Review

Effect of soy isoflavones on C-reactive protein in chronic inflammatory disorders

A systematic review and meta-analysis

Ali Gholami[1,2], Fatemeh Darudi[1], Hamid Reza Baradaran[3,4], and Mitra Hariri[1]

[1] Noncommunicable Diseases Research Center, Neyshabur University of Medical Sciences, Neyshabur, Iran
[2] Department of Epidemiology and Biostatistics, School of Public Health, Neyshabur University of Medical Sciences, Neyshabur, Iran
[3] Department of Epidemiology, School of Public Health, Iran University of Medical Sciences, Tehran, Iran
[4] Ageing Clinical and Experimental Research Team, Institute of Applied Health Sciences, School of Medicine, Medical Sciences and Nutrition University of Aberdeen, UK

Abstract: New evidence suggests that soy products might reduce chronic systemic inflammation. Therefore, we aimed to summarize the effect of soy isoflavones on serum concentration of C-reactive protein (CRP) among participants with chronic inflammatory disorders by conducting this study. Cochrane Library, Scopus, ISI Web of Science, clinicaltrials.gov, and PubMed were searched to identify randomized clinical trials (RCTs) published up to December 2020. The effect size was calculated by the mean change from baseline in concentrations of CRP and its standard deviation for both intervention and comparison groups. DerSimonian and Laird random-effects model was used when the heterogeneity test was statistically significant. In total, thirteen RCTs involving 1213 participants and ten RCTs involving 1052 participants were eligible for our systematic review and meta-analysis respectively. Study duration ranged from 4 to 96 weeks and soy isoflavones dose varied from 33 to 132 mg/day. Overall effect size indicated a non-significant effect on serum concentration of CRP following soy isoflavones intake (weighted mean differences (WMD)=−0.15 mg/L, 95% confidence interval (CI): −0.54, 0.23; p=0.430). Subgroup analysis revealed that soy isoflavones significantly reduced serum concentration of CRP in studies among participants with age >57 years and baseline CRP levels >3.75 mg/L. The present study proposed that soy isoflavones could not significantly reduce serum CRP levels. It seems more RCTs on participants with age more than 57 years and higher levels of CRP is necessary.

Keywords: Soy isoflavones, systematic review, meta-analysis, C-reactive protein

Introduction

New evidence suggests that chronic systemic inflammation has a substantial role in the pathogenic of many chronic diseases [1, 2]. Among many inflammatory mediators, elevated expression and concentration of C-reactive protein (CRP), as a key cytokine, is mainly correlated with several human chronic diseases, including type 2 diabetes mellitus (T2DM), obesity, metabolic syndrome [3], non-alcoholic fatty liver disease (NAFLD), cancer, cardiovascular diseases (CVDs), and others [4, 5]. Scientists suggested that the reduction of inflammation has an important role in reducing the risk of these chronic diseases [6]. Hence, it might be possible that dietary antioxidant supplements due to scavenging free radicals and restoring antioxidant defense could suppress inflammatory responses [7, 8].

Soy which has been most commonly consumed in Asian countries for hundreds of years might have potential cardioprotective effects in general populations, particularly in those with increased risk of heart disease [9]. The results of observational studies proposed that dietary isoflavone was associated with a significantly lower risk of CVD in women [10]. Furthermore, some clinical trials have revealed the beneficial effects of soy isoflavones on vascular endothelial function, lipid profiles, and blood pressure [11, 12, 13].

Soy isoflavones suppress cell-mediated inflammatory responses and inhibit cytokine-induced signal transduction in immune cells [3, 14]. Cell culture studies also have shown that soy isoflavones via anti-oxidant activities can conduct anti-inflammatory effects [15]. Therefore, scientists have considered isoflavones as possible anti-inflammatory agents.

However, the results of human studies concerning soy isoflavones role in anti-inflammatory effects are far from conclusion [16]. This is mainly due to their bioavailability

and equol production from daidzein as one of the soy isoflavones [17], differences in the baseline concentration of CRP, participants with different diseases, and etc [18].

Regarding the effect of soy isoflavones on serum concentration of CRP among patients with chronic inflammatory disorders, some randomized clinical trials (RCTs) have supported the beneficial effect of soy isoflavones while others have not. It might be possible that the small sample size in most of these RCTs caused an insufficient statistical power to find significant differences. Therefore, we aimed to summarize the effect of soy isoflavones on serum concentration of CRP among adults with chronic inflammatory disorders by conducting a systematic review and meta-analysis of RCTs to enhance the sample size and therefore, to increase the statistical power.

Material and methods

All steps of our systematic review and meta-analysis were conducted according to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) checklists [19].

Literature search

To identify RCTs exploring the effect of soy isoflavones on CRP levels among participants with chronic inflammatory disorders, a systematic search was performed by searching databases including Clinicaltrials.gov, PubMed, ISI Web of Science, Scopus and Cochrane Library up to December 2020. To identified more eligible studies, the search was manually done in the reference list of all the included relevant studies (research articles, review, and meta-analysis) on soy products.

In this study, taking natural or commercial soy isoflavones was the exposure variable of interest, and change in serum concentration of CRP after soy isoflavones intake was investigated as outcome. Therefore, the search terms of "soy foods", "isoflavones", "c-reactive protein", and "clinical trials" as main components of this study were found in the MeSH (Medical Subject Headings) section of the PubMed database.

