SYSTEMATIC REVIEW

The impact of equol-producing status in modifying the effect of soya isoflavones on risk factors for CHD: a systematic review of randomised controlled trials

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(Received 9 February 2016 – Final revision received 19 April 2016 – Accepted 25 April 2016)

Journal of Nutritional Science (2016), vol. 5, e30, page 1 of 21 doi:10.1017/jns.2016.18

Abstract

Recent studies suggest that the ability to produce equol, a metabolite of the soya isoflavone daidzein, is beneficial to coronary health. Equol, generated by bacterial action on isoflavones in the human gut, is biologically more potent than dietary sources of isoflavones. Not all humans are equol producers. We investigated whether equol-producing status is favourably associated with risk factors for CHD following an intervention by dietary soya isoflavones. We systematically reviewed randomised controlled trials (RCT) that evaluated the effect of soya isoflavones on risk factors for CHD and that reported equol-producing status. We searched PubMed, EMBASE, Ovid Medline and the Cochrane Central Register for Controlled Trials published up to April 2015 and hand-searched bibliographies to identify the RCT. Characteristics of participants and outcomes measurements were extracted and qualitatively analysed. From a total of 1671 studies, we identified forty-two articles that satisfied our search criteria. The effects of equol on risk factors for CHD were mainly based on secondary analyses in these studies, thus with inadequate statistical power. Although fourteen out of the forty-two studies found that equol production after a soya isoflavone intervention significantly improved a range of risk factors including cholesterol and other lipids, inflammation and blood pressure variables, these results need further verification by sufficiently powered studies. The other twenty-eight studies primarily reported null results. RCT of equol, which has recently become available as a dietary supplement, on CHD and its risk factors are awaited.

Key words: Equol; Soya isoflavones; CHD; Risk factors

CHD is the leading cause of morbidity and mortality in the USA(1) and worldwide(2). Nutrition is an important determinant for the risk of developing CHD; poor dietary habits are estimated to account for 20 % of CHD cases in the US adult population(1). Soya foods are a potential nutritional source for modifying biomarkers of CHD(3,4). One of the main components of soya that may exert protective cardioprotective effects are isoflavones, bioactive phyto-oestrogens found in soyabeans(3). The predominant soya isoflavones are genistein, daidzein and glycitein. Isoflavones may reduce the risk of CHD by: (1) their action via oestrogen receptor β, due to their structural similarity to oestradiol, leading to decreased vasodilation and inflammation(4–7); (2) their antioxidant activity, which may prevent the oxidative damage

Abbreviations: HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; RCT, randomised controlled trial.

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to LDL-cholesterol (LDL-C) that contributes to atherogenesis\(^8\); and (3) modulating the vascular system, reducing atherosclerotic lesions and improving vascular reactivity and vascular stiffness\(^9,10\).

Although there are clear cardiovascular benefits of isoflavones in vitro and in animal studies\(^3,11,17\), the evidence in humans is conflicting\(^12–14\). A growing hypothesis is that the ability of humans to metabolise daidzein to equol, referred to as ‘equol producers’, may contribute to the protective effects of soya\(^3,15,16\). Equol has a greater affinity for oestrogen receptors than its precursor daidzein\(^17\), a longer half-life and bioavailability in plasma than daidzein and genistein\(^3,18\), and more potent antioxidant activity than any other isoflavone\(^3\). Therefore, the potential beneficial effects of soya isoflavones for CHD and its risk factors may be greater among equol producers. While all tested animals, including rodents and monkeys, can produce equol, not all humans have the gut microflora required to convert daidzein to equol, a bioactive metabolite\(^15,19\).

Equol is a promising candidate for hindering the initiation and progression of atherosclerosis due to its ability to induce vasorelaxation and its anti-inflammatory and antioxidant activity\(^20\). Specifically, it induces vasorelaxation through enhancing the production of endothelium nitric oxide synthase-derived NO\(^21\). It can also inhibit NO derived by inducible nitric oxide synthase, expressed by immune cells during host defence, which is linked to atherosclerosis development\(^22\). Furthermore, equol prevents lipid and lipoprotein peroxidation, a crucial process in the pathogenesis of atherosclerosis\(^23,24\).

The purpose of the present review is to examine if there is a difference in the cardioprotective effect of soya isoflavones in humans based on the hosts’ ability to produce equol. No previous reviews have thoroughly examined the impact of equol-producing status on risk factors for CHD. We conducted a comprehensive search of the scientific literature to identify randomised controlled trials (RCT) that evaluated the effects of soya isoflavones on risk factors for CHD and selected studies that included analyses based on equol producer status.

Methods

Literature search

The systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines\(^25\). We initially searched PubMed (1950 to April 2015), EMBASE through Embase.com (1966 to April 2015), Ovid Medline (1946 to April 2015) and the Cochrane Library (Cochrane Central Register of Controlled Trials, 1999 to April 2015) for papers in any language using one or more textual or medical subject heading (MESH) terms for isoflavones (isoflavones, isoflavonoids, genistein, daidzein, equol), risk factors for CHD (cardiovascular disease, coronary heart disease, myocardial infarction, lipids, low-density lipoprotein-cholesterol, triglyceride, lipoproteins, hypercholesterolemia, lipid metabolism, blood pressure, glucose, vital signs, arterial stiffness, vascular stiffness, intima-media thickness, inflammation, endothelial function, endothelium, adipocytes) and RCT (randomised control study, clinical trial, placebo, intervention studies, pilot projects, sampling studies, twin studies, prospective studies, double blind study, single blind study, epidemiologic research design). We reviewed the reference lists of the collected articles to identify additional potentially relevant papers not identified by the original keyword search.

Study selection

Studies were selected for the systematic review if they met the following criteria: (1) RCT; (2) full-text was published in English; (3) analysed adult subjects who ingested soya with isoflavones or isolated isoflavones as an intervention; (4) analysed traditional risk factors for CHD (including lipids, inflammatory, blood pressure, glycaemic and body composition variables) as outcome measurements; (5) determined the equol producer status of the participants; and (6) stratified the outcome measurements by equol producer status. The exclusion criteria included reviews or commentaries.

Data synthesis and quality assessment

Searching, data extraction and the quality assessment were completed by two authors independently according to the inclusion criteria. Discrepancies were resolved by consensus. For each RCT, extracted data included sample size, baseline characteristics of the participants (sex, mean age, health status, demographics, equol producer status), study design, treatment regimen (dose, duration, isoflavone content, and type of soya intervention), and the assessment of the risk factor(s) for CHD.

The quality of the RCT methodology was graded using a fourteen-point evaluation tool for controlled clinical trials developed by the National Heart, Lung, and Blood Institute\(^26\). Questions were answered with a ‘yes’, ‘no’, ‘not reported’, ‘cannot determine’ or ‘not applicable’. The evaluation was based on the primary outcome measurements of the RCT. The RCT were given an overall rating of ‘good’, ‘fair’ or ‘poor’ at the discretion of the reviewers based on the guidelines provided by this tool.

Results

Search results

A total of 1671 papers were collected and, of these, 829 were excluded because they were not RCT, did not measure the traditional risk factors for CHD, or were not published in English (Fig. 1). Of the remaining 247 papers screened, forty-two met the selection criteria for this review. An outline of our search strategy using PubMed is provided in Supplementary Table S1.

Study characteristics

Study characteristics are summarised in Tables 1 and 2, and Supplementary Table S2. Thirty studies included only female participants\(^14,27–55\), eleven studies included both males and females\(^56–66\), and one study had only male participants\(^67\). Of the forty-one studies involving women, thirty-four
The age of the participants ranged from 27 to 73 years. Participants were hypercholesterolaemic in seven studies (56–58,60,64–66), hyperlipidaemic in two studies (58,66), prehypertensive or hypertensive in five studies (39–41,55,59), had type 2 diabetes in two studies (30,61), had the metabolic syndrome in two studies (27,63), and considered healthy in twenty-three studies (14,29,31,34–38,42–54,60,67). Diet interventions in nineteen studies used soya protein isolate with isoflavone flour, or powder, or tablets (31,33,37–42,48–51,37,58,61,62,65,67), fifteen used soya and isoflavone-enriched milk or foods (14,27,30–36,40–42,45,47,55–59,63–67), and nine used isolated isoflavone tablets or capsules (28,29,42–45,52–54), with Gardiner et al. (57) using interventions that covered two categories. Isoflavone doses ranged from approximately 40 to 120 mg/d, with one dose particularly high at 900 mg/d (44). Twenty-three studies examined cholesterol markers (27,28,31,32,35,38,39,42,43,48,52,56–57), twenty-one examined other lipid variables (27,28,31,32,35,38,39,42,43,47,48,52,56,58,60,61,62,66–67), eighteen examined blood pressure and vascular variables (14,27,30–34,37,39,40,41,43,48,49,51,55,56,59,63,66), seventeen examined inflammatory markers (27,33,34,42,44–46,48,50,53,54,56,63,65–67), ten examined glucose and insulin variables (27,29,35,39,43,48,57,62,63,65) and five examined body composition variables (27,41,43,65,66). There were numerous methods and standards used to distinguish equol producers from non-equol producers, including sampling from urine or serum, different threshold levels for differentiation, and various analytical techniques.

Synthesis of results

We categorised both the effects of soya isoflavones and equol producer status on the examined CHD risk factors as beneficial, negligible, or adverse (Tables 3–8). We analysed each risk factor independently; therefore the RCT were potentially categorised more than once. Twenty-two studies found statistically significant improvements in the risk factors for CHD after the soya isoflavone intervention compared with placebo. Of these, equol producer status further improved risk factors for CHD in six studies (including LDL-C, TAG, systolic blood pressure, diastolic blood pressure, flow-mediated dilation, soluble intercellular adhesion molecule-1, platelet-selectin and C-reactive protein). Equol producer status was comparable to the soya intervention in sixteen studies (including total cholesterol, LDL-C, HDL-cholesterol (HDL-C), TAG, apoB, systolic blood pressure, diastolic blood pressure, nitrate and nitrite, systemic arterial compliance, peak flow velocity, aortic augmentation index and IL–6).

