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Effects of soy isoflavone extract supplements on blood pressure in adult humans: systematic review and meta-analysis of randomized placebo-controlled trials

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Objective **Reported effects of different soy products on blood pressure vary. This systematic review and meta-analysis was performed to clarify the effects of soy isoflavone extract supplements on systolic and diastolic blood pressure (SBP and DBP) in adult humans.**

Methods ***PubMed, CENTRAL, ICHUSHI,*** **and** ***CNKI*** **were searched in June 2009 for relevant randomized placebo-controlled trials. Study data and indicators of methodological validity were independently extracted by two authors using predefined data fields. Meta-analysis was carried out in Review Manager 5.0.22.**

Results **Searches identified 3740 articles, of which 14 randomized controlled trials (789 participants) were included. Daily ingestion of 25–375 mg soy isoflavones (aglycone equivalents) for 2–24 weeks significantly decreased SBP by 1.92 mmHg (95% confidence interval −3.45 to −0.39; $P=0.01$) compared with placebo (heterogeneity $P=0.39$, fixed effect model) in adults with normal blood pressure and prehypertension. The effect was not lost on sensitivity analysis. Subgroup analyses suggest greater effects in studies longer than 3 months, in Western populations, at lower doses, and in studies at lower risk of bias. Soy isoflavones did not affect DBP [−0.13 (95% confidence interval −1.03 to 0.78) mmHg, $P=0.78$; heterogeneity $P=0.20$, fixed effect model].**

Conclusion **Soy isoflavone extracts significantly decreased SBP but not DBP in adult humans, and no dose–response relationship was observed. Further studies are needed to address factors related to the observed effects of soy isoflavones on SBP and to verify the effect in hypertensive patients.** ***J Hypertens*** **28:1971–1982 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Keywords: blood pressure, human, hypertension, meta-analysis, soy isoflavone supplements, systematic review

Abbreviations: BP, blood pressure; CVD, cardiovascular disease; RCT, randomized controlled trial; SPI, soy protein isolate; WMD, weighted mean difference

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Introduction

From 2000 to 2006, 48% of Japanese men and 43% of Japanese women over 30 years of age had systolic blood pressure (SBP) over 140 mmHg and diastolic blood pressure (DBP) over 90 mmHg, or were taking antihypertensive drugs, and the number of people with hypertension is predicted to increase as the proportion of elderly people in the population grows [1]. Elevated blood pressure (BP) is positively related to cardiovascular morbidity and mortality [2–5] and, in people over 50 years of age, SBP over 140 mmHg is a more important cardiovascular disease (CVD) risk factor than DBP. In fact, the risk of CVD doubles with each increase of 20/10 mmHg (SBP/DBP), beginning at 115/75 mmHg. Individuals with SBP of 120–139 mmHg or DBP of 80–89 mmHg should be considered as prehypertensive and, therefore, require health-promoting lifestyle modifications to prevent CVD [6].

Epidemiological studies have reported mixed effects of diets rich in flavonoids on CVD risk. Randomized controlled trials (RCTs) evaluating the effects of various flavonoids on BP were statistically summarized in a recent systematic review and meta-analysis [7]. The effects of soy protein isolate (SPI), soy foods, and soy isoflavone extracts on BP differed. A meta-analysis of nine RCTs revealed that ingestion of SPI compared with control over 4–24 weeks significantly reduced DBP by 1.99 mmHg [95% confidence interval (CI) −2.86 to −1.12; heterogeneity $P=0.39$], but did not affect SBP. Data from seven RCTs of soy isoflavone extracts compared with placebo suggested a reduction

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in SBP (−2.60 mmHg, 95% CI −5.20 to 0.00, close to statistical significance; heterogeneity $P = 0.71$), but no effect on DBP.

The increasing use of various soy isoflavone supplements for myriad purposes underscores the importance of accurate assessments of their efficacy. Whether or not soy isoflavone extracts, which are widely available from health food stores, have beneficial effects in reducing BP remains unclear. Several RCTs that evaluated the effects of soy isoflavone extracts on BP were published after the above-mentioned meta-analysis [7]. The current systematic review and meta-analysis of randomized placebo-controlled trials was performed to clarify the effect of soy isoflavone extract supplements on SBP and DBP in adult humans.

Methods

Study identification and selection

The protocol of this systematic review and meta-analysis was based on Cochrane Handbook guidelines [8]. *PubMed*, *CENTRAL*, *ICHUSHI*, and *CNKI* were searched for published RCTs in June 2009, using complex search strategies containing text and indexing terms (Fig. 1). The search strategies for *PubMed* and *CENTRAL* excluding *PubMed* were developed using the Cochrane Handbook guidelines [8]. For the *PubMed* search, free keywords for soy isoflavones were mapped to appropriate indices terms. Reference lists of included RCTs and relevant systematic reviews and meta-analyses [7,9] were manually searched. We also contacted some authors to ask about additional studies.

RCTs published in English, Chinese, or Japanese were included in the review if they met all of the following criteria: parallel-group design or crossover design without significant carryover, period effects, or both; evaluated ingestion of soy isoflavone extract supplements (not soy protein, soy foods, or soymilk, and not products containing isoflavones from nonsoy sources) with a clearly described dose in adult humans; contained at least one relevant pairwise comparison of intervention arms (i.e., soy isoflavone extract vs. placebo or both plus a nonestrogen add-on); the placebo did not contain isoflavones/phytoestrogens or estrogens and was identical or similar in appearance and taste to the soy isoflavone supplement; and postintervention or change from baseline SBP, DBP, or both data (sample size, mean, and SD) were available. When two or more reports were published from the same trial, only the report with the largest sample size was included in the meta-analysis. Two researchers independently reviewed and evaluated the inclusion and exclusion of relevant RCTs, and consensus was reached by discussion when there were disagreements.

Data extraction and quality assessment

Data on study design, number of participants, intervention, SBP and DBP outcome, or change from baseline were independently extracted by two reviewers and were compared and confirmed. When necessary, data on BP outcomes were obtained from graphs. Wherever possible, we obtained necessary unreported data by contacting the authors. As postintervention and change from baseline data can be combined in the same meta-analysis [8], we extracted both types of data if the required means and SDs were available. If there was more than one dose of

Fig. 1

PubMed
#1: soy* OR isoflavone OR phytoestrogen OR genistein OR genistin OR daidzein OR daidzin OR glycitein OR glycitin
#2: randomized controlled trial [pt]
#3: random [tiab] OR randomized [tiab] OR randomly [tiab]
#4: control [tiab] OR controlled [tiab] OR placebo [tiab]
#5: #2 OR (#3 AND #4)
#6: #1 AND #5

CENTRAL excluding PubMed
#1: (soy* OR isoflavone OR phytoestrogen OR genistein OR genistin OR daidzein OR daidzin OR glycitein OR glycitin) in Clinical Trials
#2: "accession number" near pubmed in Clinical Trials
#3: (#1 NOT #2)

ICHUSHI
#1: 大豆[1]/TH or 大豆蛋白質[2]/TH or 大豆[1]/AL or だいず[1]/AL or ダイズ[1]/AL or イソフラボン[3]/AL or Isoflavones/TH or Genistein/TH or Daidzein/TH or Glycitein/TH or Genistin/TH or Daidzin/TH or Glycitin/TH or soy/AL or isoflavone/AL or genistein/AL or daidzein/AL or glycitein/AL or genistin/AL or daidzin/AL or glycitin/AL
#2: RD=ランダム化比較試験[4],準ランダム化比較試験[5]
#3: (random/TA or randomized/TA or randomly/TA or ランダム/TA or 無作為[6]/TA or 割り付[7]/TA or 割付[7]/TA or blind/TA or blinded/TA or mask/TA or masked/TA or double-blind/TA or double-blinded/TA or single-blind/TA or single-blinded/TA or single-mask/TA or single-masked/TA or double-mask/TA or double-masked/TA or 盲検[8]/TA or 遮蔽[9]/TA) and (control/TA or controlled/TA or placebo/TA or 対照[10]/TA or プラセボ[11]/TA or 偽薬[11]/TA)
#4: #2 or #3
#5: #1 and #4

CNKI
SU=soy+soya+soybean+isoflavone+phytoestrogen+daizein+daidzin+genistein+genistin+glycitein+glycitin+大豆[12]+黄豆[12]+染料木[13]+金雀异黄素[13]+异黄酮[14] and
SU=random+control+placebo+随机[15]+盲[16]+对照[17]+安慰剂[18]+伪药[18]

Search strategy for *PubMed*, *CENTRAL* excluding *PubMed*, *ICHUSHI*, and *CNKI*. [pt] and [tiab] are *PubMed* search field tags indicating publication type and title/abstract, respectively; +, the Boolean term; *CENTRAL*, Cochrane Central Register of Controlled Trials (http://www.mrw.interscience.wiley.com/cochrane/cochrane_clcentral_articles_fs.html); *CNKI*, China National Knowledge Infrastructure (http://www.cnki.net); *ICHUSHI*, Japana Centra Revuo Medicina (http://www.jamas.or.jp); SU, search tag including Chinese, English title, or both, keywords, and abstracts fields; /TH, /AL, RD, and /TA are search field tags of thesaurus terms, all fields, research design, and title/abstract, respectively. Japanese search terms: [1]soybean, [2]soy protein, [3]isoflavone, [4]randomized controlled trial, [5]quasi-randomized controlled trial, [6]random, [7]assign, [8]blind, [9]mask, [10]control, [11]placebo. Chinese search terms: [12]soybean, [13]genistein, [14]isoflavone, [15]random, [16]blind, [17]control, [18]placebo.

soy isoflavones, data for all isoflavone arms were combined to create one isoflavone group to compare with the placebo [8].

