Does genotype and equol-production status affect response to isoflavones? Data from a pan-European study on the effects of isoflavones on cardiovascular risk markers in post-menopausal women

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The increase in CVD incidence following the menopause is associated with oestrogen loss. Dietary isoflavones are thought to be cardioprotective via their oestrogenic and oestrogen receptor-independent effects, but evidence to support this role is scarce. Individual variation in response to diet may be considerable and can obscure potential beneficial effects in a sample population; in particular, the response to isoflavone treatment may vary according to genotype and equol-production status. The effects of isoflavone supplementation (50 mg/d) on a range of established and novel biomarkers of CVD, including markers of lipid and glucose metabolism and inflammatory biomarkers, have been investigated in a placebo-controlled 2 × 8-week randomised cross-over study in 117 healthy post-menopausal women. Responsiveness to isoflavone supplementation according to (1) single nucleotide polymorphisms in a range of key CVD genes, including oestrogen receptor (ER) \( \alpha \) and \( \beta \) and (2) equol-production status has been examined. Isoflavones supplementation was found to have no effect on markers of lipids and glucose metabolism. Isoflavones improve C-reactive protein concentrations but do not affect other plasma inflammatory markers. There are no differences in response to isoflavones according to equol-production status. However, differences in HDL-cholesterol and vascular cell adhesion molecule 1 response to isoflavones vs. placebo are evident with specific ER\( \beta \) genotypes. In conclusion, isoflavones have beneficial effects on C-reactive protein, but not other cardiovascular risk markers. However, specific ER\( \beta \) gene polymorphic subgroups may benefit from isoflavone supplementation.

**Isoflavones: CVD: Post-menopausal women: Oestrogen receptor \( \beta \): Single nucleotide polymorphisms: Nutrient–gene interaction**

Oestrogen loss at the menopause has been associated with increased risk of CVD that is partly attributed to an adverse lipoprotein profile and arterial dysfunction. Hormone-replacement therapy (HRT) has been widely used to counteract the adverse effects of oestrogen deficiency. However, the recently reported lack of HRT efficacy in relation to CVD progression (Nelson et al. 2002) and evidence of adverse effects (Cushman et al. 1999; Manning et al. 2002) has led to increased interest in alternative therapies such as isoflavones. Isoflavones are phyto-oestrogens bearing a similar structure to mammalian oestrogen and, therefore, could act as oestrogen mimics or selective oestrogen-receptor (ER) modulators. It is not clear from the present literature whether isoflavones have lipid-lowering properties or if they have any effect on novel cardiovascular risk factors such as inflammatory biomarkers or markers of endothelial dysfunction. Furthermore, it is not known whether variability in response to

**Abbreviations:** CRP, C-reactive protein; ER, oestrogen receptor; HDL-C, HDL-cholesterol; HRT, hormone-replacement therapy; VCAM-1, vascular cell adhesion molecule 1.

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isoflavones exists among individuals according to genotype or according to their capacity for equol production, since equol is thought to be one of the active isoflavone metabolites.

The present paper will give a brief review of the current literature concerning the effects of isoflavones on markers of lipid and glucose metabolism and inflammatory biomarkers in men and women, and will present the results of a pan-European study (ISOHEART) that has investigated the effects of isoflavone supplementation on established and novel risk markers for CVD in a large group of healthy post-menopausal women, including the analysis of differences in response to isoflavones according to single nucleotide polymorphisms in key genes relevant to CVD and according to equol-production status.

CVD and menopause

Susceptibility to CVD can be partially characterised by a range of risk factors, including impaired lipid and glucose metabolism and endothelial dysfunction. For example, elevated triacylglycerol and LDL-cholesterol and decreased HDL-cholesterol (HDL-C) levels are associated with increased risk of CVD (Hopkins et al. 2005). High lipoprotein (a) concentrations also seem to be an independent risk factor for CVD (Burman et al. 2004). Disturbed glucose and insulin metabolism, which is a feature of the metabolic syndrome, is associated with subsequent development of diabetes and CVD (Lakka et al. 2002). Endothelial dysfunction, characterised by a chronic vascular inflammation and increased expression of cell adhesion molecules such as intracellular cell adhesion molecule 1, vascular cell adhesion molecule 1 (VCAM-1), E-selectin and chemokines such as monocyte chemotactic protein 1, is also considered to be predictive of cardiovascular risk (Hwang et al. 1997). Other markers of inflammation, including C-reactive protein (CRP) and von Willebrand factor (which plays an important role in platelet aggregation and adhesion), are also considered to be predictive biomarkers of coronary risk (Blann et al. 1993; Ridker et al. 2000). Furthermore, increased concentrations of the vasoconstrictor molecule, endothelin 1, indicate endothelial dysfunction associated with chronic inflammation (Tousoulis et al. 2005).

