Reprint from The Review of DIABETIC STUDIES Vol 15 2019

The Review of DIABETIC STUDIES

REVIEW

Systematic Review and Meta-Analysis of the Effects of Soy on Glucose Metabolism in Patients with Type 2 Diabetes

Soheil Soltanipour[1], Tolou Hasandokht[1], Robabeh Soleimani[2], Marjan Mahdavi-Roshan[1], and Mir Mohammad Jalali[3]

[1] *School of Medicine, Guilan University of Medical Sciences, Rasht, Guilan, Iran.* [2] *Kavosh Cognitive Behavior Sciences and Addiction Research Center, School of Medicine, Shafa Hospital, Guilan University of Medical Sciences, Rasht, Guilan, Iran.* [3] *Otorhinolaryngology Research Center, School of Medicine, Amiralmomenin Hospital, Guilan University of Medical Sciences, Rasht, Guilan, Iran.*
Address correspondence to: Mir Mohammad Jalali,e-mail: mmjalali@gmail.com

Manuscript submitted September 4, 2019; resubmitted October 10, 2019; accepted October 10, 2019

■ Abstract

OBJECTIVE: This study aimed to assess the effects of soy consumption on glucose metabolism in patients with type 2 diabetes. **METHODS**: A systematic review was performed in accordance with the Preferred Reporting Items for Systematic Review (PRISMA) principles. Literature published between 1990 and 2019 was searched. Primary outcomes were the effect of soy on fasting plasma glucose (FPG), insulin, and HbA1c. The data were pooled using random effects models. Heterogeneity was assessed using Cochran's Q and I^2 statistics. Also, the Cochrane Collaboration's tool for assessing risk of bias was used, and sensitivity analysis and meta-regression were conducted. Publication bias was evaluated using Egger and Begg tests. **RESULTS**: Sixteen randomized clinical trials (RCTs) with a total of 471 participants were regarded as eligible and included in the study. Soy consumption had no significant effects on FPG, insulin, and HbA1c. After the "trim-and-fill" method was applied, soy revealed a significant effect size on FPG (adjusted Cohen's d: -0.18; p = 0.03). Also, subgroup analyses using studies with parallel design showed a significant improvement (moderate effect size) in FPG and insulin. Sensitivity analysis indicated the robustness of our findings. Among secondary outcomes, the results showed a significant effect of soy on HOMA-IR and total cholesterol levels. **CONCLUSIONS**: Although this systematic review and meta-analysis indicated no beneficial effects of soy consumption on FPG, insulin, and HbA1c in patients with type 2 diabetes, pooling of parallel studies showed different results from crossover studies. The quality of evidence revealed low levels of confidence for primary outcomes. Therefore, further research is recommended.

Keywords: diabetes · soy · blood glucose · HbA1c · insulin · meta-analysis · systematic review

1. Introduction

The prevalence of diabetes is constantly increasing throughout the world. While the disease affected 180 million people in 1980, the number has risen to 422 million people with diabetes in 2014 [1]. Clinical trials have reported lifestyle modifications (e.g. dietary interventions) to be even more effective than pharmacological treatment (e.g. metformin) in decreasing the risk of complications of type 2 diabetes (T2D) [2-4].

According to animal studies, soy protein and isoflavones may improve glucose metabolism (through activated protein kinase and protein kinase B pathways), decrease insulin resistance, increase insulin sensitivity (via increasing glucose uptake in muscle and liver), and enhance β-cell mass [5]. By binding to the estrogen receptor (ER), isoflavones can show either estrogen agonistic or antagonistic activity, and the nature of their activity depends on the tissue and level of both isoflavone and endogenous estradiol [6].

Abbreviations:

BMI	body mass index
CDSR	Cochrane database of systematic reviews
ER	estrogen receptor
GRADE	grading of recommendations assessment, development and evaluation
HbA1c	glycated hemoglobin
HDL	high-density lipoprotein-cholesterol
HOMA-IR	homeostatic model assessment for insulin resistance
HPLC	high performance liquid chromatographic
ICTRP	international clinical trials registry platform
LDL	low-density lipoprotein-cholesterol
PRISMA	preferred reporting items for systematic reviews and meta-analyses
RCT	randomized clinical trial
SD	standard deviation
SE	standard error
SMD	standardized mean difference
T2D	type 2 diabetes
TC	total cholesterol

Yang *et al*. conducted a systematic review in 2011 to clarify the relationship between soy product intake and risk factors in patients with T2D [7]. After searching PubMed and Cochrane Library for relevant clinical trials published up to 2010, they included seven trials, six of which had a crossover design. The authors found the use of soy products had no significant effects on insulin, fasting plasma glucose (FPG), and glycated hemoglobin (HbA1c). However, the consumption of soy products significantly decreased serum triglycerides (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL), and increased high-density lipoprotein cholesterol (HDL).

In a more recent meta-analysis, Zhang *et al*. searched several electronic databases (PubMed, EMBASE, and Cochrane) up to March 2015 [8]. Half of the 10 randomized clinical trials (RCTs) included in their study had a crossover design. The authors concluded that soy protein supplementation significantly decreased FPG and insulin. However, no significant differences were detected in HDL and TG levels with soy protein consumption. Since the authors included prediabetic and diabetic patients, pooling of the results might have weakened the conclusions drawn by this meta-analysis. This leads to an unpredictable bias in the estimate of treatment differences and exaggerates the apparent sample size. Such exaggeration leads to spuriously narrow confidence intervals and low p-values.

A greater number of studies on patients with diabetes may be available in other bibliographic databases, conference abstracts, and grey literature [9-18]. A review of more recent studies is required to enhance existing knowledge and clarify the observed inconsistencies regarding the impact of soy consumption on clinical indices in diabetic patients. Therefore, the present comprehensive meta-analysis was performed to evaluate the long-term effects of soy on clinical diabetes indices.

2. Methods

This is a systematic review and meta-analysis. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were followed for reporting this study [19]. The PRISMA checklist can be requested from the authors. The protocol of this systematic review and meta-analysis was also registered in the International Prospective Register of Systematic Reviews (Registration No: CRD42018089448).

