td>
<td>12</td>
<td>Soya beverage</td>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RC extract</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baber et al. (1999)</td>
<td>54 (so 4-1)</td>
<td>43</td>
<td>Pa</td>
<td>12</td>
<td>RC extract</td>
<td>25</td>
<td></td>
<td>→ on vaginal maturation index</td>
</tr>
<tr>
<td>Knight et al. (1999)</td>
<td>55 (so 3-6)</td>
<td>35</td>
<td>Pa</td>
<td>12</td>
<td>RC extract</td>
<td>25–100</td>
<td></td>
<td>→ on vaginal maturation index</td>
</tr>
<tr>
<td>SI extract</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scambia et al. (2000)</td>
<td>54 (so 7-1)</td>
<td>39</td>
<td>Pa</td>
<td>6</td>
<td>SI extract</td>
<td>32</td>
<td>↓54%</td>
<td>→ on vaginal maturation index, climacteric symptoms</td>
</tr>
<tr>
<td>Upmais et al. (2000)</td>
<td>55 (so 4-4)</td>
<td>112</td>
<td>Pa</td>
<td>12</td>
<td>SI extract</td>
<td>32</td>
<td>↓28%</td>
<td>→ on vaginal maturation index</td>
</tr>
<tr>
<td>Han et al. (2002)</td>
<td>49 (so 1-2)</td>
<td>80</td>
<td>Pa</td>
<td>16</td>
<td>SI extract</td>
<td>63</td>
<td></td>
<td>↓ on Kupperman (1953) scale</td>
</tr>
<tr>
<td>Faure et al. (2002)</td>
<td>53 (so 4-8)</td>
<td>55</td>
<td>Pa</td>
<td>16</td>
<td>SI extract</td>
<td>38</td>
<td>↓61%</td>
<td>↓ in climacteric symptoms</td>
</tr>
<tr>
<td>Nikander et al. (2003)</td>
<td>54 (so 6)</td>
<td>56</td>
<td>CO</td>
<td>24</td>
<td>SI extract</td>
<td>72</td>
<td></td>
<td>→ in Kupperman (1953) scale</td>
</tr>
<tr>
<td>Penotti et al. (2003)</td>
<td>52 (so 2-4)</td>
<td>56</td>
<td>Pa</td>
<td>24</td>
<td>SI extract</td>
<td>45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Petri Nahas et al. (2004)</td>
<td>52 (so 5)</td>
<td>50</td>
<td>Pa</td>
<td>24</td>
<td>Soyabean-germ extract</td>
<td>38</td>
<td>↓44%</td>
<td>→ in vaginal maturation index</td>
</tr>
<tr>
<td>Crisafulli et al. (2004)</td>
<td>52 (range 47–57)</td>
<td>90</td>
<td>Pa</td>
<td>52</td>
<td>Genistein</td>
<td>50</td>
<td>↓24%</td>
<td></td>
</tr>
</tbody>
</table>

Pa, parallel; CO, cross-over; SPI, soyabean-protein isolate; SI, soyabean isoflavones; RC, red clover (*Trifolium pratense*L.); ↓, decrease; →, no effect.

*Values shown are means and standard deviations or ranges.
only two of the studies have observed a difference in the occurrence of other climacteric symptoms (Han et al. 2002; Petri Nahas et al. 2004). No change in vaginal dryness has been observed following intervention in any of the studies.

**Health effects: breast cancer**

**Consensus statement on breast cancer: grade C recommendation.** On the basis of the available evidence the panel has concluded that there is some epidemiological evidence of an association between lifelong soyabean intake and reduced risk of breast cancer in premenopausal and post-menopausal women. Although to date three double-blind RCT have been conducted, using mammographic density as a marker of breast cancer risk, none of these studies has examined effects in only post-menopausal women. Further studies are required to address the potential effect of soyabean on breast cancer and to address the current concerns of the potential risk–benefit profile of soyabean isoflavones for breast-cancer survivors.

**Summary of data on breast cancer.** Several observational epidemiological studies have examined the relationship between soyabean and breast cancer, relying on varying measures of soyabean intake, ranging from detailed analyses to crude estimates of the consumption of tofu. Eight case–control studies have examined the association between soyabean intake and breast cancer risk in Asian women in Singapore (Lee et al. 1991), Japan (Hirose et al. 1995; Yamamoto et al. 2003) and China (Yuan et al. 1995; Shu et al. 2001; Dai et al. 2002), and in Asian-Americans (Wu et al. 1996, 2002). Results from all these studies consistently suggest an inverse association for both premenopausal and post-menopausal breast cancer. In a meta-analysis (A Wolk, unpublished results) of these studies a significant 33% decreased risk of developing breast cancer has been observed for post-menopausal women (summary odds ratio 0·67 (95% CI 0·48, 0·93)) when comparing the highest consumption with the lowest consumption in the respective study populations.