The decreased ovarian function and subsequent oestrogen deficiency at the menopause predisposes post-menopausal women to a high CVD risk (Mobasser et al. 2004) as a result of a less favourable blood lipid profile, decreased insulin sensitivity and impaired endothelial function. Previously, HRT was advocated as an effective means of delaying the progression of atherosclerosis in post-menopausal women. HRT has been shown to improve the lipoprotein profile by decreasing plasma concentrations of LDL-cholesterol and increasing concentrations of the beneficial HDL-C (Erberich et al. 2002) and improves insulin sensitivity (Sites et al. 2005). Evidence also exists that is suggestive of a protective role for HRT on endothelial function (Krasinski et al. 1997). HRT has been suggested to decrease cell adhesion molecules (Koh et al. 1997) and endothelin 1 (Wilcox et al. 1997) concentrations.

However, recent reports have shown adverse effects of HRT on the vasculature, including increased concentrations of CRP (Cushman et al. 1999; Manning et al. 2002) and increased risk of thrombosis (Nelson et al. 2002). In addition to the latter, the reported association between hormone-dependent cancers and HRT (Beral et al. 2005), as well as unexpected reports of increased CVD rates with HRT (Grady et al. 2002), has led to investigation of alternative therapies to counteract oestrogen deficiency at menopause.

Isoflavones

Interest has been focused on isoflavones as a natural alternative to traditional oestrogen therapies, and in particular as a means of delaying CVD incidence in post-menopausal women. Isoflavones are plant-derived compounds with structural similarity to oestrogen (phyto-oestrogens), able to bind to ER and therefore induce transcription of oestrogen-responsive genes (Kuiper et al. 1998). The most important dietary source of isoflavones is soyabean. Epidemiological evidence in human subjects suggests that increased soyabean consumption is cardioprotective. This effect may be a result of the ability of the isoflavones found in soyabean (genistein, daidzein and glycitein) to act as oestrogen mimics or selective ER modulators. Although evidence from in vitro studies suggests that isoflavones can be cardioprotective through a range of mechanisms, the current data from human intervention studies are insufficient to support this role.

Isoflavones and cardiovascular health: the existing evidence

Isoflavones and markers of lipid and glucose metabolism

It is currently uncertain whether isoflavones can exert beneficial effects on lipoprotein profile similar to that reported for oestrogen. The small number of studies that have investigated the effects of isolated isoflavones on lipoprotein profiles have to date produced negative results (for example, see Nestel et al. 1997; Hodgson et al. 1998; Simons et al. 2000; Dewell et al. 2002; Nikander et al. 2004), although there is evidence from a single study for beneficial effects of isoflavones on lipoprotein profiles (Han et al. 2002). Only a few studies have investigated the effects of pure isoflavones on insulin concentrations, giving equivocal findings (Blakesmith et al. 2003; Cheng et al. 2004; Nikander et al. 2004). Some of these studies have low statistical power, have administered isoflavones as capsules and only a handful have included a randomised double-blind placebo-controlled cross-over design. Thus, the question of the effect of isoflavones on lipoproteins and on glucose metabolism remains open. A summary of human intervention studies on the effect of isolated isoflavones on circulating lipoproteins is shown in Table 1.