Combining the mentioned terms, a proper electronic search strategy was built. The following search terms (MeSH and non-MeSH) were used in this review: "C-Reactive Protein", "Protein-C Reactive", "CRP", "Interleukin-6", "Interleukin6", "IL6", "IL-6", "Tumor Necrosis Factor-alpha", "Tumor Necrosis Factor", "Tumor Necrosis Factorα", "TNFα", "TNF-α", "Vascular Cell Adhesion Molecule-1", "Vascular Cell Adhesion Molecule1", "Vascular Cell Adhesion Molecule", "VCAM-1", "VCAM1", "Intercellular Adhesion Molecule-1", "Intercellular Adhesion Molecule1", "Intercellular Adhesion Molecule", "ICAM-1", "ICAM1", "E-Selectin", "Selectin E", "SelectinE", "ESelectin", "Texturized Soy Protein", "Texturized Soy Proteins", "Texturized Vegetable Protein", "Soya", "Natto", "Tempeh", "Tofu", "Soy Cheese", "Soy Cheeses", "Soy Foods", "Soy Food", "Soy, Food", "Soy, Foods", "Soyfood", "Soyfoods", "Foods Soy", "Soy Sauce", "Soysauce", "Soy Bean Curd", "Miso", "Soy Beverage", "Soy Beverages", "Soy, Beverage", "Soy Milk", "Milk Soy", "Milk, Soy", "Soybeans", "Soybean", "Soy Bean", "Soy Beans", "Glycine max", "Soybean Proteins", "Soy Bean Proteins", "Soy Protein", "Soy Proteins", "Proteins Soy", "Protein Soy", "Genistein", "Soy Products", "Isoflavones", "Isoflavone", "Homoisoflavones", "3-Benzylchroman-4-Ones", "Phytoestrogens", "Phytoestrogen", "Phyto-Estrogen", "Plant Estrogen", "Plant Estrogens", "Equol", "Clinical Trials", "Clinical Trial", "RCT". Since few articles might be missed out searching only "CRP", the search strategy was established to use the most important inflammatory mediators rather than a focus on CRP. There was no time limitation on publications and any discrepancy was resolved in a group discussion.

Study eligibility criteria

To formulate the eligibility criteria in this systematic review and meta-analysis, PICOS (Patient/Population, Intervention, Comparison, Outcome, Study types) framework was used : I) Population: Participants had to be adults with chronic inflammatory conditions; II) Intervention: Soy isoflavones; III) Comparison: Control group; IV) Outcome: the baseline and final CRP levels in intervention and comparison group had to be reported; V) Study design: RCTs with either parallel or crossover design had to be included. The studies were reviewed to be excluded for the following criteria: 1) the studies in which other nutrient supplements besides soy isoflavones in intervention group were taken; 2) there was no information reported the serum concentration of CRP at baseline or after the intervention and subsequently, no data how it was calculated in both intervention and comparison groups; 3) the trials that were planned without the use of any comparison group; 4) the dose of soy isoflavones in natural soy product was not reported; 5) the serum concentration of CRP was reported in figures; 6) the articles were published in non-English languages.

Data management and extraction

Two reviewers (MH, AGh) independently conducted the initial search. The found articles in different databases were imported into the EndNote X9. After excluding duplicated articles, they read titles and abstracts and assessed the full

text of the papers that met our inclusion criteria. Two researchers (MH and AGh) independently extracted the following information from the original studies including first author's name, publication year, country, sample size, participants' age, sex, and body mass index (BMI), trial design (parallel or crossover), soy isoflavones dose and source, intervention duration, serum concentration of CRP before and after the intervention. CRP measurements converted to the same unit (mg/L). Any discrepancies were resolved in a group discussion. The studies with more than one intervention or comparison group were considered as separate studies in our meta-analysis. An email was sent to the corresponding author when there was any unclear information.

Quality assessment

Two independent reviewers (MH and AGh) carried out the risk of bias assessment for the included studies according to the items outlined in the Cochrane Handbook for Systematic Reviews of Interventions (20). The criteria included: 1) performing random sequence generation; 2) conducting allocation concealment; 3) blinding participants and personnel; 4) blinding outcome assessment; 5) reporting incomplete outcome data; 6) reporting selective outcome. The quality of each article was scored based on a judgment for each criteria. We classified included studies in this systematic review as "good", "fair", and "weak" quality if there was a low risk for at least three items, two items, and less than two items, respectively.

Data synthesis and statistical analysis

This meta-analysis was performed using mean differences (MDs) and their standard deviations (SDs) for CRP (mg/L). For papers in which the authors didn't report the mentioned data, we calculated them from available data. The effect size for each article was calculated based on mean changes of CRP from the baseline and its corresponding SDs for both intervention and comparison groups [20]. In studies in which median or range are reported, the mean concentration of CRP was estimated by a method developed by Hozo et al. [21]. Furthermore, SD was obtained from the standard errors (SEs) by multiplying SE in the square root of the sample size in articles that SE was reported instead of SD. The overall effects and heterogeneity were computed by DerSimonian and Laird random effects model. To determine the statistical heterogeneity of intervention effects, Cochran's Q test and I-squared statistic were used. Heterogeneity was considered substantial if the p-value for the Cochran's Q test was $\leq$0.10 or the value of the I-squared statistic was $\geq$50% [22].

In order to examine the sources of heterogeneity, subgroup analyses were done based on isoflavones dose, trial design, intervention duration, baseline CRP levels, sample size, geographical region, participants' age, sex, and BMI, quality assessment, and study publication year. The effect of previously named variables on the effect sizes via meta-regression analysis was adjusted. Moreover, the heterogeneity across studies was explored by conducting a meta-regression analysis. In presence of publication bias, Begg's funnel plot and Eggar's weighted regression test were used [23]. Sensitivity analysis was performed to check the effect of each study on the overall effect size. Ninety-five percent confidence intervals were provided for all calculated effect sizes. Analyses were performed in STATA 15 software (Stata Corp, College Station, TX).