Forty studies found no association between soya isoflavones and risk factors for CHD compared with placebo. Of these, equol producer status significantly improved risk factors for CHD in seven studies (including total cholesterol, LDL-C, TAG, apoA-I, apoB, lipoprotein (a), blood pressure, diastolic blood pressure, mean arterial pressure, carotid to femoral pulse wave velocity). As with the soya isoflavone intervention, equol producer status was insignificant in thirty-two studies and was adverse in one study.

Three studies found that soya isoflavones had a negative effect on the risk factors of CHD. Of these, equol producer status was negligible in two studies and magnified the adverse outcomes of the soya isoflavone intervention in one study (isoprostane excretion). Equol producer status was also associated with the adverse outcome of an increase in insulin-like growth factor binding protein-3.

Five studies were comprised of participants who were all equol producers (Table 8); two of the studies found statistically significant beneficial effects of the isoflavone interventions on risk factors of CHD (including LDL-C, high-sensitivity C-reactive protein, TAG, inflammatory gene expression) while four studies observed negligible effects.

The RCT varied in quality, with the overall scores provided in Table 1 and the ratings summarised in Supplementary Table S3. Failure to report sample size calculations, details on the randomisation and allocation concealment procedures, and lack of intention-to-treat analyses or other suitable statistical method of dealing with participant drop-out were the most frequent flaws. Six RCT were given a ‘good’ rating, twenty were given a ‘fair’ rating and sixteen were given a ‘poor’ rating.

The heterogeneity of the studies in terms of populations, treatment regimens, intended duration, and outcomes prevented us from quantitatively synthesising the evidence in the form of a meta-analysis. Besides, the total number of participants included in all forty-two of the studies together was 3796, which, along with varying interventions and populations, probably provides insufficient statistical power to quantitatively measure the effect of dietary interventions. Further, most of these forty-two studies were small and had fewer than fifty participants, and only eighteen out of the forty-two studies qualified to be ‘fair’ or ‘good’ quality. The six ‘good’-quality papers (Hodis et al. (18), Liu et al. (39–41), van der Velpen et al. (53,54)) come from three different trials – while the Hodis study examined carotid artery intima-media thickness.

Fig. 1. Study flow diagram of screened, excluded and analysed publications.
| First author, year | Country | Sex (no. M/no. F) | Mean age (years) | No. of EP (no. EP/no. NEP) | Guidelines for determining EP status | Subjects’ characteristics | Study design | Quality rating* |
|--------------------|---------|-------------------|------------------|--------------------------|---------------------------------------|--------------------------|-------------|----------------|
| Acharjee et al. (2015)†| USA | 60 F | MetS: 54.1 (sd 6.5), without MetS: 54 | 35 EP/25 NEP | Urinary equol concentration >1000 nmol/l Equol concentration > five times baseline Urinary equol concentrations >1 mg/ml | Postmenopausal, with and without MetS Postmenopausal breast cancer survivors | CO | Fair |
| Badeau et al. (2007)‡ | Finland | 30 F | | 15 EP/15 NEP | | Healthy | CO, DB | Fair |
| Campbell et al. (2004)†| UK | 23 F | Premenopausal: 34, postmenopausal: 57 | 7 EP/9 NEP in premenopausal group, 1 EP/6 NEP in postmenopausal group | | Healthy | CO, DB | Fair |
| Clerici et al. (2007)§ | Italy | 25 M/37 F | Control: 52.0 (SEM 2.4), intervention: 58.1 (SEM 2.2) | 20 EP/9 NEP (of intervention group) | | Hypercholesterolaemic, adhering to Italian Heart Association Step II diet | CO, P, B | Fair |
| Curtis et al. (2013)§ | UK | 118 F | Control: 63.0 (SEM 0.8), intervention: 62.1 (SEM 0.7) | 17 EP/30 NEP (of intervention group) | | Not reported | DB | Poor |
| Gallagher et al. (2004)¶ | USA | 65 F | | 36 EP/29 NEP | | Serum equol >10 ng/ml | DB | Poor |
| Gardner et al. (2007)¶ | USA | 6 M/22 F | | 9 EP/19 NEP | | Plasma equol >50 nM | CO, SB | Poor |
| Greany et al. (2004)§ | USA | 37 F | | 8 EP/29 NEP | | Plasma equol concentrations >15 nmol/l and urinary excretion >1500 nmol/24 h | CO | Poor |
| Greany et al. (2008)§ | USA | 34 F | 57.7 (sd 6.0) | 6 EP/28 NEP | | Plasma equol concentration >15 nmol/l and urinary equol excretion >1500 nmol/d 24 h urinary equol concentration during the isoflavone intervention >936 nmol/l 24 h urinary equol concentration during the isoflavone intervention >936 nmol/l 24 h urinary equol concentration during the isoflavone intervention >936 nmol/l | CO | Poor |
| Hall et al. (2005)¶ | UK, Germany, Denmark, Italy | 117 F | 57.7 (sd 5.4) | 33 EP/84 NEP | | Not reported | CO, DB | Fair |
| Hall et al. (2006)¶ | UK, Germany, Denmark, Italy | 117 F | 57.7 (sd 5.4) | 33 EP/84 NEP | | Not reported | CO, DB | Fair |
| Hallund et al. (2006)¶ | Denmark, UK, Germany, Italy | 28 F | 57 (sd 5) | 6 EP/22 NEP | | Not reported | CO, DB | Fair |
| Hodis et al. (2011)¶ | USA | 350 F | 60.9 | 39 consistent EP/35 intermittent EP/76 NEP | | Consistent EP: plasma equol >20 nmol/l at all visits, intermittent EP: plasma equol >20 nmol/l at some visits, NEP: plasma equol never >20 nmol/l | DB | Good |
| Kreijkamp-Kaspers et al. (2005)¶ | Netherlands | 175 F | Control: 66.8 (sd 4.7), intervention: 66.6 | 26 EP/62 NEP (of intervention group) | | Not reported | DB | Fair |
| Study/Region | Country | Gender | Sample Size | Age (mean, SD) | Intervention | Control | Outcome | Result |
|--------------|---------|--------|-------------|----------------|-------------|---------|---------|--------|
| Kreijkamp-Kaspers et al. (2004) | Netherlands | 175 F | Control: 66-7 (sd 4-8), intervention: 66-5 (sd 4-7) | 26 EP/62 NEP (of intervention group) | Plasma equol concentration >83 nmol/l | Postmenopausal | DB | Fair |
| Liu et al. (2014) | China | 287 F | Control: 58-5 (sd 4-7), whole soya: 57-6 (sd 5-3), daidzein: 57-7 (sd 5-0) | 287 EP/0 NEP | 24 h urinary log10 S-equol: daidzein ratio > 1.75 after daidzein challenge | Postmenopausal, prehypertensive | DB | Good |
| Liu et al. (2015) | China | 265 F | Control: 58-5 (sd 4-7), whole soya: 57-6 (sd 5-3), daidzein: 57-7 (sd 5-0) | 265 EP/0 NEP | 24 h urinary log10 S-equol: daidzein ratio > 1.75 after daidzein challenge | Postmenopausal, prehypertensive or untreated hypertensive | P, DB | Good |
| Liu et al. (2013) | China | 253 F | Control: 58-5 (sd 4-7), whole soya: 57-6 (sd 5-3), daidzein: 57-7 (sd 5-0) | 253 EP/0 NEP | 24 h urinary log10 S-equol: daidzein ratio > 1.75 after daidzein challenge | Postmenopausal, prehypertensive | DB | Good |
| Ma et al. (2005) | USA | 70 M/89 F | Control: 56-9 (sd 6-1), soya protein and isoflavone: 72-3 (sd 5-7), soya protein and isoavone: 73-0 (sd 5-7) | 56 (8.46) | Serum equol concentration >20 ng/ml | Hyperlipidaemic | DB | Fair |
| Mangano et al. (2013) | USA | 97 F | Control: 72-9 (sd 6-1), soya protein: 74-0 (sd 6-2), isoflavone: 72-3 (sd 5-7), soya protein and isoavone: 73-0 (sd 5-7) | 25 EP/26 NEP | 12-month serum concentration of S-equol 20 nmol/l (5 µg/l) | Postmenopausal | DB | Poor |
| McVeigh et al. (2017) | Canada | 35 M | Control: 27-9 (sd 5-7) | 12 EP/23 NEP | Urinary equol >1000 nmol/24 h | Healthy | CO, B | Poor |
| Meyer et al. (2004) | Australia | 13 M/10 F | Control: 54-0 (sd 1.8) | 8 EP/15 NEP | Equol detected in the plasma or urine | Postmenopausal, hypercholesterolaemic and/or hypertensive | CO | Poor |
| Nestel et al. (2004) | Australia | 46 M/34 F | Control: 58 (sd 7), Females: 58 (sd 6) | 15 EP/65 NEP | Excretion of equol >1000 nmol/24 h | Postmenopausal | CO, P, DB | Fair |
| Nikander et al. (2004) | Finland | 56 F | Control: 54 (sd 6) | 8 EP/40 NEP | EP: equol concentration >83 nmol/l, NEP: equol concentration <40 nmol/l | Postmenopausal | CO, DB | Fair |
| Pipe et al. (2009) | Canada | 16 M/13 F | Control: 60-1 (sd 9.64) | 6 EP/23 NEP | Urinary equol > 1000 nmol/24 h | Postmenopausal, diet-controlled type 2 diabetic | CO, DB | Poor |
| Pop et al. (2008) | USA | 30 F | Placebo: 53.50 (se 1.06), intervention: 56.78 (se 1.25) | 6 EP/23 NEP/1 intermediate EP | EP: plasma equol concentrations >20 µg/l; intermediate EP: ≥10 to ≤20 µg/l; NEP: plasma equol concentration <10 µg/l | Postmenopausal | DB | Poor |
| Pusparini & Hidayat (2015) | Indonesia | 182 F | Control EP: 54-3 (sd 3-42), control NEP: 52.2 (sd 3-24), intervention EP: 53-3 (sd 34-6), intervention NEP: 53-7 (sd 3-65) | 110 EP/72 NEP | Baseline blood equol concentration >5 ng/ml | Postmenopausal | DB | Fair |
| Qin et al. (2014) | China | 91 M/86 F | Control: 52.9 (sd 6-0), low daidzein: 54-5 (sd 6-6), high daidzein: 53-4 (sd 6-4) | 106 EP/71 NEP | Urinary equol concentration >1000 nmol/l, log10-transformed urinary S-equol:daidzein ratio > –1.75 after daidzein intervention | Hypercholesterolaemic | DB | Fair |
| Reimann et al. (2006) | Denmark, UK | 89 F | Control: 59 (sd 5) | 29 EP/59 NEP | Urinary equol concentration >936 nmol/l urine Equol/daidzein ≥0.018 with a daidzein threshold of ≥2 nmol/ mg creatinine | Postmenopausal, MetS | CO, DB | Poor |
| Reveri et al. (2015) | USA | 5 M/12 F | Control: 56 (sd 5) | 8 EP/9 NEP | | Postmenopausal, MetS | CO | Poor |