2.1 Data sources and searches

The PICOS criteria were used to define the research question for this review as follows:

Population:	Patients with type 2 diabetes.
Intervention:	Soy, soy protein, isoflavones, soy products.
Control:	Placebo.
Outcome:	FPG, insulin, HbA1c.
Study design:	RCT.

We adopted a five-stage strategy to retrieve relevant English language articles published from January 1, 1990 to April 1, 2019:

1. Four general databases: Medline (PubMed), Google Scholar (the first 200 citations), BIOSIS (Web of Science), and Cochrane Library. One specific database: EMBASE (Ovid).
2. Search in the American Journal of Clinical Nutrition, British Journal of Nutrition, and the Journal of Nutrition.
3. The Cochrane Database of Systematic Reviews (CDSR) and the references of the selected papers, related reviews, or systematic reviews.
4. Grey literature, ProQuest, and Scopus.
5. Clinical.trial.gov and the International Clinical Trials Registry Platform (ICTRP).

2.2 Study selection

Only RCTs, either with or without blinding, were included. All subjects had type 2 diabetes. Studies were included if primary outcomes on

FPG, insulin, or HbA1c were reported. Secondary outcomes addressed in this meta-analysis included homeostatic model assessment for insulin resistance (HOMA-IR), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TG), and systolic and diastolic blood pressure. The following studies were excluded:

- Administered dose not determined
- Use of soy polysaccharides, soy fiber, or pinitols derived from soy
- Other potentially active ingredients mixed with soy
- Isoflavones extracted from sources other than soy.

Duplicate publications and studies lacking adequate data for calculating mean and standard deviation (SD) or standard error (SE) values were also excluded.

2.3 Data extraction and quality assessment

All researchers independently assessed the titles and abstracts of the selected papers and then evaluated the full texts of potentially eligible papers to confirm their eligibility. Any disagreement over the eligibility of particular studies was settled through consensus. Authors were contacted via e-mail when relevant data could not be extracted or data were missing. Studies were excluded if authors did not respond to the e-mail.

The Cochrane Collaboration's tool for assessing risk of bias was used to evaluate the quality of the selected papers [20]. Four researchers independently evaluated the quality of each study and resolved any case of disagreement by consensus.

2.4 Data synthesis and statistical analysis

The main outcome measure for the meta-analysis was effect size (Cohen's d). The standardized mean difference (SMD) was computed. The SMD measure is a point estimate of the effect of a treatment. The magnitude of 0.2, 0.5, and 0.8 was considered as small, medium, and large effect size, respectively. We extracted data on SD differences from two trials [13, 14] to calculate the correlation coefficients. An r of 0.5 was finally used in the analysis of FPG, LDL, TG, and systolic and diastolic blood pressure. An r of 0.8 was used for other outcomes. A sensitivity analysis was performed by using different correlation coefficients (0.2, 0.5, and 0.8) to test the stability of the data results.

The overall effect size was estimated by entering the individual effect size for each study into a random effect model with DerSimonian and Laird weighting [21]. Statistical heterogeneity was assessed using Cochran's Q and I^2 test with random inverse-variance heterogeneity [22]. I^2 values >50% were considered as moderate heterogeneity. Subgroup analysis was performed based on study design (parallel and crossover), ethnicity (American, European, and Asian), gender distribution (≥50% men and <50% men), duration of diabetes (≤6 years and >6 years), intervention period (≤8 weeks and >8 weeks), treatment dose (≤20 and >20 g soy protein per day, ≤80 and >80 mg isoflavones per day), and risk of bias (low risk and unclear compared to high risk). The median of each classification was used as the basis of categorizing duration of diabetes, intervention period, and treatment dose for the subgroup analysis.

The method proposed by Altman and Bland was adopted for the analysis of interactions between subgroups [23]. To find sources of heterogeneity in the effect sizes, meta-regression analyses were performed by considering the participants' age, BMI, duration of diabetes, intervention period, and treatment dose.

The "leave-one-out" method was used for sensitivity analysis and determination of the effects of each particular study on the pooled results, measurement of the reliability of the meta-analysis results, and assessment of the robustness of the findings regarding the primary endpoint. In cases of moderate or high heterogeneity ($I^2 \geq 50\%$), the "hetred" command in Stata was used to evaluate the change in between-study heterogeneity when one or more outlier studies were excluded from the calculations [24].

Funnel plots were created for the graphical assessment of the presence and extent of publication bias. Statistical analysis of the publication bias was conducted using Egger's weighted regression and Begg's rank correlation tests. In the case of statistical evidence of funnel plot asymmetry, the "trim-and-fill" method was applied [25]. Analyses were performed using Stata/MP v.13.0 software (Stata Corporation, College Station, Texas) and RevMan version 5.3 software. All tests were two-tailed and p-values less than 0.05 were considered significant.

2.5 Evidence quality

The Grading of Recommendations Assessment, Development, and Evaluation (GRADE) system was used to evaluate the quality of the evidence

Table 1. Overview of studies investigating the effects of soy consumption on clinical indices in patients with type 2 diabetes