Results

Study selection

Briefly, a total of 4387 articles were identified using databases search. After removing duplicate articles, 2955 potentially relevant papers were remained to be screened by titles and abstract. Then 2833 articles were excluded due to being non-human studies, using soy as placebo, using soy oil as intervention, study protocol, congress abstract, review, and cross-sectional studies. Thus, 122 full-text articles were retrieved and carefully reviewed to meet the inclusion and exclusion criteria of this study, among which, 109 articles were excluded after a full-text review for the following reasons: 1) seventeen articles studied on a mixture of soy intake with other dietary regimens; 2) twenty five studies were excluded due to taking soy protein or the combination of soy isoflavones and soy protein by participants; 3) forty six articles did not measure the CRP; 4) three studies didn't involve any comparison group; 5) sixteen studies were conducted on healthy participants; 6) Two studies did not report the isoflavones dose. The flowchart of the selection of reviewed articles is given in Figure 1. In all, thirteen RCT were remained to be included in this systematic review [24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36], among which, one article did not report enough data for meta-analysis [36], and two articles had very large effect size [34, 35]; hence, ten articles met all of our specified criteria in this meta-analysis.

Study characteristics

Ten out of thirteen trials were conducted on females [24, 26, 28, 29, 30, 31, 32, 33, 35, 36] and three trials were administrated on both males and females [25, 27, 34].

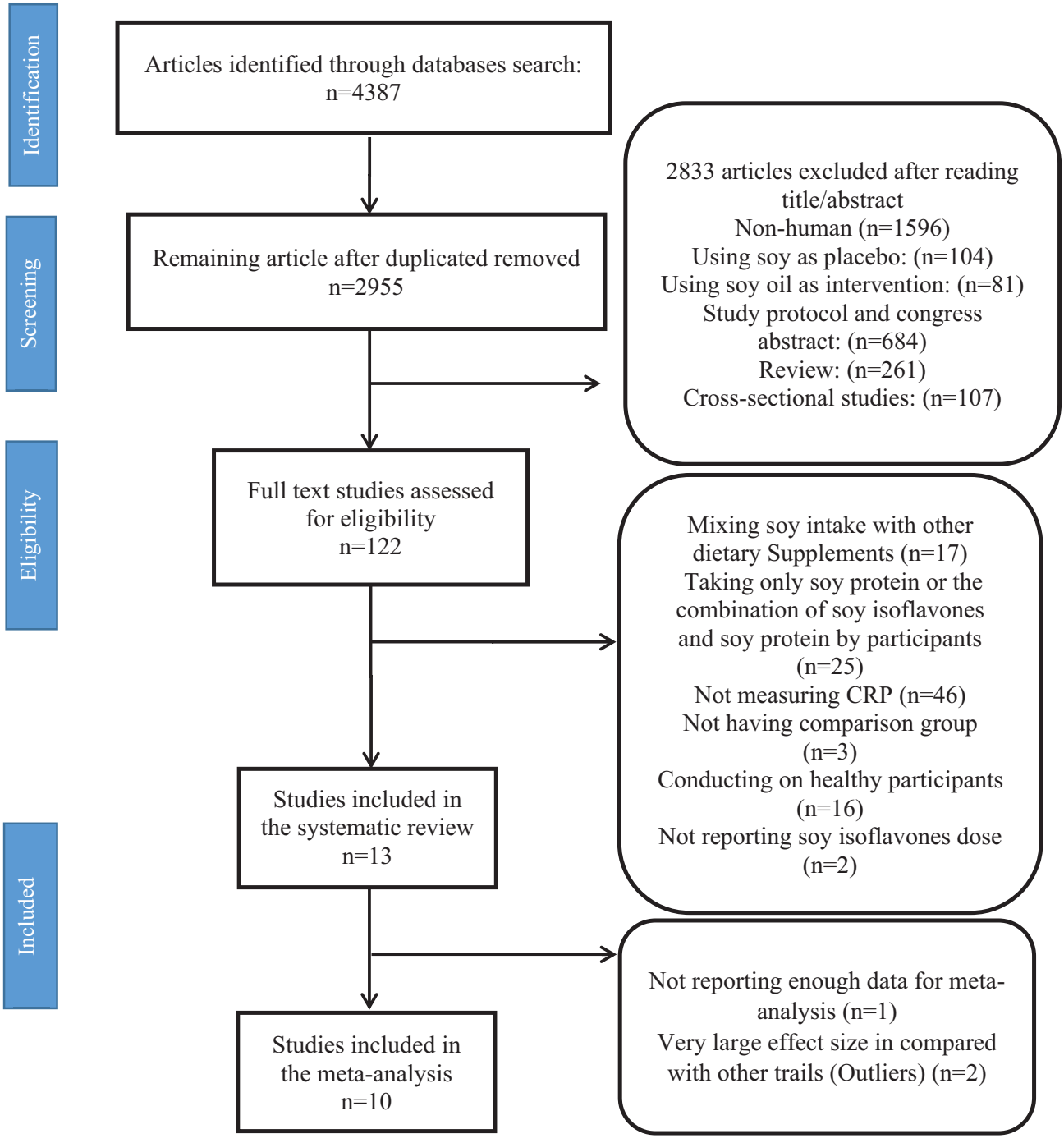

Figure 1. Flowchart of study selection process.

Regarding study design, three trials had crossover design [26, 32, 34] and ten trials had parallel design [22, 25, 27, 28, 29, 30, 31, 33, 35, 36]. Study duration ranged from 4 to 96 weeks. Soy isoflavones dose varied from 33 to 132 mg/day. More than one effect size was extracted from studies with more than one intervention or comparison group [27]. The studies which reported an intervention effect in different treatment durations [24, 29, 31] were considered as separate studies in Table 1. In total, sixteen datasets from 10 studies were analyzed in our meta-analysis.