Continued
| First author, year | Country | Sex (no. M/no. F) | Mean age (years) | No. of EP (no. EP/no. NEP) | Guidelines for determining EP status | Subjects’ characteristics | Study design | Quality rating* |
|--------------------|---------|------------------|------------------|---------------------------|--------------------------------------|--------------------------|-------------|----------------|
| Sen et al. (2012)† | USA     | 82 F             | 39.2 (SD 6.1)    | 43 EP/39 NEP             | Urinary daidzein excretion ≥2 nmol/mg creatinine, urinary equol:daidzein ≥0.018; participants who meet both criteria at least once during the study considered EP | Premenopausal            | CO          | Poor           |
| Steinberg et al. (2003)† | USA | 28 F             | 54.9 (SEM 1.0)   | 10 EP/18 NEP             | Urinary log₁₀ S-equol:daidzein value > −1.75 after soya or daidzein intervention | Postmenopausal; using tibolone | CO, DB      | Poor           |
| Thorp et al. (2008)† | Australia | 33 M/58 F       | 52.7 (SD 1.0)    | 30 EP/61 NEP             | >4-fold rise in serum equol concentration >4-fold rise in serum equol concentration >4-fold rise in serum equol concentration | Hypercholesterolaemic; using tibolone | CO, DB      | Poor           |
| Törmälä et al. (2008)† † | Finland | 36 F             | 57.7 (SEM 0.8)   | 16 EP/20 NEP             | Equol concentration > five times baseline after soya isoflavone challenge | Postmenopausal, using tibolone | CO          | Fair           |
| Törmälä et al. (2008)† † | Finland | 36 F             | 57.7 (SEM 0.8)   | 16 EP/20 NEP             | Equol concentration > five times baseline after soya isoflavone challenge | Postmenopausal, using tibolone | CO, DB      | Fair           |
| Törmälä et al. (2007)† † | Finland | 33 F             | 57.7 (SEM 0.8)   | 14 EP/19 NEP             | Equol concentration > five times baseline after soya isoflavone challenge | Postmenopausal, using tibolone | CO, DB      | Fair           |
| Törmälä et al. (2006)‡ | Finland | 30 F             | 56 (SD 6)        | 15 EP/15 NEP             | Equol concentration > five times baseline after soya isoflavone challenge | Postmenopausal, history of breast cancer | CO, DB      | Fair           |
| van der Velpen et al. (2014)† | Netherlands | Low genistein group (LG): 24 F; high genistein group (HG): 31 F | LG: 63.2 (SD 5.5); HG: 63.0 (SD 5.5) | LG: 7 EP/17 NEP; HG: 8 EP/23 NEP | Log₁₀-transformed urinary S-equol:daidzein ratio > 1.75 | Postmenopausal | CO, DB      | Good           |
| van der Velpen et al. (2013)† | Netherlands | 30 F             | 61.1 (SD 5.8)    | 30 EP/0 NEP             | Log₁₀-transformed urinary S-equol:daidzein ratio > −1.75 post-isoflavone or daidzein challenge | Postmenopausal | CO, DB      | Good           |
| Welty et al. (2007)† | USA     | 60 F             | Normotensive: 53.5 (SD 5.3), hypertensive: 58.3 (SD 6.5) | 35 EP/25 NEP             | Urinary equol concentration greater than 1000 nmol/l | Postmenopausal; hypertensive, prehypertensive, or normotensive | CO          | Fair           |
| West et al. (2005)† † | USA     | 14 M/18 F        | Males: 57.36 (SE 1.43), females using HRT: 57.17 (SE 2.18), females not using HRT: 59.08 (SE 1.54) | 11 EP/21 NEP             | High concentrations of equol in urine | Postmenopausal, hypercholesterolaemic, adhering to National Cholesterol Education Program Step I diet | CO, DB      | Fair           |
Table 2. Characteristics of the soya isoflavone interventions used in the randomised controlled trials examining the effect of equol producer (EP) status on the risk factors for CHD

| First author, year | Source of isoflavones | Control | Isoflavone dose/d | Constituents of isoflavone dose | Duration of trial |
|--------------------|-----------------------|---------|-------------------|-------------------------------|------------------|
| Acharjee et al.    | TLC diet with soya nuts | TLC diet without soya nuts | 101 mg isoflavones (AG)/d | 30 mg daidzein, 61 mg genistein, 10 mg glycitein | 16 weeks |
| Badeau et al.      | Isoflavone tablet      | Placebo tablet           | 114 mg isoflavones/d      | 41 mg daidzein, 7 mg genistein, 66 mg glycitein | 6 months |
| Campbell et al.    | Isoflavone tablet      | Placebo tablet           | 86 mg red clover isoflavones/d | 43 mg total isoflavones: 4 mg genistein, 5 mg daidzein, 25 mg biochanin, 8 mg formononetin | 2 months |
| Clerici et al.     | Soya germ-enriched pasta | Conventional pasta | 33 mg of isoflavones (AG)/d | Predominantly daidzein, genistein, glycitein | 8 weeks |
| Curtis et al.      | Flavonoid-enriched chocolate | Placebo chocolate | 100 mg isoflavones (AG)/d | Predominantly daidzein | 1 year |
| Gallagher et al.   | SPI powder with isoflavones (SPI 96 or SPI 50) | SPI without isoflavones (SPI 4) | 52 mg isoflavones/d | SPI 96: 28 mg daidzein, 52 mg genistein; SPI 50: 20 mg daidzein, 28 mg genistein; SPI 4: 0 mg daidzein, 4 mg genistein | 19 months (soya for an additional 6 months) |
| Gardner et al.     | WB milk, SPI milk      | Dairy milk               | WB: 125 (so 17) isoflavones (AG)/d; SPI milk: 39 (so 1) isoflavones (AG)/d | WB milk: 56-4 (so 6-4) mg daidzein, 65-5 (so 9-7) mg genistein, 2-9 (so 0-4) mg glycitein; SPI milk: 12-9 (so 0-2) mg daidzein, 24-7 (so 0-3) mg genistein, 1-0 (so 0-2) mg glycitein | 12 weeks |
| Greany et al.      | SPI, SPI plus probiotic capsules | MPI powder | 44 (SEM 8) mg isoflavones/d | 34 % daidzein, 57 % genistein, 9 % glycitein | 24 weeks |