Fewer epidemiological studies have been conducted in Caucasian women, given their limited exposure to soya-based products. In one case–control study of premenopausal women from Germany (Linseisen et al. 2004) and one prospective study of post-menopausal women from The Netherlands (den Tonkelaar et al. 2001) summary estimates of risk are odds ratios of 0·61 (95% CI, 0·43, 0·86) and 0·83 (95% CI, 0·45, 1·50) respectively. A recent Italian study has reported no association between isoflavones and breast cancer risk in premenopausal or post-menopausal women (Bosetti et al. 2005). The summary risk estimate indicates no association for post-menopausal women (summary odds ratio 0·96 (95% CI 0·89, 1·04) based on the Dutch (den Tonkelaar et al. 2001) and Italian (Bosetti et al. 2005) studies). The summary risk estimate from the meta-analysis of two prospective studies of total breast cancer (premenopausal and post-menopausal women combined) among women from UK (Grace et al. 2004) and The Netherlands (Keinan-Boker et al. 2004) as well as a case–control study from Australia (Ingram et al. 1997) is an odds ratio of 0·96 (95% CI, 0·71, 1·29).

To date two studies, one based in China (Shu et al. 2001) and the other in the USA (Wu et al. 2002), have assessed the impact of adolescent dietary exposure in Asian women on breast cancer risk. Both studies have found a strong significant inverse association between dietary soyabean and post-menopausal breast cancer risk, with odds ratios of 0·49 (95% CI 0·33, 0·74) and 0·41 (P = 0·007 for trend).

Two prospective nested case–control studies of pretreatment urine in Caucasian women have shown conflicting results; a non-significant inverse association between excreted genistin and breast cancer in Dutch women (den Tonkelaar et al. 2001), and a positive association between excreted equol and breast cancer risk (odds ratio 1·34 (95% CI 1·06, 1·70)) in English women (Grace et al. 2004). Case–control studies analysing urine collected after breast-cancer diagnosis have shown significant inverse associations; one for the metabolite equol (Ingram et al. 1997) and the other (in Asian women) in relation to total isoflavone excretion (Dai et al. 2002; odds ratios 0·27 (95% CI 0·1, 0·69) and 0·46 (95% CI 0·22, 0·95) respectively).

Two cross-sectional studies (Maskarinec & Meng, 2001; Jakes et al. 2002) have looked specifically at soyabean intake and its association with breast tissue density. Maskarinec & Meng (2001) have reported a higher density with higher soyabean intake and a non-significant reduction in dense area, while Jakes et al. (2002) have shown that higher soyabean intake is associated with lower risk mammographic patterns (odds ratio 0·44 (95% CI 0·18, 0·96).

To date three double-blind RCT have been conducted, two in premenopausal women (Maskarinec et al. 2003, 2004) and one with combined menopausal status (Atkinson et al. 2004b). No significant differences in mammographic characteristics that could be attributed to the soyabean intervention were observed. However, it is possible that the association between soyabean intake and breast density is different in post-menopausal women, among those women with a strong family history of the disease or in women with polymorphisms in genes that encode oestrogen-metabolising enzymes.

**Health effects: colon cancer**

**Consensus statement on colon cancer: grade C recommendation.** The inconsistent results obtained by epidemiological studies provide no general support for the hypothesis that frequent ingestion of soyabean reduces the risk of colo-rectal cancer.

**Summary of the data on colon cancer.** Although the incidence of colo-rectal cancer is markedly lower in many Asian countries compared with Western populations, there is limited support from epidemiological studies for a potential protective role for soyabean and its isoflavones. Numerous epidemiological studies, predominantly using a case–control design, have examined the relationship between intake of different soyabean foods and colo-rectal cancer risk, but the data are contradictory (Haenszel et al. 1973; Watanabe et al. 1984; Tajima & Tominaga, 1985; Hirayama, 1990; Hu et al. 1991; Hoshiyama et al. 1993;

In relation to colonic adenoma development two studies have examined the effect of the consumption of soyabean food on polyp growth (Kono et al. 1993; Witte et al. 1996). Although the findings from both studies are suggestive of an inverse relationship between the intake of miso soup (Kono et al. 1993) or tofu (Witte et al. 1996) and the development of colo-rectal adenomas, the relationship was not found to be significant.