Quality assessment

Based on quality assessment, one out of the thirteen RCTs included in the systematic review were scored as "fair" [34], one RCT as "weak" [27], and eleven RCTs as "good"

Table 1. Randomized controlled trial studies included in the systematic review and meta-analysis

Code Author (year) (country)	Subjects	Age and BMI (mean±SD)	RCT	Intervention	Placebo	Duration (week)	Values of CRP, mg/L in intervention group (Baseline vs after intervention) P-value	Values of CRP, mg/L in comparison group (Baseline vs after intervention) P-value	CRP change P-value
1.1 Aubertin-Leheudre, M. 2007 Canada [24]	Obese postmenopausal women N=20	Age: 58±5 BMI: 30±5	Randomized, double-blind, controlled trial	70 mg/day isoflavones (44 mg of diadzein, 16 mg of glycitein, and 10 mg of genistein)	NR	24	(4.5 vs 6.3) P>0.05	(2.5 vs 3.8) P>0.05	–
1.2 Aubertin-Leheudre, M. 2007 Canada [24]	Obese postmenopausal women N=20	Age: 58±5 BMI: 30±5	Randomized, double-blind, controlled trial	70 mg/day isoflavones (44 mg of diadzein, 16 mg of glycitein, and 10 mg of genistein)	NR	48	(4.5 vs 4.2) P>0.05	(2.5 vs 3.8) P>0.05	–
2 Chan, Y. H. 2008 China [25]	Patients with ischaemic stroke N=102	Age: 66.8±9.6 BMI: 26.2±4.1	Randomized, double-blinded, controlled clinical trial	80 mg/day soy isoflavones	Cellulose	12	–	–	−1.7 0.033
3 Clerici, C. 2007 Italy [34]	Adults with hypercholesterolemia N=62	Age: 58.1±2.2 BMI: 26.6±0.8	Randomized blinded parallel single-crossover	Pasta containing 33 mg/day soy isoflavones	Pasta without soy isoflavones	4	NR	NR	NR
4 Gonźalez, S 2007 UK [26]	Postmenopausal women with diet-controlled type 2 diabetes N=26	Age: NR BMI: 30.7±5.5	Randomized, double-blind, placebo-controlled, crossover	132 mg/day isoflavones (53% genistein, 37% daidzein, and 10% glycitein)	Microcrystalline cellulose	12	(5.4 vs 6.2) P>0.05	(5.1 vs 6.4) P>0.05	–
5 Jamilian, M. 2016 Iran [35]	Women with polycystic ovary syndrome N=70	Age: 27.5±6.4 BMI: 24.9±5.6	Randomized, double-blinded, controlled clinical trial	50 mg/d soy isoflavones	NR	12	(4.8 vs 4.6) P>0.05	(5.3 vs 5.5) P>0.05	–
6 Lebon, J. 2014 Canada [36]	Overweight and obese postmenopausal women N:29	Age: 59.5±4.5 BMI: 29.5±3.8	Randomized, double-blind, controlled trial	70-mg/day daily dose of isoflavones (containing 44 mg of daidzein, 16 mg of glycitein, and 10 mg of genistein)	Cellulose	24	NR	NR	NR
7.1 Li, Y. 2017 China [27]	Women with ischemic stroke N=71	Age: NR BMI: NR	Randomized clinical trial	80 mg/day soy isoflavones	NR	24	–	–	−1.76 0.020
7.2 Li, Y. 2017 China [27]	Men with ischemic stroke N=123	Age: NR BMI: NR	Randomized clinical trial	80 mg/day soy isoflavones	NR	24	–	–	−1.83 0.020

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Table 1. (Continued)