Continued
Table 2. Continued

| First author, year | Source of isoflavones | Control | Isoflavone dose/d | Constituents of isoflavone dose | Duration of trial |
|--------------------|-----------------------|---------|-------------------|-------------------------------|------------------|
| Greany et al. (2008) | SPI powder            | MPI powder | 44 (so 8) mg isoflavones/d | 34 % daidzein, 57 % genistein, 9 % glycitein | 24 weeks |
| Hall et al. (2005) | Isoflavone-enriched cereal bars | Placebo cereal bars | 50 mg isoflavones/d | Genistein:daidzein ratio of 2 | 16 weeks |
| Hall et al. (2006) | Isoflavone-enriched cereal bars | Placebo cereal bars | 50 mg isoflavones/d | Genistein:daidzein ratio of 2 | 16 weeks |
| Hallund et al. (2006) | Isoflavone-enriched cereal bars | Placebo cereal bars | 50 mg isoflavones/d | Genistein:daidzein ratio of 2 | 16 weeks |
| Hodis et al. (2011) | Powdered soya beverage or food bars | Total milk protein beverage or food bars | 91 mg isoflavones/d (154 mg total isoflavone conjugates plus AGs) | 36 mg AG daidzein 36 mg (61 mg total), 52 mg AG genistein (88 mg total), 3 mg AG glycitein (5 mg total) | 2.5–3 years |
| Kreijkamp-Kaspers et al. (2005) | Soya protein powder | Total milk protein powder | 25.6 g of isoflavone-rich soya protein/d | 41 mg daidzein, 52 mg genistein, 6 mg glycitein (AG) in 36.5 g soya-protein powder | 12 months |
| Kreijkamp-Kaspers et al. (2006) | Soya protein powder | Total milk protein powder | 25.6 g of isoflavone-rich soya protein/d | 41 mg daidzein, 52 mg genistein, 6 mg glycitein (AG) in 36.5 g soya-protein powder | 12 months |
| Liu et al. (2014) | Whole soya group: soya flour; daidzein group: daidzein and milk powder | Low-fat milk powder | Whole soya group: 40 g soya with 49.8 mg total isoflavones/d; daidzein group: 63 mg daidzein/d | Whole soya group: 23.2 mg daidzein, 19.4 mg genistein; daidzein group: 63 mg daidzein | 6 months |
| Liu et al. (2015) | Whole soya group: soya flour; daidzein group: daidzein and milk powder | Low-fat milk powder | Whole soya group: 49.3 mg isoflavones/d; daidzein group: 63 mg daidzein/d | Whole soya group: 23.2 mg daidzein, 19.4 mg genistein; daidzein group: 63 mg daidzein | 6 months |
| Liu et al. (2013) | Whole soya group: soya flour; daidzein group: daidzein and milk powder | Low-fat milk powder | Whole soya group: 40 g soya with 49.8 mg total isoflavones/d; daidzein group: 63 mg daidzein/d | Whole soya group: 23.2 mg daidzein, 19.4 mg genistein; daidzein group: 63 mg daidzein | 6 months |
| Ma et al. (2005) | Soya protein powder | Milk protein powder | Whole soya group: 40 g soya with 49.8 mg total isoflavones/d; daidzein group: 63 mg daidzein/d | Whole soya group: 23.2 mg daidzein, 19.4 mg genistein; daidzein group: 63 mg daidzein | 6 months |
| Mangano et al. (2013) | SPI: soya protein and isoflavone tablets, SPP: soya protein and placebo tablets, CPI: control protein and isoflavone tablets | Low-iso SPI: 61.7 (so 8.35) mg isoflavones (AG)/d | SPP: control tablet, CPI: control tablet, SPI: SPI with daidzein (DAI40 and DAI80) supplementation | Primarily daidzein, genistein, glycitein and their β-glycosides | 1 year |
| McVeigh et al. (2006) | Low-iso SPI: low-isoflavone SPI, high-iso SPI: high-isoflavone SPI | MPI powder | Low-iso SPI: 1-64 (so 0.19) mg isoflavones (AG)/d; high-iso SPI: 61.7 (so 8.35) mg isoflavones (AG)/d | Not reported | 171 days |
| Meyer et al. (2004) | Soya milk, soya yogurt | Dairy milk, dairy yogurt | 80 mg isoflavones/d | Soya milk: 8.8 mg isoflavones/100 g, soya yogurt: 8.8 mg isoflavones/100 g | 10 weeks |
| Nestel et al. (2004) | Red clover pill (B or F preparations) | Placebo pill | 40 mg isoflavones/d of B or F preparations | Red clover B: <1 % daidzein, 4 % genistein, red clover F: <1 % daidzein and genistein | 12 weeks |
| Nikander et al. (2004) | Isoflavonoid tablets | Placebo tablets | 114 mg isoflavonoids/d | 41 mg daidzein, 7 mg genistein, 66 mg glycitein | 6 months |
| Pipe et al. (2009) | SPI powder | MPI powder | 88 mg isoflavones (AG)/d | 27 mg daidzein, 57 mg genistein, 4 mg glycitein | 114 days |
| Pop et al. (2008) | Isoflavone capsules | Placebo capsule | 900 mg isoflavones/d | 296 mg daidzein, 558 mg genistein, 44 mg glycitein | 84 days |
| Pusparini & Hidayat (2015) | Soya isoflavone tablets | Placebo tablet | 40 mg isoflavones/d | 16 mg daidzein, 22.4 mg genistein, 1.2 mg glycitein | 6 months |
| Qin et al. (2014) | SPI with daidzein (DAI40 and DAI80) supplementation | SPI without daidzein supplementation | 0.7 mg isoflavones/d supplemented with 40 mg/d daidzein (DAI40) or 80 mg/d daidzein (DAI80) | DAI40: 40 mg daidzein, DAI80: 80 mg daidzein | 6 months |
| Reimann et al. (2006) | Isoflavone-enriched fruit cereal bars | Fruit cereal bar without isoflavones | 50 mg isoflavones/d | Genistein:daidzein ratio of 2:1 | 16 weeks |
| Study                  | Design/Intervention                                                                 | Isoflavone Content (mg/d) | Duration |
|------------------------|--------------------------------------------------------------------------------------|---------------------------|----------|
| Reverri et al. (2015)  | Soya nuts Cookies supplemented with whey protein and fibre                           | 101 (AG)                  | 8 weeks  |
| Sen et al. (2012)      | High-soya group: two servings of soya foods/d; low-soya group: three servings of soya/week | >40 mg isoflavones/d      | Not reported |
| Steinberg et al. (2003)| Soya+: SPI with isoflavones, soya-: SPI with trace amounts of isoflavones          | 107-67 (AG)               | 18 weeks |
| Thorp et al. (2008)    | Diet S: food with soya protein, diet SD: food with soya and dairy protein            | 71.4 (SEM 1.9) mg isoflavones (AG)/d; diet SD: 76 (SEM 1.5) mg isoflavones (AG)/d; diet D: 0.5 (SEM 0.1) mg isoflavones (AG)/d | Not reported |
| Törmälä et al. (2008) | Soya protein powder Milk protein powder                                             | 112 mg isoflavones (AG)/d | 16 weeks |
| Törmälä et al. (2008) | Soya protein powder Milk protein powder                                             | 112 mg isoflavones/d      | 16 weeks |
| Törmälä et al. (2007) | Soya protein powder Milk protein powder                                             | 112 mg isoflavones/d      | 16 weeks |
| Törmälä et al. (2006) | Isoflavone tablet Placebo tablet                                                    | 114 mg isoflavones/d      | 6 months |
| van der Velpen et al. (2014) | Isoflavone capsule Placebo capsule | Low genistein (LG): 100 mg isoflavones (AG)/d; high genistein (HG): 104 mg isoflavones/d | 16 weeks |
| van der Velpen et al. (2013) | Isoflavone capsule Placebo capsule | 94 mg isoflavones (AG)/d | 16 weeks |
| Welty et al. (2007)    | TLC diet with soya nuts TLC diet without soya nuts                                  | 101 mg isoflavones (AG)/d | 16 weeks |
| West et al. (2005)     | SPI powder MPI powder                                                              | 90 mg isoflavones/d       | Not reported |
| Wong et al. (2012)     | Soya food with isoflavones (three different diet protocols)                       | N/A                       | 4–8 weeks |

TLC, therapeutic lifestyle changes; AG, aglycone; SPI, soya protein isolate; WB, whole bean soya; MPI, milk protein isolate; N/A, not applicable.

* Studies that are or potentially using shared study participants.
† Studies that are or potentially using shared study participants.
‡ Studies that are or potentially using shared study participants.
§ Studies that are or potentially using shared study participants.
¶ Studies that are or potentially using shared study participants.
** Studies that are or potentially using shared study participants.
Table 3. Randomised clinical trial results reporting the effect of soya isoflavone interventions and equal producer (EP) status on cholesterol and other lipid parameters