**Health effects: cognitive function**

Consensus statement on cognitive function: grade B recommendation. As there are few available studies, it is not possible to draw a conclusion on the effect of soyabean products or isoflavones on cognitive function in post-menopausal women.

**Summary of the data on cognitive function.** To date, three studies (Kritz-Silverstein et al. 2003; Howes et al. 2004; Kreijkamp-Kaspers et al. 2004) have examined the potential effects of phyto-oestrogens on cognitive function in post-menopausal women (Table 9). The study duration ranged from 6 to 12 months and the dose of isoflavones fed daily ranged from 32 to 69 mg aglycone equivalents. The study with red clover (Kreijkamp-Kaspers et al. 2004) and the study with SPI (Howes et al. 2004) do not show any improvement in a range of cognitive tests such as verbal memory, digit span (a measure of short-term memory) and verbal fluency. The findings of the study performed with soyabean isoflavone extracts suggest an improvement in the category fluency (Kritz-Silverstein et al. 2003).

**Health effects: diabetes**

Consensus statement on the effect on diabetes: grade C recommendation. There are limited studies that have specifically focused on diabetic women, but the available evidence suggests that there may be an effect of soyabean on diabetes. However, more studies of longer duration are needed to confirm this finding and to determine what active components may be causing the changes.

**Summary of data on diabetes.** The prevalence of diabetes is reaching epidemic proportions and is increasing in parallel with obesity. Cardiovascular mortality is up to five times higher in women with diabetes compared with those without diabetes, and the cardiovascular mortality rate is increasing in diabetic women (Hu et al. 2001; Collins et al. 2003; Bibbins-Domingo et al. 2004). Women who develop diabetes lose their cardiovascular protection from oestrogen, and post-menopausal oestrogen loss may contribute to a high risk of accelerated CVD.

Ten studies have examined the effect of phyto-oestrogens on post-menopausal diabetes. Of these studies only four (Hermansen et al. 2001; Jayagopal et al. 2002; Howes et al. 2003; Li et al. 2005) met the inclusion criteria of being focused on glycaemic control, but each study used different variables and they are therefore difficult to compare (Table 10). In addition, two studies used a mixed population of men and women (Hermansen et al. 2001; Li et al. 2005), whilst only two studies have focused on
<table>
<thead>
<tr>
<th>Reference</th>
<th>Age (years)</th>
<th>Subjects completed</th>
<th>Subjects characteristics</th>
<th>Study design</th>
<th>Duration</th>
<th>Isoflavone source</th>
<th>Lipid profile</th>
<th>Insulin resistance markers</th>
<th>Other changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hermansen et al. (2001)</td>
<td>63·6±7·5</td>
<td>20</td>
<td>Fourteen M, six W T2DM (mean duration 3·0 (so 2·7) years</td>
<td>RDB CO</td>
<td>15 weeks</td>
<td>50 g soyabean protein/ d+&gt;165 mg isoflavones’ d=20 g soyabean-cotyledon fibre/d v=50 g casein/d +20 g cellulose</td>
<td>↓ in triacylglycerols ↓ in LDL cholesterol ↓ in apoB100 ↓ in LDL : HDL NC in HDL, apoA1 or total cholesterol</td>
<td>NC in insulin, glucose levels or HbA1c ↓ in homocysteine NC in PA1-1, fibrinogen, factor VIIc or vWfactor</td>
<td></td>
</tr>
<tr>
<td>Jayagopal et al. (2002)</td>
<td>62·5±6·8</td>
<td>32</td>
<td>Thirty-two W (post-menopausal) T2DM on diet alone Last period &gt;1 year ago</td>
<td>RDB CO</td>
<td>26 weeks</td>
<td>30 g soyabean protein/d+132 mg soyabean isoflavones’ d v=30 g microcrystalline cellulose</td>
<td>↓ in total cholesterol ↓ in LDL-cholesterol ↓ LDL : HDL NC in triacylglycerols or HDL</td>
<td>↓ in fasting insulin ↓ in HOMA-IR NC in glucose levels ↓ in serum free thyroxine</td>
<td></td>
</tr>
<tr>
<td>Howes et al. (2003)</td>
<td>62·0±2·0</td>
<td>16</td>
<td>Nineteen W T2DM on diet or oral hypoglycaemic agents</td>
<td>RDB CO</td>
<td>8 weeks</td>
<td>50 mg red clover isoflavones v: placebo</td>
<td>NC in plasma lipoproteins</td>
<td>NC in HbA1c</td>
<td>↓ in systolic and diastolic BP ↑ in forearm vascular resistance after LNMMA</td>
</tr>
<tr>
<td>Li et al. (2005)</td>
<td>55·2±9·8</td>
<td>77</td>
<td>Thirty-one W, fifty-two M T2DM on oral antidiabetic therapy</td>
<td>R Pa</td>
<td>12 months</td>
<td>Soya-based meal replacement v: individualised diet plan</td>
<td>↓ in plasma glucose ↓ in HbA1c at 3 and 6 months but not at 12 months</td>
<td>NC in CRP</td>
<td></td>
</tr>
</tbody>
</table>