Code Author (year) (country)	Subjects	Age and BMI (mean±SD)	RCT	Intervention	Placebo	Duration (week)	Values of CRP, mg/L in intervention group (Baseline vs after intervention) P-value	Values of CRP, mg/L in comparison group (Baseline vs after intervention) P-value	CRP change P-value
8 Liu, Z. M. 2014 Hong Kong [28]	prehypertensive postmenopausal women N=270	Age: 57.6±5.3 BMI: 23.2±3.5	Randomized, double-blind, controlled trial	40 g low-fat milk powder +63 mg daidzein	40 g low-fat milk powder	24	(1.26 vs 2.01) P=NR	(1.69 vs 165) P=NR	–
9.1 Liu, Z. M. 2012 Hong Kong [29]	Prediabetes postmenopausal women N=180	Age: 56.4±4.7 BMI: 24.1±3.8	Randomized, double-blind, controlled trial	100-mg isoflavone (35 mg daidzin, 59 mg genistin and 4 mg glycitin)	15 g/day milk protein	12	(1.14 vs 1.09) P=NR	(1.24 vs 1.08) P=NR	–
9.2 Liu, Z. M. 2012 Hong Kong [29]	Prediabetes postmenopausal women N=180	Age: 56.4±4.7 BMI: 24.1±3.8	Randomized, double-blind, controlled trial	100-mg isoflavone (35 mg daidzin, 59 mg genistin and 4 mg glycitin)	15 g/day milk protein	24	(1.14 vs 1.15) P=NR	(1.24 vs 1.11) P=NR	–
10 Llaneza, P. 2011 Spain [30]	Obese postmenopausal women N=87	Age: 56.1±3.51 BMI: 35.2±4.78	Single blind randomized clinical trial	80 mg/day isoflavone (60.8mg of genistein, 16mg of daidzein and 3.2mg of glicitein)	Nothing	24	(2.14 vs 2.42) P>0.05	(1.5 vs 1.7) P>0.05	–
11.1 Llaneza, P. 2012 Spain [31]	Obese postmenopausal women N=65	Age: 56.7±3.5 BMI: 30.6±4.7	Single blind randomized clinical trial	80 mg of isofl avones (60.8 mg genistein, 16 mg daidzein and 3.2 mg glicitein)	Nothing	24	(0.3 vs 0.4) P>0.05	(0.3 vs 0.4) P>0.05	–
11.2 Llaneza, P. 2012 Spain [31]	Obese postmenopausal women N=65	Age: 56.7±3.5 BMI: 30.6±4.7	Single blind randomized clinical trial	80 mg of isofl avones (60.8 mg genistein, 16 mg daidzein and 3.2 mg glicitein)	Nothing	48	(0.3 vs 0.6) P>0.05	(0.3 vs 0.4) P>0.05	–
11.3 Llaneza, P. 2012 Spain [31]	Obese postmenopausal women N=65	Age: 56.7±3.5 BMI: 30.6±4.7	Single blind randomized clinical trial	80 mg of isofl avones (60.8 mg genistein, 16 mg daidzein and 3.2 mg glicitein)	Nothing	72	(0.3 vs 0.4) P>0.05	(0.3 vs 0.7) P>0.05	–
11.4 Llaneza, P. 2012 Spain [31]	Obese postmenopausal women N=65	Age: 56.7±3.5 BMI: 30.6±4.7	Single blind randomized clinical trial	80 mg of isoflavones (60.8 mg genistein, 16 mg daidzein and 3.2 mg glicitein)	Nothing	96	(0.3 vs 0.3) P>0.05	(0.3 vs 0.3) P>0.05	–
12 Nikander, E. 2003 Finland [32]	Postmenopausal women with a history of breast Cancer N=56	Age: 54±6 BMI: NR	Randomized, double-blind, placebo-control, crossover trial	58 mg/day soy isoflavones	NR	12	(1.16 vs 1.10) P>0.05	(1.10 vs 1.10) P>0.05	–
13 Riesco, E. 2012 Canada [33]	Overweight or obese postmenopausal women N=52	Age: 56.2 (52.7–59.7)* BMI: 28.8 (25.2–34.2)	Randomized, double-blind, placebo-control, clinical trial	70 mg/day soy isoflavones	Cellulose	24	–	–	–0.9 P<0.001

Notes. BMI: Body Mass Index; CRP: C-reactive protein; NR: Not reported; RCT: Randomized clinical trial. *Means (95% confidence interval).

Table 2. Quality of bias assessment of the included studies according to the Cochrane guidelines

Author name, year of publication, reference	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Overall quality
Aubertin-Leheudre, 2007 [24]	U	H	L	U	L	L	Good
Chan, 2008 [25]	L	L	L	L	U	U	Good
Clerici, 2007 [34]	U	U	L	U	L	H	Fair
Gonźalez, 2007 [26]	L	H	U	U	L	L	Good
Jamilian, 2016 [35]	L	U	L	L	L	L	Good
Lebon, 2014 [36]	L	U	L	L	U	L	Good
Li, 2017 [27]	U	H	H	H	H	L	Weak
Liu, 2014 [28]	L	L	L	L	L	L	Good
Liu, 2012 [29]	L	U	L	L	L	L	Good
Llaneza, 2011 [30]	L	U	L	L	L	L	Good
Llaneza, 2012 [31]	U	H	L	L	L	U	Good
Nikander, 2003 [32]	L	U	L	U	H	L	Good
Riesco, 2012 [33]	U	L	L	L	L	L	Good

L: low risk of bias; H: high risk of bias; U: unclear risk of bias.

[24, 25, 26, 28, 29, 30, 31, 32, 33, 35, 36]. Of these, one article had a high risk of bias for blinding of participants and personals [27] and one had a high risk of bias for blinding of outcome assessment [27]. Lack of allocation concealment was the source of risk of bias in four studies [24, 26, 27, 31]. Two studies had high risk of bias due to incomplete outcome data [27, 32]. One study detected a high risk of bias regarding selective reporting [34]. The assessment of the quality of included RCTs is detailed in Table 2.

Pooled estimate and subgroup analysis of the effect of soy isoflavones on CRP concentration

Ten RCTs with sixteen effect sizes assessed the effect of soy isoflavones on serum concentration of CRP (Figure 2A). We found a non-significant effect of soy isoflavones compared with comparison group on serum concentration of CRP (weighted mean differences (WMD)=−0.15 mg/L, 95% confidence interval (CI): −0.54, 0.23; p>0.05). There was a substantial heterogeneity among studies (Cochrane's Q test, p<0.001; I^2=82.2%). The results of subgroup analysis revealed that the soy isoflavones significantly reduced the serum concentration of CRP in studies among participant aged >57 years (WMD=−0.69 mg/L, 95% CI: −1.32, −0.06; p=0.033; I^2: 49.6%) and baseline CRP levels >3.75 mg/L (WMD=−0.77 mg/L, 95% CI: −1.52, −0.01; p=0.046; I^2: 39.6%) (Table 3).

Meta-regression analysis, sensitivity analysis, and publication bias of the effect of soy isoflavones on CRP concentration

In order to find the possible sources of heterogeneity and characteristics of participants or trials with effective treatment effects, a meta-regression analysis was used. The results of univariate meta-regression analysis could not find a significant linear association between soy isoflavones dose and effect size (Coefficient=0.004, 95% CI: −0.04, 0.05; P>0.05) (Table 4 and Figure 2B). Other studied variables also did not show any significant association with the effect size of soy isoflavones effect on CRP levels in univariate meta-regression analyses (Table 4). The dose of soy isoflavones did not report any linear association with the effect size of soy isoflavones effect on CRP levels even after adjustment for article publication year, quality assessment, BMI, sex, age, geographical region, sample size, baseline CRP levels (mg/L), study duration and design (Coefficient=0.005 95% CI: −0.25, 0.27 P>0.05). The funnel plot visually suggested evidence of publication bias, however; there was no evidence of publication bias according to the result of Egger test (Egger test p-value>0.05) (Figure 3). Sensitivity analyses indicated that the summary effects were not influenced by any particular study.