Study	Country	Design	n (m/f)	POM (%)	Age (yr)	BMI (kg/m^2)	Duration of diabetes (yr)	Case/ control	Length (wk)	ISP (g)	ISF (mg)	Quality score
Azadbakht, 2003	Iran	crossover	14 (10/4)	-	62.5±12.1	26.6±4.0	10.0±4.0	14/14	7	17.8	42.3	high
Azadbakht, 2008	Iran	parallel	41 (18/23)	-	62.0±12.0	-	10.0±3.0	20/21	204	16	43	high
Ble-Castilo, 2010	Mexico	crossover	28 (4/24)	-	51.7±5.6	34.9±2.3	-	28/28	4	4.92	-	high
Chang, 2008	Korea	parallel	20 (8/12)	-	55.8±2.7	24.8±0.2	6.4±1.5	10/10	4	23.9	-	high
Curtis, 2012	UK	parallel	93 (0/93)	100	62.6±7.5	32.3±9.5	6.4±8.5	47/46	48	-	100	high
Daoroong, 2009	Thailand	parallel	38 (16/22)	-	61.3±9.2	25.3±2.1	-	19/19	6	30	32	unclear
Gobert, 2010	Canada	crossover	29 (16/13)	-	60.1±9.6	29.6±4.1	3.4±4.8	29/29	8.14	40	88	high
Gonzalez, 2007	UK	crossover	26 (0/26)	100	-	-	-	26/26	12	-	132	high
Hermansen, 2001	Denmark	crossover	20 (14/6)	-	63.6±7.5	30.2±4.2	3.0±2.7	20/20	6	50	165	high
Jayagopal, 2002	UK	crossover	32 (0/32)	-	62.5±6.8	32.2±5.0	2.6±2.7	32/32	12	30	132	low
Konya, 2014	UK	parallel	37 (27/10)	100	64.1±7.6	30.2	4.3±3.7	26/11	8	10.5	32	unclear
Miraghajani, 2012	Iran	crossover	25 (10/15)	24	51.0±10.0	28.0±4.0	-	25/25	4	6	-	high
Salari Moghaddam, 2014	Iran	crossover	30 (0/30)	0	45.7±3.8	29.5±3.9	-	30/30	6	-	-	high
Setchell, 2015	Italy	crossover	10 (5/5)	-	62.7±7.3	28.8±3.2	-	10/10	8	.8	32	high
Teixiera, 2004	USA	crossover	14 (14/0)	-	-	29.8±0.8	-	14/14	8	-	-	high
Vaisman, 2009	Brazil, Israel, Netherlands, UK, USA,	parallel	14	-	76.2±12.8	26.9±4.0	9.0±7.8	7/7	12	4.3	-	high

Legend: POM: postmenopausal, BMI: Body mass index, ISP: isolated soy protein, ISF: isoflavones.

for each primary outcome across all selected studies. Two reviewers (RS and MMJ) independently evaluated the level of confidence in estimates of effect for each outcome based on the GRADE criteria.

3. Results

3.1 Study characteristics

A total of 16 studies [9-18, 26-34], including six parallel and 10 crossover RCTs, was identified for inclusion in the study (**Table 1**). The results of three RCTs were published in several articles [15, 16, 26, 27, 32, 33]. Also, Konya described more than two intervention groups in her RCT [14]. The study selection process is depicted in **Figure 1**. Among the selected studies, six trials were conducted in Asia, six in Europe, and three in North America. Only one trial was multi-centric.

In total, data from 471 participants, including 315 females (68.9%), were pooled and evaluated. In most studies, females slightly outnumber males. Four studies noted that enrolled women were postmenopausal [11, 13, 15, 35]. In total, about 43% of women were in the postmenopausal period. The mean age of the patients in the selected studies ranged between 45.7 and 76.2 years. All studies enrolled overweight or obese participants with diabetes (BMI range: 24.8-34.9 kg/m^2). Six studies involved patients with diabetic complications (such as nephropathy, retinopathy, or hypertension) [12, 15, 26, 27, 30, 34]. One study included 25 patients with diabetes on tube feeding [18].

The included trials used different types and/or doses of soy products (**Table 1**). In nine out of 16 included studies, both isolated soy protein and isoflavones were used [12, 14, 17, 18, 26, 27, 29-31]. Also, the amount of soy protein or isoflavone levels varied from 0.8 to 50 g and from 32 to 165 mg, respectively. The duration of intervention periods ranged from four weeks [9, 10, 15] to four years [27]. In the crossover studies, the range of washout period was 0-4 weeks. Different methods,