Isoflavones and inflammatory biomarkers

The effect of isoflavones on novel cardiovascular risk markers, such as inflammatory markers, is unclear. Although evidence from in vitro studies suggests that
<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects and study design</th>
<th>Duration</th>
<th>Isoflavone supplementation (mg/d)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nestel et al. (1997)</td>
<td>Seven peri-menopausal W, fourteen PMW RSBP, CO</td>
<td>15 weeks per arm</td>
<td>80 isoflavones (45 genistein, 33 daidzein, 2 glycitein), in tablets</td>
<td>NC in TC, LDL-C, HDL-C and TAG</td>
</tr>
<tr>
<td>Hodgson et al. (1998)</td>
<td>Forty-six middle-aged M, thirteen PMW RDBP, Pa</td>
<td>8 weeks</td>
<td>55 clover (<em>Trifolium subterraneum</em>) isoflavones (16 biochanin A, 30 genistein, 8 formononetin, 1 daidzein), in tablets</td>
<td>NC in TC, LDL-C, HDL-C or TAG</td>
</tr>
<tr>
<td>Nestel et al. (1999)</td>
<td>Twenty-one menopausal and peri-menopausal W RDBP, Pa</td>
<td>15 weeks</td>
<td>RC isoflavones, in tablets; placebo, 40 isoflavone and 80 isoflavone sequentially for 5 weeks (n 14) or placebo for 15 weeks (n 3)</td>
<td>NC in TC, LDL-C, HDL-C and TAG</td>
</tr>
<tr>
<td>Howes et al. (2000)</td>
<td>Sixty-six HC PMW, sixty-six on increasing dose treatment, nine on placebo RDBP</td>
<td>5 weeks on each dose</td>
<td>Placebo followed by 43.5 and then 87 (mg/g; 600 biochanin A, 370 formononetin, 20 genistein, 10 daidzein), in tablets</td>
<td>NC in TC, TAG, HDL-C or LDL-C</td>
</tr>
<tr>
<td>Simons et al. (2000)</td>
<td>Twenty PMW RDBP, CO</td>
<td>8 weeks</td>
<td>80, in tablets</td>
<td>NC in TC, LDL-C, HDL-C or TAG</td>
</tr>
<tr>
<td>Han et al. (2002)</td>
<td>Eighty menopausal W RDBP, Pa</td>
<td>16 weeks</td>
<td>100 (70 genistein, 19 daidzein, 11 glycitein), in capsules</td>
<td>▼ in TC and LDL-C</td>
</tr>
<tr>
<td>Dewell et al. (2002)</td>
<td>Thirty-six HC PMW RP, Pa</td>
<td>24 weeks</td>
<td>150 (90 aglycones (40 genistein, 50 daidzein and glycitein), 60 glycosides), in tablets</td>
<td>NC in TC, HDL-C or TAG</td>
</tr>
<tr>
<td>Squadrito et al. (2002)</td>
<td>Sixty PMW RDBP, Pa</td>
<td>6 months</td>
<td>54 genistein, in tablets</td>
<td>NC in TC, HDL-C, LDL-C or TAG</td>
</tr>
<tr>
<td>Squadrito et al. (2003)</td>
<td>Seventy-nine PMW RDBP, Pa</td>
<td>12 months</td>
<td>54 genistein</td>
<td>NC in TC, HDL-C, LDL-C or TAG</td>
</tr>
<tr>
<td>Nikander et al. (2004)</td>
<td>Fifty-six PMW RDBP, Pa</td>
<td>3 months</td>
<td>114 (66 glycitein, daidzein, 6 genistein), in tablets</td>
<td>NC in TC, HDL-C, LDL-C or TAG</td>
</tr>
<tr>
<td>Atkinson et al. (2004)</td>
<td>Twenty-eight premenopausal W, twenty-six peri-menopausal, 117 PMW RSBP, Pa</td>
<td>12 months</td>
<td>43.5 RC isoflavones (26 biochanin, 16 formononetin, 1 genistein, 0.5 daidzein), in tablets</td>
<td>NC in TC, LDL-C, HDL-C ▼ in TAG (perimenopausal W only)</td>
</tr>
<tr>
<td>Campbell et al. (2004)</td>
<td>Sixteen premenopausal W, 7 PMW RDBP, CO</td>
<td>28 d</td>
<td>86 RC isoflavones (50 biochanin, 16 formononetin, 8 genistein, 10 daidzein), in pills</td>
<td>▼ in HDL-C</td>
</tr>
</tbody>
</table>

NC, no change; HC, hypercholesterolaemic; M, men; W, women; PMW, post-menopausal women; RDBP, randomised double-blind placebo-controlled; RSBP, randomised single-blind placebo-controlled; RP, randomised placebo-controlled; CO, cross-over design; Pa, parallel design; RC, red clover (*Trifolium pratense L*); TC, total cholesterol; LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol; TAG, triacylglycerol; ▼, decrease.
isoflavones possess anti-inflammatory effects, including inhibition of cell adhesion molecule expression (Gottstein et al. 2003; Rimbach et al. 2004), there have been few human intervention studies reporting on the effects of isoflavones on inflammatory biomarkers for cardiovascular risk. Some studies have reported no effect for fibrinogen, CRP, E-selectin and NO (Nikander et al. 2003; Teede et al. 2003; Atkinson et al. 2004), but others have shown beneficial effects of isoflavones on plasminogen activator inhibitor 1, endothelin 1, VCAM-1 and NO (Squadrito et al. 2002, 2003; Teede et al. 2003, Atkinson et al. 2004). A summary of human isoflavone supplementation studies on inflammatory biomarkers is shown in Table 2.