| First author, year | CHD risk factor measured | Effect of isoflavone on CHD risk factors | Result marker† | Effect of EP status on CHD risk factors | Result marker‡ |
|--------------------|--------------------------|----------------------------------------|----------------|----------------------------------------|----------------|
| Acharjee et al. (2015) | TAG                       | Reduction in TAG in women with MetS (17.8 %, \(P = 0.04\)) after the soya intervention compared with placebo, unlike in women without MetS | + | Reduction in TAG in EP with MetS (22.9 %, \(P = 0.02\)) after the soya intervention compared with placebo. There were NS effects on NEP with or without MetS in TAG | + |
| Clerici et al. (2007)(5a) | LDL-C, isoprostane excretion | Reduction in LDL-C (8.6 %, \(P = 0.002\)) compared with placebo after the soya intervention. Isoprostane excretion reduced from 58 (SEM 6) ng/l at baseline to 39 (SEM 4) ng/l after 4 weeks in the soya group \((P < 0.001)\) (not measured in placebo group) | + | LDL-C reduced 15 (SEM 7) mg/dl more in EP than in NEP \((P = 0.042)\) after the soya intervention. Isoprostane excretion decreased in both EP and NEP, though more significantly in EP \((P = 0.012)\) than NEP \((P = 0.038)\) | + |
| Hall et al. (2006)(4) | %sdLDL-C                  | The isoflavone intervention was associated with a greater reduction of %sdLDL-C compared with placebo \((24.14 (so 14.26) and 22.22 (so 11.67), \text{respectively}; \(P = 0.044\)) | + | The interaction between positive EP status and treatment was significant for %sdLDL-C \((P < 0.05)\) | + |
| Hall et al. (2006)(4) | Lp(a)                     | The isoflavone intervention had a NS effect on Lp(a) compared with placebo | 0 | There was an interaction between EP status and treatment for Lp(a) \((P < 0.05, \text{data highly skewed})\) | + |
| Manganese et al. (2013)(2) | TC:HDLC, LDL-C:HDL-C | The soya intervention had a NS effect on the risk factors compared with placebo | 0 | EP had lower TC:HDLC and LDL-C:HDL-C compared with NEP \((P = 0.018\) and 0.043, respectively) after the isoflavone intervention | + |
| McVeigh et al. (2006)(2) | LDL-C                     | The soya intervention had a NS effect on LDL-C compared with placebo | 0 | EP status associated with a significant decrease in LDL-C on the low-isoflavone diet \((P = 0.035)\) and high-isoflavone diet \((P = 0.041)\) compared with placebo | + |
| Meyer et al. (2004)(19) | TC, LDL-C, LDL-C:HDL-C, TAG, Lp(a) | The soya intervention had a NS effect on the risk factors compared with placebo | 0 | EP status associated with significant reductions \((P < 0.001)\) in TC \((8.5 \%)\), LDL-C \((10 \%)\), LDL-C:HDL-C ratio \((13.5 \%)\), TAG \((21 \%)\) and Lp(a) \((11 \%)\) after the soya intervention, unlike NEP | + |
| Pipe et al. (2009)(41) | TC, apoB                  | The isoflavone intervention had a NS effect on the risk factors compared with placebo | 0 | There was an interaction between EP status and TC \((P = 0.05)\) and apoB \((P = 0.04)\) after the soya intervention. There were NS effects of the soya intervention on TC or apoB in EP or NEP when analysed separately | + |
| Wong et al. (2012)(5a) | HDL-C, apoA-I             | The soya interventions had a NS effect on the risk factors compared with placebo | 0 | Apo A-I reduced in NEP but not EP \((−0.08 (se 0.02)\) and \(−0.02 (se 0.02)\) g/l, respectively; \(P = 0.010\) and HDL-C reduced in NEP but not EP \((−0.07 (se 0.02)\) and 0 (se 0.03) mmol/l, respectively; \(P = 0.036\) after the soya interventions | + |
| Badeau et al. (2007)(20) | Pre-(β) HDL-C             | Pre-(β) HDL-C increased by 18 % \((P < 0.05)\) after the isoflavone treatment | + | EP status had a NS effect on pre-(β) HDL-C levels after the isoflavone intervention | 0 |
| Clerici et al. (2007)(5a) | TC                        | TC reduced after the soya intervention compared with placebo \((7.3 \%, \(P = 0.001)\) | + | TC reduction was greater in EP than NEP \((P = 0.103)\) after the soya intervention | 0 |
| Gardner et al. (2007)(21) | LDL-C                     | LDL-C decreased after both soya interventions compared with placebo \((161 (so 20), 161 (so 26), and 170 (so 24) mg/dl for the WB soya milk, SPI milk, and dairy milk, respectively; \(P = 0.02\) for each soya milk v. dairy milk) | + | EP status had a NS effect on LDL-C after either soya milk intervention | 0 |
| Greany et al. (2004)(22) | TC, LDL-C, HDL-C, TAG     | Reductions in TC \((−2.2 \%, \(P = 0.02\)\), LDL-C \((−3.5 \%, \(P = 0.006\)\) and TAG \((−8.8 \%, \(P = 0.07\)\) while HDL-C increased \((4.2 \%, \(P = 0.006\)\) after the soya intervention compared with control | + | EP status had a NS effect on the risk factors on in all subjects, hypercholesterolaemic subjects alone, or normocholesterolaemic subjects alone after the soya intervention | 0 |
| Author(s)                  | Treatment | Lipid Changes                                                                 | Significance | Notes                                                                 |
|---------------------------|-----------|-------------------------------------------------------------------------------|--------------|----------------------------------------------------------------------|
| McVeigh et al. (2006)     | TC:HDL-C, LDL-C:HDL-C, apoB:apoA-I | Reductions in TC:HDL-C, LDL-C:HDL-C, apoB:apoA-I after the soya diets (P = 0.031, 0.006, 0.011, respectively in the low-soya diet, P = 0.054, 0.012, 0.005, respectively in the high-soya diet) compared with control | +            | Interaction of EP status and treatment was NS for the risk factors   |
| Nestel et al. (2004)      | LDL-C     | LDL-C reduced after the genistin-rich (biochanin) isoflavone intervention compared with placebo (P = 0.026) | +            | EP status had a NS effect on LDL-C after the isoflavone interventions |
| Pipe et al. (2009)        | LDL-C, LDL-C:HDL-C, apoB:apoA-I | Reductions in LDL-C (P = 0.04), LDL-C:HDL-C (P = 0.04), and apoB:apoA-I (P = 0.05) after the isoflavone intervention compared with placebo | +            | EP status had a NS effect on the risk factors after the isoflavone intervention |
| Qin et al. (2014)         | TAG       | Reduction in the low- and high-daidzein interventions compared with placebo in TAG (−0.15 (SEM 0.062) and 0.24 (SEM 0.61) mmol/l, respectively; P < 0.05) | +            | EP status had a NS effect on TAG after the isoflavone intervention   |
| Thorp et al. (2008)       | TC, TAG   | The soya diet caused a 3 % greater reduction in TC (−0.17 (SEM 0.06) mmol/l, P < 0.05) and 4 % greater reduction in TAG (−0.14 (SEM 0.05) mmol/l, P < 0.05) compared with control | +            | NS interaction between EP status and diet treatment on the risk factors (P > 0.68 for all) |
| Wong et al. (2012)        | LDL-C, apoB | Reductions in LDL-C and apoB after the soya treatments compared with placebo (P values not provided) | +            | EP status had a NS effect on the risk factors after the soya treatments |
| Acharjee et al. (2015)    | TC, LDL-C, HDL-C | The soya intervention had a NS effect on the risk factors compared with placebo | 0            | EP status had a NS effect on the risk factors compared with placebo |
| Badeau et al. (2007)      | ABCA1-dependent cholesterol efflux, TC, HDL-C, HDL-2, HDL-3, TC:HDL-C, non-HDL-C, TAG, apoB- | The isoflavone intervention had a NS effect on the risk factors compared with placebo | 0            | EP status had a NS effect on the lipid risk factors. ABCA1-dependent cholesterol efflux values were higher in EP than NEP (3.4 (so 1.4) % and 2.7 (so 0.6) %, respectively), though NS, after the isoflavone intervention |
| Gallagher et al. (2004)   | TC, LDL-C, HDL-C, TAG, apoA-I, apoB | The isoflavone intervention had a NS effect on the risk factors compared with placebo | 0            | NS differences in percentage change between equal levels and the risk factors after the isoflavone intervention |
| Hall et al. (2006)        | TC, LDL-C, HDL-C, TAG, TC: HDL-C, TC: HDL-C, LDL-C, TAG, Lp(a) | The isoflavone intervention had a NS effect on the risk factors compared with placebo | 0            | EP status had a NS effect on the risk factors after the isoflavone intervention |
| Kreijkamp-Kaspers et al. (2004) | TC, HDL-C, LDL-C, TAG | The isoflavone intervention had a NS effect on the risk factors compared with placebo | 0            | EP status had a NS effect on the risk factors after the isoflavone intervention |
| Ma et al. (2005)          | TC, HDL-C, Lp(a) | The isoflavone intervention had a NS effect on the risk factors compared with placebo | 0            | EP status had a NS effect on the risk factors after the isoflavone intervention |
| Mangano et al. (2013)     | TC, LDL-C, HDL-C | The isoflavone intervention had a NS effect on the risk factors compared with placebo | 0            | EP status had a NS effect on the risk factors after the isoflavone intervention |
| McVeigh et al. (2006)     | TC, HDL-C, non-HDL-C, TAG, apoA-I, apoB | The isoflavone intervention had a NS effect on the risk factors compared with placebo | 0            | EP status had a NS effect on the risk factors after the isoflavone intervention |
| Meyer et al. (2004)       | HDL-C     | The isoflavone intervention had a NS effect on HDL-C compared with placebo | 0            | EP status had a NS effect on the risk factors after the isoflavone intervention |
| Nestel et al. (2004)      | LDL-C     | The intervention of isoflavones isolated from red clover enriched in formononetin had a NS effect on LDL-C compared with placebo | 0            | EP status had a NS effect on the risk factors after the isoflavone intervention |
| Nikander et al. (2004)    | TC, LDL-C, HDL-C, TAG, apoA-I, apoB, Lp(a) | The isoflavonoid intervention had a NS effect on the risk factors compared with placebo though in women with baseline levels of LDL-C above the median LDL-C, it increased (P = 0.009) | 0            | EP status had a NS effect on the risk factors after the isoflavonoid intervention |
| Pipe et al. (2009)        | HDL-C, non-HDL-C, TAG, apoA-I, TC: HDL-C, TAG:HDL-C, non-HDL: HDL-C | The isoflavonoid intervention had a NS effect on the risk factors compared with placebo | 0            | EP status had a NS effect on the risk factors after the isoflavonoid intervention |

Continued
Table 3. Continued

| First author, year | CHD risk factor measured | Effect of isoflavone on CHD risk factors | Result marker† | Effect of EP status on CHD risk factors | Result marker‡ |
|-------------------|--------------------------|------------------------------------------|----------------|-----------------------------------------|----------------|
| Qin et al. (2014)  | HDL-C, LDL-C, apoA-I, apoB, Lp(a) | The isoflavone intervention had a NS effect on the risk factors compared with placebo | 0 | EP status had a NS effect on the risk factors after the isoflavone intervention | 0 |
| Reveri et al. (2015) | OxLDL-C | The soya intervention had a NS effect on oxLDL-C compared with placebo | 0 | EP status had a NS effect on oxLDL-C after the soya intervention | 0 |
| Steinberg et al. (2003) | TC, LDL-C, HDL-C, TC:HDL-C, TAG, CD formation | The isoflavone intervention had a NS effect on the risk factors | 0 | EP status had a NS effect on the risk factors after the soya interventions | 0 |
| Thorp et al. (2008) | LDL-C, HDL-C, TC:HDL-C | The soya intervention had a NS effect on the risk factors | 0 | NS interaction between EP status and diet treatment on the risk factors after the soya intervention \( (P > 0.68 \text{ for all}) \) | 0 |
| Törmälä et al. (2006) | TC, HDL-C, LDL-C, TAG, apoA-I, apoB, serum cholesterol efflux capacity | The isoflavone intervention had a NS effect on the risk factors compared with placebo | 0 | EP status had a NS effect on the risk factors after the isoflavone intervention | 0 |
| West et al. (2005) | HDL-C, LDL-C, apoA-I, apoB, Lp(a) | The soya intervention had a NS effect on the risk factors compared with placebo | 0 | EP status had a NS effect on the risk factors after the soya intervention | 0 |
| Wong et al. (2012) | TC, TC:HDL-C, LDL-C:HDL-C, TAG, apoB:apoA-I | The soya interventions had a NS effect on the risk factors on the risk factors compared with placebo | 0 | EP status had a NS effect on the risk factors after the soya treatments | 0 |
| Sen et al. (2012) | Isoprostane excretion | There was a positive association between isoprostane excretion and isoflavones after the high soya diet intervention \( (P = 0.02) \) | – | There was a positive association between isoprostane excretion and the isoflavone intervention for EP \( (P = 0.03) \) but not NEP \( (P = 0.32) \) after the high-soya diet intervention | – |

MetS, metabolic syndrome; NEP, non-equol producer; LDL-C, LDL-cholesterol; sLDL-C, small dense LDL-C; Lp, lipoprotein; TC, total cholesterol; HDL-C, HDL-cholesterol; WB, whole bean soya; SPI, soya protein isolate; ABCA1, adenosine triphosphate-binding cassette A1; CD, conjugated diene formation; OxLDL-C, oxidised LDL-C.* Results are first stratified by the impact of EP status and then the impact of the soya isoflavone interventions on each of the lipid risk factors.

†+, Beneficial effect of soya isoflavones on risk factors of CHD; 0, negligible effect of soya isoflavones on risk factors of CHD; –, adverse effect of soya isoflavones on risk factors of CHD.