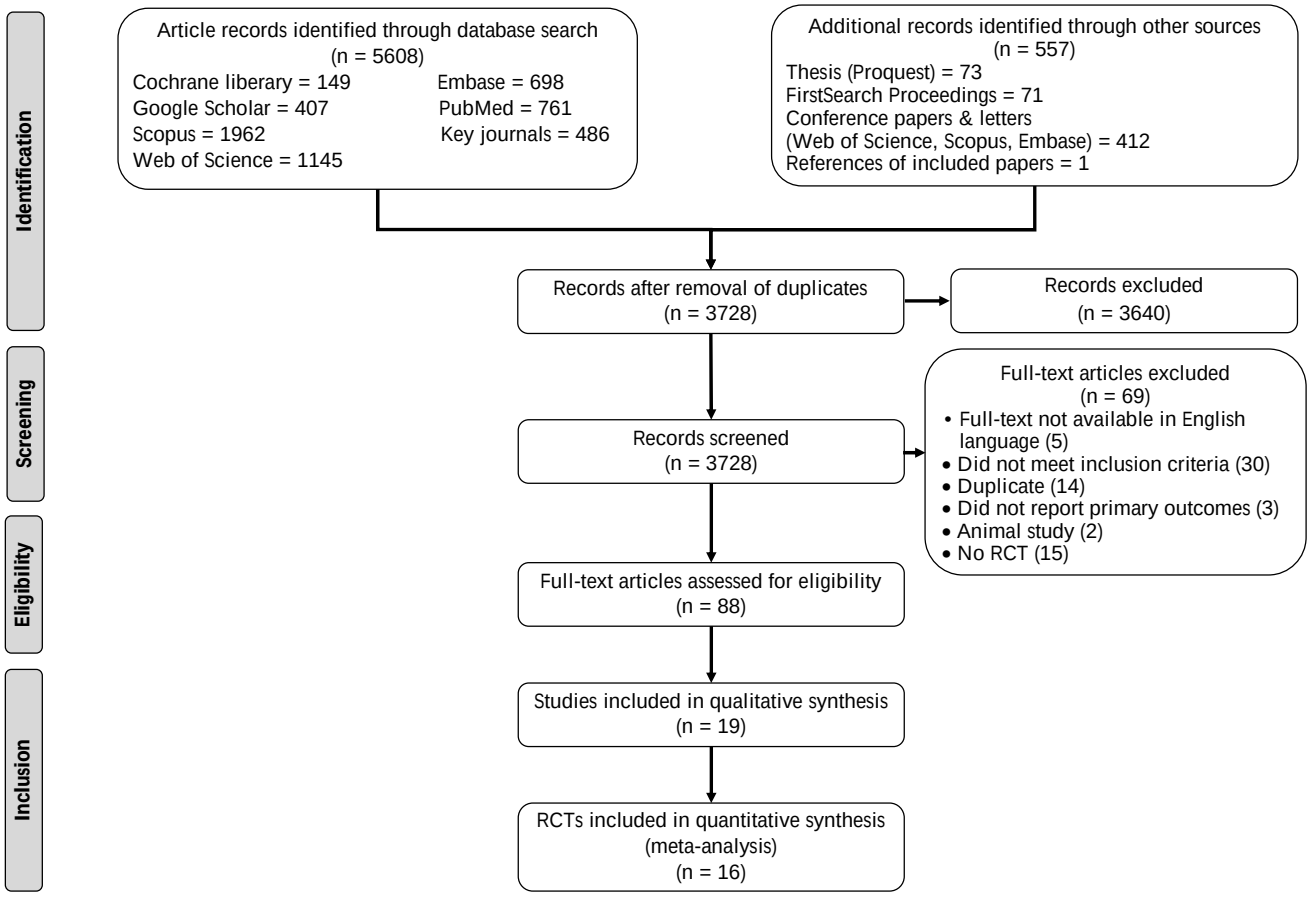

Figure 1. Flowchart of the inclusion and exclusion criteria applied in the study.

from subjective assessment to the measurement of isoflavone and its metabolites [3, 11, 34] or phytoestrogen [27, 35], were applied to determine adherence to treatment.

3.2 Risk of bias

Among the 16 selected RCTs, only one had low and two had an unclear risk of bias. The risk of bias was high in the remaining articles. Except for four studies, all the other studies had a low or unclear risk of bias in the sequence of generation domain. Only two studies had a high risk of bias in the allocation concealment domain. Furthermore, seven RCTs reported incomplete outcome data.

3.3 Primary outcomes

According to the data from 14 RCTs, soy consumption had no significant effect on FPG levels (Cohen's d: -0.14; 95% CI: -0.29 to 0.02; p = 0.09; I^2 = 0.0%). Three parallel and eight crossover RCTs evaluated the impact of soy on insulin. The pooling of these studies revealed no significant differences between the intervention and control groups (Cohen's d: -0.11; 95% CI: -0.32 to 0.11; p = 0.34; I^2 = 39.2%). Five parallel and eight crossover RCTs reported the relation between soy consumption and HbA1c. Data pooled from these studies showed no significant differences in HbA1c levels between the intervention and placebo groups (Cohen's d: -0.22; 95% CI: -0.54 to 0.10; p = 0.18; I^2 = 71.8%). **Figure 2** shows forest plots for the impact of soy consumption on primary outcomes. Sensitivity analysis using the "leave-one-out" method indicated the robustness of the effect sizes of the primary outcomes.

Although the results of Egger's linear regression test and Begg's rank correlation test did not suggest the presence of a publication bias in the

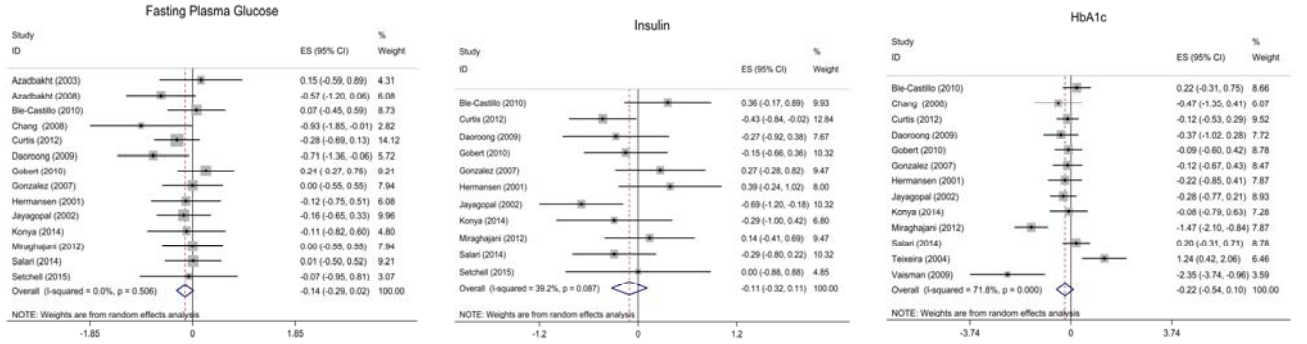

Figure 2. Forest plot showing standardized mean difference (Cohen's d) and 95% confidence intervals (95% CI) for the impact of soy consumption on primary outcomes (FPG, insulin, and HbA1c).

meta-analysis of FPG outcome, the funnel plot of SE by effect size was slightly asymmetric. Using the "trim-and-fill" method and imputation of one potentially missing study on the left side of the plot, an adjusted effect size (Cohen's d) of -0.18 was obtained (95% CI: -0.33 to -0.02; p = 0.03).

Using the hetred command in Stata to reduce the heterogeneity of meta-analysis, as measured by I^2, showed that the exclusion of two studies (Miraghjani *et al.* [32] and Vaisman *et al.* [18]) decreased the heterogeneity of the pooled estimate of HbA1c to 31.1%. However, their exclusion did not change the pooled effect of this primary outcome (Cohen's d: -0.03; 95% CI: -0.24-0.18; p = 0.75; I^2 = 31.1%). Sensitivity analysis for different correlation coefficient values (i.e. 0.20, 0.50, and 0.80) showed no statistically significant differences in the primary outcomes.

3.4 Evaluation of heterogeneity

The overall Cohen's d for HbA1c was -0.22 with significant heterogeneity between studies (p < 0.01; I^2 = 71.8%). Consequently, we used meta-regression with Knapp-Hartung modification for our meta-analysis. No statistically significant source of heterogeneity was identified in a meta-regression analysis of age, BMI, duration of diabetes, intervention period, and treatment dose (isolated soy protein or isoflavones) (p > 0.10 for each).