### Inter-individual variability in response to isoflavones

The efficacy of isoflavones could vary between individuals, and studies are needed to identify and verify the factors that define responsiveness to isoflavones. Factors that may predict responsiveness to isoflavone supplementation include equol-production status and genotype. Equol, the gut bacterial metabolite of daidzein, is a molecule with great biological importance since it exhibits higher binding affinity to ER and greater antioxidant capacity than the parent compound (Rimbach et al. 2003; Muthyala et al. 2004). Epidemiological and observational studies suggest a relationship between equol production and decreased risk of certain diseases (Atkinson et al. 2003). Evidence shows that there is an inter-individual variability in equol-synthesising capacity and studies have suggested that the response to isoflavone supplementation may vary according to an individual’s equol-synthesising capacity (Rowland et al. 2000; Setchell et al. 2002).

In addition, there is emerging evidence from genetic studies to suggest that the response to diet can be affected by inter-individual differences in the genetic background of a population (Masson & McNeil, 2005). Thus, variability in response to isoflavones may be observed in relation to single nucleotide polymorphisms in cardiovascular risk genes or genes relevant to oestrogen action, as has been shown for the HDL-C and E-selectin response to HRT therapy for ER polymorphisms (Herrington et al. 2002a,b).

### Materials and methods

#### Study protocol

Each study centre obtained ethical approval from their local ethics and research committees and written consent was obtained from all volunteers before the beginning of the study. Healthy post-menopausal women (117; 45–70 years old) were recruited from the surrounding areas of the University of Reading (Reading, UK), the German Institute of Human Nutrition (Nuthetal, Germany), the Royal Veterinary and Agricultural University (Copenhagen, Denmark) and the Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione (Rome, Italy). Participants were randomly assigned to isoflavones (50 mg/d) or placebo by stratified randomisation according to baseline age, BMI and triacylglycerol in a placebo-controlled 2 × 8-week double-blind cross-over design with an 8-week washout period. The

### Table 2. Human intervention studies on isoflavones and inflammatory biomarkers

<table>
<thead>
<tr>
<th>Reference</th>
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<th>Outcome</th>
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<tbody>
<tr>
<td>Squadrito et al. (2002)</td>
<td>Sixty PMW RDBP, Pa</td>
<td>6 months</td>
<td>54 genistein, in tablets</td>
<td>↑ in nitrates or nitrates and ↓ in ET-1</td>
</tr>
<tr>
<td>Squadrito et al. (2003)</td>
<td>Seventy-nine PMW RDBP, Pa</td>
<td>12 months</td>
<td>54 genistein</td>
<td>↑ in nitrates or nitrates and ↓ in ET-1</td>
</tr>
<tr>
<td>Nikander et al. (2003)</td>
<td>Fifty-six PMW RDBP, CO</td>
<td>3 months</td>
<td>114 (66 glycitein, 42 daidzein, 6 genistein), in tablets</td>
<td>NC in CRP, E-selectin and nitrates or nitrates ↓ in VCAM-1 (following formotenin)</td>
</tr>
<tr>
<td>Teede et al. (2003)</td>
<td>Thirty-four PMW RDBP, CO.</td>
<td>6 weeks</td>
<td>80 RC isoflavones (80 biochanin or formotenin), in tablets</td>
<td>NC in fibrinogen and ↓ in PAI-1 (in peri-menopausal women only)</td>
</tr>
<tr>
<td>Atkinson et al. (2004)</td>
<td>Twenty-six peri-menopausal women, 117 PMW RSBP, Pa</td>
<td>12 months</td>
<td>43.5 RC isoflavones (26 biochanin, 16 formotenon, 1 genistein, 0.5 daidzein), in tablets</td>
<td></td>
</tr>
</tbody>
</table>

NC, no change; PMW, post-menopausal women; RDBP, randomised double blind placebo controlled; RSBP, randomised single blind placebo controlled; CO, cross-over design; Pa, parallel design; RC, red clover (Trifolium pratense L.); CRP, C-reactive protein; ET-1, endothelin 1; VCAM-1, vascular cell adhesion molecule 1; PAI-1, plasminogen activator inhibitor 1; ↑, increase; ↓, decrease.
source of isoflavones was an extract (Solgen 40; Solbar Plant Extracts Ltd, Ashdod, Israel; genistein:daidzein 2:1) that was incorporated into cereal bars. The method used to determine the isoflavone content of the enriched and unenriched cereal bars has been described by Hall et al. (2005). The average nutrient content of cereal bars was (g): energy 652 kJ, protein 2.6, carbohydrate 17.3, fat 8.5, fibre 1.8, Na 0.012. Food diet diaries (3 d) were used to evaluate the dietary intake at baseline and midway during each intervention period at 4 weeks.