Although the Jadad *et al.* [10]'s scale has often been used to assess the quality of RCTs in meta-analyses, we used the three-category grading system (A, B, and C) used in the Agency for Healthcare Research and Quality [9] report on soy to denote the methodological quality of each study. Category A studies have the least bias and results are considered valid; category B studies are susceptible to some bias, but not sufficient to invalidate the results; and category C studies have significant bias that may invalidate the results (e.g., dropout rate >20%, missing baseline data, or irreconcilable apparent differences between data in figures, tables, and text). Category C studies were defined as low quality in this meta-analysis. Concealment of treatment allocation in RCTs was assessed as adequate, inadequate, or unclear [11]. In addition, methods of randomization and double blinding, and description of withdrawals and dropouts were evaluated based on the Jadad *et al.* [10]'s scale.

Study data and indicators of methodological validity were independently extracted by two authors using predefined data fields, and consensus was reached by discussion when there were disagreements.

Meta-analysis and statistical analysis

Meta-analysis was carried out using Review Manager 5.0.22 (Nordic Cochrane Center, Oxford, England). Of the 14 trials, five were crossover trials; and none of these five studies reported BP data for the first period or paired analysis. In order to include some crossover trials in the meta-analysis, it was necessary to impute missing SEs of mean differences between the two intervention arms. Because none of the trials reported data that would allow calculation of the correlation coefficients for associations between baseline and postintervention values or between the two crossover intervention arms, we estimated the SEs of mean differences between comparative arms for crossover design trials, using the most conservative imputation of the three levels of the correlation coefficient (0.25, 0.5, and 0.75), that is, the lowest correlation (0.25) [12]. We performed a meta-analysis to determine the overall effect of soy isoflavones on BP using the generic inverse variance method in Review Manager. The treatment effect of each trial was estimated as the mean difference between postintervention measurements or changes from baseline in BP for each comparison intervention arm (calculated as data for participants ingesting soy isoflavones – data for those ingesting placebo). The point estimate of mean difference for a crossover paired analysis is the same as for a parallel-group analysis (the mean of the differences is equal to the difference in means). The SEs of the mean difference for parallel-group trials were obtained with the use of Review Manager [8]. When trials contained repeated measurements of BP, we used the final data as long as the data from various time points had the same dropout rates; otherwise, the data with the lowest dropout were used. The data for other time points were used for sensitivity analyses. I^2 (0–40%, might not be important) was used to quantify inconsistency across studies [8].

We used both a fixed effect model and a random effects model to calculate weighted mean differences (WMDs), 95% CIs for each comparison, a combined overall effect with P value, and the P value for testing heterogeneity (where $P < 0.1$ was considered statistically significant). When there was significant heterogeneity across included trials, the results based on the random effects model were reported [8,13].

We conducted sensitivity analyses to evaluate the effects of degree of correlation between two postintervention values for crossover trials (imputing the correlation as 0.5 and 0.75), time point of measurement (using data for midterm reported in trials with repeated measurements on participants), and study quality (eliminating C-category trials). We also performed meta-analysis (using the WMD) incorporating crossover trials with parallel-group trials by using data from isoflavone periods and placebo periods as if the trial were a parallel-group trial of isoflavones and placebo [8].

If meta-analysis of at least 10 trials revealed a significant effect of soy isoflavones on BP, subgroup analyses and meta-regressions were performed to investigate possible factors that might relate to the various effects of soy isoflavones across included trials, on the basis of five prespecified factors: study design, intervention duration, participants' region, soy isoflavone dose, and study quality. We used a cutoff point of 75 mg/day in the subgroup analysis for isoflavone dose because a daily isoflavone intake of up to 75 mg (aglycone equivalent) is considered well tolerated by the Japan Food Safety Commission. Tests for heterogeneity based on χ^2 statistics were performed to investigate differences between the two subgroups [8].

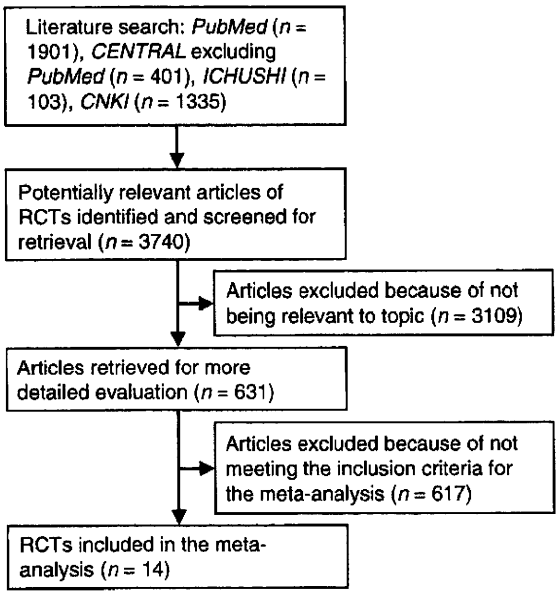
We examined potential publication bias by using funnel plots and by performing Egger's test to assess the asymmetry of funnel plots. Meta-regressions and tests for asymmetry of funnel plots were performed with Stata 10.1 for Windows (StataCorp LP, College Station, Texas, USA) user-written 'metareg' and 'metabias' commands; the 'metainf' command was used to investigate the influence of each trial on the overall meta-analysis estimate.

Results

Characteristics of the studies

The search strategy (Fig. 1) resulted in 3740 potentially relevant articles. Fourteen randomized placebo-controlled trials [14–27] met all the inclusion criteria and were included in the systematic review (Fig. 2). Characteristics of the 14 trials are summarized in Table 1. Nine

Fig. 2



Selection of randomized placebo-controlled trials. Among 631 full-text articles assessed for eligibility, 617 articles that did not meet all of the inclusion criteria (described in the second paragraph of the Methods section) were excluded from the meta-analysis. Articles were excluded as they were not RCTs; were published in a language other than English, Chinese, or Japanese; did not test soy isoflavone extracts; or did not meet another of the five inclusion criteria (many studies did not meet several inclusion criteria).

trials used a parallel-group design and the remaining five used a crossover design. Study duration ranged from 2 to 24 weeks (1 month was approximately converted to 4 weeks; median, 12 weeks; mean, 13 weeks). Two trials [23,27] did not clearly report the number of posttreatment participants analyzed, so we considered them to be the same as at baseline. Two trials [16,27] included men and women, the remaining 12 trials included only perimenopausal, postmenopausal women, or both. Four trials [15,22,25,27] did not clearly describe the form of isoflavones used; we assumed them to be aglycone equivalents for calculating average dosage of isoflavones for all included trials. If the studies did not report detailed composition of soy isoflavones used, we calculated the aglycone equivalent value by multiplying the total isoflavones by 0.61 [28]. Soy isoflavone dose ranged from 25 to 375 mg/day (median, 80 mg; mean, 95 mg). Only two [19,24] of the 14 trials reported significantly different postintervention SBP between soy isoflavone and placebo intervention arms.

Of the 14 included trials, 13 reported only postintervention BP data, and the remaining trial reported only change from baseline data. Therefore, we decided to combine the postintervention data and the change from baseline data in the meta-analysis. One trial [14] reported results of both per-protocol and intention-to-treat analyses, and the data from the intention-to-treat analysis were used for meta-analysis. For one study [21] that used two doses of isoflavones, data for both doses were combined to create one isoflavone arm to compare with placebo. For trials [20,26] with more than two intervention arms, data for relevant pairwise comparative intervention arms (i.e., soy isoflavone extracts vs. placebo) were used. For one trial [26] with repeated measurements, data from the final assessment at 6 months were used.