Discussion

The results of our systematic search revealed that this is the first systematic review and meta-analysis which reported the effect of soy isoflavones on serum CRP levels among subjects with chronic inflammatory disorders. Thirteen and ten articles are included in our systematic review and meta-analysis, respectively. The results indicated that soy isoflavones could not significantly reduce serum levels of CRP. There was significant heterogeneity among studies.

The Observational and experimental evidence demonstrated that natural isoflavones played an important role in protecting against chronic diseases. In humans, the epidemiologic results of studies indicated that the prevalence

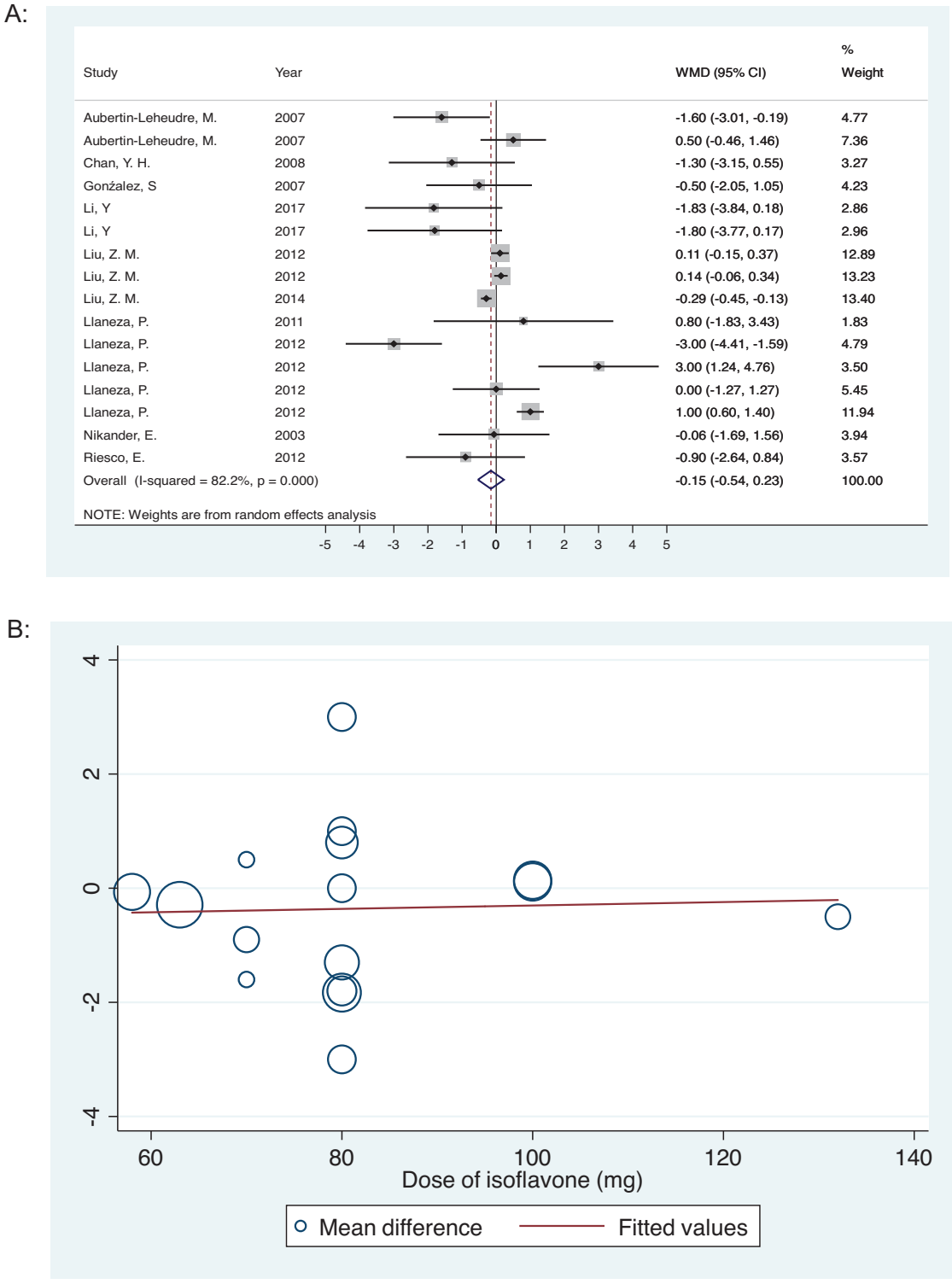

Figure 2. A: Forest plot of the effect of soy isoflavones consumption on serum CRP concentrations. B: Meta-regression plot of the effect of soy isoflavones dose on isoflavones effect on CRP. Values are in mg/L.

of some common types of cancer and coronary heart diseases was higher in western populations with limited amounts of soybean isoflavones in the diet [37]. Isoflavones were identified as phytoestrogens because they were structurally similar to the estrogen-like compound of 17β-estradiol especially equol which was produced by one of the soy isoflavones known as daidzein [17]. Consequently, isoflavones could have estrogenic or anti-estrogenic effects;

Table 3. Results of subgroup analyses for studies evaluating the effect of soy isoflavones on serum concentration of CRP