3.5 Secondary outcomes

Data from eight studies were pooled and the effects of soy and placebo consumption on HOMA-IR were compared. A significant reduction was observed in the SMD (Cohen's d: -0.25; 95% CI: -0.44 to -0.06; p < 0.01; I^2 = 0.0%). The data from the 14 studies showed a stronger TC reduction in subjects who consumed soy compared to those who received placebo (Cohen's d: -0.47; 95% CI: -0.72 to -0.21; p < 0.01; I^2 = 59.8%). Soy consumption appeared to be beneficial in decreasing LDL, TG, and systolic blood pressure, but had no significant effects on HDL or diastolic blood pressure. No evidence of publication bias was found for studies that examined soy consumption and secondary outcomes.

3.6 Sensitivity analysis and publication bias

Based on participant characteristics and study design, subgroup analysis was performed with seven items including participants' ethnicity, sex, duration of diabetes, dosage and duration of intervention, and quality of the study (**Table 2**). Fourteen studies reported the effect of soy consumption on FPG. Subgroup analysis was performed according to study design, including 5 parallel studies with 185 participants and 9 crossover studies with 185 participants. Cohen's d for FPG was -0.43 (95% CI -0.70, -0.17) in parallel studies and 0.02 (95% CI -0.17, 0.21) in crossover studies. Interaction analysis suggested that the study design had a significant interaction effect on FPG (p < 0.01). Compared to participants of crossover studies, subjects in parallel studies were older (61.8 ± 8.7 versus 56.0 ± 10.0 years, p < 0.001) and had longer diabetes duration (6.7 ± 0.7 versus 3.7 ± 0.4 years, p = 0.003).

The evaluation of subgroups regarding insulin yielded the following results: while parallel studies with 133 participants revealed statistical differences between intervention and control groups (Cohen's d: -0.37; 95% CI -0.68, -0.05), crossover studies with 177 participants did not. Studies with a low risk of bias (3 studies) also revealed significant differences (Cohen's d: -0.47; 95% CI -0.82, -

Table 2. Determination of the effects of soy consumption on clinical indices and subgroup analysis

Parameter	Value	FPG (n = 14)			Insulin (n = 11)			HbA1c (n = 13)		
		No. of studies	Pooled Cohen's d (95% CI)	I^2	No. of studies	Pooled Cohen's d (95% CI)	I^2	No. of studies	Pooled Cohen's d (95% CI)	I^2
Study design	Parallel	5	-0.43(-0.70, -0.17)	0.0%	3	-0.37(-0.68, -0.05)	0.0%	5	-0.44(-0.55, 0.30)	58.5%
	Crossover	9	0.02(-0.17, 0.21)	0.0%	8	-0.01(-0.29, 0.26)	46.2%	8	0.15(-0.47, 0.77)	88.7%
Ethnicity	Americans	2	0.16(-0.21, 0.52)	0.0%	2	0.10(-0.40, 0.60)	46.2%	3	1.38(-0.28, 0.24)	94.5%
	Europeans	6	-0.15(-0.38, 0.08)	0.0%	6	-0.16(-0.52, 0.20)	55.5%	5	-0.17(-0.40, -0.07)	0.0%
	Asians	6	-0.29(-0.62, 0.04)	37.0%	3	-0.14(-0.46, 0.19)	0.0%	4	-0.52(-1.28, 0.24)	81.8%
Sex	< 50% men	9	-0.21(-0.41, -0.02)	11.1%	4	-0.14(-0.44, 0.15)	53.2%	8	-0.27(-0.62, 0.08)	67.4%
	≥ 50% men	5	0.05(-0.25, 0.35)	0.0%	7	-0.02(-0.34, 0.31)	0.0%	4	0.85(-0.44, 2.15)	92.3%
Duration of diabetes	≤ 6 years	4	-0.02(-0.30, 0.26)	0.0%	4	-0.20(-0.65, 0.24)	57.2%	4	-0.14(-0.54, 0.25)	0.0%
	> 6 years	4	-0.36(-0.71, -0.00)	21.8%	1	-0.43(-0.84, -0.02)	-	3	-0.79(-1.83, 0.25)	78.2%
Intervention period	≤ 8 weeks	9	-0.13(-0.34, 0.08)	0.0%	7	0.02(-0.21, 0.25)	0.9%	8	-0.33(-0.85, 0.19)	76.2%
	> 8 weeks	5	-0.14(-0.29, 0.02)	13.8%	4	-0.27(-0.64, 0.11)	57.8%	5	0.11(-0.59, 0.81)	88.2%
Treatment dose (soy protein)	≤ 20 g / day	6	-0.09(-0.34, 0.18)	0.0%	4	0.12(-020, 0.43)	0.0%	3	-0.44(-1.49, 0.61)	88.4%
	> 20 g / day	5	-0.26(-0.64, 0.13)	48.4%	4	-0.20(-0.64, 0.23)	57.1%	5	-0.25(-0.52, 0.02)	0.0%
Treatment dose (isoflavones)	≤ 80 mg / day	5	-0.33(-0.65, 0.00)	2.6%	3	-0.22(-0.64, 0.20)	0.0%	2	-0.24(-0.71, 0.24)	0.0%
	> 80 mg/ day	5	-0.09(-0.31, 0.14)	0.0%	5	-0.15(-0.53, 0.23)	63.7%	5	-0.16(-0.39, 0.06)	0.0%
Risk of bias	low risk & unclear	3	-0.30(-0.66, 0.05)	6.4%	3	-0.47(-0.82, -0. 12)	0.0%	3	-0.26(-0.60, 0.08)	0.0%
	high risk	11	-0.09(-0.27, 0.08)	0.0%	8	0.00(-0.23, 0.23)	31.2%	10	-0.07(-0.63, 0.50)	87.5%
Overall		14	-0.14(-0.29, 0.02)	0.0%	11	-0.11(-0.32, 0.11)	39.2%	13	-0.22(-0.54, 0.10)	83.5%

0.12). This result was different from the overall pooled analysis (**Table 2**).