Participants in the comparative intervention arms had similar physical activity patterns and habitual dietary intakes of soy isoflavones. Most of the studies were designed to maintain the participants' usual diets, lifestyles, and body weights. Adverse events were generally similar for both the isoflavone and placebo arms and no serious adverse events were noted in the included trials, although they were not well addressed in several trials. In one study [19], an extremely high dose of soy isoflavones (375 mg/day) seemed to increase SBP.

Study validity was not high. Two trials [18,23] were assessed as having 'adequate' concealment of treatment allocation, one of which was rated 'A' (at low risk of bias), the other 'B' (at moderate risk of bias). The remaining trials were assessed as 'unclear' regarding allocation concealment due to insufficient information, of which four were rated 'A', four 'B', and four 'C' (at high risk of bias).

Effect on SBP

Meta-analysis of the 14 trials with 789 participants using the fixed effect model resulted in nonsignificant heterogeneity ($P = 0.39$, $I^2 = 6\%$), and revealed that daily ingestion of 25–375 mg soy isoflavones (aglycone equivalent) for 2–24 weeks significantly decreased SBP by 1.92 mmHg (95% CI −3.45 to −0.39, $P = 0.01$) compared with placebo (Fig. 3). No trial seemed to clearly influence the overall combined effect (Fig. 4). Results of meta-analysis using the random effects model were −2.02 mmHg (95% CI −3.65 to −0.39, $P = 0.02$).

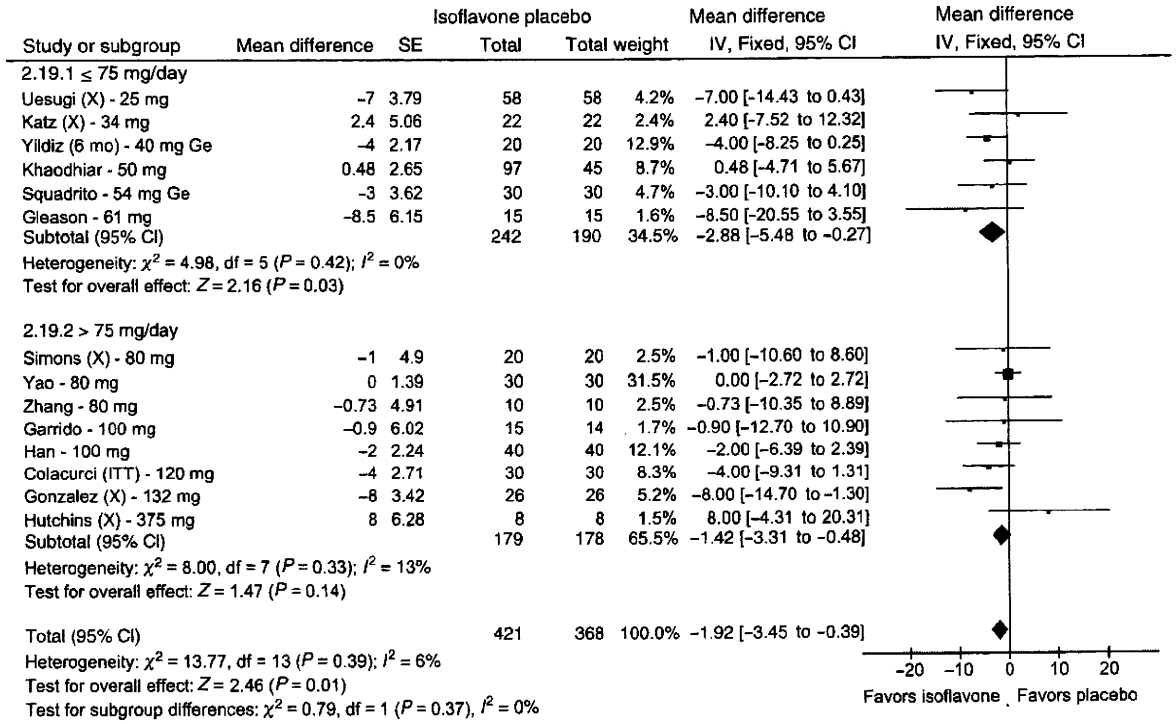
The following prespecified sensitivity analyses were performed: assuming the level of correlation coefficient between isoflavone and placebo intervention arm to be 0.5 [combined overall effect, −2.02 (95% CI −3.49 to −0.54) mmHg, $P = 0.007$; heterogeneity $P = 0.17$, $I^2 = 26\%$, fixed effect model] and 0.75 [−2.12 (95% CI −4.28 to 0.04) mmHg, $P = 0.006$; heterogeneity $P = 0.008$, $I^2 = 54\%$, random effects model]; using data for another time point of 3-month in one trial [26] with repeated measurements on participants [−2.85 (95% CI −3.45 to −0.25) mmHg, $P = 0.02$; heterogeneity $P = 0.34$, $I^2 = 10\%$, fixed effect model]; eliminating trials of C category [−3.44 (95% CI −5.47 to −1.41) mmHg, $P = 0.0009$; heterogeneity $P = 0.81$, $I^2 = 0\%$ fixed effect model] [16,19,21,25]; and incorporating crossover trials with parallel-group trials by using data from isoflavone

Table 1 Characteristics for randomized placebo-controlled trials of soy isoflavone extracts

Reference	Design[a]	Duration	Participants[b]	Intervention[c]	Blood pressure (mmHg)[d]	Quality[e]
Colacurci *et al.* [14]	P; R+, WD	6 months	60/57 (5%) healthy Post; age, 55.2 years; BMI (kg/m^2), 25.9; Italy	120 mg SI (Estromineral; AE; De/Ge, 60/60 mg) vs. placebo; tablet	122/79→119/78 vs. 119/77→121/79	A; unclear
Garrido *et al.* [15]	P; R, DB+	12 weeks	29/29 healthy Post; age, 53.5 years; BMI (kg/m^2), 26.9; Chile	100 mg SI (SoyLife) vs. placebo; capsule	119.0/76.2→116.2/78.4 vs. 124.0/78.3→117.1/75.2	B ($n<30$); unclear
Gleason *et al.* [16]	P; R, DB+, WD	6 months	34/30 healthy men and Post; age, 73.7 years; USA	100 mg SI glycoside (Novasoy; 61 mg AE; ~85% De & Ge) vs. placebo (maltodextrin and caramel food color); tablet	NA/NA→NA/NA (−5.8/−5.0) vs. NA/NA→NA/NA (2.7/−0.6)	C (missing baseline data); unclear
Gonzalez *et al.* [17]	X; R+, DB+, WD	12 weeks × 2; 4-week washout	32/26 (19%) white Post with type 2 diabetes; BMI (kg/m^2), 31; UK	132 mg SI (Essential Nutrition; AE; De/Ge/Gle, 37%/53%/10%) vs. placebo (microcrystalline cellulose); tablet	130/73→129/74 vs. 133/75→137/76	A; unclear
Han *et al.* [18]	P; R+, DB+, WD	4 months	82/80 (2%) Post; age, 48.5 years; BMI (kg/m^2), 24.9; Brazil	100 mg SI (Eugenbio Co. Ltd; AE; De/Ge/Gle, 19%/70%/11%) + 151 mg SP vs. placebo (100 mg glucose + 151 mg SP); capsule	131/84→131/85 vs. 133/84→133/84	A; Adequate
Hutchins *et al.* [19]	X; R, DB, WD	2 weeks × 4; 2-week washout	10/8 (20%) healthy Post; age, 56 years; BMI (kg/m^2), 28; USA	375 mg SI (Novasoy; AE; De/Ge/Gle, 28%/63%/9%) vs. placebo (95% white flour and 5% malic acid, or corn flour) vs. vitamin C vs. vitamin C + SI; capsule	NA/NA→125¶/72 vs. NA/NA→117/70	C (missing baseline data); unclear
Katz *et al.* [20]	X; R, DB, WD	6 weeks × 3; 6-week washout	25/22 (12%) healthy Post; age, 58.5 years; BMI (kg/m^2), 27.6; USA	55 mg daidzin and genistin (Maxisoy; 34 mg AE) vs. placebo vs. raloxifene; capsule	126.9/71.5→129.5/69.9 vs. 126.9/71.5→127.1/70.5	B ($n<30$); Adequate
Khaodhiar *et al.* [21]	P; R, DB, WD	12 weeks	191/142 (26%) Peri and Post; age, 53.1 years; BMI (kg/m^2), 28.6; USA	40 mg SI (AglyMax; AE; De/Ge/Gle, 70%/10%/20%) vs. 60 mg SI vs. placebo; capsule	122/76→119/75 vs. 121/78→116/74 vs. 120/76→117/74	C (drop >20%); unclear
Simon *et al.* [22]	X; R, DB+, WD	8 weeks × 2; 8-week washout	23/20 (13%) healthy Post; age, 59 years; BMI (kg/m^2), 26.8; Australia	80 mg SI (PhytoLife 1) vs. placebo; tablet	135/85→125/80 vs. 135/85→126/80	B ($n<30$); unclear
Squadrito *et al.* [23]	P; R, DB	6 months	60/unclear healthy Post; age, 56 years; Italy	54 mg Ge (Lab. Plants; ≈98% pure) vs. placebo; tablet	113/80→110/79 vs. 112/77→113/78	A; unclear
Uesugi *et al.* [24]	X; R, DB+	4 weeks × 2; no washout	58/58 Peri and Post; age, 58 years; BMI (kg/m^2), 23; Japan	40 mg SI (Fuji Oil Company Institute; 25 mg AE; De/Ge/Gle, 46%/13%/41%) vs. placebo (the same matrix without SI); tablet	140/84→127#¶/78# vs. 140/84→134#/80#	A; unclear
Yao *et al.* [25]	P; R, DB	12 weeks	60/60 healthy Post; age, 40–55 years; China	80 mg SI vs. placebo (starch); capsule	NA/NA→124.5/78.7 vs. NA/NA→124.5/78.0	C (missing baseline data); unclear
Yildiz *et al.* [26]	P; R, patient-blind, WD	3, 6 months	80/80 (0%) healthy Post; age, 50 years; BMI (kg/m^2), 27.2; Turkey	40 mg Ge vs. placebo vs. raloxifene vs. estradiol valerate + dienogest	123.0/77.0→119.5/76.5 (3 mo)→126.8/81.8 (6 mo) vs. 130.0/78.1→126.3/77.5→130.8/81.8	B (not double-blind); unclear
Zhang and Pu [27]	P; R	6 weeks	20/unclear MetS (male/female, 15/5); age, 38–55 years; Australia	80 mg SI (Novogen Ltd.) vs. placebo	132.79/70.29→130.13/68.29 vs. 131.57/68.45→130.86/69.55	B (unclear dropout, $n<30$); unclear