‡+, Beneficial effect of EP status on risk factors of CHD after soya intervention; 0, negligible effect of EP status on CHD risk factors after soya intervention; –, adverse effect of EP status on risk factors of CHD after soya intervention.
Table 4. Randomised clinical trial results reporting the effect of soya isoflavone interventions and equol producer (EP) status on blood pressure and vasculature parameters*

| First author, year | CHD risk factor measured | Effect of isoflavone on CHD risk factors | Result marker† | Effect of EP status on CHD risk factors | Result marker† |
|--------------------|--------------------------|------------------------------------------|----------------|----------------------------------------|----------------|
| Acharjee et al. (2015)(27) | DBP | Reduction in DBP in women with and without MetS (5-4 %, $P = 0.03$ and 3-4 %, $P = 0.0008$, respectively) after the soya intervention | + | EP with and without MetS had reduced DBP (7.7 %, $P = 0.02$ and 3.3 %, $P = 0.02$, respectively) after the soya intervention compared with placebo. There were NS effects on NEP with or without MetS in DBP | + |
| Clerici et al. (2007)(56) | FMD | Increase in FMD (2 (SEM 0-8); $P = 0.012$) after the soya intervention compared with placebo | + | Increase in FMD in EP from baseline concentrations ($P = 0.03$) after the soya intervention, unlike in NEP | + |
| Welty et al. (2007)(58) | SBP | Reduction in SBP in hypertensive women (9-9 %, $P = 0.003$) and normotensive women (5-2 %, $P < 0.001$) after the soya intervention compared with the placebo | + | EP compared with NEP had reduced BP ($P = 0.01$), DBP (EP: $-2.24$ (SE 1-31) mmHg; NEP: $1.00$ (SE 0.89) mmHg; $P < 0.01$), MAP (EP: $-1.24$ (SE 1-30) mmHg; NEP: $1.90$ (SE 1-08) mm Hg; $P = 0.01$) and PWV (EP: $-0.68$ (SE 0.40) m/s; NEP: $0.32$ (SE 0.55) m/s; $P = 0.001$). In EP, an inverse correlation between DBP and urinary equol concentrations was observed ($r = 0.80$; $P = 0.02$) | + |
| Curtis et al. (2013)(30) | BP, DBP, MAP, PWV | The flavonoid intervention had a NS effect on BP and PWV compared with placebo. The flavonoid intervention had a NS greater reduction compared with placebo in DBP ($P = 0.06$) and MAP ($P = 0.06$) | 0 | EP compared with NEP had reduced BP ($P = 0.01$), DBP (EP: $-2.24$ (SE 1-31) mmHg; NEP: $1.00$ (SE 0.89) mmHg; $P < 0.01$), MAP (EP: $-1.24$ (SE 1-30) mmHg; NEP: $1.90$ (SE 1-08) mm Hg; $P = 0.01$) and PWV (EP: $-0.68$ (SE 0.40) m/s; NEP: $0.32$ (SE 0.55) m/s; $P = 0.001$). In EP, an inverse correlation between DBP and urinary equol concentrations was observed ($r = 0.80$; $P = 0.02$) | + |
| Acharjee et al. (2015)(27) | SBP | Reduction of SBP in women with and without MetS (5-9 %, $P < 0.001$ and 6-7 %, $P = 0.01$, respectively) after the soya intervention compared with placebo | + | SBP changed in both EP (6-4 %, $P < 0.001$) and NEP (5-4 %, $P = 0.003$) in women without MetS compared with placebo. In women with MetS, NS change in SBP in EP or NEP | 0 |
| Hallund et al. (2006)(55) | NMD, NOx, NOx:ET-1, SAC | Reductions in NMD (15-5 % v. 12-4 %, $P = 0.03$), NOx ($P = 0.003$), NOx:ET-1 ($P = 0.005$) and SAC ($P = 0.04$) after the soya intervention compared with placebo | + | NS interaction between EP status and vascular responses to isoflavones and placebo treatment | 0 |
| Reveri et al. (2015)(63) | Alx | Reduction in Alx after the soya intervention compared with placebo ($P = 0.03$) | + | EP status had a NS effect on Alx after the soya intervention | 0 |
| Steinberg et al. (2003)(40) | PFV | Reduction in PFV after the soya intervention compared with placebo (37 %; $P = 0.03$) | + | EP status had a NS effect on PFV after the soya intervention | 0 |
| Welty et al. (2007)(58) | DBP | Reduction in DBP after the soya intervention in hypertensive women (6-8 % mmHg, $P = 0.001$) and normotensive women (2-9 %, $P = 0.02$) compared with the placebo | + | EP status had a NS effect on DBP after the soya intervention | 0 |
| Wong et al. (2012)(56) | SBP, DBP | Reductions in DBP and SBP after the soya treatments compared with placebo (P values not provided) | + | EP status had a NS effect on the risk factors after the soya treatments | 0 |
| Curtis et al. (2013)(30) | SBP, total plasma NO concentrations, ET-1 | The flavonoid intervention had a NS effect on the risk factors compared with placebo. There was a NS greater decrease in SBP the flavonoid group compared with placebo ($P = 0.07$) | 0 | EP status had a NS effect on the risk factors after the flavonoid intervention | 0 |
| Hall et al. (2005)(34) | BP, ET-1, vWF | The isoflavone intervention had a NS effect on the risk factors compared with placebo | 0 | EP status had a NS effect on the risk factors after the isoflavone intervention | 0 |
| Hall et al. (2006)(34) | Mean SBP, Mean DBP | The isoflavone intervention had a NS effect on the risk factors compared with placebo | 0 | EP status had a NS effect on the risk factors after the isoflavone intervention | 0 |
| Hallund et al. (2006)(34) | FMD, ET-1, BP, IAC, arterial volume, arterial distensibility, SVR | The isoflavone intervention had a NS effect on the risk factors compared with placebo. There was a NS greater increase in SVR after the isoflavone intervention compared with placebo ($P = 0.06$) | 0 | NS interaction between EP status and the risk factors after the isoflavone intervention | 0 |

Continued
### Table 4. Continued

| First author, year | CHD risk factor measured | Effect of isoflavone on CHD risk factors | Result marker† | Effect of EP status on CHD risk factors | Result marker‡ |
|--------------------|--------------------------|-----------------------------------------|----------------|----------------------------------------|---------------|
| Hodis et al. (2011) | CIMT                     | There was a NS greater reduction in CIMT progression after the isoflavone intervention compared with control (16%; $P=0.038$) | 0              | EP status had a NS effect on CIMT progression rate after the isoflavone intervention | 0             |
| Kreijkamp-Kaspers et al. (2005) | DBP, %FMD                  | The soya intervention had a NS effect on the risk factors compared with placebo | 0              | EP status had a NS effect on the risk factors after the soya intervention | 0             |
| Meyer et al. (2004) | HDL-C, MAP, SBP, DBP, arterial compliance | The soya intervention had a NS effect on the risk factors compared with placebo | 0              | EP status had a NS effect on the risk factors after the soya intervention | 0             |
| Nikander et al. (2004) | BP                          | The isoflavonoid intervention had a NS effect on the risk factors compared with placebo | 0              | EP status had a NS effect on the risk factors after the isoflavonoid intervention | 0             |
| Pusparini & Hidayat (2015) | NO                         | The isoflavone intervention had a NS effect on NO compared with placebo | 0              | EP status had a NS effect on NO after the isoflavone intervention | 0             |
| Reverri et al. (2015) | Reactive hyperemia index  | The soya intervention had a NS effect on the risk factor compared with placebo | 0              | EP status had a NS effect on the risk factor after the soya intervention | 0             |
| Steinberg et al. (2003) | Brachial artery vessel diameter, ET-1, total NO | The soya interventions had a NS effect on the risk factors | 0              | EP status had a NS effect on the risk factors after the soya interventions | 0             |
| Törmälä et al. (2008) | AIX, EFI                   | The soya intervention had a NS effect on the risk factors compared with placebo | 0              | EP status had a NS effect on the soya intervention. EP taking tibolone had lower AIX ($P=0.01$) and EFI ($P=0.009$) compared with NEP | 0             |
| Törmälä et al. (2007) | SBP, DBP, MAP              | The soya intervention had a NS effect on the risk factors compared with placebo | 0              | EP status had a NS effect on the risk factors after the soya intervention. EP women taking tibolone had lower SBP ($P=0.02$), DBP ($P=0.01$) and MAP ($P=0.007$) which was maintained after the soya intervention | 0             |
| Kreijkamp-Kaspers et al. (2005) | SBP                       | Increase in SBP after the soya intervention compared with placebo (4.3 mmHg; $P=0.004$) | –              | EP status had a NS effect on the risk factors after the soya intervention | 0             |

DBP, diastolic blood pressure; MetS, metabolic syndrome; NEP, non-equal producer; FMD, flow-mediated dilation; SBP, systolic blood pressure; LDL-C, LDL-cholesterol; BP, blood pressure; MAP, mean arterial pressure; PWV, carotid to femoral pulse wave velocity; NMD, nitroglycerine-mediated endothelium-independent vasodilation; NOx, nitrate and nitrite; ET-1, endothelin-1; SAC, systemic arterial compliance; AIX, augmentation index; PFV, peak flow velocity; vWF, von Willebrand factor; IAC, isobaric arterial compliance; SVR, systemic vascular resistance; CIMT, carotid artery intima-media thickness; HDL-C, HDL-cholesterol; EFI, endothelial function index.

†, Beneficial effect of soya isoflavones on risk factors of CHD; 0, negligible effect of soya isoflavones on risk factors of CHD; –, adverse effect of soya isoflavones on risk factors of CHD.