	Subgroup	No of trial	Change in CRP (95% CI)	P-value	I^2 (%)	$P_{heterogeneity}$
Total	–	16	−0.15 (−0.54, 0.23)	0.430	82.2	<0.001
Isoflavones dose (mg)	≤80 mg/d	13	−0.36 (−1.01, 0.30)	0.287	84.4	<0.001
	>80 mg/d	3	0.12 (−0.03, 0.28)	0.124	0.00	0.719
Trial design	Parallel	14	−0.15 (−1.41, 0.83)	0.476	84.5	<0.001
	Cross-over	2	−0.29 (−1.41, 0.83)	0.608	0.00	0.704
Intervention duration	≤168 day	12	−0.10 (−0.37, 0.17)	0.462	54.5	0.012
	>168 day	4	−0.16 (−2.39, 2.06)	0.886	93.4	<0.001
Baseline CRP (mg/L)	≤3.75 mg/L	8	0.11 (−0.35, 0.56)	0.647	89.8	<0.001
	>3.75 mg/L	8	−0.77 (−1.52, −0.01)	0.046	39.6	0.115
Sample size	≤68 Persons	8	−0.17 (−1.24, 0.90)	0.754	86.2	<0.001
	>68 Persons	8	−0.13 (−0.43, 0.18)	0.426	67.6	0.003
Geographical region	Americas	3	−0.57 (−1.96, 0.82)	0.420	68.6	0.042
	Europe	7	0.14 (−1.10, 1.38)	0.829	84.8	<0.001
	Asia	6	−0.15 (−0.48, 0.18)	0.375	76.4	0.001
Age	≤57 years	8	0.21 (−0.33, 0.75)	0.446	84.8	<0.001
	>57 years	7	−0.69 (−1.32, −0.06)	0.033	49.6	0.064
	Unknown	1	−0.50 (−2.05, 1.05)	0.527	–	–
Sex	Female	14	−0.06 (−0.45, 0.33)	0.762	83.6	<0.001
	Male	1	−1.83 (−3.84, 0.18)	0.075	–	–
	Both	1	−1.30 (−3.15, 0.55)	0.168	–	–
BMI	≤29	8	−0.16 (−0.47, 0.15)	0.307	68.2	0.003
	>29	8	−0.01 (−1.09, 1.07)	0.988	85.4	<0.001
	Good	14	−0.05 (−0.43, 0.34)	0.811	83.4	<0.001
Quality assessment	Fair	0	–	–	–	–
	Weak	2	−1.81 (−3.22, −0.41)	0.012	0.00	0.983
Study publication year	≤2010	5	−0.47 (−1.33, 0.38)	0.277	44.2	0.127
	>2010	11	−0.06 (−0.50, 0.38)	0.774	86.9	<0.001

CRP: C-reactive protein; BMI: body mass index; mg: milligram; mg/L: milligram per liter; mg/d: milligram per day; CI: confidence interval.

Table 4. Univariate meta-regression analysis of the association of intervention or participant characteristics with the effect size (effect of soy isoflavones on serum CRP) in chronic inflammatory disorders

	No of trial	Coefficient (95% CI)	P-value
Isoflavones dose (mg)	16	0.004 (−0.04, 0.05)	0.860
Trial design	16	0.015 (−2.33, 2.36)	0.989
Intervention duration	16	0.194 (−1.50, 1.89)	0.810
Baseline CRP (mg/L)	16	−0.900 (−2.30, 0.50)	0.191
Sample size	16	−0.282 (−1.76, 1.20)	0.689
Geographical region	16	−0.120 (−1.14, 0.88)	0.788
Age	15	−1.080 (−2.55, 0.39)	0.136
Sex	16	−0.765 (−2.27, 0.74)	0.293
BMI	16	0.566 (−0.89, 2.02)	0.417
Quality assessment	16	−0.842 (−2.03, 0.35)	0.151
Publication year of article	16	0.341 (−1.27, 1.96)	0.658

CRP: C-reactive protein; BMI: body mass index; mg: milligram; mg/L: milligram per liter; CI: confidence interval.

therefore, scientists believed that these properties of isoflavones might be responsible for their beneficial effects [38]. The effect of soy isoflavones on the activation of intracellular pathways such as protein tyrosine kinase, phospholipase C, and mitogen-activated protein kinase (MAPK) might also cause soy health benefits [39].

Soy isoflavones also might reduce the gene expression of inflammatory mediators via the modulation of the nuclear factor-κB (NF-κB) pathway [40]. NF-κB is the most important regulator of pro-inflammatory mediator production that has a key role in regulating the immune response. Incorrect regulation of NF-κB has been linked