M, men; W, women; T2DM, type 2 diabetes; CO, cross-over; R, randomised; RDB, randomised double-blind; Pa, parallel; NC, no change; HbA1c, glycosylated Hb; vWF von Willebrand factor; HOM-IR, homeostasis model assessment index of insulin resistance; PAI-1, plasminogen-activator inhibitor; BP, blood pressure; LNMMA, NG-monomethyl-L-arginine acetate; CRP, C-reactive peptide.
post-menopausal women alone (Jayagopal et al. 2002; Howes et al. 2003), and these latter two studies used markedly different isoflavone preparations. Hermansen et al. (2001), using 50 g soyabean protein plus 160 mg isoflavone, have shown no change in glycaemic indices, whilst Howes et al. (2003) have shown no change in overall glycaemic control using 50 mg red-clover isoflavone alone. Conversely, Jayagopal et al. (2002), using 30 g soyabean protein plus 132 mg isoflavone, have shown a reduction in fasting insulin, glycosylated Hb and insulin resistance. Li et al. (2005), using a soyabean-based meal replacement, have shown a reduction in plasma glucose and glycosylated Hb at 3 and 6 months, an effect that is lost at 12 months. These studies suggest that a combination of soyabean protein and isoflavones could have a positive effect on diabetes control, although isoflavones alone may not be effective and not all studies are positive. It is unclear which component(s) is active, and indeed it may be the soluble fibre alone that is beneficial (Chandalia et al. 2000).

Summary and conclusions

The panel has concluded that because of the limited number of appropriately-designed studies and differences in methodological approaches there is no conclusive evidence on the different health aspects. However, the revision and weighing of the available evidence has led the panel to provide the following conclusions and recommendations, each one with its associated grading:

1. there is a suggestion but no conclusive evidence that isoflavones from the sources studied so far have a beneficial effect on bone health: grade A;
2. the consumption of whole-soyabean foods and SPI has beneficial effects on lipid markers of cardiovascular risk in healthy post-menopausal women. The consumption of isolated isoflavones does not affect blood lipid levels or blood pressure, although it may improve endothelial function: grade A;
3. for menopausal symptoms, there is currently limited evidence that SPI, soyabean foods or red-clover extract are effective but soyabean-isoflavone extracts may be effective in reducing hot flushes: grade A;
4. there is some epidemiological evidence of an association between lifelong soyabean intake and reduced risk of breast cancer in premenopausal and post-menopausal women. Although to date three double-blind RCT have been conducted, using mammographic density as a marker of breast cancer risk, none of these studies has examined effects in only post-menopausal women: grade C;
5. based on available evidence the consumption of soyabean does not lead to reduced risk of colo-rectal cancer: grade C;
6. based on available evidence soyabean products or isoflavones do not have an effect on cognitive function in post-menopausal women: grade B;
7. based on available evidence soyabean consumption may reduce the risk of diabetes: grade C.

In conclusion, the use of soyabean products and soyabean isoflavones may be beneficial in post-menopausal women for bone, cardiovascular risk and hot flushes. However, the benefits are subtle and do not appear in all individuals. There are too few RCT studies to reach conclusions on the effects of isoflavones on breast cancer, colon cancer, diabetes or cognitive function.

Recommendations for research

Understanding the bioavailability of isoflavones in a given foodstuff will be important in the interpretation of the results of studies of clinical effects. Most importantly, it is essential to measure accurately the amount and form of isoflavones at the point of consumption during the study, and to check this information at regular intervals. Together, factors such as glycoside v. aglycone, food source, the ‘equol-producer’ state of the volunteers and the influence of other foods eaten during the study can determine whether a significant biological or clinical effect is observed, or not.

Adequately-powered human intervention studies that can definitely establish the benefits of either encapsulated isoflavones or isoflavone-fortified foods on clinical outcomes such as the incidence of heart disease and bone fractures are needed.

Appropriately-designed studies are required to examine the effects of soyabean and soyabean isoflavones on breast cancer, diabetes, colo-rectal cancer and cognitive function.

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