[a] DB, double-blinded; DB+, double-blinded by appropriate method; P, parallel; R, randomized; R+, randomized by appropriate method; WD, withdrawals and dropouts described; X, crossover. [b] Randomized/completer number (dropout rate) of participants; MetS, patients with metabolic syndrome; Peri, perimenopausal women; Post, postmenopausal women. [c] Daily soy isoflavone (SI) dose; AE, aglycone equivalents; De, daidzein; Ge, genistein; Gle, glycitein; SP, soy protein. [d] Baseline→posttreatment (change) SBP/DBP for SI intervention vs. that for placebo. ¶, significantly different with placebo; #, significantly different from baseline; NA, not available. [e] Three-category grading system (A, B, and C) [9]; concealment of treatment allocation (adequate, inadequate, and unclear) [11].

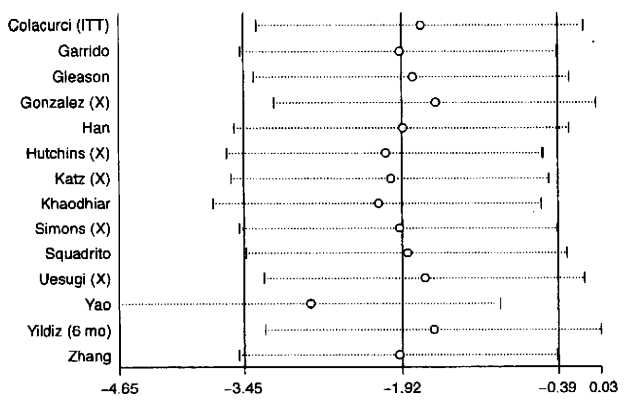
Fig. 3



Effects of soy isoflavones on SBP (mmHg). ■, effect estimate of each study (size of the square corresponds to its weight), horizontal line denote the 95% CI; ◆, combined overall effect; CI, confidence interval; fixed, fixed effect model; ITT, intention-to-treat analysis data were used; mean difference, mean of postintervention or change from baseline SBP for soy isoflavones minus that for placebo; X, crossover design.

periods and placebo periods as if the trial were a parallel-group trial of isoflavones and placebo [−1.86 (95% CI −3.42 to −0.30) mmHg, $P = 0.02$; heterogeneity $P = 0.55$, $I^2 = 0\%$ fixed effect model]. An additional sensitivity analysis excluding one 2-week trial [19] that used an extremely high dose of soy isoflavones revealed that daily ingestion of 25–132 mg (median, 80 mg; mean, 74 mg) soy isoflavones (aglycone equivalent) for 4–24 weeks (median, 12 weeks; mean, 14 weeks) significantly decreased SBP by 2.08 mmHg (95% CI −3.62 to −0.53, $P = 0.008$; heterogeneity $P = 0.51$, $I^2 = 0\%$, using either the fixed effect model or the random effects model).

Fig. 4



Influence of each study on the overall effect on SBP (mmHg). The vertical centerline and the two lines on both sides of it, respectively, denote the combined overall effect and 95% CI for all included studies (fixed effect model). The open circles (○) and horizontal dotted lines, respectively, denote the combined overall effect and 95% CI when each study is omitted, thereby demonstrating the influence of this study on the overall effect. CI, confidence interval; ITT, intention-to-treat analysis data were used; X, crossover design.

Results of subgroup analyses of the effects of soy isoflavones on SBP based on the five prespecified factors and participants' menopausal status (menopausal women only vs. both men and women) are shown in Table 2. Using the fixed effects model, subgroup analyses of trials conducted in Western participants, of trials using 75 mg or less soy isoflavones per day, of trials with more than 3-month duration, of A and B-category trials, or of trials including menopausal women only, revealed significant effects of soy isoflavones on SBP. However, subgroup analyses of trials conducted in Asian participants, of trials using more than 75 mg soy isoflavones per day, of trials with 3 months or less duration, of C-category trials, or of trials including both men and women did not reveal significant effects of soy isoflavones on SBP. None of the six subgroup analyses based on the six factors (study

Table 2 Subgroup analyses of the effects of soy isoflavones on SBP

Factors	Trials	*n*	Heterogeneity	Mean difference (mmHg) [95% CI] Fixed effect model	Random effects model
Study design					
Parallel-group [14–16,18,21, 23,25–27]	9	521	$P=0.71$, $I^2=0\%$	−1.62 [−3.29 to 0.05], $P=0.06$	−1.62 [−3.29 to 0.05], $P=0.06$
Cross-over [17,19,20,22,24]	5	268	$P=0.11$, $I^2=47\%$	−3.51 [−7.34 to 0.33], $P=0.07$	−2.45 [−7.90 to 2.99], $P=0.38$
Subgroup difference				$P=0.38$	
Participants' region					
Asian [24,25]	2	176	$P=0.08$, $I^2=67\%$	−0.83 [−3.39 to 1.73], $P=0.52$	−2.61 [−9.25 to 4.02], $P=0.44$
Western [14–23,26,27]	12	613	$P=0.56$, $I^2=0\%$	−2.53 [−4.44 to −0.62], $P=0.009$	−2.53 [−4.44 to −0.62], $P=0.009$
Subgroup difference				$P=0.30$	
Isoflavone dose					
≤75 mg/day [16,20,21,23,24,26]	6	432	$P=0.42$, $I^2=0\%$	−2.88 [−5.48 to −0.27], $P=0.03$	−2.88 [−5.48 to −0.27], $P=0.03$
>75 mg/day [14,15,17–19,22,25,27]	8	357	$P=0.33$, $I^2=13\%$	−1.42 [−3.31 to 0.48], $P=0.14$	−1.64 [−3.84 to 0.57], $P=0.15$
Subgroup difference				$P=0.37$	
Intervention duration					
≤3 months [15,17,19–22,24–27]	10	559	$P=0.20$, $I^2=27\%$	−1.30 [−3.20 to 0.61], $P=0.18$	−1.784 [−4.37 to 0.81], $P=0.18$
>3 months [14,16,18,23,26]	5	270	$P=0.88$, $I^2=0\%$	−3.45 [−5.88 to −1.02], $P=0.005$	−3.45 [−5.88 to −1.02], $P=0.005$
Subgroup difference[a]				$P=0.11$	
Study quality					
A and B category [14,15,17,18, 20,22–24,26,27]	10	541	$P=0.81$, $I^2=0\%$	−3.44 [−5.47 to −1.41], $P=0.0009$	−3.44 [−5.47 to −1.41], $P=0.0009$
C category [16,19,21,25]	4	248	$P=0.31$, $I^2=16\%$	0.07 [−2.26 to 2.39], $P=0.96$	0.08 [−2.92 to 3.08], $P=0.96$
Subgroup difference				$P=0.04$	
Participants' menopausal status					
Menopausal women only	12	739	$P=0.32$, $I^2=12\%$	−1.84 [−3.40 to −0.28], $P=0.02$	−2.01 [−3.77 to −0.24], $P=0.03$
Both men and women	2	50	$P=0.32$, $I^2=0\%$	−3.75 [−11.28 to 3.77], $P=0.33$	−3.75 [−11.28 to 3.77], $P=0.33$
Subgroup difference				$P=0.63$	

[a]Excluded data at 3-month for one study [26] with repeated measurements from ≤3 months subgroup.