Sample collection and biochemical analysis

Fasting blood samples were taken at weeks 0, 4 and 8 of the isoflavone and placebo interventions. The protocols and methods used for blood collection and subsequent biochemical analysis have been described elsewhere (Hall et al. 2005; WL Hall, K Vafeiadou, J Hallund, S Bugel, C Koebnick, F Zunft, M Ferrari, F Branca, D Talbot, J Powell, AM Minihane, A Cassidy, M Nilsson, K Dahlman-Wright, J-Å Gustafsson and CM Williams, unpublished results). Briefly, fasting blood samples were collected for the determination of total cholesterol, HDL-C, triacylglycerol, lipoprotein(a), LDL subclasses, glucose, NEFA, endothelin 1, CRP, von Willebrand factor, insulin, E-selectin, VCAM-1, intracellular cell adhesion molecule 1 and monocyte chemotactic protein 1. A formula was used to describe insulin resistance, i.e. the homeostasis model assessment: insulin resistance = (fasting glucose × fasting insulin)/22.5 (Matthews et al. 1985).

Genotyping

Genotypes for single nucleotide polymorphisms in ERα (XbaI and PvuII), ERβ (AluI and ERjcx Tsp509I), endothelial NO synthase (Glu298Asp), apoE (apoE2, E3 and E4), cholesteryl ester transfer protein (TaqIB) and leptin receptor (Gln223Arg) were characterised using methods described in detail elsewhere (Hall et al. 2005; WL Hall, K Vafeiadou, J Hallund, S Bugel, C Koebnick, F Zunft, M Ferrari, F Branca, D Talbot, J Powell, AM Minihane, A Cassidy, M Nilsson, K Dahlman-Wright, J-Å Gustafsson and CM Williams, unpublished results).

Statistical analysis

SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses (for details, see Hall et al. 2005). Data are expressed as the means and standard deviations, except in Figs. 1 and 2, in which the means with their standard errors are given. SPSS for windows (version 12.0.1; SPSS Inc., Chicago, IL, USA) was used to calculate the difference between dietary intakes at baseline, mid-isoflavone (week 4) and mid-placebo (week 4) intervention arms using repeated-measures ANOVA. P<0.05 was considered significant.

Results

No changes in body weight were evident following the dietary intervention. Subject mean age, BMI, blood pressure and fasting plasma lipids and glucose at baseline were as follows: age 57.7 (sd 5.4) years, BMI 25.0 (sd 2.9) kg/m², systolic blood pressure 120.6 (sd 15.4) mmHg.
diastolic blood pressure 76.1 (SD 8.3) mmHg, total cholesterol 5.88 (SD 0.93) mmol/l, LDL-cholesterol 3.59 (SD 0.80) mmol/l, HDL-C 1.79 (SD 0.38) mmol/l, triglyceride 1.10 (SD 0.47) mmol/l, fasting glucose 5.17 (SD 0.47) mmol/l.

Compliance was assessed using study diaries, number of empty cereal-bar packets and serum and urinary isoflavone analysis. Following the isoflavone treatment plasma genistein and daidzein concentrations increased 20-fold and 36-fold respectively, with no significant increase in concentrations evident following placebo treatment.

The classification of volunteers as ‘equol producers’ has been described elsewhere (Hall et al. 2005). Thirty-three of the 117 subjects (28.2%) were classified as equol producers.

Dietary intake was assessed at baseline and week 4 of the intervention and placebo arms. There were no significant differences in energy intake or macronutrient intake across the treatments nor compared with baseline. Urinary isoflavones concentrations, equal production and differences in nutrient intake after both placebo and isoflavone periods are reported elsewhere (Hall et al. 2005).

**Effects of isoflavones on markers of lipid and glucose metabolism and inflammatory biomarkers**

There were no significant differences in plasma biomarkers of lipid and glucose metabolism or in the majority of plasma inflammatory biomarkers after the two intervention periods. However, there was a beneficial effect of isoflavones intake on CRP concentrations ($P < 0.05$; Hall et al. 2005; WL Hall, K Vafeiadou, J Hallund, S Bugel, C Koebnick, F Zunft, M Ferrari, F Branca, D Talbot, J Powell, AM Minihane, A Cassidy, M Nilsson, K Dahlman-Wright, J-A Gustafsson and CM Williams, unpublished results).