According to interaction analysis, "country" had a significant interaction effect on HbA1c. We also conducted a meta-regression analysis of six covariates to determine sources of heterogeneity. None of the evaluated variables served as a confounding factor in the pooled estimate effect of FPG. However, the results showed that about one-fifth of the reduction in the heterogeneity of insulin outcome depended on the intervention period.

3.7 Quality assessment of evidence

Quality assessment based on the GRADE approach suggested low quality of evidence for primary outcomes. This finding was caused by the risk of bias and imprecision in each primary outcome. Inconsistency was also present in the case of HbA1c. A summary of the results of the quality assessment is provided in **Table 3**.

4. Discussion

The most important finding of this meta-analysis was that soy consumption did not significantly improve FPG or insulin in patients with T2D compared with placebo. In contrast, soy consumption had a favorable effect on FPG or insulin in the pooled effect sizes of parallel studies.

The principal problem associated with the included crossover studies was the short washout period (0-4 weeks) which carried the risk of carry-over effects, i.e. the effects of an intervention given in one period persist into a subsequent period, thus interfering with the effects of the second intervention. Food and nutrition research experts recommend that the length of the washout period should be the same as the feeding period to allow measured outcomes to return to baseline values or become re-stabilized [36].

Also, important differences existed in demographic characteristics between the two different types of trials. Crossover trials included younger participants with less duration of diabetes. It is therefore advisable to meta-analyze parallel and crossover trials in separate subgroups, irrespective of whether they are also combined [22]. Another important issue that should be noted is that after soy ingestion, bacterial β-glucosidases in the intestinal wall generate biologically active aglycones (genistein, daidzein, and glycitein). About 30%-50% of humans can metabolize daidzein into equol [37]. Although equol exhibited higher affinity to estrogen receptor α than any of the isoflavones originating from soy [38], serum or urinary levels of equol were not measured in most RCTs administering soy isoflavones.

Similarly to FPG and insulin, HbA1c was not significantly affected by soy consumption. It is noteworthy that there was a large heterogeneity in this outcome. The included trials used different methods for HbA1c measurement. High performance liquid chromatography (HPLC) measures all types of hemoglobin and has been recommended as

Table 3. Meta-analysis grades (question: does soy compared to placebo improve clinical indices in patients with type 2 diabetes?)

Certainty assessment							No. of patients		Effect	Certainty	Importance
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Soy	placebo	Absolute (95% CI)		
Fasting plasma sugar (follow-up: median 8 weeks; scale from: -0.93 to 0.24)											
14	Randomized trials	Serious	Not serious	Not serious	Serious	None	336	321	Cohen's d **0.14 SD lower** (0.29 lower to 0.02 higher)	Low	Not important
Insulin (follow-up: median 8 weeks; scale from: -0.43 to 0.36)											
11	Randomized trials	Serious	Not serious	Not serious	Serious	None	339	322	Cohen's d **0.11 SD lower** (0.32 lower to 0.11 higher)	Low	Not important
HbA1c (follow-up: median 8 weeks; scale from: -1.56 to 4.6)											
13	Randomized trials	Serious	Serious	Not serious	Serious	None	313	297	Cohen's d **0.22 SD lower** (0.54 lower to 0.1 higher)	Very low	Not important

an acceptable standard. Turbidimetric immunoassays that use the HbA1c antibody have lower precision than HPLC [39]. On the other hand, two studies involved patients with diabetic nephropathy [15, 40]. In a study on patients with uremia and normal glucose tolerance, de Boer *et al*. found that HbA1c measured by ion exchange chromatography was significantly elevated irrespective of the degree of glucose intolerance [41].

We observed a significant interaction effect between soy consumption and HbA1c, ethnicity ($p < 0.001$) and male percentage ($p = 0.02$). Only Curtis *et al*. measured urinary levels of equol in participants [11]. It is well known that western adults are capable of producing significantly lower equol levels than Asian adults (20%-30% vs. 50%-60%) [42]. A majority of studies were conducted in postmenopausal women and in men. Postmenopausal women have low amounts of estrogen and may benefit most from the consumption of isoflavones. In men, however, because of faster excretion rates, higher concentrations may be needed to exert the same biological effects [43].

Although there was significant heterogeneity in the pooled estimate of HbA1, we could not identify a significant source in the meta-regression analysis. Since meta-regression is an observational link between trials, the relationships between effect size and covariates within trials may not be the same as such relationships across trials. This might be caused by confounding effects at either the trial level or the individual level (aggregation bias) [44].

Assessment of secondary outcomes showed significant reductions in serum HOMA-IR, TG, LDL, TC, and systolic blood pressure following the consumption of soy products. These findings are in line with previous meta-analyses in diabetic populations [7, 8]. No significant associations were observed between either HDL or diastolic blood pressure and soy consumption.

5. Relation to previous literature

Yang *et al*. performed a meta-analysis of seven trials with 172 participants and reported the absence of significant relationships between soy consumption and changes in FPG, insulin, and HbA1c levels in patients with T2D [7]. A more recent meta-analysis of 10 trials with 290 participants indicated that soy consumption significantly reduced these parameters in patients with T2D or metabolic syndrome [8]. However, a problem with this meta-analysis was a unit-of-analysis error in the effects of soy consumption on clinical indices.

In our meta-analysis, 16 trials with 471 participants were included and subgroup analysis and meta-regression were applied to detect the sources of heterogeneity. The results demonstrated the stability of the pooled effect sizes. Our results are in agreement with the first systematic review and meta-analysis that showed no association between soy consumption and changes in FPG and insulin in the overall pooled data [7]. Similarly to our meta-analysis, most included studies in Yang's meta-analysis had a crossover design. When we

conducted a subgroup analysis by study design, the effect of soy on FPG and insulin was significantly different from that of placebo in the parallel studies.

Assessments based on GRADE criteria revealed low levels of confidence for each primary outcome. Therefore, these findings should be interpreted with caution. Further high-quality RCTs are required to clarify the impact of soy/isoflavones on improving clinical indices in T2D patients.