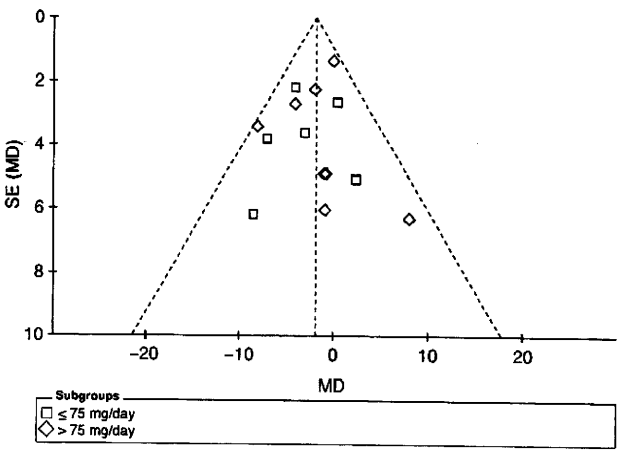
design, participants' region, isoflavone dose intervention duration, study quality, and participants' menopausal status) resulted in significantly different effects of soy isoflavones on SBP between the two comparative subgroups of trials, with the exception of that resulting from the subgroup analysis including A and B-category trials, which was significantly ($P=0.04$) different from the effect resulting from the subgroup analysis including C-category trials. Meta-regressions analyzing each of the five prespecified factors and participants' menopausal status did not reveal these factors to be significantly associated with the observed effects of isoflavones on SBP across the included 14 trials (data not shown), except that the factor of study quality seemed to significantly ($P=0.046$) relate to the observed effects of soy isoflavones.

The funnel plots (Fig. 5) and Egger's test ($P=0.605$) did not indicate obvious publication bias.

Effect on DBP

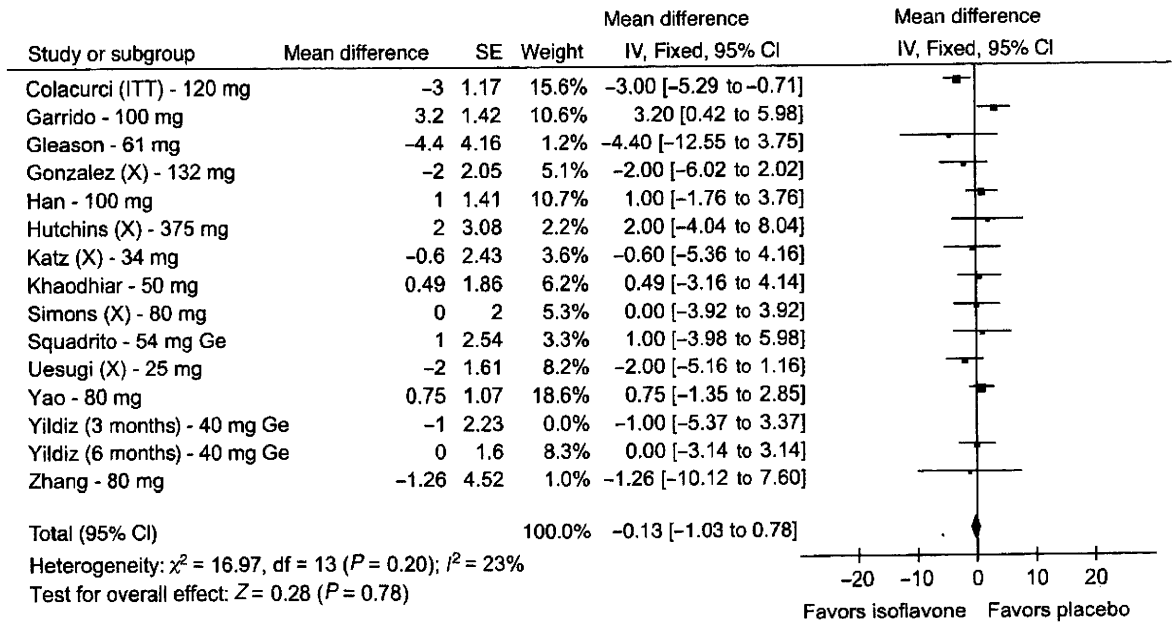
One study [15] reported much smaller SD values for DBP than other included trials, and so carried 63.5% of the weight of the meta-analysis using the fixed effect model (heterogeneity $P<0.00001$, $I^2=73\%$). In addition, obvious publication bias was also detected by the funnel plots and Egger's test ($P=0.002$). Correcting the reported SD data to SE data or excluding this study removed this anomaly. The SD data were thus assumed to be SE data. We performed the meta-analysis by correcting the reported SD data to SE data and conducted extra sensitivity analyses by using the originally reported values as SD or by excluding this trial. Meta-analysis of the 14 trials with 789 participants using the fixed effects model did not result in significant heterogeneity ($P=0.20$, $I^2=23\%$), and revealed that daily ingestion of 25–375 mg soy isoflavones (aglycone equivalent) for 2–24 weeks did not significantly affect DBP

Fig. 5



Funnel plots of effects of soy isoflavones on SBP (mmHg). The vertical center broken line and two broken lines on both sides of it, respectively, denote the combined overall effect and 95% CI (fixed effect model). CI, confidence interval; MD, mean postintervention or change from baseline SBP for soy isoflavones minus that for placebo; SE (MD), standard error of MD.

Fig. 6



Effects of soy isoflavones on DBP (mmHg). ■, effect estimate of each study (size of the square corresponds to its weight), horizontal line denote the 95% CI; ◆, combined overall effect; CI, confidence interval; ITT, intention-to-treat analysis data were used; mean difference, mean postintervention or change from baseline DBP for soy isoflavones minus that for placebo; random, random effects model; X, crossover design.

(−0.13 mmHg, 95% CI −1.03 to 0.78 mmHg, $P=0.78$) compared with placebo (Fig. 6). Meta-analysis using the random effects model resulted in a nonsignificant decrease of −0.13 mmHg (95% CI −1.21 to 0.96, $P=0.82$) in DBP.

The following sensitivity analyses were performed: assuming the level of correlation coefficient between isoflavone and placebo intervention arm to be 0.5 [combined overall effect −0.21 (95% CI −1.07 to 0.65) mmHg, $P=0.63$; heterogeneity $P=0.15$, $I^2=28\%$, fixed effect model] and 0.75 [−0.25 (95% CI −1.28 to 0.78) mmHg, $P=0.63$; heterogeneity $P=0.08$, $I^2=38\%$, random effects model]; using data for another time point of 3-month in one trial [26] with repeated measurements on participants [−0.18 (95% CI −1.10 to 0.74) mmHg, $P=0.70$; heterogeneity $P=0.19$, $I^2=24\%$, fixed effect model]; eliminating trials [16,19,21,25] of C category [−0.18 (95% CI −1.09 to 0.74) mmHg, $P=0.70$; heterogeneity $P=0.17$, $I^2=27\%$, fixed effect model]; incorporating crossover trials with parallel-group trials by using data from isoflavone periods and placebo periods as if the trial were a parallel-group trial of isoflavones and placebo [−0.08 (95% CI −1.01 to 0.85) mmHg, $P=0.87$; heterogeneity $P=0.23$, $I^2=20\%$, fixed effect model]; without correcting the originally reported SD data to be the SE data [−0.06 (95% CI −1.63 to 1.52) mmHg, $P=94$; heterogeneity $P<0.00001$, $I^2=73\%$, random effects model] or excluding the study [15] with extremely smaller SD data [−0.52 (95% CI −1.48 to 0.43) mmHg, $P=0.28$; heterogeneity $P=0.54$, $I^2=0\%$, fixed effect model]. An additional sensitivity analysis excluding one trial [19] that used an extremely high dose of soy isoflavones revealed that daily ingestion of 25–132 mg (median, 80 mg; mean, 74 mg) soy isoflavones (aglycone equivalent) for 4–24 weeks (median, 12 weeks; mean, 14 weeks) did not significantly affect DBP [−0.18 (95% CI −1.09 to 0.74) mmHg, $P=0.70$; heterogeneity $P=0.17$, $I^2=27\%$, fixed effect model].