‡, Beneficial effect of EP status on risk factors of CHD after soya intervention; 0, negligible effect of EP status on CHD risk factors after soya intervention; –, adverse effect of EP status on risk factors of CHD after soya intervention.
Table 5. Randomised clinical trial results reporting the effect of soya isoflavone interventions and equol producer (EP) status on inflammation and DNA damage parameters*

| First author, year       | CHD risk factor measured | Effect of isoflavone on CHD risk factors | Result marker† | Effect of EP status on CHD risk factors | Result marker† |
|--------------------------|--------------------------|----------------------------------------|----------------|-----------------------------------------|----------------|
| Acharjee et al. (2015)   | CRP, sICAM-1             | Reduction in CRP in women with and without MetS (11.8%, \(P = 0.04\) and 30%, \(P = 0.01\), respectively) after the soya intervention compared with placebo. In women with MetS, reduction in sICAM-1 (5.2%, \(P = 0.04\)) compared with placebo, unlike in women without MetS | +             | Reduced CRP (21.4%; \(P = 0.01\)) and sICAM-1 (7.3%, \(P = 0.03\)) in EP with MetS compared with placebo after the soya intervention. Reduced CRP (30%; \(P = 0.04\)) in EP without MetS compared with placebo. There were NS effects on NEP with or without MetS in any of these variables | +             |
| Clerici et al. (2007)    | hsCRP                    | Reduction in hsCRP (2.2 SEM 0.9 mg/l, \(P = 0.03\)) after the soya intervention compared with placebo | +             | After the soya intervention, hsCRP decreased 0.9 (SEM 0.5) mg/l more in EP than NEP (\(P = 0.025\)) | +             |
| Pusparini & Hidayat      | MDA                      | Reduction in MDA after the soya intervention (\(P = 0.021\)) | +             | After the soya intervention, EP had a greater decline in MDA than NEP | +             |
| Törmälä et al. (2008)   | P-selectin               | P-selectin decreased by 10.3% (\(P = 0.002\)) after the soya intervention compared with placebo | +             | EP had a greater decline in P-selectin (13.5%; \(P = 0.007\)) than NEP (7.7%; NS) after the soya intervention | +             |
| Mangano et al. (2013)    | IL-6                     | The percentage change of IL-6 declined from baseline after the soya intervention compared with placebo (\(P = 0.007\)) | +             | EP status had a NS effect on percentage change of IL-6 after the soya intervention | 0             |
| Qin et al. (2014)        | Uric acid                | Reductions in the low and high daidzein isoflavone interventions compared in placebo in uric acid (−23 (SD 47) and −29 (SD 0.44) μmol/l, respectively; \(P < 0.05\)) | +             | EP status had a NS effect on uric acid after the isoflavone intervention | 0             |
| Greany et al. (2008)     | Hcy, CRP, E-selectin, VCAM-1, ICAM-1 | The isoflavone intervention had a NS effect on the risk factors compared with placebo | 0             | EP status had a NS effect on the risk factors after the soya intervention | 0             |
| Hall et al. (2005)       | MCP-1, CRP, VCAM-1, ICAM-1, E-selectin, hsCRP | The isoflavone intervention had a NS effect on the risk factors compared with placebo | 0             | EP status had a NS effect on the risk factors after the isoflavone intervention | 0             |
| Mangano et al. (2013)    | hsCRP                    | The isoflavone intervention had a NS effect on hsCRP compared with placebo | 0             | EP status had a NS effect on hsCRP after the soya intervention | 0             |
| McVeigh et al. (2006)    | CRP                      | The isoflavone intervention had a NS effect on CRP compared with placebo | 0             | NS interaction with EP status and the soya intervention with CRP | 0             |
| Pop et al. (2008)        | Neutrophil count, DNA damage markers (AP-site assay, comet assay), apoptosis markers (TUNEL assay, caspase-3 activation) | The isoflavone intervention had a NS effect on the risk factors compared with placebo | 0             | EP status had a NS effect on the risk factors after the isoflavone intervention. Activated caspase-3 was higher in treated EP on day 1 but decreased through day 84, while it increased in NEP in this time period | 0             |
| Pusparini & Hidayat      | VCAM-1                   | The isoflavone intervention had a NS effect on VCAM-1 compared with placebo | 0             | EP status had a NS effect on VCAM-1 after the soya intervention | 0             |
| Reimann et al. (2006)    | Hcy, ADMA                | The isoflavone intervention had a NS effect on the risk factors compared with placebo | 0             | EP status had a NS effect on the risk factors after the isoflavone intervention | 0             |
| Reverri et al. (2015)    | CRP, TNF, IL-6, IL-18, IL-10 | The isoflavone intervention had a NS effect on the risk factors compared with placebo | 0             | EP status had a NS effect on the risk factors after the soya intervention | 0             |
| Steinberg et al. (2003)  | VCAM-1, ICAM-1, E-selectin | The isoflavone intervention had a NS effect on the risk factors | 0             | EP status had a NS effect on the risk factors after the soya intervention | 0             |
| Törmälä et al. (2008)   | CRP, ICAM-1, VCAM-1      | The isoflavone intervention had a NS effect on the risk factors compared with placebo. There was a NS increase in VCAM-1 after the soya intervention compared with placebo (9.2%; \(P = 0.06\)) | 0             | EP status had a NS effect on the risk factors after the soya intervention | 0             |
| West et al. (2005)       | VCAM-1, P-selectin       | The isoflavone intervention had a NS effect on the risk factors compared with placebo | 0             | EP status had a NS effect on the risk factors after the soya intervention | 0             |

Continued
## Table 5. Continued

| First author, year | CHD risk factor measured | Effect of isoflavone on CHD risk factors | Result marker† | Effect of EP status on CHD risk factors | Result marker‡ |
|--------------------|--------------------------|----------------------------------------|----------------|----------------------------------------|----------------|
| Wong et al. (2012)  | CRP                      | The soya interventions had a NS effect on CRP | 0              | EP status had a NS effect on CRP after the soya intervention | 0              |
|                    |                          |                                        |                |                                        |                |
|                    |                          |                                         |                |                                        |                |

Table 5. Continued

progression among equol producers and non-producers, Liu et al. and van der Velpen et al. examined their intervention only among equol producers. Liu et al. examined the effect of soya on risk factors such as lipid markers\(^{39–41}\), while van der Velpen et al.\(^{53,54}\) examined the effect of soya on the expression of inflammatory genes. Given these varying outcomes, we have chosen to not perform a meta-analysis in our present review.

### Discussion

Though the overall effect of equol producer status during a dietary soya intervention on risk factors of CHD is inconclusive, we found evidence of a favourable effect of equol producer status in fourteen of the forty-two studies\(^{27,30,35,39,42,48,50,54,56,59,61,66,67}\) regardless of the success of the soya intervention. Equol production was associated with positive changes in cholesterol\(^{35,39,42,56,59,61,66,67}\) and other lipid variables\(^{27,35,39,56,61,66}\) and inflammatory markers\(^{27,39,45,50,54,56}\). The effect of equol producer status was insignificant on CHD risk factors in forty studies\(^{14,27–46,48–47}\) and adverse in two studies\(^{29,47}\). We did not find consistent evidence of equol production affecting specific risk factors for CHD. The heterogeneity of the CHD risk factors analysed, sample size, study designs and quality, and definition of equol producers prevented quantitative synthesis of the results.

The majority of the studies in the present review retrospectively categorised study participants by equol producer status and conducted a secondary analysis of the effect of equol on the risk factors for CHD. Therefore, these RCT were very unlikely to be sufficiently powered to detect a difference in CHD risk factors between equol producers and non-equol producers. We identified ten studies with study designs that included enrolment criteria based on equol producer status\(^{28,39–41,49–54}\). Of these, three found equol producer status improved several CHD risk factors (LDL-C, LDL-C:HDL-C, TAG, platelet-selectin and inflammatory markers)\(^{27,30,35}\) after the soya intervention\(^{29,45,50,54}\) while the remaining associations measured in the RCT were negligible.

There are numerous differences in the experimental design of the RCT that could explain the inconsistency in the outcomes. The isoflavone dose ranged in both quantity and consistency between RCT. In particular, the amount of daidzein in the intervention formulations, which indicates the magnitude of equol that could be metabolised from daidzein and bioavailable in equol producers, largely varied between studies. Additionally, the duration and frequency of exposure to the intervention were inconsistent. Curtis et al.\(^{50}\) found that improvements in blood pressure, mean arterial pressure, and pulse wave velocity measures in equol producers were seen after 1 year but not at 6 months, suggesting that long-term exposure to isoflavones may be more beneficial.

The criteria used to define equol producers differed across the RCT included in our review, with variability in the biological samples used to measure equol, the concentration cutoffs selected to distinguish equol producers from non-equol producers, and the analytical methods used to measure equol. Setchell & Cole\(^{58}\) proposed classifying equol producers
Table 6. Randomised controlled trial results reporting the effect of soya isoflavone interventions and equol producer (EP) status on glucose and insulin parameters*

| First author, year | CHD risk factor measured | Effect of isoflavone on CHD risk factors | Result marker† | Effect of EP status on CHD risk factors | Result marker‡ |
|--------------------|--------------------------|-----------------------------------------|---------------|----------------------------------------|---------------|
| Acharjee et al.    | Glucose                  | The soya intervention had a NS effect on glucose compared with placebo | 0             | EP status had a NS effect on glucose compared with placebo | 0             |
| Campbell et al.    | IGF-1, IGF-BP1           | The isoflavone intervention had a NS effect on the risk factors compared with placebo | 0             | EP status had a NS effect on the risk factors after the isoflavone intervention | 0             |
| Gardner et al.     | Glucose, insulin         | The soya intervention had a NS effect on the risk factors compared with placebo | 0             | EP status had a NS effect on the risk factors after the soya intervention | 0             |
| Hall et al.        | Glucose, insulin         | The isoflavone intervention had a NS effect on the risk factors compared with placebo | 0             | EP status had a NS effect on the risk factors after the isoflavone intervention | 0             |
| Nikander et al.    | Glucose, insulin         | The isoflavonoid intervention had a NS effect on the risk factors compared with placebo | 0             | EP status had a NS effect on the risk factors after the isoflavonoid intervention | 0             |
| Qin et al.         | Glucose, insulin, glycated Hb | The isoflavone intervention had a NS effect on the risk factors compared with placebo | 0             | EP status had a NS effect on the risk factors after the isoflavonoid intervention | 0             |
| Reverri et al.     | Glucose, insulin, SHBG   | The soya intervention had a NS effect on insulin compared with placebo | 0             | EP status had a NS effect on insulin after the soya intervention | 0             |
| Törmälä et al.     | SHBG                     | The soya intervention had a NS effect on SHBG compared with placebo | 0             | EP status had a NS effect on SHBG after the soya intervention | 0             |
| West et al.        | Glucose                  | The soya intervention had a NS effect on glucose compared with placebo | 0             | EP status had a NS effect on glucose after the soya intervention | 0             |
| Reverri et al.     | Glucose                  | Glucose decreased after both snack interventions but decreased more after the control compared with the soya intervention (P = 0·02) | –             | EP status had a NS effect on the risk factor after the soya intervention | 0             |
| Campbell et al.    | IGF-BP3                  | The isoflavone intervention had a NS effect on IGF-BP3 compared with placebo | 0             | Equol excretion was positively associated with IGF-BP3 concentrations in postmenopausal women at the end of the placebo phase (r 0·895; P = 0·04) and isoflavone intervention (r 0·984; P = 0·002) | –             |

IGF, insulin-like growth factor; IGF-BP1, insulin-like growth factor binding protein-1; SHBG, sex hormone binding globulin; IGF-BP3, insulin-like growth factor binding protein-3.