6. Limitations

This meta-analysis had several limitations which should be considered in the interpretation of the results. Six studies involved patients with diabetic complications. Differences in the methods of measurement, inclusion criteria (e.g. age range and health situation), and exclusion criteria (e.g. disease status and medication use) may have been responsible for the observed heterogeneity in HbA1c. Also, most of the included trials were judged to have a high risk of bias. This is largely due to selection bias and attrition bias. Serum or urinary levels of equol were not measured in most studies. Further robust RCTs are, therefore, warranted to validate the effects of soy on primary outcomes and to determine the impact of equol production and the optimal dose of soy/isoflavones.

7. Conclusions

In this study, multiple databases were searched to identify relevant studies. We identified studies from America, Europe, and Asia, which increased the generalizability of the results. A relatively large number of subjects provided sufficient evidence for the reliability of the results. Sensitivity analysis using the "leave-one-out" method showed the reliability of the results of the meta-analysis, and the robustness of the findings in terms of the primary endpoint. No evidence of publication bias of the studies was found using Egger's regression test.

Although this systematic review and meta-analysis indicated no beneficial effects of soy consumption on FPG, insulin, and HbA1c in patients with T2D, subgroup analyses using studies with parallel design showed a significant improvement (moderate effect size) in FPG and insulin. Considering the high heterogeneity in HbA1c and possible covariates such as age, equol production, and soy dosage between studies, more high-quality articles are required to reach a more reliable conclusion.

Author contributions: All authors contributed to the study concept and design, literature search, and article evaluation. RS and MMJ performed statistical analysis of the data and wrote the first draft. SS, TH, and MM-R finalized the manuscript. All authors participated in research methodology and data interpretation.

Disclosures: The authors report no conflict of interests.

■ References

1. **Roglic G.** WHO global report on diabetes: a summary. *Int J Noncommun Dis* 2016. 1:3-8.
2. **Krook A, Holm I, Pettersson S, Wallberg-Henriksson H.** Reduction of risk factors following lifestyle modification programme in subjects with type 2 (non-insulin dependent) diabetes mellitus. *Clin Physiol Funct Imaging* 2003. 23:21-30.
3. **Cater NB, Garg A.** The effect of dietary intervention on serum lipid levels in type 2 diabetes mellitus. *Curr Diab Rep* 2002. 2:289-294.
4. **Knowler W, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM, Diabetes Prevention Program Research Group.** Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002. 346(6):393-403.
5. **de Campos Zani SC, Wu J, Chan CB.** Egg and soy-derived peptides and hydrolysates: a review of their physiological actions against diabetes and obesity. *Nutrients* 2018. 10(5):549.
6. **Cederroth CR, Nef S.** Soy, phytoestrogens and metabolism: a review. *Mol Cell Endocrinol* 2009. 304:30-42.
7. **Yang B, Chen Y, Xu T, Yu Y, Huang T, Hu X, Li D.** Systematic review and meta-analysis of soy products consumption in patients with type 2 diabetes mellitus. *Asia Pac J Clin Nutr* 2011. 20:593-602.
8. **Zhang XM, Zhang YB, Chi MH.** Soy protein supplementation reduces clinical indices in type 2 diabetes and metabolic syndrome. *Yonsei Med J* 2016. 57(3):681-689.
9. **Ble-Castillo JL, Aparicio-Trapala MA, Francisco-Luria MU, Cordova-Uscanga R, Rodriguez-Hernandez A, Mendez JD, Diaz-Zagoya JC.** Effects of native banana starch supplementation on body weight and insulin sensitivity in obese type 2 diabetics. *Int J Environ Res Public Health* 2010. 7(5):1953-1962.
10. **Chang JH, Kim MS, Kim TW, Lee SS.** Effects of soybean supplementation on blood glucose, plasma lipid levels, and erythrocyte antioxidant enzyme activity in type 2 diabetes mellitus patients. *Nutr Res Pract* 2008. 2(3):152-157.
11. **Curtis PJ, Sampson M, Potter J, Dhatariya K, Kroon PA, Cassidy A.** Chronic ingestion of flavan-3-ols and isoflavones improves insulin sensitivity and lipoprotein status and attenuates estimated 10-Year CVD risk in medicated postmenopausal women with type 2 diabetes. *Diabetes Care* 2012. 35(2):226-232.
12. **Daoroong K, Oranong K, Kulwara M.** Effects of soy