Discussion

This meta-analysis of 14 randomized placebo-controlled trials with 789 participants found that daily ingestion of 25–375 (median 80) mg soy isoflavones (aglycone equivalent) for 2–24 weeks significantly decreased SBP by 1.92 mmHg compared with placebo in adults with normal BP and prehypertension (Fig. 3). The nonsignificant heterogeneity ($P=0.39$, $I^2=6\%$) indicated that the 14 included trials reported similar effects of soy isoflavone extracts on SBP. Results of sensitivity analyses indicated that the effect of soy isoflavones on SBP was robust, although the validity of the included studies was not high. The reduction in SBP resulting from soy isoflavone extracts was similar to that resulting from decreasing daily salt intake by 2 g [29] or decreasing body weight by 2 kg [30]. A daily intake of 80 mg (the median value of the studies included in this review) of soy isoflavones is approximately equivalent to 1.7 times the amount consumed habitually in Japan (mean daily intake is 47.2 mg

soy isoflavones) [31]. Our meta-analysis did not reveal a significant effect of soy isoflavone extracts on DBP.

Although 10 of the 14 included trials in this meta-analysis showed decreases in SBP resulting from soy isoflavones compared with placebo, the 95% CI often included zero (Fig. 3). However, the combined overall effect was significantly different from zero, which indicated that limited sample sizes often prevent detection of significant effects in individual studies. The results of our meta-analysis (including 14 RCTs) of soy isoflavone extracts on SBP and DBP are consistent with those reported by the recent meta-analysis (including seven RCTs); furthermore, the significant effect on SBP and increase in trial number and, therefore, sample size led to strengthened statistical power in the current meta-analysis compared with the previous meta-analysis (the results of which were close to significance) [7]. Uesugi *et al.* [24] reported that isoflavone ingestion significantly decreased both SBP and DBP levels from baseline. The decrease was greater in hypertensive (SBP > 130 mmHg, DBP > 85 mmHg) patients than that in normotensive participants, and there was a significant difference between the isoflavone and placebo interventions. Only five [17,18,22,24,27] of the 14 RCTs included prehypertensive participants according to the baseline mean BP (SBP > 130 mmHg but ≤140 mmHg, whereas none of the included studies included participants with DBP > 85 mmHg) and the remaining RCTs included participants with normal BP. This may explain why our meta-analysis including participants with above-mentioned baseline BP revealed a small but significant effect of soy isoflavone extracts on SBP but not on DBP. Further studies are needed to verify the effect on BP in those with differing baseline BP values. The lack of studies on those with hypertension suggests that studies of soy isoflavone supplements in conjunction with antihypertensive treatment may provide useful data.

Whether the lack of effect of isoflavones on DBP is due to greater errors in measurement of DBP than SBP is unclear. None of the 14 RCTs included in the current meta-analysis addressed this problem. Among the included 14 RCTs, coefficients of variation (calculated as mean value divided by its SD) for baseline SBP and DBP measurements (mean ± SD, 2.25 ± 0.59 vs. 2.11 ± 0.88%, $P = 0.55$), posttreatment measurements (2.30 ± 0.99 vs. 2.06 ± 1.03%, $P = 0.38$), and both baseline and posttreatment measurements (2.28 ± 0.84 vs. 2.08 ± 0.96%, $P = 0.28$) were calculated. These data indicated that the measurement errors of DBP were similar to those of SBP, and that the lack of effect on DBP is likely due to other factors.

The mechanism by which soy isoflavones decrease SBP is not yet well understood, but it may involve their chemical and biological similarity to mammalian estrogens, which have been shown to reduce BP in women [32]. Isoflavones are phytoestrogens that possess estrogen-like activities due to their ability to bind to the estrogen receptor, and they may trigger many of the biological responses that are evoked by physiological estrogens [33]. For example, infusion of the isoflavone genistein was shown to produce a dose-dependent increase in forearm blood flow in men and premenopausal women, and equimolar concentrations of 17β-estradiol caused similar vasodilation [34]. Responses to genistein and 17β-estradiol were inhibited to the same degree by the nitric oxide synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA). A threshold dose of genistein potentiated the endothelium-dependent vasodilator acetylcholine but not the endothelium-independent vasodilator nitroprusside. Ingestion of soy isoflavones significantly improved endothelium-dependent vasodilatation but had no impact on endothelial-independent arterial diameter and flow [14]. Intraarterial infusion of L-NMMA inhibited the effects of isoflavones on endothelium-mediated vasodilatation. Finally, a recent meta-analysis [35] demonstrated that oral isoflavone supplementation significantly increased flow-mediated dilation (FMD) in postmenopausal women.

A number of different and possible mechanisms regarding BP-lowering effects of isoflavones could be related to their properties as flavonoids [7,36]. Flavonoids are thought to influence FMD through effects on the acute response cell-signaling pathways that increase nitric oxide production [7]. The isoflavone-associated improvement in endothelial function is particularly relevant in light of the hypothesis that these compounds may act directly on endothelial cells, causing them to release nitric oxide. The direct impact of isoflavones on endothelial function is further supported by data showing that they are associated with improvement in endothelium-dependent responses, as demonstrated by inhibition of brachial artery changes due to the simultaneous presence of L-NMMA [14,34]. Among the 14 RCTs included in our meta-analysis, two studies [23,25] addressed the effects of soy isoflavones on nitric oxide in healthy postmenopausal women, with both reporting a significant increase in nitric oxide. One [23] of the two studies revealed that genistein ingestion improved flow-mediated vasodilation, and that this improvement might be mediated by a direct effect of genistein on vascular function, possibly as a result of an increased ratio of nitric oxide to endothelin (the decrease in endothelin by genistein was also significant). In addition, changes in plasma nitric oxide products and changes in brachial artery blood flow were positively correlated with genistein plasma levels [23]. Thus, it is reasonable to conclude that ingestion of soy isoflavones, a subclass of flavonoids, increases nitric oxide release and inhibits nitric oxide inactivation, similar to estrogen [37] and raloxifene [38], and the improved nitric oxide status induces vasodilation and hence decreases BP [39].

Isoflavones significantly reduced plasma levels of intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin [14]. The finding of a trend toward lower plasma P-selectin levels suggests a reduction of platelet activation, and may be related to the antithrombotic and platelet aggregation inhibition effects of isoflavones [40]. Both genistein and, to a lesser extent, daidzein have antiaggregatory activity in human platelets *in vitro* [41], and these results are consistent with earlier reports of lower platelet aggregation in rats treated with isoflavones [42]. The mechanisms through which isoflavones may enhance nitric oxide release by endothelium and reduce circulating adhesion molecules are not fully known, but it is possible that they may involve genomic (estrogen receptor activation) or alternatively nongenomic (inhibition of tyrosine kinase and antioxidant properties) effects of isoflavones [14,43].

A dose–response effect of soy isoflavones on SBP was not found in our meta-analysis, which might be due to the different chemical forms and proportions of individual soy isoflavones provided in the various studies [39,44–46]. Some of the included trials used soy isoflavone extracts rich in genistein, whereas some used products rich in daidzein, and some used pure genistein (Table 1). An intake of about 50 mg/day of genistein has been reported to have biological effects [47]. Thus, trials that used soy isoflavone extracts containing genistein at doses over 50 mg/day might be expected to show stronger effects on SBP than extracts containing genistein at doses less than 50 mg/day (although the total soy isoflavones could still be over 50 mg/day). It was impossible to evaluate the effect of each individual soy isoflavone (genistein, daidzein, or glycitein) on the combined effect estimate resulting from soy isoflavone extracts in this systematic review because not all studies clearly reported the dose of each individual soy isoflavone.

Another factor to be considered when interpreting our results is the potential impact of isoflavone metabolites. Isoflavone glycosides are not absorbed intact across the enterocytes of healthy adults, and their bioavailability requires initial hydrolysis by intestinal β-glucosidases for uptake to the peripheral circulation [46]. Asian and Western populations are reported to have differences in the capacity of intestinal flora to convert daidzein to its metabolite, equol [48]. Equol is easily absorbed and possesses substantial estrogenic activity because of its affinity for both the estrogen α and β receptors [44]. Equol has been suggested to be an important factor that influences the clinical efficacy of soy isoflavones in terms of cardiovascular health [49]. Thus, the influence of equol production on BP should be studied further. Further research is needed to clarify the particular factors that may increase or decrease the magnitude of the effects of soy isoflavone extracts.