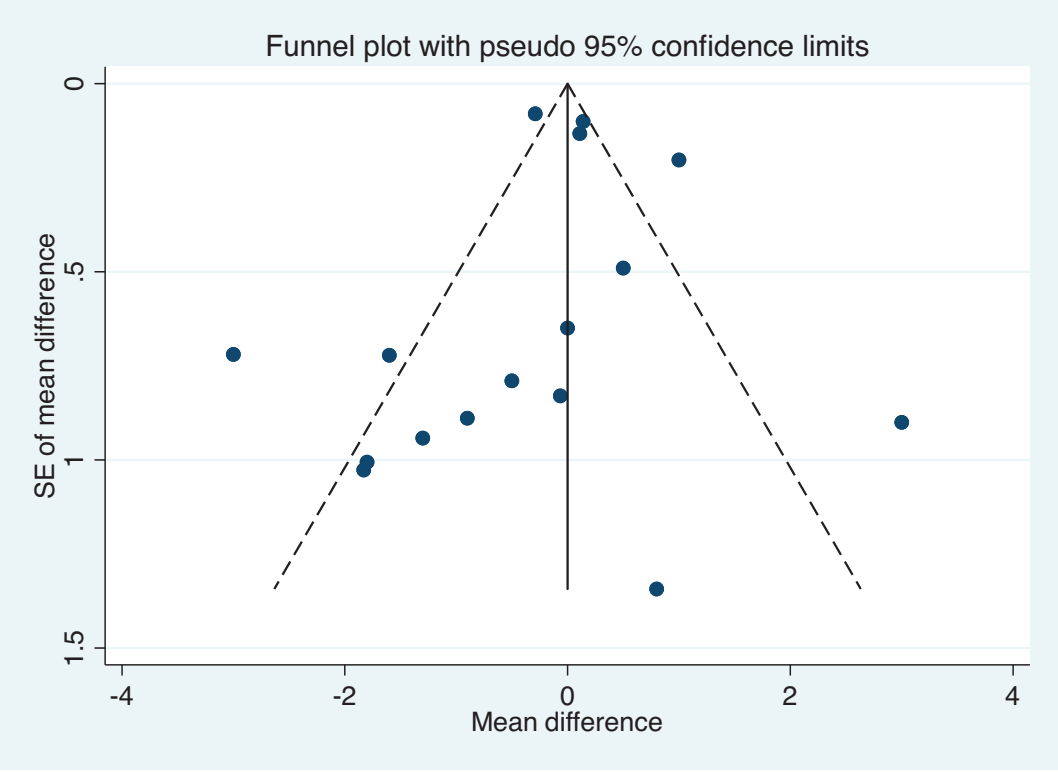

Figure 3. Funnel plots for the studies of the effects of soy isoflavones consumption on serum CRP concentrations.

to inflammatory and autoimmune diseases [41]. Consistent evidence has indicated that soy isoflavones can inhibit the activity of the nuclear translocation of NF-κB and reduce the gene expression of inflammatory mediators [42].

The absence of an intervention effect concerning isoflavones effect may be explained by several factors including participants' age, baseline CRP concentration, and equol-producer status.

In this study, we included all studies in which the participants suffered from chronic inflammatory disorders with a slight increase in CRP levels. Therefore, it was impossible that the serum concentration of CRP was significantly reduced after taking soy isoflavones. Concerning the results of our subgroup analyses, soy isoflavones could reduce serum levels of CRP in subjects with baseline CRP concentrations higher than 3.75 mg/L, hence, baseline CRP level is very likely to change the magnitude of treatment effect.

Another mechanism for the anti-inflammatory effects of soy isoflavones would be due to their antioxidant activity. If this is true, the absence of soy isoflavones effect in this study might have been due to participants' inability to produce equol. Compared to the other isoflavones, equol which is produced from one of the soy isoflavones named daidzein has stronger antioxidant activity [43]. Scientists have indicated that less than 30% of the adult populations in Western countries can produce equol and almost 60% of adults from China, Japan, and Korea are reported to be equol producers [44]. In this article only six out of 16 effect sizes were from Asian countries; therefore, if our study showed a non-significant effect, it might be due to the inability to produce in equol.

According to the results of our subgroup analysis, soy isoflavones could reduce serum concentration of CRP among subjects aged 57 years. Since a large body of clinical and preclinical evidence suggested that estrogen might have an anti-inflammatory effect [45], it might be possible by aging when sex hormones are reduced, phytoestrogens apply an anti-inflammatory effect much stronger than young age. Phytoestrogen also might be ineffective if serum concentration of endogenous estrogen was high, or they may compete for binding to receptors; therefore, the effectiveness of soy isoflavones decreased.

A significant heterogeneity was observed among studies that might be related to the subjects' genetic background, various sources of soy isoflavones, participants' health condition, and different medicines which were taken besides intervention in a few studies.

This study has some limitations that must be kept in mind when interpreting our findings. Firstly, most of the reviewed articles had no information about the serum or urine levels of isoflavones; therefore, no information was found about

the actual absorbed values of soy isoflavones in the included studies. Secondly, no information was provided on confounding factors such as smoking and alcohol consumption which may change the levels of inflammation; therefore, we could not adjust these confounders in our meta-analysis. Thirdly, we excluded the studies that tend to have a very large effect size [34, 35]. However, the exclusion of these studies did not change our results. Fourthly, soy isoflavones were received from different sources and we couldn't consider the possible effect of various sources of soy isoflavones on treatment effects. Fifthly, we found substantial heterogeneity among the reviewed studies.

Our study had a number of strengths. First of all, we used a broad search term to find eligible RCTs. Secondly, we conducted the meta-regression analyses to identify possible sources of heterogeneity and the subgroup analyses based on isoflavones dose, study design, intervention duration, baseline CRP levels, sample size, geographical region, participants' age, sex, and BMI, quality assessment, and study publication year. Thirdly, included trials used only soy isoflavones and trials which used other food supplements besides soy isoflavones were excluded. Fourthly, we did not have any limitation on publication time.

In conclusion, the present study proposed that soy isoflavones could not significantly reduce CRP concentration among participants with chronic inflammatory disorders; however, the baseline serum levels of CRP and participants' age seem to be strong predictors of soy isoflavones effect on serum CRP levels. Therefore, more intervention studies, especially those conducted on old subjects and subjects with moderate to high rates of chronic inflammatory disorders are recommended to confirm the effects of soy isoflavones on serum CRP levels.

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The authors declare that there are no conflicts of interest.

Authorship
AGh found key words and designed search terms. MH, AGh searched data bases, read titles and abstracts, and found relevant RCTs. They also read articles full text, excluded irrelevant RCTs, and extracted data. AGh did statistical analysis. MH, AGh and FD wrote the first version of manuscript. HRB approved the final version of article. Discrepancies in every part of work solved through group discussions.

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Mitra Hariri
Noncommunicable Diseases Research Center
Neyshabur University of Medical Sciences
Janbazan Ave
Neyshabur, Iran
haririm1@nums.ac.ir