**Effect of genotype on response to isoflavone supplementation**

Isoflavones–genotype interactions were evident for polymorphisms in ERβ gene. The change in plasma HDL-C (week 8 – baseline) was significantly different according to the ERβ Tsp509I genotype ($P < 0.01$; Fig. 1; WL Hall, K Vafeiadou, J Hallund, S Bugel, C Koebnick, F Zunft, M Ferrari, F Branca, D Talbot, J Powell, AM Minihane, A Cassidy, M Nilsson, K Dahlman-Wright, J-A Gustafsson and CM Williams, unpublished results). The change in plasma VCAM-1 was significantly different according to the ERβ AluI genotype ($P < 0.05$; Fig. 2; Hall et al. 2005).

**Effect of equal-production status on response to isoflavone supplementation**

There were no differences between the responses of plasma markers of glucose and lipid metabolism and inflammatory biomarkers to isoflavones and those to the placebo between equol producers ($n$ 33) and non-equol ($n$ 84) producers ($P < 0.05$; Hall et al. 2005; WL Hall, K Vafeiadou, J Hallund, S Bugel, C Koebnick, F Zunft, M Ferrari, F Branca, D Talbot, J Powell, AM Minihane, A Cassidy, M Nilsson, K Dahlman-Wright, J-A Gustafsson and CM Williams, unpublished results).

**Discussion**

The ISOHEART study was a pan-European study on the cardiovascular effects of dietary soyabean isoflavones. The aims of ISOHEART were to determine the impact of isoflavones on a range of established and novel biomarkers for cardiovascular risk in healthy post-menopausal women and to investigate whether genotype or equal production status can affect the response to isoflavone supplementation. The results obtained from the ISOHEART study demonstrate few beneficial effects of isoflavone supplementation on biomarkers of lipoprotein and glucose metabolism or on the majority of inflammatory markers in a group of healthy post-menopausal women. However, data from the ISOHEART study suggest that isoflavones improve CRP concentrations. The response to isoflavones was not affected by equol-production status. However, when the response to isoflavones was evaluated according to genotype, significant isoflavones-genotype associations were evident for polymorphisms in ERβ gene.

The ISOHEART study has shown that isoflavones have no beneficial effects on plasma lipids, including total cholesterol, HDL-C, LDL-cholesterol or triglyceride, in healthy post-menopausal women. The outcome confirms previous findings that consumption of isolated isoflavones has no effect on plasma lipids (for example, see Nestel et al. 1997; Hodgson et al. 1998; Dewell et al. 2002; Nikander et al. 2004). Previous studies have shown that isolated isoflavones have no effect on lipoprotein(a) (Hodgson et al. 1998; Simons et al. 2000; Blakesmith et al. 2003; Nikander et al. 2004), a finding that has been confirmed by the results of the ISOHEART study. The ISOHEART study is the first to examine the effect of isoflavone consumption on LDL subclass distribution and the results suggest that isoflavones have no effect on percentage small dense LDL. In addition, the ISOHEART study suggests that isoflavones have no effect on plasma insulin and glucose, which is in agreement with the limited number of previous studies that have addressed this issue (Blakesmith et al. 2003; Nikander et al. 2004).

Before the unequivocal findings of the present study it was difficult to draw conclusions about the effect of isoflavones on lipoprotein profile, since a number of the previous studies lacked adequate statistical power and appropriate design. In addition, in most cases encapsulated isoflavones were administered, which raised the possibility of poor intestinal absorption in the absence of a food vehicle. The ISOHEART study was a well-powered randomised double-blind placebo-controlled cross-over design in which isoflavones were administered within a food and analysis of isoflavones in serum and urine has confirmed that isoflavones were well-absorbed.