protein isolate supplementation on insulin resistance, fasting blood glucose and lipid profile in type 2 diabetic outpatients at public health center 66, health department, bangkok metropolitan administration. Chulalongkorn University, 2009. Thesis.
13. **Gonzalez S, Jayagopal V, Kilpatrick ES, Chapman T, Atkin SL.** Effects of isoflavone dietary supplementation on cardiovascular risk factors in type 2 diabetes. *Diabetes Care* 2007. 30:1871-1873.
14. **Konya J.** The influence of dietary and other environmental changes on vascular risk markers in type 2 diabetes. University of Hull and the University of York, 2014. Thesis.
15. **Miraghajani MS, Esmaillzadeh A, Najafabadi MM, Mirlohi M, Azadbakht L.** Soy milk consumption, inflammation, coagulation, and oxidative stress among type 2 diabetic patients with nephropathy. *Diabetes Care* 2012. 35(10):1981-1985.
16. **Salari Moghaddam A, Entezari MH, Iraj B, Askari GR, Maracy MR.** The effects of consumption of bread fortified with soy bean flour on metabolic profile in type 2 diabetic women: a cross-over randomized controlled clinical trial. *Int J Prev Med* 2014. 5(12):1529-1536.
17. **Setchell KD, Nardi E, Battezzati PM, Asciutti S, Castellani D, Perriello G, Clerici C.** Novel soy germ pasta enriched in isoflavones ameliorates gastroparesis in type 2 diabetes: A pilot study. *Diabetes Care* 2013. 36(11):3495-3497.
18. **Vaisman N, Lansink M, Rouws CH, van Laere KM, Segal R, Niv E, Bowling TE, Waitzberg DL, Morley JE.** Tube feeding with a diabetes-specific feed for 12 weeks improves glycaemic control in type 2 diabetes patients. *Clin Nutr* 2009. 28(5):549-555.
19. **Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA, PRISMA-P Group.** Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev* 2015. 4:1.
20. **Higgins J, Altman D, Sterne J.** Cochrane handbook for systematic reviews of interventions version 5.2 (updated June 2017). Cochrane, 2017 Higgins J, Churchill R, Chandler J, Cumpston M (eds.) Available from www.training.cochrane.org/handbook. Accessed 4/6/2018.
21. **Hedges LV, Vevea JL.** Fixed-and random-effects models in meta-analysis. *Psychol Methods* 1998. 3(4):486-504.
22. **Higgins JP, Green S.** Cochrane handbook for systematic reviews of interventions version 5.1.0 (updated 2011 Mar). John Wiley and Sons, 2011.
23. **Altman DG, Bland JM.** Interaction revisited: the difference between two estimates. *BMJ* 2003. 326(7382):219.
24. **Patsopoulos NA, Evangelou E, Ioannidis JP.** Sensitivity of between-study heterogeneity in meta-analysis: proposed metrics and empirical evaluation. *Int J Epidemiol* 2008. 37(5):1148-1157.
25. **Duval S, Tweedie R.** Trim and fill: a simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. *Biometrics* 2000. 56(2):455-463.
26. **Azadbakht L, Shakerhosseini R, Atabak S, Jamshidian M, Mehrabi Y, Esmaill-Zadeh A.** Beneficiary effect of dietary soy protein on lowering plasma levels of lipid and improving kidney function in type II diabetes with nephropathy. *Eur J Clin Nutr* 2003. 57(10):1292-1294.
27. **Azadbakht L, Atabak S, Esmaillzadeh A.** Soy protein intake, cardiorenal indices, and C-reactive protein in type 2 diabetes with nephropathy. *Diabetes Care* 2008. 31(4):648-654.
28. **Azadbakht L, Esmaillzadeh A.** Soy-protein consumption and kidney-related biomarkers among type 2 diabetics: a crossover, randomized clinical trial. *J Ren Nutr* 2009. 19(6):479-486.
29. **Gobert CP, Pipe EA, Capes SE, Darlington GA, Lampe JW, Duncan AM.** Soya protein does not affect glycaemic control in adults with type 2 diabetes. *Br J Nutr* 2010. 103(3):412-421.
30. **Hermansen K, Sondergaard M, Hoie L, Carstensen M, Brock B.** Beneficial effects of a soy-based dietary supplement on lipid levels and cardiovascular risk markers in type 2 diabetic subjects. *Diabetes Care* 2001. 24(2):228-233.
31. **Jayagopal V, Albertazzi P, Kilpatrick ES, Howarth EM, Jennings PE, Hepburn DA, Atkin SL.** Beneficial effects of soy phytoestrogen intake in postmenopausal women with type 2 diabetes. *Diabetes Care* 2002. 25(10):1709-1714.
32. **Miraghajani MS, Najafabadi MM, Surkan PJ, Esmaillzadeh A, Mirlohi M, Azadbakht L.** Soy milk consumption and blood pressure among type 2 diabetic patients with nephropathy. *J Ren Nutr* 2013. 23(4):277-282.
33. **Salari Moghaddam A, Entezari MH, Iraj B, Askari G, Sharifi Zahabi E, Maracy MR.** The effects of soy bean flour enriched bread intake on anthropometric indices and blood pressure in type 2 diabetic women: a crossover randomized controlled clinical trial. *Int J Endocrinol* 2014. 2014:240760.
34. **Teixeira SR, Tappenden KA, Carson L, Jones R, Prabhudesai M, Marshall WP, Erdman JW Jr.** Isolated soy protein consumption reduces urinary albumin excretion and improves the serum lipid profile in men with type 2 diabetes mellitus and nephropathy. *J Nutr* 2004. 134(8):1874-1880.
35. **Konya J, Rigby A, Sathyapalan T, Kilpatrick ES, Atkin SL.** Effects of different soy preparations on glycaemic control and cardiovascular disease risk in type 2 diabetes: A randomised placebo-controlled double-blind parallel study. *Diabet Med* 2014. 31:63-64.
36. **Agriculture and Agri-food Canada.** Best practice for food-based clinical trials: guidance for planning, conducting and reporting on human studies to support health claims. Ottawa, Minister of Agriculture and Agri-food Canada, 2013. Catalogue No: A72-119/2103E-PDF.
37. **Atkinson C, Frankenfeld CL, Lampe JW.** Gut bacterial metabolism of the soy isoflavone daidzein: exploring the relevance to human health. *Exp Biol Med (Maywood)* 2005. 230:155-170.
38. **Wang S, Wang Y, Pan MH, Ho CT.** Anti-obesity molecular mechanism of soy isoflavones: weaving the way to new therapeutic routes. *Food Funct* 2017. 8:3831-846.
39. **Ozcelik F, Yiginer O, Serdar MA, Kurt I, Oztosun M, Arslan E, Orhun A.** Comparison of three methods for measurement of HbA1c. *Turk Biyokim Derg* 2010. 35: 344-349.
40. **Teixeira S, Tappenden K, Marshall W, Carson L, Ringenberg M, Erdman J.** Effects of soy protein on diabetic nephropathy and blood lipids in type 2 diabetes mellitus. *J Nutr* 2002. 132(3):584S-585S.
41. **De Boer MJ, Miedema K, Casparie A.** Glycosylated haemoglobin in renal failure. *Diabetologia* 1980. 18(6):437-440.

42. **Setchell KD, Cole SJ.** Method of defining equol-producer status and its frequency among vegetarians. *J Nutr* 2006. 136(8):2188-2193.
43. **Orgaard A, Jensen L.** The effects of soy isoflavones on obesity. *Exp Biol Med (Maywood)* 2008. 233(9):1066-1080.
44. **Thompson SG, Higgins JP.** How should meta-regression analyses be undertaken and interpreted? *Stat Med* 2002. 21(11):1559-1573.