Subgroup analyses (Table 2) and meta-regressions did not reveal significant relationships between the observed effects of soy isoflavone extracts on SBP across the 14 included trials and study design, intervention duration, participants' region, isoflavone dose, or menopausal status. Although significant effects of soy isoflavones on SBP were seen in subgroup analyses of trials conducted in Western participants, studies using 75 mg or less soy isoflavones/day, studies of more than 3-month duration, or studies in which participants were menopausal women only, and the magnitude of the effect for each of those subgroups was larger than that for comparative subgroups, none of these subgroup analyses revealed significant differences between two comparative subgroups (Table 2). The effect on SBP resulting from studies of low or moderate risk of bias (subgroup A and B-category trials) was significant and was significantly more pronounced than that resulting from lower quality (subgroup of C category) trials. The explanation for the significant relationship between the effect on SBP and study quality is unclear, but might be caused by the limited number of trials in each subgroup, and might also be caused by differences in other factors such as habitual dietary intake of soy isoflavones [50], participants' age and ethnicity, baseline BP status, chemical forms and proportions of individual soy isoflavones, urinary isoflavone excretion, and equol producer status. Because of the limited number of randomized placebo-controlled trials and insufficient available data, our meta-analysis was unable to evaluate the possible influences of those factors.

Among the 14 RCTs included for the current meta-analysis, there were five studies reporting plasma concentrations of genistein, other isoflavones, or both after soy isoflavone extracts ingestion (Garrido *et al.* [15], total isoflavones; Gleason *et al.* [16], total isoflavones, genistein, daidzein, and equol; Khaodhiar *et al.* [21], daidzein, genistein, glycitein, and equol; Simons *et al.* [22], daidzein and genistein; Squadrito *et al.* [23], genistein). Unfortunately, none of the five studies addressed the effects of plasma levels of isoflavones on BP. We were unable to stratify our results considering this with the use of subgroup analysis and meta-regression due to the small number (<10) of available studies. Although preliminary meta-regressions were conducted to investigate the relationships between soy isoflavone extract effect estimates and plasma total isoflavones (for the five RCTs) and genistein (for four RCTs excluding Garrido *et al.* [15]) levels, none were statistically significant.

Very few studies have addressed the possible interactions between the different components (e.g., soy isoflavones, soy protein *per se*, soy fibers, and soy saponins) actively affecting BP. The methodology used in this current meta-analysis did not allow for elucidation of interactions between soy isoflavones and other active components. However, of the 14 RCTs included in the meta-analysis, one study [14] failed to reveal a significant effect of soy isoflavone extracts on BP and suggested that the hypotensive effects of soy may be related to the ingestion of

phytoestrogens in combination with other soy components.

Since the publication of a recent meta-analysis [7], we have identified several other RCTs that may meet the inclusion criteria for evaluating effects of soy isoflavone extracts, soy foods, and isolated soy protein ingestion on BP. Evaluation of these studies is complicated by the variety of soy products, interventions, and controls used. We conducted the current meta-analysis to clarify the specific effects of soy isoflavone extracts on BP compared with placebo, eliminating the possible effects resulting from other active confounding components in soy foods or SPIs. Further research is needed to clarify the effects of soy foods and isolated soy proteins.

In conclusion, this meta-analysis revealed that daily ingestion of soy isoflavone extract supplements (25–375 mg aglycone equivalent) for 2–24 weeks significantly decreased SBP by 1.92 mmHg but had no effect on DBP in normal or prehypertensive adult humans, with no observation of a dose–response relationship. Further studies are needed to address factors related to the effects of soy isoflavones on SBP and to verify the lack of effect on DBP, especially in hypertensive patients. The isoflavone-associated increase in nitric oxide release may play an important role in decreasing BP, although the mechanisms by which soy isoflavones lower BP need further investigation.

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Mini Review

イソフラボン

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はじめに

イソフラボンの健康効果と安全性については、これまでに多くの報告と議論がなされてきた。本ミニレビューでは、イソフラボンの基礎と最近の話題を紹介する。

1. イソフラボンについて

イソフラボンは大豆に含まれるフラボノイドで、大豆１ｇ当たりアグリコン換算で２～4mg 含まれている。イソフラボンには異性体があり、大豆に含まれるイソフラボンの 50%はゲニステイン型、40%はダイゼイン型、10%がグリシテイン型である。通常の食品中には配糖体として存在しているが、納豆などの発酵食品では糖を含まないアグリコン型の含量が高い（図 1）。日本人のイソフラボン摂取量は中央値で約 18mg/日、平均値で約 25mg/ 日である[1]。一方、欧米人の摂取量は約 3mg/ 日にすぎない。イソフラボンは、エストロゲン受容体に対してエストロゲンの 1/1,000 ～ 1/10,000 の親和性で結合し、弱いエストロゲン様作用を示す。生体内にエストロゲンが存在する場合にはエストロゲンに対して拮抗的に働くと考えられるが、その濃度関係は不明である。その他、イソフラボンには、抗酸化作用やチロシンキナーゼ活性抑制作用もある[2]。

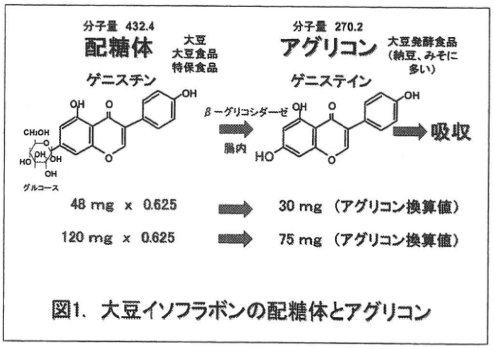

図1. 大豆イソフラボンの配糖体とアグリコン

2. イソフラボンの代謝

大豆に含まれている主なイソフラボンは、ダイゼイン、ゲニステイン、グリシテインであるが、中でもダイゼインは腸内細菌によってエストロゲン活性のより強いエクオール、あるいは活性の弱い O- デスメチルアンゴレンシン（O-DMA）に代謝される（図 2）。

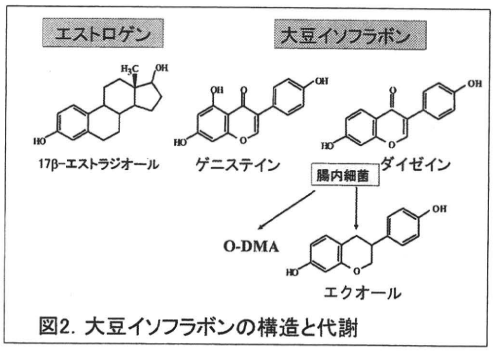

図2. 大豆イソフラボンの構造と代謝

エクオールはヒト以外の動物では個体差なく産生されるが、ヒトでは個人差や人種差がある。欧米人のエクオール産生者はおよそ30%であり、一方、日本人の閉経後女性では約50%であるとの報告がある[3]。近年、ダイゼインの骨量減少抑制作用は、その代謝産物の産生能の有無に依存する可能性が示唆されている。最も有力な代謝産物はエクオールであり、Shechellら[3]及び我々の日本人を対象としたイソフラボンの介入試験[4]においても、エクオール産生者は、非産生者に比べてイソフラボンの骨量減少抑制作用が有意に強く認められた。

エクオール産生能は、食事により影響されることが分かっている。現在までの観察研究により、大豆のほか、不飽和多価脂肪酸、炭水化物、食物繊維の多い食事とエクオール産生能の関連が示唆されている[5]。動物試験では、フラクトオリゴ糖などの難消化性糖質がエクオールの産生を亢進し、イソフラボンの生体利用性を高めることが確認されている。エクオール産生を亢進する腸内細菌として、乳酸菌ラクトコッカス20-92株が健常な日本人の糞便中から単離されている[6]。興味深いことに、我々のイソフラボン介入試験に参加した閉経後女性の35%がこの乳酸菌を保有していたが、保有者は必ずしもエクオール産生者ではなかった。現在のところ、エクオール産生能は特定の産生菌と腸内環境の両方に依存すると考えられている。なお、エクオール含有食品の無作為割付比較試験において、閉経後女性の尿中の骨吸収マーカーが有意に低下することが確認されている[7]。