* Results are first stratified by the impact of EP status and then the impact of the soya isoflavone interventions on each of the lipid risk factors.

† †, Beneficial effect of soya isoflavones on risk factors of CHD; 0, negligible effect of soya isoflavones on risk factors of CHD; –, adverse effect of soya isoflavones on risk factors of CHD.

‡ ‡, Beneficial effect of EP status on risk factors of CHD after soya intervention; 0, negligible effect of EP status on CHD risk factors after soya intervention; –, adverse effect of EP status on risk factors of CHD after soya intervention.
Table 7. Randomised controlled trial results reporting the effect of soya isoflavone interventions and equol producer (EP) status on body composition variables

| First author, year | CHD risk factor measured | Effect of isoflavones on CHD risk factors | Result marker† | Effect of EP status on CHD risk factors | Result marker‡ |
|--------------------|--------------------------|------------------------------------------|----------------|----------------------------------------|----------------|
| Acharjee et al. (2015) (39) | BMI | The soya intervention had a NS effect on BMI compared with placebo | 0 | EP status had a NS effect on BMI compared with placebo | 0 |
| Nikander et al. (2004) (53) | BW | The isoflavonoid intervention had a NS effect on BW compared with placebo | 0 | EP status had a NS effect on BW after the isoflavonoid intervention | 0 |
| West et al. (2005) (53) | BW | The soya intervention had a NS effect on BW compared with placebo | 0 | EP status had a NS effect on BW after the soya intervention | 0 |
| Wong et al. (2012) (53) | BW, BMI, waist circumference | The soya interventions had a NS effect on the risk factors compared with placebo | 0 | EP status had a NS effect on the risk factors after the soya treatments | 0 |

BW, body weight.
† +, Beneficial effect of soya isoflavones on risk factors of CHD; 0, negligible effect of soya isoflavones on risk factors of CHD; −, adverse effect of soya isoflavones on risk factors of CHD.
‡ +, Beneficial effect of EP status on risk factors of CHD after soya intervention; 0, negligible effect of EP status on CHD risk factors after soya intervention; −, adverse effect of EP status on risk factors of CHD after soya intervention.

Table 8. Results of the randomised clinical trials examining the effect of soya isoflavone interventions on the risk factors for CHD in equol producers (EP) only

| First author, year | CHD risk factor measured | Effect of EP status on CHD risk factors | Result marker* |
|--------------------|--------------------------|----------------------------------------|----------------|
| Liu et al. (2014) (53) | LDL-C, LDL-C:HDL-C, hsCRP, TAG, TC, HDL-C, glucose, NEFA, CIMT | Reductions from baseline after the whole soya intervention in LDL-C (−0.25 mmol/l; 95 % CI: −0.19, −0.014), LDL-C:HDL-C (0.157; 95 % CI: −0.318, 0.004) and hsCRP (−0.054 mg/l; 95 % CI: −0.199, 0.012) compared with placebo. TAg were reduced at 6 months in the whole soya group compared with placebo (P < 0.05) | + |
| Liu et al. (2015) (53) | 24 h, daytime, and night time DBP, SBP, MAP, %FMD | The soya and daidzein interventions had a NS effect on the risk factors compared with placebo | 0 |
| Liu et al. (2013) (53) | BW, BMI, waist and hip circumferences, waist:hip ratio, body fat percentage, fat mass, free-fat mass | The soya and daidzein interventions had a NS effect on the risk factors compared with placebo | 0 |
| Van der Velpen et al. (2014) (53) | Expression of inflammatory genes | Expression of inflammatory-related genes in the adipose tissue was up-regulated in EP and down-regulated in NEP in both isoflavone interventions. Further analysis identified a predominance of anti-inflammatory gene expression in EP | 0 |
| van der Velpen et al. (2013) (53) | Expression of inflammatory genes | The expression of 357 genes on a gene chip encoding 19 738 gene identifiers (1.8 %) significantly changed after isoflavone intervention in peripheral blood mononuclear cells of EP. There was a down-regulation of gene sets related to inflammation, driven by reduced TLR4, TIRAP and IL-1β gene expression and complement and coagulation gene sets | + |

LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol; hsCRP, high-sensitivity C-reactive protein; TC, total cholesterol; CIMT, carotid artery intima-media thickness; DBP, diastolic blood pressure; SBP, systolic blood pressure; MAP, mean arterial pressure; FMD, flow-mediated dilation; BW, body weight; NEP, non-equol producers; TLR4, Toll-like receptor 4; TIRAP, toll-interleukin 1 receptor domain-containing adaptor protein.
* +, Beneficial effect of EP status on risk factors of CHD after soya intervention; 0, negligible effect of EP status on CHD risk factors after soya intervention; −, adverse effect of EP status on risk factors of CHD after soya intervention.

by a threshold log_{10}-transformed ratio of Δ(-)equol, a diastereoisomer of equol produced by the intestinal bacteria in humans, to its precursor daidzein of −1–75 in urine after a 3 d soya isoflavone challenge. This accounts for inconsistency in the technical measurements of equol and avoids classifying equol producers based on absolute measurements of equol, which exhibit greater variability (68). Nine studies used this approach (39–41,53,56,62,64,66), with four finding a beneficial effect of equol producer status on risk factors of CHD (39,54,56,66) and eight finding a negligible effect (39–41,53,56,62,64,66).

Further complicating the interpretation of the data are the potential sex differences in the metabolism of soya (69), which could
affect the bioavailability of isoflavone metabolites between males and females. In a meta-analysis examining the effects of soya isoflavones on lipids, subjects with hypercholesterolaemia had greater reductions in men than in women\(^{(15)}\). While there were studies of mixed sex (\(n = 11\)) or of only males (\(n = 1\)), the present review consisted primarily of female-only RCT, which may have masked the effects of equol producer status on the outcome measurements. Nestel et al\(^{(60)}\) found that LDL-C was significantly reduced after supplementation with biochanin (a precursor of genistein) compared with placebo (\(P = 0.026\)); when results were stratified based on sex, males showed a significant reduction in median LDL-C levels of 9.5% while females had no measurable difference. Equol producer status did not further reduce LDL-C, which the authors speculated was due to the small sample size of fifteen equol producers, with seven included in the biochanin intervention group\(^{(60)}\).

The source of soya may also contribute to the variability in its effectiveness. The type of processing used for soya products during production can affect the isoflavone content\(^{(13)}\) and modify other components of soya\(^{(70)}\). Additionally, soya protein isolate primarily contains isoflavone glucosides while fermented soya foods contain isoflavones mainly in the aglycone form\(^{(15,71)}\). Isoflavone aglycones are absorbed more efficiently than isoflavone glucosides in humans and may therefore be more effective in CHD prevention\(^{(72)}\). Daidzein in the aglycone form is also more readily converted to equol\(^{(15)}\). Clerici et al\(^{(56)}\) found that pasta enriched in isoflavone aglycones significantly reduced total cholesterol, LDL-C, high-sensitivity C-reactive protein, and arterial stiffness compared with placebo in study participants, with effects more pronounced in equol producers. Of the fourteen RCT that found a positive association between equol producer status and CHD risk factors, seven used interventions of foods and milk enriched with soya\(^{(70)}\).

Furthermore, baseline age and the health status of the participants may contribute to variability in the outcome measurements. Oestrogen receptor B has been found to be enhanced in extracted arteries from postmenopausal CHD patients compared with normal subjects, with enhanced dilution in response to isoflavones\(^{(37)}\). Hodis et al. found that isoflavone supplementation failed to prevent the progression of subclinical atherosclerosis in healthy postmenopausal women overall; a subanalysis indicated, however, that healthy women within 5 years of becoming postmenopausal had a significantly reduced mean carotid artery intima-media thickness progression rate of 68% compared with placebo\(^{(74)}\). Previous meta-analyses have also found lipid variables to be more positively affected by soya interventions in hypercholesterolaemic patients than in healthy subjects\(^{(72,75)}\). We identified thirty-five RCT that only used postmenopausal women; all of the studies that found a favourable association of equol producer status on risk factors of CHD had postmenopausal participants. There were a relatively equal number of RCT using healthy participants (\(n = 20\)) \(v\). participants with underlying health issues or a history of illness (\(n = 22\)); of the fourteen studies that found equol producer status to improve risk factors for CHD, five had healthy participants\(^{(53,42,45,54,67)}\) while nine had participants with underlying health issues related to CHD\(^{(27,30,39,50,55,56,59,61,66)}\).

In the present systematic review, electronic databases were extensively searched following our defined set of guidelines and used to extract relevant data. Our results may imply that equol is beneficial on cardiovascular health, yet the interpretation is limited largely because of the secondary analysis of equol producers in RCT of dietary sources of isoflavones. Recently, equol itself has become available as a dietary supplement. Orally administered equol has greater plasma accumulation than other dietary sources of isoflavones\(^{(76)}\) and has the potential for enhanced therapeutic effects due to its more potent antioxidant properties and bioactivity among all isoflavones. In fact, one RCT of equol on risk factors of CHD has been conducted. Usui et al\(^{(77)}\) found a statistically significant improvement in LDL-C, glycated HbA1c levels, and cardio-ankle vascular index scores, a measure of vascular stiffness, in overweight and obese patients after dietary equol supplementation, particularly for non-equol producers. This study is limited by its small sample size and short duration of the intervention. We recommend additional RCT of equol itself as an intervention to directly assess its effects on CHD risk factors and potentially CHD.

**Supplementary material**

The supplementary material for this article can be found at http://dx.doi.org/10.1017/jns.2016.18

**Acknowledgements**

We thank Barb Folb for her expertise and guidance in designing the systematic review.

The present review was supported by the National Institutes of Health (R01 HL068200).

The authors’ responsibilities were as follows: A. S. designed the study; R. L. B. conducted the research; A. S., A. V. and R. L. B. analysed the data; A. S., V. A., A. V., R. W. E., Y. M., K. M., T. U. and R. L. B. drafted the manuscript; R. L. B. conducted the research; A. S., A. V. and R. W. E. conducted the systematic review. We thank Barb Folb for her expertise and guidance in designing the systematic review.

None of the authors reported a conflict of interest related to the present review.

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