Increased production of inflammatory factors associated with endothelial dysfunction is integral to the progression of atherosclerosis. Although isoflavones have been suggested to attenuate inflammation of the endothelium *in vitro* (Rimbach et al. 2004), the ISOHEART study has
found no beneficial effects of isoflavone supplementation on circulating biomarkers of endothelial function including intracellular cell adhesion molecule 1, VCAM-1, E-selectin, monocyte chemotactic protein 1 and endothelin 1. Previously, data on the effects of isoflavones on markers of endothelial function have been equivocal. Nikander et al. (2003) have shown that 114 mg isoflavones/d in capsules has no effect on plasma E-selectin concentrations. However, Teede et al. (2003) have observed a decrease in VCAM-1 following 80 mg formononetin/d, a precursor of daidzein found in red clover (Trifolium pratense L.), in a large group of subjects. An important factor that could explain the discrepancy between the studies is that the dose of daidzein used in the ISOHEART study was much lower than that used by Teede et al. (2003; mg/d; 17 daidzein, 33 genistein). Genistein intake (54 mg/d) has been shown previously to reduce endothelin 1 concentrations in post-menopausal women following 6 and 12 months of supplementation (Squadrito et al. 2002, 2003). The results from the ISOHEART study do not support these findings, perhaps because of a lower dose of genistein or a shorter duration of intervention. To the authors’ knowledge only one study in the past has investigated the effects of isoflavones on von Willebrand factor, an important indicator of endothelial dysfunction. The results from the ISOHEART study are in agreement with findings from the study by Hermansen et al. (2001), which show that isoflavones have no beneficial effect on von Willebrand factor.

Although isoflavones did not improve the majority of the inflammatory biomarkers, the results of the ISOHEART study suggest that isoflavone intake may improve CRP concentrations. CRP has been shown to increase after HRT (Manning et al. 2002), but CRP concentrations were not increased following supplementation in the ISOHEART study, and therefore isoflavones do not appear to mimic oestrogen action, at least as far as the effects on CRP are concerned. On the contrary, although the data for CRP was highly skewed and the statistical processing was problematic, thorough statistical analysis of the data by logistic regression has demonstrated that isoflavone consumption has a beneficial effect on CRP concentrations compared with the placebo treatment (see Hall et al. 2005). This result does not agree with the findings from a recent study in which CRP was not found to change following isoflavone supplementation (Nikander et al. 2004). The discrepancy between the two studies may be because Nikander et al. (2004) used an isoflavone supplement with a different aglycone profile from the one used in the ISOHEART study, providing (mg/d) 66 glycitein, 42 daidzein and only 6 genistein, in the form of capsules. In the ISOHEART study 50 mg isoflavones (genistein:daidzein 2:1)/d were consumed by subjects as part of a food vehicle.

There are several factors to consider in interpreting the observed lack of efficacy of isoflavones on the majority of the cardiovascular risk factors in the ISOHEART study. First, the period of exposure to isoflavones may have been too short to observe any beneficial outcomes. Epidemiological data has suggested that countries that habitually consume higher amounts of isoflavones show lower rates of heart disease. The volunteers in the ISOHEART study consumed isoflavones twice daily for 8 weeks. Whilst this intervention period is regarded as a biologically-acceptable length of time for a clinical intervention study, it may not be enough to observe benefits from weak oestrogenic plant compounds compared with the lifetime exposure observed in epidemiological studies. Second, it is possible that the dose of isoflavones used in the ISOHEART study (50 mg/d, with genistein:daidzein 2:1), although representative of a typical dietary intake in countries where soyabean is a staple, is not sufficient to exert any major beneficial effects during the 8-week exposure. Third, the study group consisted of healthy post-menopausal women; it is possible that isoflavones may have beneficial cardiovascular effects on subgroups of population that are in greater risk of developing CVD, such as individuals who are dyslipidaemic or insulin-resistant.

Evidence from in vivo studies is supportive of an association between equol production and health benefits (Atkinson et al. 2005). In addition, data from in vitro studies suggests that equol is biologically more active than the parent isoflavones (Morito et al. 2001; Muthyala et al. 2004). Since exposure to equol might have biological effects, the ISOHEART study examined whether response to isoflavone supplementation differed according to equol-production status. Of the post-menopausal women 28% were defined as ‘equol producers’, a rate that is in agreement with the prevalence rate that has previously been reported (30%) for other populations (Lampe et al. 1998). However, equol-production status was not found to be a determinant of response to isoflavone supplementation in the ISOHEART study. This finding is in contrast to recent evidence that suggests that responsiveness to isoflavones may vary according to an individual’s equol-synthesising capacity (Sethell et al. 2002). The lack of any effect of equol-production status on the response to isoflavones in the ISOHEART study may again be because the exposure to isoflavones lasted for only 8 weeks, compared with lifelong exposure in certain Asian populations in whom the strongest associations between equol production capacity and beneficial health effects have been reported (Nagata et al. 2001).