3. イソフラボンの生理作用

3.1 骨代謝調節作用

骨粗鬆症モデル動物において、イソフラボンが骨量減少を抑制するという報告は多い[8]。観察研究では、上海在住の40～70歳の女性75,000人を対象にした前向きコホート研究において、大豆たん白質及びイソフラボンの摂取量と骨折率が負の相関を示すことが報告されている[9]。一方、閉経後女性を対象とした無作為割付比較試験では、対象となる人種や摂取期間によりイソフラボンの骨密度に対する効果は一致していない[10]。ヒトを対象とした無作為割付比較試験の最近のメタ解析においても、結果は分かれている。イソフラボンの骨密度に対する効果が一致しない理由として、使われた食品の形態（食品かまたは濃縮物か等）、含有されるイソフラボンの種類、エクオール産生の有無等が挙げられる[4,11]。わが国では、大豆イソフラボンは「骨が気になる方」のための特定保健用食品の関与成分として健康強調表示が許可されているが、これはイソフラボンの摂取により尿中の骨吸収マーカーが低下することを科学的根拠としている。Natural Medicine Comprehensive Databaseでは、イソフラボンの骨粗鬆症予防効果は、効くとは断言できないが、効果の可能性が科学的に示唆されていると記載されている[12]。

これらのことを総合すると、イソフラボンを含む大豆の長期間の摂取は、骨代謝に対して好い影響を及ぼすが、1～3年間の介入試験では、個人差や人種差により、必ずしも骨密度を改善するまでには至らないものと考えられる。

イソフラボンの骨に対する効果の作用機序として、エストロゲンと同様に骨吸収を司る破骨細胞のアポトーシスを促進する可能性、また、チロシンキナーゼ活性を抑制することにより、破骨細胞の活性を低下させる可能性等が考えられている[13]。

3.2 その他の生理作用

大豆の摂取量と生活習慣病の罹患率が負の相関を示すという疫学データは多い[14]。実際、アジア人は欧米人に比べてホルモン依存性のがんや心疾患の罹患率が低いこと、更年期症状が軽いこと等が報告されている。イソフラボンは、このような大豆の健康効果の一旦を担っていると考えられるが、イソフラボンが直接これらに関連するという科学的根拠は十分に蓄積されていない。これらについては、今後の研究の発展が期待されるところである。

4. イソフラボンの安全性

イソフラボンは弱いエストロゲン様作用を示すことから、生殖系への影響が懸念されている。植物性エストロゲンの一種であるクメステロールを含むクローバーを大量に摂取した羊が不妊症を呈したという50年前の報告がある[15]。大豆イソフラボンについては、骨への作用は子宮肥大化作用の約1/10量で発現されることが動物試験でわかっている[16]。内閣府食品安全委員会は、特定保健用食品またはイソフラボンが濃縮・強化された食品からの大豆イソフラボンの安全な一日上乗せ摂取上限量を30mg/日、また、大豆イソフラボンの一日摂取目安量の上限値を75mg/日と結論した[1]。これは、若年女性がアグリコン換算で57 mgのイソフラボンを含む豆乳を2月経周期毎日摂取した結果、月経周期が数日間延長したこと、また、閉経後女性が150mg/日のイソフラボンアグリコンのサプリメントを5年間摂取した結果、子宮内膜症の発症がプラセボ群に比べて僅かに高かったという報告が主な科学的根拠になっている[1]。さらに、濃縮・強化された食品からのイソフラボンの摂取は、妊娠中の女性や、乳幼児、小児については推奨できないとされた[1]。また、乳がん等、ホルモン依存性のがんに既往歴がある場合も注意を要する。我々が136名の閉経後女性を対象に実施した1年間の大豆イソフラボンの介入試験では、食事からの摂取量との合計で75mg/日（アグリコン換算）のイソフラボンの摂取は、血中エストロゲン、FSH、LH、プロゲステロン、甲状腺ホルモン濃度に影響を及ぼさなかった[17]。この結果は、大豆イソフラボンの一日摂取目安量の上限値の閉経後女性における安全性が確認されたことを示唆するものである。

おわりに

イソフラボンには弱い女性ホルモン様作用があることから、閉経後女性の健康に維持にある程度役立つことは十分に考えられる。近年、大豆イソフラボンを含む「健康食品」が市場に出ているが、その内容は様々である[18]。大豆には、イソフラボンのほかに血中のコレステロールを低下させる大豆たんぱく質、お腹の調子を整える食物繊維や大豆オリゴ糖、さらにカルシウムやビタミンB1等、栄養成分や機能性成分等が豊富に含まれていることから、イソフラボンはできるだけ豆腐、納豆、豆乳、大豆菓子等の大豆食品から日常的に摂取したいものである。

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平成21年度食品の安心・安全確保推進研究推進事業
【 若手研究者育成活用事業 】

研　究　報　告　書

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２．リサーチ・レジデント期間
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５．研究課題
大豆イソフラボンの安全性評価に関する研究

６．研究活動の概要
平成２１年７月１日より上記４の研究指導者の下で「大豆イソフラボンの安全性評価に関する研究」の研究課題について、大豆イソフラボンの安全性を評価するための科学的根拠を提供するための研究に従事した。具体的には、乳児用調製粉末大豆乳や大豆たんぱく質強化食品中のイソフラボン含量を定量した。

大豆イソフラボンはエストロゲン様作用により骨代謝調節、抗酸化作用等その機能性が注目される一方で、安全性に関しては十分に検討されていない。2006 年、内閣府食品安全委員会は「大豆イソフラボンを含む特定保健用食品の安全性評価の基本的な考え方」を報告した。その中で、「妊娠、胎児、乳児に対しては大豆イソフラボンを上乗せ摂取することは推奨できない」としている。

他方、大豆イソフラボンが濃縮・強化されていない食品については、表示義務がないため、大豆たんぱく質強化食品からイソフラボンを多量に摂取している可能性が考えられる。例えば、乳児用調製粉末大豆乳中のイソフラボン含量は不明であり、それ

らの食品をホルモン感受性の高い乳児期に摂取した場合の安全性は評価されていない。加えて、大豆たんぱく質が強化されたスポーツ選手用の食品、いわゆる大豆プロテインパウダーには、大豆たんぱく質とともに大豆イソフラボンが濃縮されていることが予想されるが、その表示は義務づけられていない。

そこで、本年度は大豆イソフラボンの安全性を評価するための科学的な根拠を提供するため、「乳児用調製粉末大豆乳」や「大豆たんぱく質を含有する健康食品（大豆たんぱく質強化食品）」中のイソフラボン含量を定量することを試みた。

【目的】

市販されている「乳児用調製粉末大豆乳」や大豆たんぱく質を強化した「健康食品（大豆たんぱく質強化食品）」中のイソフラボン含量を、高速液体クロマトグラフ（HPLC）法にて定量し、イソフラボン含量を明らかにすることを目的とした。

【方法】

1．試料の調製

食品中の大豆イソフラボンの定量は、厚生労働省が通知した「大豆イソフラボンを含む特定保健用食品等の取扱いに関する指針（食安発第 0823001 号）」別紙の方法に従って行った。15 種類の大豆イソフラボン成分を分析した。液状食品は、食品を 100ml 容メスフラスコに正確に分取し、70%エタノールで 100ml に定量して試験溶液とした。粉末状食品は、食品を 50ml ビーカーに精密に秤量し、70%エタノール 25ml を加えて溶解させた。沈殿物が析出した場合には、必要に応じて浸透を行った後に遠心分離し、上清を試験溶液とした。完全に溶解させた後、100ml 容メスフラスコに移し変え、70%エタノールで 100ml に定容して試験溶液とした。試料はすべて 0.45μm のメンブランフィルターでろ過した後、分析に供した。

2．分析

HPLC 法により定量した。分析条件を以下に示す。

カラム；YMC-Pack　ODS-AM-303（size 内径 4.6×250mm）（YMC 社、京都）

移動相；A　アセトニトリル/水/酢酸　混液（15：85：0.1）（V/V/V）、

B　アセトニトリル/水/酢酸　混液（35：65：0.1）（V/V/V）

濃度勾配；A から B までの直線濃度勾配を 70 分間行う。

流速；1ml / min，カラム温度：35℃，測定波長：254nm 試料注入量：10μl

3．食品中の大豆イソフラボン量の算出

試料中の大豆イソフラボン濃度は、厚生労働省の「大豆イソフラボンを含む特定保健用食品等の取扱いに関する指針（食安発第 0823001 号）」別紙に従い、分子量の比を用いて全てアグリコン当量として求めた。CV 値（%）は、測定間で 2.2%であった。

【結果】

1．表示と食品中のイソフラボン量

今回対象としたすべての食品には、大豆イソフラボン含有量に関する表示がなかった。乳児用調製粉末大豆乳の一日あたりの摂取目安量に含まれる大豆イソフ