Differences in response to isoflavone supplementation may be related to variation in the genetic background of a population; for example, single nucleotide polymorphisms in key cardiovascular genes. Apart from one study that has examined the effect of apoE genotype on lipid response to isoflavones (Atkinson et al. 2004), the ISOHEART is the first to investigate the effect of various polymorphisms in genes relevant to oestrogen action and lipoprotein metabolism on the response to isoflavone supplementation. In the ISOHEART study preliminary evidence for diet–gene interactions has been observed for isoflavones and Alu and Tsp509I polymorphisms in ERβ gene. More specifically, isoflavones reduce plasma VCAM-1 in the variant AA genotype but not the other genotypes (GG or GA) of ERβ Alu polymorphism. Given the fact that VCAM-1 expression has been shown to be regulated by oestrogen via ER-mediated mechanisms (Mori et al. 2004), it may be speculated that variation in the function of the
ER as a result of differences in ligand–receptor activity may influence the expression of VCAM in response to oestrogen or phyto-oestrogens, including isoflavones. It should, however, be noted that the ERβ AluI polymorphism is positioned in the non-coding 3′ untranslated region in exon 8 of the ERβ gene, and therefore the functional implications are unclear (Rosenkranz et al. 1998). It is possible that the ERβ AluI polymorphism may be in linkage disequilibrium with polymorphisms of other, as yet unidentified, genes flanking ERβ that modulate VCAM-1 expression directly or indirectly.

The second evident ERβ genotype–isoflavone association has been observed in women with the ERβ Tsp509I AA genotype, who show a greater than 3-fold increase in HDL-C following isoflavone supplementation compared with the placebo, which is not observed in the other genotypes (see Hall et al. 2005; WL Hall, K Vafeiadou, J Hallund, S Bugel, C Koebnick, F Zunft, M Ferrari, F Branca, D Talbot, J Powell, AM Minihane, A Cassidy, M Nilsson, K Dahlman-Wright, J-Å Gustafsson and CM Williams, unpublished results). It is a rather complex task to attempt an interpretation of this putative ERβ Tsp509I genotype–isoflavone association, since the effect of ERβ isoform in the cardiovascular system is currently unknown. ERβ Tsp509I polymorphism is located in exon 9 of ERβ, a splice variant of ERβ that utilises an alternative exon of the gene (Nilsson et al. 2004). The importance of ERβ Tsp509I has been emphasised previously in a study in which it has been shown to influence the response to anti-oestrogen therapy, therefore acting as a potential predictive molecule in the response to oestrogen-like compounds (Palmieri et al. 2004). ERβ Tsp509I lacks the amino acid residues of the ERβ that are required for optimal oestrogen binding and subsequent oestrogen-induced transcriptional activation (Ogawa et al. 1998). The ISOHEART data suggest that isoflavones act on the Tsp509I AA variant to a greater extent than the other genotypes of ERβ (GG or GA). As the ERβ Tsp509I polymorphism is positioned in the 3′ untranslated region, which is a non-coding region, it is possible that this area influences the translational efficiency, e.g. by affecting mRNA stability (Nilsson et al. 2004). Another explanation could be that the ERβ Tsp509I polymorphism may be in linkage disequilibrium with another gene variant that may be involved in the metabolic pathways of HDL synthesis, such as apoA-I synthesis and hepatic lipase activity (Brinton,1996). The isoflavone–ERβ genotype associations observed in the ISOHEART study are a potential novel area of research and clearly warrant further investigation.

Conclusions

In summary, in the ISOHEART study it has been shown that isoflavone–enriched foods do not have any beneficial effect on markers of lipoprotein and glucose metabolism and cannot improve the concentrations of the majority of circulating inflammatory biomarkers in healthy post-menopausal women. However, isoflavones may have a beneficial effect on CRP concentrations. Although isoflavones fail to improve the concentrations of the majority of cardiovascular risk factors, the findings for beneficial effects of isoflavones on CRP suggest that there may be some basis for the recommendation of isoflavone supplements to healthy post-menopausal women for the reduction of inflammatory cardiovascular risk factors. Most importantly, certain subgroups may respond more beneficially to isoflavone supplementation, as already demonstrated by the decrease in plasma VCAM-1 concentrations in one of the genotypes of the ERβ AluI polymorphism and an increase in HDL-C in one of the genotypes of the ERβ Tsp509I polymorphism. Isoflavones can act as oestrogen mimics and show high binding affinity for ERβ. It is biologically plausible that variance in ERβ genotype, and consequent variation in isoflavone interactions with the receptor, contributes to inter-individual differences in response to isoflavones. The isoflavone–genotype associations observed in the ISOHEART study are of particular interest and deserve further investigation.

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References


